Connective tissue growth factor: just another factor in renal fibrosis?

Roel Goldschmeding¹, Jan Aten², Yasuhiko Ito², Ingrid Blom¹, Ton Rabelink³ and Jan J. Weening²

Departments of ¹Pathology and ³Vascular Medicine, UMC Utrecht and Department of ²Pathology, AMC Amsterdam, The Netherlands and ⁴Department of Nephrology, Chubu Rousai Hospital, Nagoya, Japan

Introduction

Connective tissue growth factor (CTGF) is a novel growth factor, that occurs in a broad range of species, including man, pig, rat, mouse, cow, newt and frog. Its amino acid sequence is over 90% identical among mammals. In their quest for the genes involved in development of atherosclerosis and diabetic nephropathy, differential cloning strategies applied by Oemar et al. [1], Mason et al. [2] and Murphy et al. [3] independently revealed strong overexpression of this growth factor in atherosclerotic aorta, and in mesangial cells after prolonged culture in high concentrations of glucose, respectively. Previously, CTGF had been cloned from HUVEC by Bradham et al. Its mouse ortholog (fisp-12) had been identified in serum-parietal epithelial cells, and some interstitial cells.

Growth factors in renal fibrosis

With respect to the kidney, CTGF represents the latest addition to the list of growth factors that might be involved in renal fibrosis. Does the present knowledge justify further analysis of its biological significance by researchers in nephrology?

Among previously identified growth factors, TGFβ is generally considered to be of major importance in the development of fibrosis. Blocking TGFβ with neutralizing antibody or decorin proved effective in reducing glomerulosclerosis in experimental models. However, it is well known that TGFβ also has antiproliferative (tumour suppressor) and anti-inflammatory activities. A possible danger of blocking TGFβ activity is exemplified by the hyperinflammatory (lethal) phenotype of TGFβ knock-out mice. Therefore, identification of more specific targets for antifibrotic therapy is needed.

CTGF might well be such a target, as it seems to be an important downstream mediator of at least part of the pro-fibrotic activity of TGFβ. However, it lacks, for example, the anti-proliferative effect of TGFβ on mink lung epithelial cells. CTGF expression has thus far not been reported in cells of the immune system and no data are available on involvement of CTGF in modulation of inflammation or immune reactions.

CTGF-expression in renal disease

Fibrosis is a final common pathway of renal diseases of diverse aetiology, including inflammatory, haemodynamic, and metabolic injury. To answer the question in which of these conditions CTGF expression might be altered, Ito et al. [5] performed CTGF mRNA in-situ hybridization (ISH) and morphometry in 65 human renal biopsies. In control human kidney, CTGF mRNA was mainly expressed in visceral epithelial cells, parietal epithelial cells, and some interstitial cells. CTGF mRNA was strongly upregulated in visceral epithelial and parietal epithelial cells and in extracapillary and severe mesangial proliferative lesions of crescentic glomerulonephritis, IgA nephropathy, focal and segmental glomerulosclerosis and, notably, diabetic nephropathy. In contrast, CTGF expression appeared not to be increased in glomerular diseases characterized by non-inflammatory lesions and proteinuria, such as minimal change nephrotic syndrome and membranous nephropathy, nor in acute exsudative postinfectious glomerulonephritis, which tend to heal without excessive scarring. The number of cells expressing CTGF mRNA was strongly correlated with the degree of injury at sites of chronic tubulointerstitial damage.

Unlike PDGF and TGFβ, CTGF appeared to be expressed only in resident cells of the kidney; double-staining for CD68 excluded co-expression of CTGF in monocytes or macrophages. The mRNA data were later confirmed at the protein level by immunohistochemistry [6](Figure 1).

These (ISH) observations were the first indication that CTGF overexpression might be involved in renal fibrosis, a notion further substantiated in the subsequent study of two rat models of renal wound repair and scarring, one initiated by acute, transient inflammation (anti-Thy1 nephritis), the other by chronic hypertension (in uninephrectomized spontaneously hypertensive rats). In anti-Thy1 nephritis, CTGF mRNA was already upregulated in mesangial cells and podocytes before glomerular expression of αSMA did appear. Glomerular expression of CTGF mRNA peaked at day 7, when damage was maximal, and returned to near-background levels at day 14 when tissue repair was almost completed [7]. In the chronic
hypertension model, significant upregulation of CTGF mRNA was observed in damaged glomeruli undergoing sclerosis and in fibrotic interstitium. Moreover, also epithelial cells lining atrophic tubuli expressed CTGF mRNA [8]. Stenson et al. [9] and Taal et al. [10] reported upregulation of CTGF in 5/6 nephrectomy, which was attenuated by the ACE inhibitor enalapril.

Another important in-vivo observation was made by Riser et al. [11] who, in agreement with Ito’s ISH results in human renal biopsies, measured a two-fold increase of CTGF transcripts in renal cortex, and even a 42-fold increase in microdissected glomeruli of obese db/db rats suffering from diabetic glomerulopathy. Makino et al. [12] reported less impressive (i.e. 1.7-fold) upregulation of CTGF mRNA in glomeruli of Wistar rats 4 weeks after treatment with STZ.

Thus, results obtained from both human biopsies and experimental models indicate that upregulation of CTGF in damaged glomeruli and (tubulo-) interstitial areas is a common phenomenon in diseases that lead to renal scarring. Moreover, the kinetics and topography of its expression following renal damage, suggest that prolonged overexpression of CTGF might be actively involved in glomerular and tubulointerstitial scarring.

Regulation of (renal) CTGF expression

TNF-z, cAMP and recently NO [13] have been observed to suppress CTGF mRNA transcription. TGF/β thus far seems to be the most important inducer of CTGF mRNA, which involves protein kinase A activity and a unique TGF/β response element located at positions −157 to −145 of the CTGF promoter sequence (reviewed in [4]).

Coordinate expression of TGFβ2 protein and CTGF mRNA in anti-Thy1 nephritis suggested that, at least in this model, increased TGFβ might have induced the upregulation of CTGF mRNA [7]. Dammeier et al. observed that systemic dexamethasone treatment increased CTGF mRNA in kidney, heart, and skin. It is of interest that dexamethasone treatment of NIH3T3 cells increased CTGF mRNA, but downregulated TGFβ mRNA (reviewed in [4]). Because of the extensive use of steroids in the treatment of renal diseases, it will be important to establish the net effect of their apparently opposite action on CTGF and TGFβ expression.

In vitro, Ito et al. [7] have observed that TGFβ1, 2 and 3, but not PDGF, stimulate CTGF expression in serum-starved mesangial cells and glomerular visceral epithelial cells. Riser et al. [14], Murphy et al. [3] and (earlier) Mason and co-workers [2], have independently detected upregulation of CTGF transcripts in mesangial cells upon prolonged culture in high-glucose medium. This upregulation could be partially blocked by anti-TGFβ1 antibody and by inhibition of protein kinase C activity. Riser et al. [14] reported blocking of high glucose induction of CTGF by TGFβ-neutralizing antibody. CTGF was also upregulated in renal epithelial monolayers after scrape-wounding or after exposure to calcium-oxalate-monohydrate crystals [5,6]. Again, this may have been due to the increased level of TGFβ detected under both conditions.

It thus appears that TGFβ can be a major stimulator of CTGF upregulation in renal fibrosis, but a role for other factors has not been excluded. Recent reports suggest a possible role for HGF, IL-1β, IL-4, TNF-α etc.
CTGF would significantly inhibit the profibrotic action of TGFB in the kidney.

**Biological effects of CTGF**

In-vivo data on biological effects of CTGF in renal tissue are not available. Outside the kidney, CTGF induced angiogenesis in corneal micropocket implants and chorioallantoic membrane, and granulation tissue formation and fibrosis upon subcutaneous injection [18,19] and (reviewed in [4]).

With respect to renal fibrosis, important processes in which CTGF might be involved include interstitial, extravascular, and mesangial accumulation of cells and extracellular matrix, and vascular sclerosis. In the accumulation of cells, the following have been implicated: proliferation and migration of mesangial cells, (cortical interstitial) fibroblasts, possibly circulating fibroblast progenitor cells, and trans-differentiation of mesangial cells and tubular epithelial cells to obtain a myofibroblastic phenotype. Indeed, Blom et al. [20] observed that CTGF, like TGFB, improved wound-healing of scraped mesangial cell monolayers, but that the response to CTGF was not affected by anti-TGFB antibody, and vice versa. Neither CTGF, nor TGFB induced proliferation of mesangial cells. Moreover, CTGF increased fibronectin (FN) expression in cultured mesangial cells (as was also observed by Murphy et al. [3] and by Riser et al. [12]) but not that of aSMA. In contrast, TGFB did increase expression of both FN and aSMA.

These data suggest that CTGF can contribute to increased mesangial cellularity (by stimulating migration, and possibly hypertrophy) and synthesis of extracellular matrix, but also that CTGF seems not to be directly involved in the acquisition of the myofibroblast phenotype. Also, proliferation of mesangial cells was not altered by CTGF or CTGF neutralizing antibody. Possible effects on matrix-modulating protease and protease-inhibitor activity, and on other renal cell types, have not yet been investigated.

Data obtained in other cells and tissues yield clues about further biological effects of CTGF. In-vitro effects of CTGF on fibroblasts, endothelial cells and vascular smooth muscle cells include adhesion, proliferation, chemotaxis, FN and collagen type I production, integrin α5β1 expression, migration, (endothelial) tube formation, and prevention of apoptosis. In TGFB-activated NRK (normal rat kidney) fibroblasts, CTGF controls a cell cycle restriction point in late G1 (reviewed in [4]).

**Mechanism of CTGF action**

Little is known about how CTGF influences cellular behaviour and metabolism. CTGF can bind to extra-cellular matrix molecules and serve as an adhesion molecule, but it is still largely unclear how. Integrins αVβ3 and αIIβ3 have been implicated in CTGF-binding and CTGF-induced migration of endothelial cells. Binding studies with labelled CTGF have demonstrated specific high- and low-affinity binding sites on fibroblasts and chondrocytes. Co-precipitation revealed a 280 kDa cross-linked CTGF complex, but evidence for a signal transducing receptor is still lacking (reviewed in [4]).

**CTGF levels in blood and body fluids**

Finally, soluble CTGF, and 10–24 kDa isoforms thereof, have been detected in serum, in uterine luminal flushings, and in follicular, amniotic, peritoneal and cerebrospinal fluids. In biliary atresia, serum levels were up to 8-fold higher than those in controls (reviewed in [4]). Although serum and urine levels have not yet been investigated in renal disease, it is challenging to speculate about the potential value of such measurements for diagnosis or prognosis.

**Conclusion**

CTGF can be considered an interesting target for future antifibrotic strategies. Its angiogenic potency and possible involvement in development of atherosclerosis extend the potential importance of CTGF in nephropathy to renal arteriosclerosis, chronic transplant nephropathy, shunt-vasculopathy and possibly CAPD-related modification of the peritoneal membrane. The anti-apoptotic action on endothelial cells, and transient upregulation in anti-Thy1 nephritis suggest that properly controlled expression of CTGF might be beneficial in tissue repair and adaptation to stress. An important goal for the near future is to identify the receptor(s) that mediate CTGF effects, and to establish whether modification of CTGF activity in vivo affects the natural course of tissue repair and/or the development of fibrotic scarring after renal injury.

**References**

6. Ito Y, Joles J, Bende R, Chand MA, Aten J, Kley L, Rabelink...