Histol Histopathol (2012) 27: 1131-1141
DOI: 10.14670/HH-27.1131

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Paracrine role for TGF-ß-induced CTGF and VEGF in mesangial matrix expansion in progressive glomerular disease

Hyun Soon Lee
Department of Pathology, Seoul National University College of Medicine, Seoul, Korea

Summary. Transforming growth factor-ß (TGF-ß) is a key regulator of extracellular matrix (ECM), and may mediate the development of glomerulosclerosis with accumulation of mesangial matrix. Mesangial cells secrete TGF-ß in response to common in vitro fibrogenic stimuli. Yet mesangial immunostaining for active TGF-ß1 is frequently negative in chronic glomerular disease. TGF-ß is rather expressed and/or activated by podocytes in both mesangial and podocyte diseases. Activated TGF-ß/Smad signaling by podocytes may induce connective tissue growth factor (CTGF or CCN2) and vascular endothelial growth factor (VEGF) expression. Podocyte CTGF seems to have paracrine effects on mesangial cells to stimulate CTGF expression. CTGF appears to stimulate the fibronectin-matrix assembly via enhanced cell-surface expression of α5ß1 integrin in the mesangium of diseased glomeruli. Podocyte VEGF-A overexpression also seems to play a paracrine role on mesangial cells to upregulate VEGF/VEGF receptor systems and to overproduce matrix proteins. Thus, paracrine CTGF and VEGF may contribute to mesangial matrix accumulation in chronic glomerular disease, culminating in the development of glomerulosclerosis. Together, these data bring new mechanistic insights into our understanding of the pathogenic role of TGF-ß-induced CTGF and VEGF in mesangial matrix expansion in chronic progressive glomerular disease.

Key words: α5ß1 integrin, CCN2, Chronic glomerular disease, Fibronectin, Mesangial matrix, Podocyte TGF-ß, VEGF/VEGF receptor system

Offprint requests to: Hyun Soon Lee, M.D., Department of Pathology, Seoul National University College of Medicine, Chongno-gu, Yongon-dong 28, Seoul 110-799, Korea. e-mail: hyunsoon@plaza.snu.ac.kr

Introduction

Glomerulosclerosis frequently complicates most renal diseases, and is characterized by the collapse of the glomerular tuft and mesangial matrix accumulation. Transforming growth factor-ß (TGF-ß) is a key regulator of extracellular matrix (ECM), and is secreted as latent complexes. Mesangial cells secrete TGF-ß in response to common in vitro fibrogenic stimuli (Lee and Song, 2009a). Yet mesangial immunostaining for active TGF-ß1 is frequently negative in chronic glomerular disease, possibly because large latent TGF-ß complexes secreted by mesangial cells are stored in the mesangial matrix (Lee and Song, 2009b). TGF-ß is rather expressed and/or activated by podocytes in both chronic mesangial and podocyte diseases. This podocyte TGF-ß may induce connective tissue growth factor (CTGF or CCN2) and vascular endothelial growth factor (VEGF) in chronic glomerular disease (Lee and Song, 2009b; Lee, 2011a, 2012).

It is postulated that podocyte-derived VEGF and CTGF stimulated by TGF-ß could act upon the mesangial cells to stimulate mesangial matrix synthesis, culminating in glomerulosclerosis in chronic glomerular disease (Lee and Song, 2009b; Lee, 2011a, 2012). This review will discuss the mechanisms by which TGF-ß-induced CTGF and VEGF by podocytes could contribute to mesangial matrix accumulation in chronic glomerular disease. In addition, therapeutic strategies to inhibit CTGF- and VEGF-induced mesangial matrix expansion will be discussed.

Activation of latent TGF-ß in chronic glomerular disease

TGF-ß is secreted as latent complexes, which are

stored in the ECM. Most cells secrete TGF-ß as part of a large latent complex, in which latent TGF-ß binding protein (LTBP) is linked to the latency-associated peptide (LAP)-TGF-ß complex. Multiple proteinases of the serine protease family, such as plasmin, first cleave LTBP. Then soluble large latent complex is released from the ECM, which is subsequently activated by another proteolytic event that releases TGF-ß from LAP (Koli et al., 2001, 2008).

Latent TGF-ß is activated *in vivo* by several mechanisms, such as proteolysis, thrombospondin-1 (TSP-1), reactive oxygen species (ROS), and some integrins (for review, see Lee and Song, 2009b).

Incomplete activation of latent TGF-ß by mesangial cells in the diseased glomeruli

Mesangial cells secrete TGF-ß in response to common in vitro fibrogenic stimuli (Lee and Song, 2009a), and produce matrix proteins in chronic glomerular disease. Mesangial immunostaining for active TGF-ß1, however, is frequently negative in chronic glomerular disease, such as IgA nephropathy (IgAN), focal segmental glomerulosclerosis (FSGS) and diabetic nephropathy. Instead, podocytes covering the sclerotic segments exhibit increased expression of TGF-ß1 protein (Kim et al., 2002, 2003; Wahab et al., 2005a).

Plasmin can release the large latent TGF-ß complex from the mesangial matrix by cleaving LTBP at the amino terminal hinge region (Taipale et al., 1992). Plasmin-mediated TGF-ß activation, however, may be neutralized via feedback inhibition, since TGF-ß-induced production of plasminogen activator inhibitor-1 (PAI-1) decreases active plasmin formation in mesangial cells. In rats with anti-Thy 1.1 nephritis, a murine model of mesangiopathy, accumulation of mesangial matrix progressed in association with enhanced mesangial fibrin deposition (Liu et al., 2004). Thus, the mesangial cell surface surrounded by an enlarged matrix may not express sufficient plasmin to activate latent TGF-ß1.

In high glucose-treated mesangial cells, endogenous TSP-1 is the main activator of high levels of latent TGF-ß1 (Yevdokimova et al., 2001). Yet the effects of TSP-1 on TGF-ß1 activation are relatively weak in experimental diabetic nephropathy (Daniel et al., 2007; Lee and Song, 2009a,b). In addition, TGF-ß1-stimulated arteries in either plasminogen- or TSP-1-deficient mice show no difference in either active or total TGF-ß1 secretion as compared with those in wild-type animals (Otsuka et al., 2007). It is not clear whether TSP-1 alone is able to directly activate latent TGF-ß in chronic glomerular disease.

Together, large latent TGF-ß complexes secreted by mesangial cells may be stored in the mesangial matrix, from which incompletely activated latent TGF-ß may be released (Lee and Song, 2009b; Lee, 2011b). In this regard, TGF-ß may not directly induce mesangial matrix synthesis in chronic glomerular disease.

Activation of latent TGF-ß by podocytes

Unlike mesangial cells, podocytes do not overexpress TGF-ß1 in response to common *in vitro* metabolic stimuli (Iglesias-de la Cruz et al., 2002; Chen et al., 2005). Podocytes seem to respond to paracrine TGF-ß coming from the mesangial cells in chronic mesangial disease (Wolf et al., 2005; Lee and Song, 2009b). Soluble forms of latent TGF-ß complex may be localized to the podocyte surface after its release from the mesangial matrix (Lee and Song, 2009b). In addition, biomechanical strain may induce de novo TGF-ß expression by podocytes in progressive podocyte disease. Podocyte-derived plasmin, matrix metalloproteinases (MMPs) and TSP-1, and particularly angiotensin II (Ang II)-induced oxidative stress may activate the latent TGF-ß in podocytes in the diseased glomeruli (Lee, 2011a, 2012).

Altogether, expression of TGF-ß1 by podocytes is increased, not only in progressive podocyte disease, but also in chronic mesangial disease.

Mesangial matrix expansion in progressive podocyte diseases via activation of TGF-ß signaling

TGF-ß is overexpressed by podocytes in progressive podocyte diseases (Kim et al., 1999, 2003; Sayers et al., 1999; Patek et al., 2003; Wahab et al., 2005a), in which expansion of the mesangial matrix is frequently present in association with glomerulosclerosis (Lee and Koh, 1993; Kim et al., 1995; Lee and Lim, 1995; Gregory et al., 1996; Patek et al., 2003; Taneda et al., 2003).

In Smad3-knockout diabetic mice, mesangial matrix expansion is prevented (Wang et al., 2007), as shown in the anti-TGF-ß-treated or TGF-ß type II receptor-deficient diabetic mice (Ziyadeh et al., 2000; Chen et al., 2003; Kim et al., 2004). Indeed, activation of the TGF-ß/Smad signaling by podocytes in diseased glomeruli appears to lead to overproduction of ECM in the mesangial areas, resulting in the formation of glomerulosclerosis (Kim et al., 2003; Patek et al., 2003; Lee and Song, 2009b).

Cross-talk mechanisms linking the podocyte TGF-ß with mesangial matrix accumulation

The podocyte TGF-ß, the active form of which has a very short half-life in plasma, is unlikely to traverse the GBM to promote sclerosis in the adjacent mesangium. In this regard, some TGF-ß-induced humoral factors produced by podocytes seem to have fibrogenic effects on mesangial cells (Lee and Song, 2009b).

In chronic glomerular disease, podocyte-derived VEGF and CTGF stimulated by TGF-ß might be transported across the GBM to capillary lumen to act upon the mesangial cells (Lee and Song, 2009b; Lee,

2011a,b, 2012).

Role of CTGF (CCN2)

CTGF is a member of the CCN family of matricellular proteins and is one of the TGF-ß-induced immediate early genes. Among the CCN proteins, only CCN2 (CTGF) has been shown to have profibrotic properties, acting downstream of TGF-ß1 (Gortendorst, 1997). CTGF is strongly implicated in the pathogenesis of diabetic nephropathy and possibly other fibrotic disease (Wahab et al., 2001, 2005a,b).

CCN proteins appear not to have specific high-affinity receptors. CCN2 provides independent adhesive functions by acting through integrins and heparin sulfate proteoglycans and facilitating interactions with the ECM (Chen et al., 2004b).

Induction of CTGF by TGF-ß in podocytes

TGF-ß induces CTGF in podocytes (Ito et al., 2001), although induction of CTGF by TGF-ß is generally restricted to the mesenchymal cell lineages (Leask et al., 2001, 2003; Kantarci et al., 2006). TGF-ß potently induces CTGF by the classical Smad pathway via Smad-binding element (SBE) located within the CTGF promoter (Holmes et al., 2001).

Effects of CTGF on podocytes

In normal murine or human glomeruli, CTGF protein is mainly expressed by podocytes (Yokoi et al., 2008; Ito et al., 2010), although some authors could not detect it (Wahab et al., 2001; Roestenberg et al., 2006). Podocyte-specific CTGF-transgenic mice show normal renal histology without proteinuria (Yokoi et al., 2008). Thus, CTGF alone does not likely act on podocytes or other glomerular cells. Coexisting glomerular damage or co-stimulatory signals, such as TGF-ß, may be required before high levels of podocyte CTGF protein translate into overt disease (Mason, 2009).

In podocytes of diabetic humans and wild-type mice with CTGF overexpression, phosphorylated Smad1-, 5-, and -8 (pSmad1/5/8) staining is reduced. In contrast, nuclear staining of pSmad1/5/8 is preserved in podocytes of diabetic CTGF+/- mice. Bone morphogenetic protein (BMP)-7 induces phosphorylation of Smad1/5/8 and counteracts the fibrogenic action of TGF-ß. Thus, CTGF overexpression in podocytes may induce loss of BMP signaling activity in diabetic nephropathy (Turk et al., 2009).

In vitro, treatment of podocytes with recombinant CTGF modified the actin cytoskeleton, and induced the phosphorylation of focal adhesion kinase and extracellular signal-regulated kinase. It did not stimulate podocyte mRNA expression of TGF-ß1 and CTGF, but increased that of fibronectin, and collagens I, III and IV (Fuchshofer et al., 2011).

Together, TGF-ß-induced CTGF may diminish BMP signaling and stimulate ECM synthesis in podocytes in chronic glomerular disease.

Paracrine effects of podocyte CTGF on mesangial CTGF expression

In human diabetic glomeruli, CTGF is overexpressed in mesangial areas and in podocytes (Ito et al., 1998, 2010; Wahab et al., 2001, 2005a). Yet conflicting results, possibly related to podocyte loss, were also described (Baelde et al., 2007). In murine models of diabetic nephropathy, glomerular CTGF protein levels increase initially in podocytes and later in parietal epithelial and mesangial cells (Roestenberg et al., 2006) (Table 1). Besides upregulation of podocyte CTGF, mesangial immunostaining for CTGF is positive in patients with IgAN, crescentic GN and diffuse proliferative lupus nephropathy, where its intensity becomes stronger as the mesangial lesions progress (Ito et al., 2010). During the early stage of the anti-Thy-1.1 nephritis, CTGF mRNA expression is present, confined to the podocytes and glomerular parietal epithelial cells. At day 7, however, mesangial cells express CTGF mRNA (Ito et al., 2001) (Table 1). Of note, induction of diabetes in podocyte-specific CTGF-transgenic mice results in an increased mesangial CTGF expression with more severe mesangial expansion than diabetic wild-

Table 1. Expression of CTGF in glomerular disease.

Diseases	CTGF		References
	Podocyte	mesangial	
FSGS	NC	Increased	Ito et al., 1998
PA-induced FSGS	increased	Absent	Fuchshofer et al., 2011
Diabetic nephropathy	Increased	Increased	Wahab et al., 2001, 2005; Roestenberg et al., 2006; Yokoi et al., 2008; Ito et al., 2010
IgA nephropathy	Increased	Increased	Ito et al., 1998, 2010
Anti-Thy1.1 nephritis	Increased	Increased	Ito et al., 2001
DPLN	Increased	Increased	Ito et al., 2010
Crescentic GN	Increased	Increased	Ito et al., 1998, 2010

CTGF, connective tissue growth factor; DPLN, diffuse proliferative lupus nephropathy; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; NC, no comment; PA, puromycin aminonucleoside.

type mice (Yokoi et al., 2008). Treatment of cultured mesangial cells with recombinant CTGF strongly autoinduced its own message (Riser et al., 2000; Wahab et al., 2001). In sum, podocyte CTGF seems to have paracrine effects on mesangial cells to stimulate CTGF expression in chronic glomerular disease.

Effects of CTGF on mesangial matrix expansion, particularly on fibronectin matrix deposition

CTGF is increased particularly in the glomeruli of patients with mesangial matrix expansion (Ito et al., 1998, 2010; Wahab et al., 2001, 2005a; Suzuki et al., 2003). In the glomeruli of rats with chronic puromycin aminonucleoside-induced FSGS, CTGF is overexpressed by podocytes, and fibronectin and types I, III and IV collagens are increased mainly in the mesangium (Fuchshofer et al., 2011) (Table 1). Treatment with CTGF antisense oligonucleotides significantly reduced the mesangial matrix expansion in diabetic mice (Guha et al., 2007).

In mesangial cells, CTGF stimulates fibronectin (Riser et al., 2000; Blom et al., 2001; Wahab et al., 2001; Crean et al., 2002; Weston et al., 2003), collagen (Riser et al., 2000; Gore-Heyer et al., 2002), PAI-1 (Wahab et al., 2001), MMP-2, tissue inhibitor of MMP (TIMP)-1 and TIMP-3 (McLennan et al., 2004) expression. CCN2 directly binds to fibronectin and the fibronectin receptor integrins α4 ß1 and α5 and syndecan 4 (Weston et al., 2003; Chen et al., 2004b). The promoter region of fibronectin does not contain any SBEs (Wahab and Mason, 2004), and CTGF effects on fibronectin production appear to be TGF-ß independent. In contrast, the transcription of some other matrix proteins, such as collagen, PAI-1 and TIMP-1, is TGF-ß-dependent, and their promoter contains SBEs (Mason and Wahab, 2003).

Fibronectin is secreted by numerous cell types, including human mesangial cells, and is widely distributed in ECM in vivo. The soluble fibronectin is polymerized into an insoluble fibrillar matrix in a regulated stepwise process (Schwarzbauer and Sechler, 1999; Geiger et al., 2001; Schwarzbauer and DeSimone, 2011; To and Midwood, 2011). The α5ß1 integrin receptor is key to fibronectin fibril formation (Schwarzbauer and Sechler, 1999; Geiger et al., 2001). Upon binding to integrins and other cell-surface receptors, fibronectin is unfolded from its compact structure into an extended structure exposing binding sites. Then, fibronectin-fibronectin intermolecular interactions occur. Maturation of fibronectin fibrils involves gradual conversion into a detergent-insoluble, stable matrix (McKeown-Longo and Mosher, 1985; Schwarzbauer and Sechler, 1999). Once assembled, fibronectin fibrils are continuously polymerized and remodeled within the fibrillar matrix on the cell surface (Sottile and Hocking, 2002) (Fig. 1).

In cultured mesangial cells, both TGF-ß and CTGF stimulate total fibronectin synthesis, insoluble fibronectin matrix deposition and the cell-surface expression of α5ß1 integrin (Weston et al., 2003). Blocking antibody to α5ß1 integrin inhibits fibronectin matrix deposition by mesangial cells (Weston et al., 2003). CTGF also increases fibronectin expression in mesangial cells in a ß3 integrin-dependent manner, which stimulates p42/p44 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) activation (Crean et al., 2002).

Patients with various glomerular diseases show increased total fibronectin staining in the mesangium (Oomura et al., 1989; Van Vliet et al., 2001; Kim et al., 2002). Fibronectin and its receptor, α5ß1 integrin, are overexpressed in the glomeruli of patients with IgAN and diffuse proliferative lupus nephropathy (Kuhara et al., 1997). Despite heavy mesangial accumulation of fibronectin protein in IgAN, its mRNA expression is almost negligible (Kim et al., 2002). CTGF may increase fibronectin mRNA expression in kidneys of rats with unilateral ureteral obstruction (UUO) (Yokoi et al., 2002, 2004) and of diabetic mice (Guha et al., 2007). Yet it is not clear whether CTGF actively upregulates mesangial fibronectin mRNA expression in chronic glomerular disease.

To sum up, CTGF appears to stimulate the fibronectin-matrix assembly via enhanced cell-surface expression of α5ß1 integrin in the mesangium of diseased glomeruli (Fig. 1).

Fibronectin is a major ECM protein serving as a scaffold for further deposition of fibronectin molecules

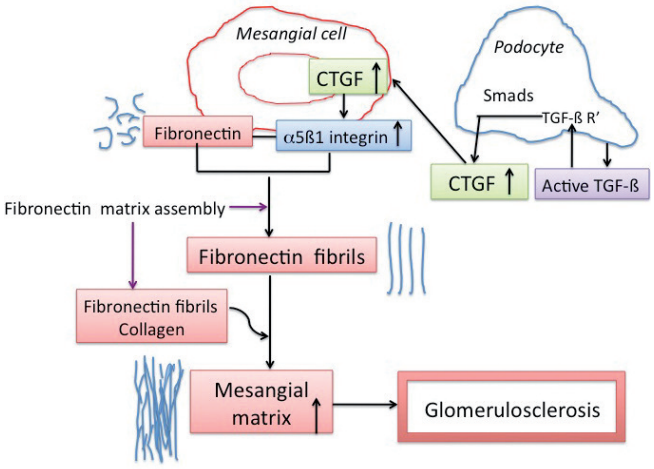

Fig. 1. Hypothetical pathway for mesangial matrix accumulation by transforming growth factor-ß (TGF-ß)-induced connective tissue growth factor (CTGF) in chronic glomerular disease. Activated TGF-ß/Smad signaling in podocytes may overproduce CTGF, which may have paracrine effects on mesangial cells to stimulate CTGF expression. In mesangial cells, CTGF stimulates the cell-surface expression of α5ß1 integrin. Through binding to integrin receptors and interactions between fibronectin molecules, fibronectin is polymerized into a fibrillar matrix. Fibronectin matrices may also act as scaffolds for further fibronectin and collagen deposition resulting in mesangial matrix accumulation. TGF-ß R', TGF-ß receptor.

and collagen (Mao and Schwarzbauer, 2005; Schwarzbauer and DeSimone, 2011). Fibronectin binds to collagen by its collagen-binding domain, located in the N-terminal part of the molecule. Pre-existing three-dimensional matrices act as scaffolds for further fibronectin deposition; new fibrils colocalize with the pre-existing matrix (Mao and Schwarzbauer, 2005).

In summary, TGF-ß-induced CTGF by podocytes may induce mesangial matrix expansion, particularly through α5ß1 integrin-dependent fibronectin matrix deposition in chronic glomerular disease, culminating in the development of glomerulosclerosis (Fig. 1).

Role of VEGF

VEGF or VEGF-A is a secreted dimeric glycoprotein. VEGF-A is almost exclusively expressed by podocytes in the developing and mature glomerulus, and plays an essential role in the formation and maintenance of a functional glomerular filtration barrier (Eremina et al., 2003). In addition, VEGF-A seems to participate in TGF-ß1-induced glomerular fibrosis (Wang et al., 2004; Chen et al., 2005). There are many isoforms of VEGF-A that are named according to their number of amino acids, i.e. $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$. Two receptor tyrosine kinases have been identified for VEGF: VEGF receptor-1 (VEGFR-1; Flt-1, fms-like tyrosyl kinase-1 where fms refers to feline McDonough sarcomavirus) and VEGFR-2 (human KDR, kinase domain receptor; mouse Flk1, fetal liver kinase-1). In normal kidneys, VEGFR-2 is expressed mainly by endothelial cells (Ferrara et al., 2003; Foster, 2009; Sison et al., 2010), but podocytes of adult mice also express VEGFR-2 (Ku et al., 2008; Veron et al., 2010). The most abundant isoform of VEGF-A expressed by kidney is $VEGF_{165}$ ($VEGF_{164}$ in mouse) (Schroppel et al., 1998), which predominantly signals through VEGFR-2 (Ferrara et al., 2003). Paracrine VEGF-A signaling seems to occur between podocytes and adjacent endothelial and mesangial cells, which express VEGF receptors (Thomas et al., 2000; Ferrara et al., 2003; Foster, 2009; Sison et al., 2010). In addition, VEGF-A/VEGFR-2 autocrine loop appears to exist in podocytes (Ku et al., 2008; Veron et al., 2010).

Induction of stimulatory $VEGF_{165}$ in damaged podocytes in chronic glomerular disease

Both TGF-ß1 and Ang II stimulate VEGF expression in cultured podocytes (Iglesias-de la Cruz et al., 2002; Chen et al., 2005). In the glomeruli of diabetic animals, VEGF is increased in the podocytes (Cooper et al., 1999; Wendt et al., 2003; Sung et al., 2006) (Table 2). Yet its expression is also decreased in the podocytes of patients with diabetic nephropathy, diffuse proliferative lupus nephropathy, crescentic glomerulonephritis and membranous nephropathy (Shulman et al., 1996; Honkanen et al., 2003) (Table 2). The downregulation of podocyte VEGF-A could be attributed to podocyte loss in chronic glomerular disease (Bailey et al., 1999; Baelde et al., 2007).

$VEGF_{165}$b cannot phosphorylate VEGFR-2 effectively, and consequently blocks $VEGF_{165}$A activation of VEGFR-2. Differentiated human podocytes secrete significant amounts of $VEGF_{165}$b protein. When podocytes dedifferentiate or are injured in glomerular disease, there is a switch from inhibitory $VEGF_{165}$b expression to stimulatory $VEGF_{165}$A expression (Cui et al., 2004).

Wilms' tumour suppressor gene, WT1, is essential for normal podocyte function. Mutations of WT1 induce Denys-Drash syndrome (DDS) characterized by diffuse mesangial sclerosis. Podocytes obtained from patients with DDS express high levels of $VEGF_{165}$, but lack $VEGF_{165}$b (Schumacher et al., 2007). Podocytes in DDS mice show overexpression of TGF-ß in association with intraglomerular hypertension or other second injury (Patek et al., 2003). Injured podocytes in other podocyte diseases also exhibit TGF-ß1 overexpression. Thus, TGF-ß1 overexpressed by damaged or dedifferentiated

Table 2. Expression of VEGF/VEGF receptors in glomerular disease.

Diseases	Podocyte VEGF	Mesangial VEGF/VEGF receptors	References
Protein-overload nephrosis	NC	Increased	Horita et al., 1998
Membranous nephropathy	Decreased	Increased	Honkanen et al., 2003
Diabetic nephropathy	Increased or decreased	Increased	Bailey et al., 1999; Cooper et al., 1999; Tsuchida et al., 1999; Wendt et al., 2003; Sung et al., 2006
Denys-Drash syndrome	Increased	NC	Schumacher et al., 2007
IgA nephropathy	NC	Increased	Noguchi et al., 1998; Thomas et al., 2000
Anti-Thy1.1 nephritis	NC	Increased	Iruela-Arispe et al., 1995
DPLN	Decreased	NC	Shulman et al., 1996
Crescentic GN	Decreased	NC	Shulman et al., 1996

DPLN, diffuse proliferative lupus nephropathy; GN, glomerulonephritis; NC, no comment.

podocytes may induce stimulatory $VEGF_{165}$ expression in chronic glomerular disease.

Effects of podocyte VEGF on glomerular pathology

Cultured podocytes possess both VEGFR-1 and VEGFR-2 (Guan et al., 2006). Yet VEGFR-1 is not detected in podocytes of normal glomerulus (Ku et al., 2008). VEGF increases α3(IV) collagen via VEGFR-1 and PI3K in podocytes (Chen et al., 2004a).

Diabetic animals with increased VEGF expression in the podocytes show GBM thickening and mesangial expansion (Cooper et al., 1999; Wendt et al., 2003; Sung et al., 2006). Moderately increased podocyte $VEGF_{164}$ in adult transgenic mice induced VEGFR-2 phosphorylation in podocytes, glomerulomegaly, GBM thickening, mesangial expansion and glomerulosclerosis (Veron et al., 2010). Moderate upregulation of podocyte VEGF in mice at postnatal day 0 rarely led to the development of FSGS after 6 weeks of induction (Sison et al., 2010). By contrast, transgenic mice overexpressing $VEGF_{164}$ in podocytes showed global collapse of the glomerular tuft and death at 5 days of age (Eremina et al., 2003).

In summary, moderately increased podocyte VEGF-A overexpression seems to play autocrine and paracrine roles on podocytes and mesangial cells, respectively, to overproduce matrix proteins, resulting in GBM thickening, mesangial matrix expansion and glomerulosclerosis.

Upregulation of mesangial VEGF/VEGF receptor systems in chronic glomerular disease

In normal kidney, weak mesangial KDR (VEGFR-2) staining is present in humans (Thomas et al., 2000), while VEGFR-2 is not expressed in mesangial cells of mice (Sison et al., 2010). In biopsy samples from IgAN and nephrotic syndrome, strong specific staining for both Flt-1 and KDR is detected in mesangial cells (Thomas et al., 2000).

VEGF-A is not detected in mesangial cells of normal glomerulus. Cultured mesangial cells, however, express VEGF, the activity of which is enhanced by protein kinase C-activating agent or TGF-ß (Iijima et al., 1993). Upregulation of mesangial VEGF has been described in humans and animals with mesangial disease, such as IgAN (Noguchi et al., 1998), diabetic nephropathy (Tsuchida et al., 1999), and anti-Thy1.1 nephritis (Iruela-Arispe et al., 1995). Furthermore, VEGF protein expression was significantly increased in mesangial areas in human membranous nephropathy (Honkanen et al., 2003) (Table 2).

Systemic injection of albumin to rats caused podocyte abnormalities, possibly via protein overload of the cell. In rats with subtotal renal ablation, protein-laden podocytes upregulate TGF-ß1 gene, which precedes the development of glomerulosclerosis (Abbate et al., 2002). Upregulation of mesangial VEGF and KDR mRNA has also been described in rats with protein-overload nephrosis (Horita et al., 1998).

In summary, mesangial VEGF/VEGF receptor systems are upregulated in chronic glomerular diseases, in which TGF-ß1 is frequently overexpressed by podocytes, suggesting that TGF-ß1-induced VEGF may act on mesangial cells to activate paracrine VEGF-A signaling (Fig. 2).

Other experiments in association with VEGF-induced mesangial matrix expansion

In cultured mesangial cells, VEGF increases collagen and fibronectin synthesis by a MAPK-dependent mechanism (Amemiya et al., 1999; Wang et al., 2004).

Transgenic rabbits with VEGF overexpression in kidney showed mesangial matrix expansion and glomerular hypertrophy, culminating in the development of glomerulosclerosis (Liu et al., 2007), while anti-VEGF attenuates mesangial matrix expansion in diabetic mice (Flyvbjerg et al., 2002). Bortoloso et al. (2004) found that glomerular $VEGF_{121}$ mRNA levels, but not $VEGF_{165}$ mRNA levels, are directly related to mesangial matrix expansion in patients with diabetic nephropathy.

In summary, TGF-ß-induced VEGF by podocytes in chronic glomerular disease may play a paracrine role in mesangial cells to upregulate VEGF/VEGF receptor systems and to overproduce matrix proteins, resulting in mesangial matrix expansion (Fig. 2).

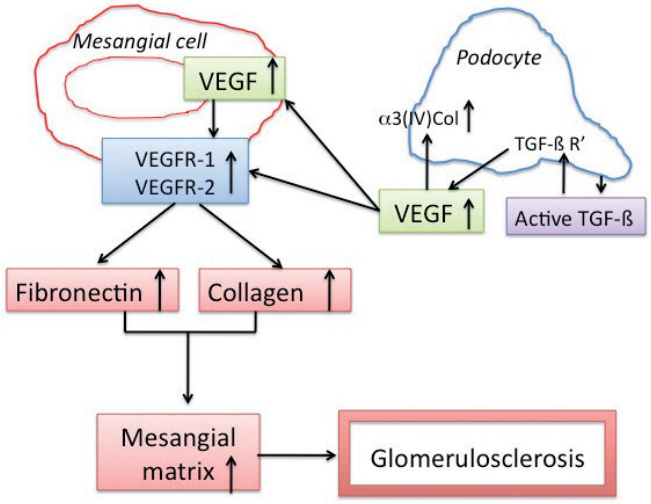

Fig. 2. Hypothetical pathway for mesangial matrix accumulation by TGF-ß-induced vascular endothelial growth factor (VEGF) in chronic glomerular disease. Activated TGF-ß signaling pathway in podocytes may overproduce VEGF, which may have paracrine effects on mesangial cells. VEGF stimulates the expression of VEGF receptor-1 (VEGFR-1) and VEGFR-2 in mesangial cells. Through binding to VEGFR-1 and VEGFR-2, VEGF stimulates fibronectin and collagen synthesis in mesangial cells resulting in mesangial matrix accumulation. Col, collagen; TGF-ß R', TGF-ß receptor.

Interaction between CTGF and VEGF

CTGF is induced by VEGF in retinal vascular cells (Suzama et al., 2000). CTGF can bind to $VEGF_{165}$, and this interaction inhibits the binding of $VEGF_{165}$ to endothelial cells and VEGFR-2. In this regard, the angiogenic activity of $VEGF_{165}$ seems to be inhibited by CTGF (Inoki et al., 2002).

$VEGF_{121}$ can also bind to the CT domain of CTGF. Despite this interaction, the binding ability of $VEGF_{121}$ to VEGFR-2 was not decreased (Inoki et al., 2002). Thus, possible fibrogenic activity of $VEGF_{121}$ (Bortoloso et al., 2004) may not be inhibited by CTGF in chronic glomerular disease.

Transportation of VEGF and CTGF from podocytes across the GBM to capillary lumen

The GBM is an array of randomly oriented fibers with fluid-filled interstices. It constitutes most of the glomerular hydraulic resistance, while being highly permeable to solutes, and does not seem to be particularly charge or size selective (Haraldsson et al., 2008b). Transport of VEGF and CTGF across the GBM is influenced by diffusive flux in and convective flux out.

The Peclet number (Pe) is a dimensional parameter that measures the importance of convection relative to diffusion (Haraldsson et al., 2008b). A Pe above unity indicates that transport occurs mainly by convection, and a Pe of less than 1 reflects diffusion-dominated transport. Pe increases with molecular size. Molecular mass of VEGF is 45 kD. The Stokes-Einstein radius of VEGF is 26 Å. The Pe in GBM for VEGF is estimated to be 0.063, indicating that VEGF is transported by diffusion across the GBM (Haraldsson et al., 2008a). In the same context, CTGF, with a molecular mass of 38 kD, may also be transported across the GBM to capillary lumen simply by diffusion. Indeed, diffusion dominates the transport of most solutes in the GBM (Haraldsson et al., 2008a).

Similar to glomerular endothelial cells, mesangial cells are connected to glomerular capillary lumen. Thus, podocyte VEGF and CTGF transported across the GBM to capillary lumen can act on mesangial cells.

Therapeutic strategies to prevent mesangial matrix expansion in chronic glomerular disease

CCN2 inhibitors

Pentoxifyline, a potent inhibitor of CTGF, inhibits CTGF expression by interfering with Smad3/4-dependent CTGF transcription, blocking the profibrogenic effects of CTGF on renal cells (Lin et al., 2005).

Treatment with CTGF antisense oligonucleotides in diabetic mice significantly reduced the mesangial matrix expansion with decreased renal cortical expression of fibronectin, type I collagen, and PAI-1 mRNAs (Guha et al., 2007). Renal fibrosis was reduced after siRNA- and antisense-mediated CTGF knockdown in rats undergoing chronic allograft nephropathy (Luo et al., 2008) and UUO (Yokoi et al., 2002, 2004). In addition, the expression of known TGF-ß targets was inhibited by antisense CTGF knockdown in the 5/6 nephrectomy model (Okada et al., 2005).

Inhibitors of fibronectin matrix deposition

Blocking antibody to the α5ß1 integrin inhibits fibronectin matrix deposition by mesangial cells (Weston et al., 2003).

Fibronectin polymerization inhibitor, pUR4, Inhibits vascular wall thickening by reducing the excess deposition of fibronectin and collagen I in arteries after induction of atherosclerosis (Chiang et al., 2009).

VEGF inhibitors

Soluble VEGFR-1 (sFlt-1), a splice variant of VEGFR-1, binds to VEGF with high affinity, and has potent and selective VEGF inhibitory action (Kendall and Thomas, 1993). Neutralization of physiologic levels of VEGF with sFlt-1 can cause renal disease (Li et al., 2007; Sugimoto et al., 2003). By contrast, inducible overexpression of sFlt-1 in podocytes of diabetic mice ameliorates mesangial matrix expansion by inhibiting podocyte-expressed VEGF-A activity (Ku et al., 2008), although systemic overexpression of sFlt-1 accelerates tubulointerstitial injury (Kosugi et al., 2010).

Monoclonal anti-$VEGF_{165}$ antibody treatment decreased albuminuria and glomerular hypertrophy in streptozotocin-induced diabetic rats (De Vriese et al., 2001). It also attenuated mesangial matrix expansion in diabetic *db/db* mice (Flyvbjerg et al., 2002).

Treatment with pan-VEGF receptor tyrosine kinase inhibitor, SU5416, did not inhibit glomerular VEGF elevation or mesangial matrix expansion in diabetic *db/db* mice, though it reduced proteinuria (Sung et al., 2006). A potent intracellular tyrosine kinase inhibitor, BIBF 1120, inhibits a variety of growth factor receptors, including VEGF receptors, which regulate fibrogenic pathways (Hilberg et al., 2008). Recently, BIFF 1120 has been shown to slow the progression of disease in patients with idiopathic pulmonary fibrosis (Richeldi et al., 2011).

Thus, targeting CTGF and VEGF may be a useful preventive measure to inhibit mesangial matrix expansion in chronic glomerular disease.

Conclusions

Activated TGF-ß/Smad signaling by podocytes may induce CTGF/CCN2 and VEGF expression in podocytes. Podocyte CTGF seems to have paracrine effects on mesangial cells to stimulate CTGF expression in chronic glomerular disease, which may induce α5ß1

integrin-dependent fibronectin matrix deposition. Podocyte VEGF-A overexpression also appears to play a paracrine role in mesangial cells to upregulate VEGF/VEGF receptor systems and to overproduce matrix proteins. Thus, paracrine CTGF and VEGF may contribute to mesangial matrix accumulation in chronic glomerular disease, culminating in the development of glomerulosclerosis. Together, these data bring new mechanistic insights into our understanding of the pathogenic role of TGF-ß-induced CTGF and VEGF in mesangial matrix synthesis in chronic progressive glomerular disease.

References

Abbate M., Zoja C., Morigi M., Rottoli D., Angioletti S., Tomasoni S., Zanchi C., Longaretti L., Donadelli R. and Remuzzi G. (2002). Transforming growth factor-ß1 is up-regulated by podocytes in response to excess intraglomerular passage of proteins: a central pathway in progressive glomerulosclerosis. Am. J. Pathol. 161, 2179-2193.

Amemiya T., Sasamura H., Mifune M., Kitamura Y., Hirahashi J., Hayashi M. and Saruta T. (1999). Vascular endothelial growth factor activates MAP kinase and enhances collagen synthesis in human mesangial cells. Kidney Int. 56, 2055-2063.

Baelde H.J., Eikmans M., Lappin D.W.P., Doran P.P., Hohenadel D., Brinkkoetter P.-T., van der Woude F.J., Waldherr R., Rabelink T.J., de Heer E. and Bruijn J.A. (2007). Reduction of VEGF-A and CTGF expression in diabetic nephropathy is associated with podocyte loss. Kidney Int. 71, 637-645.

Bailey E., Bottomley M.J., Westwell S., Pringle J.H., Furness P.N., Feehally J., Brenchley P.E.C. and Harper S.J. (1999). Vascular endothelial growth factor mRNA expression in minimal change, membranous, and diabetic nephropathy demonstrated by non-isotopic in situ hybridization. J. Clin. Pathol. 52, 735-738.

Blom I.E., van Dijk A.J., Wieten L., Duran K., Ito Y., Kleij L, deNichilo M., Rabelink T.J., Weening J.J., Aten J. and Goldschmeding R. (2001). In vitro evidence for differential involvement of CTGF, TGFß, and PDGF-BB in mesangial response to injury. Nephrol. Dial. Transplant. 16, 1139-1148.

Bortoloso E., Del Prete D., Dalla Vestra M., Gambaro G., Saller A., Antonucci F., Baggio B., Anglani F. and Fioretto P. (2004). Quantitative and qualitative changes in vascular endothelial growth factor gene expression in glomeruli of patients with type 2 diabetes. Eur. J. Endocrinol. 150, 799-807.

Chen S., Iglesias-de la Cruz M.C., Jim B., Hong S.W., Isono M. and Ziyadeh F.N. (2003). Reversibility of established diabetic glomerulopathy by anti-TGF-ß antibodies in db/db mice. Biochem. Biophys. Res. Commun. 300, 16-22.

Chen S., Kasama Y., Lee J.S., Jim B., Marin M. and Ziyadeh F.N. (2004a). Podocyte-derived vascular endothelial growth factor mediates the stimulation of α3(IV) collagen production by transforming growth factor-ß1 in mouse podocytes. Diabetes 53, 2939-2949.

Chen Y., Abraham D.J., Shi-Wen X., Pearson J.D., Black C.M., Lyons K.M. and Leask A. (2004b). CCN2 (connective tissue growth factor) promotes fibroblast adhesion to fibronectin. Mol. Biol. Cell 15, 5635-5646.

Chen S., Lee J.S., Iglesias-de la Cruz M.C., Wang A., Izquierdo-Lahuerta A., Gandhi N.K., Danesh F.R., Wolf G. and Ziyadeh F.N. (2005). Angiotensin II stimulates α3(IV) collagen production in mouse podocytes via TGF-ß and VEGF signaling: implications for diabetic glomerulopathy. Nephrol. Dial. Transplant. 20, 1320-1328.

Chiang H.-Y., Korshunov V.A., Serour A., Shi F. and Sottile J. (2009). Fibronectin is an important regulator of flow-induced vascular remodeling. Arterioscler. Thromb. Vasc. Biol. 29, 1074-1079.

Cooper M.E., Vranes D., Youssef S., Stacker S.A., Cox A.J., Rizkalla B., Casley D.J., Bach L.A., Kelly D.J. and Gilbert R.E. (1999). Increased renal expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 in experimental diabetes. Diabetes 48, 2229-2239.

Crean J.K.G., Finlay D., Murphy M., Moss C., Godson C., Martin F. and Brady H.R. (2002). The role of p42/44 MAPK and protein kinase B in connective tissue growth factor induced extracellular matrix protein production, cell migration, and actin cytoskeletal rearrangement in human mesangial cells. J. Biol. Chem. 277, 44187-44194.

Cui T.-G., Foster R.R., Saleem M., Mathieson P.W., Gillatt D.A., Bates D.O. and Harper S.J. (2004). Differentiated human podocytes endogenously express an inhibitory isoform of vascular endothelial growth factor (VEGF165b) mRNA and protein. Am. J. Physiol. Renal Physiol. 286, F767-773.

Daniel C., Schaub K., Amann K., Lawler J. and Hugo C. (2007). Thrombospondin-1 is an endogenous activator of TGF-ß in experimental diabetic nephropathy in vivo. Diabetes 56, 2982-2989.

De Vriese A.S., Tilton R.G., Elger M., Stephan C.C., Kriz W. and Lameire N.H. (2001). Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. J. Am. Soc. Nephrol. 12, 993-1000.

Eremina V., Sood M., Haigh J., Nagy A., Lajoie G., Ferrara N., Gerber H.-P., Kikkawa Y., Miner J.H. and Quaggin S.E. (2003). Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J. Clin. Invest. 111, 707-716.

Ferrara N., Gerber H.P. and LeCouter J. (2003). The biology of VEGF and its receptors. Nat. Med. 9, 669-676.

Flyvbjerg A., Dagnaes-Hansen F., De Vriese A.S., Schrijvers B.F., Tilton R.G. and Rasch R. (2002). Amelioration of long-term renal changes in obese type 2 diabetic mice by a neutralizing vascular endothelial growth factor antibody. Diabetes 51, 3090-3094.

Foster R.R. (2009). The importance of cellular VEGF bioactivity in the development of glomerular disease. Nephron Exp. Nephrol. 113, e8-e15.

Fuchshofer R., Ullmann S., Zeilbeck L.F., Baumann M., Junglas B. and Tamm E.R. (2011). Connective tissue growth factor modulates podocyte actin cytoskeleton and extracellular matrix synthesis and is induced in podocytes upon injury. Histochem. Cell Biol. 136, 301-319.

Geiger B., Bershadsky A., Pankov R. and Yamada K.M. (2001). Transmembrane extracellular matrix-cytoskeleton crosstalk. Nat. Rev. Mol. Cell Biol. 2, 793-805.

Gore-Hyer E., Shegogue D., Markiewicz M., Lo S., Hazaen-Martin D., Greene E.L., Grotendorst G. and Trojanowska M. (2002). TGF-ß and CTGF have overlapping and distinct fibrogenic effects on human renal cells. Am. J. Physiol. Renal Physiol. 283, F707-F716.

Gortendorst G.R. (1997). Connective tissue growth factor: a mediator of TGF-ß action on fibroblasts. Cytokine Growth Factor Rev. 8, 171-179.

Gregory M.C., Terreros D.A., Barker D.F., Fain P.N., Denison J.C. and Atkin C.L. (1996). Alport syndrome-clinical phenotypes, incidence,

and pathology. Contrib. Nephrol. 117, 1-28.

Guan F., Villegas G., Teichman J., Mundel P. and Tufro A. (2006). Autocrine VEGF-A system in podocytes regulates podocin and its interaction with CD2AP. Am. J. Physiol. Renal Physiol. 291, F422-F428.

Guha M., Xu Z.G., Tung D., Lanting L. and Natarajan R. (2007). Specific down-regulation of connective tissue growth factor attenuates progression of nephropathy in mouse models of type 1 and type 2 diabetes. FASEB J. 21, 3355-3368.

Haraldsson B., Barisoni L. and Quaggin S.E. (2008a). VEGF inhibition and renal thrombotic microangiopathy. N. Engl. J. Med. 359, 206-207.

Haraldsson B., Nyström J. and Deen W.M. (2008b). Properties of the glomerular barrier and mechanisms of proteinuria. Physiol. Rev. 88, 451-487.

Hilberg F., Roth G.J., Krssak M., Kautschitsch S., Sommergruber W., Tontsch-Grunt U., Garin-Chesa P., Bader G., Zoephel A., Quant J., Heckel A. and Rettig W.J. (2008). BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. Cancer Res. 68, 4774-4782.

Holmes A., Abraham D.J., Sa S., Shiwen X., Black C.M. and Leask A. (2001). CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. J. Biol. Chem. 276, 10594-10601.

Honkanen E., von Willebrand E., Koskinen P., Teppo A.-M., Törnroth T., Ruutu M. and Grönhagen-Riska C. (2003). Decreased expression of vascular endothelial growth factor in idiopathic membranous glomerulonephritis: relationships to clinical course. Am. J. Kidney Dis. 42, 1139-1148.

Horita Y., Miyazaki M., Koji T., Kobayashi N., Shibuya M., Razzaque M.S., Cheng M., Ozono Y., Kohno S. and Taguchi T. (1998). Expression of vascular endothelial growth factor and its receptors in rats with protein-overload nephrosis. Nephrol. Dial. Transplant. 13, 2519-2528.

Iglesias-de la Cruz M.C., Ziyadeh F.N., Isono M., Kouahou M., Han D.C., Kalluri R., Mundel P. and Chen S. (2002). Effects of high glucose and TGF-ß1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. Kidney Int. 62, 901-913.

Iijima K., Yoshikawa N., Connolly D.T. and Nakamura H. (1993). Human mesangial cells and peripheral blood mononuclear cells produce vascular permeability factor. Kidney Int. 44, 959-966.

Inoki I., Shiomi T., Hashimoto G., Enomoto H., Nakamura H., Makino K.-i., Ikeda E., Takata S., Kobayashi K.-I. and Okada Y. (2002). Connective tissue growth factor binds vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. FASEB J. 16, 219-221.

Iruela-Arispe L., Gordon K., Hugo C., Duijvestijn A.M., Claffey K.P., Reilly M., Couser W.G., Alpers C.E. and Johnson R.J. (1995). Participation of glomerular endothelial cells in the capillary repair of glomerulonephritis. Am. J. Pathol. 147, 1715-1727.

Ito Y., Aten J., Bende R.J., Oemar B.S., Rabelink T.J., Weening J.J. and Goldschmeding R. (1998). Expression of connective tissue growth factor in human renal fibrosis. Kidney Int. 53, 853-861.

Ito Y., Goldschmeding R., Bende R.J., Claessen N., Chand M.A., Kleij L., Rabelink T.J., Weening J.J. and Aten J. (2001). Kinectics of connective tissue growth factor expression during experimental proliferative glomerulonephritis. J. Am. Soc. Nephrol. 12, 472-484.

Ito Y., Goldschmeding R., Kasuga H., Claessen N., Nakayama M., Yuzawa Y., Sawai A., Matsuo S., Weening J.J. and Aten J. (2010). Expression patterns of connective tissue growth factor and of TGF-ß isoforms during glomerular injury recapitulate glomerulogenesis. Am. J. Physiol. Renal Physiol. 299, F545-F558.

Kantarci A., Black S.A., Xydas C.E., Murawel P., Uchida Y., Yucekal-Tuncer B., Atilla G., Emingil G., Uzel M.I., Lee A., Firatli E., Sheff M., Hasturk H., Van Dyke T.E. and Trackman P.C. (2006). Epithelial and connective tissue cell CTGF/CCN2 expression in gingival fibrosis. J. Pathol. 210, 59-66.

Kendall R.L. and Thomas K.A. (1993). Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc. Natl. Acad. Sci. USA 90, 10705-10709.

Kim K.H., Kim Y., Gubler M.C., Steffes M.W., Lane P.H., Kashtan C.E., Crosson J.T. and Mauer S.M. (1995). Structural-functional relationships in Alport syndrome. J. Am. Soc. Nephrol. 5, 1659-1668.

Kim T.S., Kim J.Y., Hong H.K. and Lee H.S. (1999). mRNA expression of glomerular basement membrane proteins and TGF-ß1 in human membranous nephropathy. J. Pathol. 189, 425-430.

Kim H.W., Moon K.C., Park S.Y., Hong H.K. and Lee H.S. (2002). Differential expression of platelet-derived growth factor and transforming growth factor-ß in relation to progression of IgA nephropathy. Nephrology 7, S131-S139.

Kim J.H., Kim B.K., Moon K.C., Hong H.K. and Lee H.S. (2003). Activation of the TGF-ß/Smad signaling pathway in focal segmental glomerulosclerosis. Kidney Int. 64, 1715-1721.

Kim H.W., Kim B.C., Song C.Y., Kim J.H., Hong H.K. and Lee H.S. (2004). Heterozygous mice for TGF-ßIIR gene are resistant to the progression of streptozotocin-induced diabetic nephropathy. Kidney Int. 66, 1859-1865.

Koli K., Saharinen J., Hyytiäinen M., Penttinen C. and Keski-Oja J. (2001). Latency, activation, and binding proteins of TGF-ß. Microsc. Res. Tech. 52, 354-362.

Koli K., Myllärniemi M., Keski-Oja J. and Kinnula V.L. (2008). Transforming growth factor-ß activation in the lung: focus on fibrosis and reactive oxygen species. Antioxid. Redox. Signal 10, 333-342.

Kosugi T., Nakayama T., Li Q., Chiodo V.A., Zhang L., Campbell-Thompson M., Grant M., Crocker B.P. and Nakagawa T. (2010). Soluble Flt-1 gene therapy ameliorates albuminuria but accelerates tubulointerstitial injury in diabetic mice. Am. J. Physiol. Renal Physiol. 298, F609-F616.

Ku C.-H., White K.E., Dei Cas A., Hayward A., Webster Z., Bilous R., Marshall S., Viberti G. and Gnudi L. (2008). Inducible overexpression of sFlt-1 in podocytes ameliorates glomerulopathy in diabetic mice. Diabetes 57, 2824-2833.

Kuhara T., Kagami S. and Kuroda Y. (1997). Expression of ß1-integrins on activated mesangial cells in human glomerulonephritis. J. Am. Soc. Nephrol. 8, 1679-1687.

Leask A., Sa S., Holmes A., Shiwen X., Black C.M. and Abraham D.J. (2001). The control of ccn2 (ctgf) gene expression in normal and scleroderma fibroblasts. Mol. Pathol. 54, 180-183.

Leask A., Holmes A., Black C.M. and Abraham D.J. (2003). Connective tissue growth factor gene regulation. Requirements for its induction by transforming growth factor-beta 2 in fibroblasts. J. Biol. Chem. 278, 13008-13015.

Lee H.S. (2011a). Pathogenic role of TGF-ß in the progression of podocyte diseases. Histol. Histopathol. 26, 107-116.

Lee H.S. (2011b). Role of TGF-ß in mesangial matrix accumulation in chronic progressive glomerular disease. In: An Update on

Glomerulopathies - Etiology and Pathogenesis. Chapter 7. Prabhakar S. (ed). InTech. Rijeka, Croatia. pp 123-140.

Lee H.S. (2012). Mechanisms and consequences of TGF-ß overexpression by podocytes in progressive podocyte disease. Cell Tissue Res. 347, 129-140.

Lee H.S. and Koh H.I. (1993). Nature of progressive glomerulosclerosis in human membranous nephropathy. Clin. Nephrol. 39, 7-16.

Lee H.S. and Lim S.D. (1995). The significance of glomerular hypertrophy in focal segmental glomerulosclerosis. Clin. Nephrol. 44, 349-355.

Lee H.S. and Song C.Y. (2009a). Oxidized low-density lipoprotein and oxidative stress in the development of glomerulosclerosis. Am. J. Nephrol. 29, 62-70.

Lee H.S. and Song C.Y. (2009b). Differential role of mesangial cells and podocytes in TGF-ß-induced mesangial matrix synthesis in chronic glomerular disease. Histol. Histopathol. 24, 901-908.

Li Z., Zhang Y., Ying Ma J., Kapoun A.M., Shao Q., Kerr I., Lam A., O'Young G., Sannajust F., Stathis P., Schreiner G., Karumanchi S.A., Protter A.A. and Pollitt N.S. (2007). Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. Hypertension 50, 686-692.

Lin S.L., Chen R.H., Chen Y.M., Chiang W.C., Lai C.F., Wu K.D. and Tsai T.J. (2005). Pentoxifyline attenuates tubulointerstitial fibrosis by blocking Smad3/4-activated transcription and profibrogenic effects of connective tissue growth factor. J. Am. Soc. Nephrol. 16, 2702-2713.

Liu N., Makino T., Nogaki F., Kusano H., Suyama K., Muso E., Honda G., Kita T. and Ono T. (2004). Coagulation in the mesangial area promotes ECM accumulation through factor V expression in MsPGN in rats. Am. J. Physiol. Renal Physiol. 287, F612-F620.

Liu E., Morimoto M., Kitajima S., Koike T., Yu Y., Shiiki H., Nagata M., Watanabe T. and Fan J. (2007). Increased expression of vascular endothelial growth factor in kidney leads to progressive impairment of glomerular functions. J. Am. Soc. Nephrol. 18, 2094-2104.

Luo G.H., Lu Y.P., Song J., Yang L., Shi Y.J. and Li Y.P. (2008). Inhibition of connective tissue growth factor by small interfering RNA prevents renal fibrosis in rats undergoing chronic allograft nephropathy. Transplant. Proc. 40, 2365-2369.

Mao Y. and Schwarzbauer J.E. (2005). Stimulatory effects of a three-dimensional microenvironment on cell-mediated fibronectin fibrillogenesis. J. Cell Sci. 118, 4427-4436.

Mason R.M. (2009). Connective tissue growth factor (CCN2), a pathogenic factor in diabetic nephropathy. What does it do? How does it do it? J. Cell Commun. Signal 3, 95-104.

Mason R.M. and Wahab N.A. (2003). Extracellular matrix metabolism in diabetic nephropathy. J. Am. Soc. Nephrol. 14, 1358-1373.

McKeown-Longo P.J. and Mosher D.F. (1985). Interaction of the 70,000-mol-wt amino-terminal fragment of fibronectin with the matrix-assembly receptor of fibroblasts. J. Cell Biol. 100, 364-374.

McLennan S.V., Wang X.Y., Moreno V., Yue D.K. and Twigg S.M. (2004). Connective tissue growth factor mediates high glucose effects on matrix degradation through tissue inhibitor of matrix metalloproteinase type 1: implications for diabetic nephropathy. Endocrinology 145, 5646-5655.

Noguchi K., Yoshikawa N., Ito-Kariya S., Inoue Y., Hayashi Y., Ito H., Nakamura H. and Iijima K. (1998). Activated mesangial cells produce vascular permeability factor in early-stage mesangial proliferative glomerulonephritis. J. Am. Soc. Nephrol. 9, 1815-1825.

Okada H., Kikuta T., Kobayashi T., Inoue T., Kanno Y., Takigawa M., Sugaya T., Kopp J.B. and Suzuki H. (2005). Connective tissue growth factor expressed in tubular epithelium plays a pivotal role in renal fibrogenesis. J. Am. Soc. Nephrol. 16, 133-143.

Oomura A., Nakamura T., Arakawa M., Ooshima A. and Isemura M. (1989). Alterations in the extracellular matrix components in human glomerular diseases. Virchows Arch. (A) 415, 151-159.

Otsuka G., Stempien-Otero A., Frutkin A.D. and Dichek D.A. (2007). Mechanisms of TGF-ß1-induced intimal growth: plasminogen-independent activities of plasminogen activator inhibitor-1 and heterogeneous origin of intimal cells. Circ. Res. 100, 1300-1307.

Patek C.E., Fleming S., Miles C.G., Bellamy C.O., Ladomery M., Spraggon L., Mullins J., Hastie N.D. and Hooper M.L. (2003). Murine Denys-Drash syndrome: evidence of podocyte de-differentiation and systemic mediation of glomerulosclerosis. Hum. Mol. Genet. 12, 2379-2394.

Richeldi L., Costabel U., Selman M., Kim D.S., Hansell D.M., Nicholson A.G., Brown K.K., Flaherty K.R., Noble P.W., Raghu G., Brun M., Gupta A., Juhel N., Klüglich M. and du Bois R.M. (2011). Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N. Engl. J. Med. 365, 1079-1087.

Riser B.L., Denichilo M., Cortes P., Baker C., Grondin J.M., Yee J. and Narins R.G. (2000). Regulation of connective tissue growth factor activity in cultured rat mesangial cells and its expression in experimental diabetic glomerulosclerosis. J. Am. Soc. Nephrol. 11, 25-38.

Roestenberg P., van Nieuwenhoven F.A., Joles J.A., Trischberger C., Martens P.P., Oliver N., Aten J., Höppener J.W. and Goldschmeding R. (2006). Temporal expression profile and distribution pattern indicate a role of connective tissue growth factor (CTGF/CCN-2) in diabetic nephropathy in mice. Am. J. Physiol. Renal Physiol. 290, F1344-F1354.

Sayers R., Kalluri R., Rodgers K.D., Shield C.F. III, Meehan D.T. and Cosgrove D. (1999). Role for transforming growth factor-ß1 in Alport renal disease progression. Kidney Int. 56, 1662-1673.

Schroppel B., Huber S., Horster M., Schlondorff D. and Kretzler M. (1998). Analysis of mouse glomerular podocyte mRNA by single-cell reverse transcription-polymerase chain reaction. Kidney Int. 53, 119-124.

Schumacher V.A., Jeruschke S., Eitner F., Becker J.U., Pitschke G., Ince Y., Miner J.H., Leuschner I., Engers R., Everding A.S., Buila M. and Royer-Pokora B. (2007). Impaired glomerular maturation and lack of VEGF165b in Denys-Drash syndrome. J. Am. Soc. Nephrol. 18, 719-729.

Schwarzbauer J.E. and DeSimone D.W. (2011). Fibronectins, their fibrillogenesis, and in vivo functions. Cold Spring Harb. Perspect. Biol. 3, a005041.

Schwarzbauer J.E. and Sechler J.L. (1999). Fibronectin fibrillogenesis: a paradigm for extracellular matrix assembly. Curr. Opin. Cell. Biol. 11, 622-627.

Shulman K., Rosen S., Tognazzi K., Manseau E.J. and Brown L.F. (1996). Expression of vascular permeability factor (VPF/VEGF) is altered in many glomerular diseases. J. Am. Soc. Nephrol. 7, 661-666.

Sison K., Eremina V., Baelde H., Min W., Hirashima M., Fantus I.G. and Quaggin S.E. (2010). Glomerular structure and function require paracrine, not autocrine, VEGF-VEGFR-2 signaling. J. Am. Soc. Nephrol. 21, 1691-1701.

Sottile J. and Hocking D.C. (2002). Fibronectin polymerization regulates

the composition and stability of extracellular matrix fibrils and cell-matrix adhesions. Mol. Biol. Cell 13, 3546-3559.

Sugimoto H., Hamano Y., Charytan D., Cosgrove D., Kieran M., Sudhakar A. and Kalluri R. (2003). Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. J. Biol. Chem. 278, 12605-12608.

Sung S.H., Ziyadeh F.N., Wang A., Pyagay P.E., Kanwar Y.S. and Chen S. (2006). Blockade of vascular endothelial growth factor signaling ameliorates diabetic albuminuria in mice. J. Am. Soc. Nephrol. 17, 3093-3104.

Suzama K., Naruse K., Suzama I., Takahara N., Ueki K., Aiello L.P. and King G.L. (2000). Vascular endothelial growth factor induces expression of connective tissue growth factor via KDR, Flt1, and phosphatidylinositol 3-kinase-akt-dependent pathways in retinal vascular cells. J. Biol. Chem. 275, 40725-40731.

Suzuki D., Toyoda M., Umezono T., Uehara G., Zhang S.-Y., Sakai T., Nishina M., Suga T., Endoh M., Yagame M. and Sakai H. (2003). Glomerular expression of connective tissue growth factor mRNA in various renal diseases. Nephrology 8, 92-97.

Taipale J., Koli K. and Keski-Oja J. (1992). Release of transforming growth factor-ß1 from the pericellular matrix of cultured fibroblasts and fibrosarcoma cells by plasmin and thrombin. J. Biol. Chem. 267, 25378-25384.

Taneda S., Pippin J.W., Sage E.H., Hudkins K.L., Takeuchi Y., Couser W.G. and Alpers C.E. (2003). Amelioration of diabetic nephropathy in SPARC-null mice. J. Am. Soc. Nephrol. 14, 968-980.

Thomas S., Vanuystel J., Gruden G., Rodriguez V., Burt D., Gnudi L., Hartley B. and Viberti G. (2000). Vascular endothelial growth factor receptors in human mesangium in vitro and in glomerular disease. J. Am. Soc. Nephrol. 11, 1236-1243.

To W.S. and Midwood K.S. (2011). Plasma and cellular fibronectin: distinct and independent functions during tissue repair. Fibrogenesis Tissue Repair 4, 21.

Tsuchida K., Makita Z., Yamagishi S., Atsumi T., Miyoshi H., Obara S., Ishida M., Ishikawa S., Yasumura K. and Koike T. (1999). Suppression of transforming growth factor beta and vascular endothelial growth factor in diabetic nephropathy in rats by a novel advanced glycation end product inhibitor, OPB-9195. Diabetologia 42, 579-588.

Turk T., Leeuwis J.W., Gray J., Torti S.V., Lyons K.M., Nguyen T.Q. and Goldschmeding R. (2009). BMP signaling and podocyte markers are decreased in human diabetic nephropathy in association with CTGF overexpression. J. Histochem. Cytochem. 57, 623-631.

Van Vliet A., Baelde H.J., Vleming L.J., de Heer E. and Bruijn J.A. (2001). Distribution of fibronectin isoforms in human renal disease. J. Pathol. 193, 256-262.

Veron D., Reidy K.J., Bertuccio C., Teichman J., Villegas G., Jimenez J., Shen W., Kopp J.B., Thomas D.B. and Tufro A. (2010). Overexpression of VEGF-A in podocytes of adult mice causes glomerular disease. Kidney Int. 77, 989-999.

Wahab N.A. and Mason R.M. (2004). Connective tissue growth factor and renal diseases: some answers, more questions. Curr. Opin. Nephrol. Hypertens. 13, 53-58.

Wahab N.A., Yevdokimova N., Weston B.S., Roberts T., Li X.J., Brinkman H. and Mason R.M. (2001). Role of connective tissue growth factor in the pathogenesis of diabetic nephropathy. Biochem. J. 359, 77-87.

Wahab N.A., Schaefer L., Weston B.S., Yiannikouris O., Wright A., Babelova A., Schaefer R. and Mason R.M. (2005a). Glomerular expression of thrombospondin-1, transforming growth factor beta and connective tissue growth factor at different stages of diabetic nephropathy and their interdependent roles in mesangial response to diabetic stimuli. Diabetologia 48, 2650-2660.

Wahab N.A., Weston B.S. and Mason R.M. (2005b). Connective tissue growth factor CCN2 interacts with and activates the tyrosine kinase receptor Trka. J. Am. Soc. Nephrol. 16, 340-351.

Wang L., Kwak J.H., Kim S.I., He Y. and Choi M.E. (2004). Transforming growth factor-ß1 stimulates vascular endothelial growth factor 164 via mitogen-activated protein kinase kinase 3-p38α and p38δ mitogen-activated protein kinase-dependent pathway in murine mesangial cells. J. Biol. Chem. 279, 33213-33219.

Wang A., Ziyadeh F.N., Lee E.Y., Pyagay P.E., Sung S.H., Sheardown S.A., Laping N.J. and Chen S. (2007). Interference with TGF-ß signaling by Smad3-knockout in mice limits diabetic glomerulosclerosis without affecting albuminuria. Am. J. Physiol. Renal Physiol. 293, F1657-F1665.

Wendt T.M., Tanji N., Guo J., Kislinger T.R., Qu W., Lu Y., Bucciarelli L.G., Rong L.L., Moser B., Markowitz G.S., Stein G., Bierhaus A., Liliensiek B., Arnold B., Nawroth P.P., Stern D.M., D'Agati V.D. and Schmidt A.M. (2003). RAGE drives the develolpment of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. Am. J. Pathol. 162, 1123-1137.

Weston B.S., Wahab N.A. and Mason R.M. (2003). CTGF mediates TGF-ß-induced fibronectin matrix deposition by upregulating active $\alpha_5\beta_1$ integrin in human mesangial cells. J. Am. Soc. Nephrol. 14, 601-610.

Wolf G., Chen S. and Ziyadeh F.N. (2005). From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. Diabetes 54, 1626-1634.

Yevdokimova N., Wahab N.A. and Mason R.M. (2001). Thrombospondin-1 is the key activatior of TGF-beta1 in human mesangial cells exposed to high glucose. J. Am. Soc. Nephrol. 12, 703-712.

Yokoi H., Mukoyama M., Sugawara A., Mori K., Nagae T., Makino H., Suganami T., Yahata K., Fujinaga Y., Tanaka I. and Nakao K. (2002). Role of connective tissue growth factor in fibronectin expression and tubulointerstitial fibrosis. Am. J. Physiol. Renal Physiol. 282, F933-F942.

Yokoi H., Mukoyama M., Nagae T., Mori K., Suganami T., Sawai K., Yoshioka T., Koshikawa M., Nishida T., Takigawa M., Sugawara A. and Nakao K. (2004). Reduction in connective tissue growth factor by antisense treatment ameliorates renal tubulointerstitial fibrosis. J. Am. Soc. Nephrol. 15, 1430-1440.

Yokoi H., Mukoyama M., Mori K., Kasahara M., Suganami T., Sawai K., Yoshioka T., Saito Y., Ogawa Y., Kuwabara T., Sugawara A. and Nakao K. (2008). Overexpression of connective tissue growth factor in podocytes worsens diabetic nephropathy in mice. Kidney Int. 73, 446-455.

Ziyadeh F.N., Hoffman B.B., Han D.C., Iglesias-de la Cruz M.C., Hong S.W., Isono M., Chen S., McGowan T.A. and Sharma K. (2000). Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-ß antibody in db/db diabetic mice. Proc. Natl. Acad. Sci. USA 97, 8015-8020.

Accepted April 24, 2012