**Imbalance of growth factor signalling in diabetic kidney disease: is connective tissue growth factor (CTGF, CCN2) the perfect intervention point?**

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**Introduction**

The prevalence of diabetes mellitus and its clinical complications are increasing rapidly worldwide. Diabetic nephropathy has already become the leading cause of end-stage renal disease in developed countries and is thus forming an increasing clinical problem [1]. Mesangial matrix accumulation and glomerular basement membrane (GBM) thickening are primary structural alterations characteristic for diabetic nephropathy [2]. These structural changes are accompanied by increased permeability of the GBM for proteins, resulting in increased urinary albumin excretion (UAE). Growth factors and cytokines such as transforming growth factor-β (TGF-β), growth hormone, insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF) play important roles in the development of diabetic nephropathy [2,3]. In addition to these ‘pro-fibrotic’ growth factors, the ‘anti-fibrotic’ growth factor bone morphogenetic protein-7 (BMP-7) has been reported to be downregulated in diabetic nephropathy, and addition of BMP-7 in experimental diabetes is capable of antagonizing renal fibrosis [4]. TGF-β is generally accepted to be the main pro-fibrotic factor in diabetic nephropathy. Several studies have reported that TGF-β is upregulated in diabetes and induces matrix accumulation in vitro and in vivo. Inhibition of TGF-β by administration of neutralizing antibodies or antisense oligonucleotides, or by blocking its downstream signalling pathway all result in prevention of the fibrotic process in diabetic nephropathy [1]. However, since TGF-β also has important anti-proliferative and anti-inflammatory effects, inhibition of TGF-β may be a double-edged sword. Therefore, alternative targets for therapeutic intervention are needed to treat this complication of diabetes mellitus. A few years ago, connective tissue growth factor (CTGF) was proposed as a potential target for anti-fibrotic strategies. The question was asked whether CTGF is just another factor in renal fibrosis, or a particularly interesting key player in tissue response to injury [5]. As will become apparent from this review, recent evidence does indeed support a prominent role for CTGF in the pathogenesis of diabetic nephropathy, both as a downstream effector of profibrotic TGF-β action and as an extracellular mediator of cross-talk between various growth factor signalling pathways implicated in diabetic kidney disease.

**Connective tissue growth factor (CTGF, CCN2)**

CTGF is involved in extracellular matrix remodelling during development and in pathological conditions, and has increasingly been recognized as a pro-fibrotic factor in diabetic renal changes. It is a 36–38 kDa, cysteine-rich, secreted protein belonging to the CCN family of matricellular proteins [6]. CTGF contains four modules: module 1 is an IGF-binding protein (IGFBP) domain, module 2 is a cysteine-rich von Willebrand type c (VWC) domain, module 3 is homologous to thrombospondin type 1 (TSP-1) and module 4 is a cysteine-rich C-terminal (CT) domain, found in several growth factors, including TGF-β [6]. CTGF’s modular structure explains its multiple interactions with the cell surface, extracellular matrix and other...
CTGF as a clinical marker for the development of diabetic nephropathy

UAE is used to identify diabetic patients who are at risk for development of nephropathy. However, recent studies show that the predictive value of microalbuminuria as a marker for the development of diabetic nephropathy is less than originally described [12]. Therefore, there is a need for additional risk markers to identify diabetic patients with incipient nephropathy. CTGF might be such a clinical marker since its expression is strongly associated with fibrotic disorders. Overexpression of CTGF in diabetic nephropathy was first described in human biopsies using in situ hybridization [13]. Subsequently, CTGF was found to be upregulated in renal cells stimulated with high glucose or advanced glycation end-products (AGEs), in glomeruli of experimental obese type 2 diabetes (i.e. the db/db mouse), and type 1 diabetes (the non-obese diabetic mouse) and in renal cortex of streptozotocin (STZ)-induced diabetic rats (reviewed in [2,5,14]). Studies in our laboratory showed that the CTGF upregulation was most prominent in the visceral epithelial cells (podocytes) of the glomeruli [15]. Glomerular CTGF mRNA levels were found to be upregulated in diabetic patients with microalbuminuria as well as in overt nephropathy [16]. In addition, CTGF mRNA levels were found to correlate with the degree of albuminuria [16]. Recently, the detection of CTGF in blood and urine of diabetic patients has been reported. Urinary CTGF levels were shown to be low in healthy volunteers and in four out of six diabetic patients without renal disease, while five out of seven patients with renal disease showed a significant increase in urinary CTGF excretion [17]. In a study of 31 type 1 diabetic patients, urinary CTGF excretion was significantly higher in patients with microalbuminuria or overt nephropathy as compared with patients with normal albuminuria [18]. Moreover, urinary CTGF excretion was closely correlated to the degree of albuminuria [18]. We observed that plasma CTGF levels of type 1 diabetic patients with nephropathy were elevated as compared to normoalbuminuric diabetic patients and correlated with both albuminuria and creatinine clearance [19]. In addition to its possible role as a marker for the development of diabetic nephropathy, determination of CTGF levels might also be useful to determine effects of treatment. Intervention studies in both type 1 and 2 diabetic patients with nephropathy showed that angiotensin II receptor blockers (ARBs) significantly reduced urinary CTGF excretion and that this reduction was correlated with a lower rate of decline of GFR [20,21]. It has to be noted, however, that all published studies in patients on the relationship between CTGF and diabetic kidney disease have been performed in relatively small numbers of subjects. Larger cross-sectional and prospective studies are currently in progress to assess whether CTGF is a useful clinical marker for the development and progression of diabetic nephropathy.

Pathogenic role of CTGF in diabetic nephropathy

CTGF has been shown to induce extracellular matrix proteins in vitro, and its expression in renal cells is upregulated by high glucose, mechanical strain, AGEs and TGF-β [2,5,14,22]. In addition, some studies performed in animal models of diabetes have provided indirect evidence for a role for CTGF in diabetic kidney disease. Accordingly, renal CTGF and fibronectin overexpression in STZ-diabetic rats was prevented by treatment with an inhibitor of AGE formation [23]. In addition, as mentioned above, our preliminary studies in diabetic patients with nephropathy showed that ARBs reduce urinary CTGF excretion and that plasma CTGF levels and urinary CTGF excretion correlate with nephropathy parameters. The only study so far reporting direct profibrotic effects of CTGF in vivo showed that CTGF is required in addition to TGF-β to induce persistent fibrosis [24]. An ongoing study in our laboratory using heterozygous CTGF +/- mice [25] shows that diabetes-induced GBM thickening was significantly attenuated in CTGF +/− mice in parallel with lower CTGF expression levels [26]. This suggests that CTGF overexpression in podocytes is critically involved in diabetes-related GBM thickening [26]. Conditional overexpression or disruption of CTGF in the context of diabetic nephropathy will ultimately give insight into the importance of CTGF as a pathogenic factor in diabetic nephropathy.

CTGF as potential target for therapeutic intervention

Due to concerns regarding possible risks of long-term TGF-β inhibition as a therapeutic approach to treat fibrotic diseases, CTGF was already recognized as a potential alternative target in 1997 [27]. In addition,
targeting of CTGF as a possible therapy specifically for diabetic nephropathy has been proposed by several investigators [5,2,14]. Some studies have been published in which CTGF inhibition was applied in an effort to attenuate renal fibrotic processes. *In vitro*, an antibody against CTGF partly inhibited the glucose-induced collagen production in human renal fibroblasts [9]. Glucose-induced elevated synthesis of fibronectin and plasminogen activator inhibitor-1 in human mesangial cell cultures was inhibited by CTGF

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**Fig. 1.** (a) Modular structure of CTGF. IGFBP = insulin-like growth factor-binding protein domain; VWC = von Willebrand type c domain; TSP-1 = thrombospondin 1 domain; CT = C-terminal domain. (b) Possible mechanism of the pro-fibrotic action of CTGF in diabetic kidney disease by modulating the signalling balance of key growth factors. CTGF contains four modules that associate with different growth factors, extracellular matrix proteins and cell surface proteins. The signalling activities of the different growth factors are influenced by binding to CTGF: IGF-I and TGF-β1 signalling activity is enhanced, while BMP-4 and VEGF signalling activity is reduced by CTGF binding. The enhanced IGF-I and TGF-β1 signalling is pro-fibrotic, while BMP signalling has been shown to reverse fibrosis (note that this has so far been shown for BMP-7 only, and binding of BMP-7 to CTGF has not been reported yet). VEGF signalling and angiogenic activity is inhibited by binding to CTGF, but is reactivated after cleavage of CTGF by MMPs. As well as the cleavage site as shown in the figure, other sites of cleavage by different proteases have been described, which are located predominantly between the modules. It thus appears that CTGF plays a key role in modulating the activity of several growth factors important in the development of diabetic kidney disease. In addition, CTGF might mediate cross-talk between signalling pathways by physical approximation of signalling receptors. MMP = matrix metalloprotease; IGF-I = insulin-like growth factor I; TGF-β = transforming growth factor-β; BMP = bone morphogenetic protein; VEGF = vascular endothelial growth factor; LRP = low-density lipoprotein receptor-related protein; HSPG = heparan sulfate proteoglycan.
antisense oligodeoxynucleotide (ODN) treatment [28]. Transfection of CTGF antisense ODN in cultured renal fibroblasts significantly attenuated TGF-β-stimulated upregulation of fibronectin [29]. The same investigators showed that CTGF antisense ODN treatment in vivo attenuated renal fibrosis in rats after unilateral ureteral obstruction [30]. Moreover, in a very recent study, administration of a neutralizing CTGF antibody to db/db mice for 2 months showed beneficial effects in terms of reduced renal hypertrophy, UAE and hyperfiltration, while glomerular hypertrophy was unchanged [31]. Further, the diabetes-induced GBM thickening was significantly attenuated in CTGF-antibody treated mice (cf. attenuated GBM thickening in CTGF +/− STZ mice in Roestenberg et al. [26]), while the diabetes-associated increase in total mesangial volume was unaffected by the treatment [31]. The safety and tolerability of the same CTGF antibody are currently being tested in a phase 1 clinical trial in patients with idiopathic pulmonary fibrosis. In addition to neutralizing antibodies and ODN, specific low molecular size inhibitors of CTGF are being developed and will be used to study the suitability of CTGF as a target for therapeutic intervention in diabetic nephropathy.

Conclusions

In the diabetic environment, the balance between the signalling activity of the different growth factors involved in renal matrix homeostasis is shifted towards a pro-fibrotic state. This leads to matrix accumulation and fibrosis, and eventually contributes to the development of diabetic nephropathy. The special role CTGF appears to play in this process most probably relates to its capacity to modulate the signalling activity of other growth factors critically involved in renal response to injury. More specifically, the CTGF-induced stimulation of IGF-I and TGF-β signalling, together with a decrease in BMP and VEGF signalling, might well contribute significantly to the diabetes-related inappropriate response to injury and adverse remodelling of the diabetic kidney. Restoring the balance of these growth factor signalling disturbances in the development of diabetic nephropathy by targeting CTGF might be more attractive than addressing individual growth factor signalling pathways. The first studies that have been performed so far suggest beneficial effects of CTGF inhibition in processes leading to renal fibrosis and abnormal glomerular permeability (see Table 1). To assess the possible suitability of CTGF as a target for therapeutic intervention in diabetic nephropathy, we need more extended studies with CTGF-neutralizing antibodies and/or ODNs, and studies in genetic animal models in which CTGF expression can be conditionally disrupted. Ultimately, such studies will reveal whether CTGF is indeed more than just another factor in diabetic kidney disease, and qualifies as a suitable target for therapeutic intervention.

## Table 1. Involvement of CTGF in hallmarks of development of diabetic nephropathy

<table>
<thead>
<tr>
<th>Hallmarks of diabetic nephropathy</th>
<th>CTGF involvement</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Glomerular hyperfiltration</td>
<td>+</td>
<td>31</td>
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<tr>
<td>Renal hypertrophy</td>
<td>+</td>
<td>31</td>
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<td>Albuminuria</td>
<td>+</td>
<td>18–21, 31</td>
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<td>Declining renal function</td>
<td>+</td>
<td>19, 21</td>
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<td>GBM thickening</td>
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<td>Mesangial matrix accumulation</td>
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<td>28</td>
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<td>Macrophage influx</td>
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Coping with the CKD epidemic: the promise of multidisciplinary team-based care

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Introduction

It is well known that late referral to a nephrologist is associated with many adverse outcomes [1–4], and indeed has been the subject of a recent review in this journal [5]. Some of the more important negative outcomes include more rapid onset of end-stage renal disease (ESRD), progression of co-morbid conditions such as anaemia and cardiovascular disease, suboptimal vascular access at initiation of dialysis, increased use of centre-based haemodialysis (HD), increased hospital utilization, increased cost and worse survival. The literature has many examples of sub-optimal chronic kidney disease (CKD) care provided by primary care physicians prior to referral, and also shows clearly that care provided by nephrologists is...