Glomerulosclerosis is characterized by mesangial matrix accumulation that is mediated primarily by activation of transforming growth factor-ß (TGF-ß). Unlike podocytes, mesangial cells secrete TGF-ß in response to common in vitro fibrogenic stimuli. However, mesangial immunostaining for active TGF-ß1 in chronic glomerular disease is almost negligible, despite increased mesangial TGF-ß1 mRNA expression, while podocytes covering the sclerotic glomerular segments exhibit increased TGF-ß1 protein expression. The mechanisms whereby TGF-ß is activated in the diseased glomeruli and how the activated TGF-ß leads to mesangial matrix overproduction are not clear. We provide evidence that TGF-ß secreted as latent complexes by mesangial cells is stored in the mesangial matrix, from which soluble forms of latent TGF-ß are released and localized to the podocyte surface in chronic glomerular disease. Podocyte-derived reactive oxygen species, plasmin and thrombospondin-1, particularly renin-angiotensin-aldosterone system-induced oxidative stress, seem to be involved in TGF-ß activation in podocytes. We also provide evidence that the TGF-ß-induced secretion of connective tissue growth factor and vascular endothelial growth factor by podocytes acts as a paracrine regulatory mechanism on mesangial cells, which may cause mesangial matrix accumulation culminating in the development of glomerulosclerosis. Collectively, these data bring new insights into our understanding of the roles of the mesangial cells and podocytes in the TGF-ß-induced mesangial matrix synthesis in chronic glomerular disease.

Key words: Chronic glomerular disease, Glomerulosclerosis, Mesangial matrix, Podocytes, TGF-ß activation
diseased glomeruli, and how activated TGF-ß could lead to enhanced mesangial matrix synthesis culminating in the development of glomerulosclerosis.

**Activation of latent TGF-ß**

The activity of all TGF-ß isoforms is under strict control during developmental and pathological processes. TGF-ß is secreted as latent complexes in which a 25 kD homodimer growth factor is noncovalently associated with a latency-associated peptide (LAP, 75 KD). Some cells secrete TGF-ß in the form of a TGF-ß/LAP complex, referred to as the small latent complex. However, most cells secrete TGF-ß as part of a large latent complex, in which latent TGF-ß binding protein (LTBP, 125-160 kD) is linked to the small latent complex. LTBP has a role in targeting the transport of latent TGF-ß complex into the ECM (Hyytiäinen et al., 2004; Koli et al., 2008). Intense mesangial immunostaining for LTBP-1 is observed in anti-Thy1.1 nephritis associated with severe but transient mesangial matrix accumulation (Porst et al., 2006).

The large latent complex is susceptible to proteolysis, within which LTBP is first cleaved at the protease sensitive hinge region. The soluble large latent complex is then released from the ECM and is activated by another proteolytic event that releases TGF-ß from LAP (Koli et al., 2001). The TGF-ß released then binds to its receptor and exerts its cellular functions.

Under in vitro conditions, latent TGF-ß is activated by heating, acid or alkaline treatment, irradiation, reactive oxygen species (ROS), proteases including plasmin, cathepsin, calpain, matrix metalloproteinase (MMP)-2 and MMP-9, some integrins, or thrombospondin-1 (TSP-1) (reviewed in Koli et al., 2001).

Several activation mechanisms may exist in vivo as reviewed below (Table 1).

**TGF-ß activation by proteolysis**

Plasmin could function as an in vivo activator of TGF-ß (Lyons et al., 1990; Edgerton et al., 2004). Plasmin can release the large latent TGF-ß complex from the ECM by cleaving LTBP at the amino terminal hinge region (Taipale et al., 1992). Furthermore, it can cleave LAP that releases active TGF-ß dimer from the large latent TGF-ß1 complex (Lyons et al., 1990; Annes et al., 2003; George et al., 2005) (Table 1).

MMP-2 and MMP-9 have also been implicated in the cleavage of LAP and the release of mature TGF-ß at the cell surface (Yu et al., 2000).

**TSP-1-mediated activation of TGF-ß**

TSP-1 is known to be a major physiologic activator of latent TGF-ß (Crawford et al., 1998). A specific peptide sequence within TSP-1, KRFK, binds to the LSKL sequence in LAP, and releases mature TGF-ß by inducing conformational changes in the protein (Crawford et al., 1998; Lawrence, 2001; Koli et al., 2008) (Table 1). However, it is not clear whether TSP-1 alone could directly activate latent TGF-ß (Abdelouahed et al., 2000; Grainger and Frow, 2000; Otsuka et al., 2007).

Overexpression of both TSP-1 and active TGF-ß occurs in podocytes in patients with focal segmental glomerulosclerosis (FSGS) (Kim et al., 2003) and diabetic nephropathy (Wahab et al., 2005). In various experimental renal disease models, TSP-1 is co-localized with TGF-ß and predicts the development of tissue fibrosis (Hugo et al., 1998).

In rats with anti-Thy1.1 nephritis, active TGF-ß1-positive area/glomerulus was 18%, while treatment with antisense TSP-1 oligodeoxynucleotides reduced it to 9% (Daniel et al., 2003). In wild-type and TSP-1 deficient diabetic mice, the TGF-ß1-positive area in the glomerulus was 5% and 3%, respectively (Daniel et al., 2007). Thus, TSP-1 seems to activate mesangial TGF-ß1 more actively in acute mesangial proliferate GN than in chronic glomerular disease.

**ROS-mediated activation of TGF-ß**

ROS produced by ionizing radiation was found to induce rapid TGF-ß activation in vivo (Barcellos-Hoff and Dix, 1996). ROS can activate TGF-ß directly through oxidation-induced conformational change in LAP, in which the unique methionine residue in the TGF-ß1/LAP functions as a redox switch center (Jobling et al., 2006), or indirectly through the activation of proteolytic enzymes (Koli et al., 2008) (Table 1).

**Integrins in TGF-ß binding and activation**

Integrins can bind to the RGD recognition domain in the LAP of TGF-ß1 and TGF-ß3. Particularly, αvβ6 and αvβ8 are known to activate the TGF-ß complex (Munger et al., 1999; Mu et al., 2002), leading to release of TGF-ß either by tractional force (αvβ6) or by MT1-MMP-dependent proteolytic activity (αvβ8) (Koli et al., 2008). By cell movement, an αvβ6–integrin-expressing cell causes sufficient traction for the release of mature TGF-

---

**Table 1. Possible mechanisms of latent TGF-ß activation in chronic glomerular disease.**

<table>
<thead>
<tr>
<th>Activators</th>
<th>Mechanisms of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmin</td>
<td>Proteolytic nicking of LTBP releasing large latent TGF-ß complex from the ECM</td>
</tr>
<tr>
<td></td>
<td>Proteolytic nicking of the LAP</td>
</tr>
<tr>
<td>TSP-1</td>
<td>Induction of conformational changes in LAP</td>
</tr>
<tr>
<td>ROS</td>
<td>Induction of conformational changes in LAP</td>
</tr>
<tr>
<td>Integrin, αvβ6</td>
<td>Release of TGF-ß from LAP by tractional force</td>
</tr>
</tbody>
</table>

TGF-ß, transforming growth factor-ß; LTBP, latent TGF-ß binding protein; ECM, extracellular matrix; TSP-1, thrombospondin-1; LAP, latency-associated peptide; ROS, reactive oxygen species.
β from the ECM-anchored large latent complex (Annes et al., 2004) (Table 1). αvβ6 integrin is expressed in the diseased kidneys confined to the distal tubules and collecting ducts (Trevisian et al., 2004).

**Expression of TGF-β in podocytes**

**Mesangial expression of TGF-β**

In the early stage of human diabetic nephropathy (Wahab et al., 2005) and IgA nephropathy (IgAN), mesangial cell proliferation (Stein-Oakley et al., 1997), some mesangial cells show immunoreactivity for TGF-β1. Nonetheless, mesangial immunostaining for active TGF-β1 is very weak or almost negligible in most human IgAN (Stein-Oakley et al., 1997; Kim et al., 2002), FSGS (Kim et al., 2003) and diabetic nodular glomerulosclerosis (Wahab et al., 2005), despite increased mesangial TGF-β1 mRNA levels (Kim et al., 2002, 2003; Wahab et al., 2005).

Mesangial expression of TGF-β isoforms is transiently upregulated in acute anti-Thy1.1 nephritis (Ito et al., 2001; Hartner et al., 2003), in which TGF-β1 expression is only segmentally and weakly distributed (Ito et al., 2001). In the glomeruli of experimental diabetic nephropathy, only a few cells show positive immunostaining for TGF-β1 (Hill et al., 2000). Mutations of the Wilms’ tumour suppressor gene, WT1, induce Denys-Drash syndrome (DDS) characterized by diffuse mesangial sclerosis. In DDS mice, no TGF-β1 expression occurred in the mesangium (Patek et al., 2003).

Altogether, immunohistochemical antigen detection for mesangial TGF-β1 might be difficult in progressive glomerular disease, suggesting that latent TGF-β complexes secreted by mesangial cells might be stored in the mesangial matrix to provide stability to the active molecule.

**Expression of TGF-β in podocytes**

In contrast to mesangial cells, podocytes covering the sclerotic segments exhibit increased expression of TGF-β1 mRNA and protein in human IgAN (Kim et al., 2002), FSGS (Kim et al., 2003) and end-stage diabetic nephropathy (Wahab et al., 2005), and in DDS mice (Patek et al., 2003).

**Profibrotic growth factors mediating the downstream fibrogenic activity of TGF-β**

**Connective tissue growth factor (CTGF)**

CTGF is a major autocrine growth factor induced by TGF-β. TGF-β1 induces CTGF mRNA and protein expression in mesangial cells (Riser et al., 2000; Blom et al., 2001; Ito et al., 2001; Chen et al., 2002; Cooker et al., 2007), podocytes (Ito et al., 2001), and parietal epithelial cells (Kanemoto et al., 2003). However, CTGF administration stimulates CTGF, but not TGF-β, expression in mesangial cells (Riser et al., 2000). Both TGF-β and CTGF independently induced collagen or fibronectin expression in mesangial cells (Riser et al., 2000; Blom et al., 2001; Gore-Hyer et al., 2002; Weston et al., 2003). CTGF can interact with, and influence the signaling of, TGF-β and bone morphogenetic proteins (BMPs) (Abreu et al., 2002; Nguyen et al., 2008), and vascular endothelial growth factor (VEGF) (Hashimoto et al., 2002). Maximal TGF-β1 induction of CTGF requires synergy between Smad and Ras/extracellular signal-regulated kinase signaling (Chen et al., 2002).

Expression of CTGF mRNA and/or protein in the mesangium and podocytes is upregulated in human chronic glomerular disease (Ito et al., 1998; Wahab et al., 2005), but not in acute postinfectious GN (Ito et al., 1998). CTGF is also overexpressed in the glomeruli of experimental diabetic glomerulosclerosis (Riser et al., 2000) and mesangial proliferative GN (Ito et al., 2001).

**VEGF**

Besides its angiogenic actions, VEGF may play an important role in TGF-β1-induced glomerular fibrosis (Wang et al., 2004; Chen et al., 2005). TGF-β1 stimulates VEGF expression in podocytes (Iglesias-de-la Cruz et al., 2002) and mesangial cells (Wang et al., 2004), although treatment with anti-TGF-β slightly prevented the enhanced VEGF mRNA expression in the db/db kidneys (Ziyadeh et al., 2000). VEGF increases type I collagen and fibronectin synthesis in mesangial cells via a TGF-β1-induced mitogen-activated protein kinase activity (Wang et al., 2004). VEGF and/or VEGF receptor expression is increased in the glomeruli of diabetic animals, particularly in the podocytes (Wendt et al., 2003; Sung et al., 2006).

**Platelet-derived growth factor (PDGF)**

PDGF-B and PDGF–D chains mediate mesangial cell proliferation and matrix accumulation in anti-Thy1.1 model (Ostendorf et al., 2006) and in diabetic nephropathy (Nakagawa et al., 2000). PDGF-B induces TGF-β in mesangial cells (Yamabe et al., 2000) or vice versa (Chow et al., 2005). PDGF-B antagonism had no effect on the glomerular expression of TGF-β system components in rat anti-Thy1.1 model despite reduced ECM protein accumulation (Ostendorf et al., 2002). Thus, PDGF-B could act downstream of TGF-β, or PDGF-B and TGF-β might act independently of each other in vivo.

**Activation of latent TGF-β in the diseased glomeruli**

**Activation of TGF-β by mesangial cells**

The latent TGF-β complexes stored in the mesangial matrix may be catabolized by plasmin. The plasmin-mediated TGF-β activation, however, may be neutralized via feedback inhibition, since TGF-β-induced
plasminogen activator (PA) inhibitor-1 production decreases the formation of active plasmin in mesangial cells (Baricos et al., 2003). Furthermore, accumulation of mesangial matrix progressed in association with enhanced mesangial fibrin deposition in rats with anti-Thy 1.1 nephritis (Liu et al., 2004). Thus, the mesangial cell surface surrounded by an enlarged matrix may not express active plasmin to liberate mature TGF-ß.

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), although albumin load or mechanical strain upregulates TGF-ß1 mRNA expression in them (Abbate et al., 2002; Durvasula et al., 2004). In this regard, Wolf et al. (2005) suggested that podocytes respond to paracrine TGF-ß coming from the mesangial cell. Even though free active TGF-ß is liberated from the mesangial cells, it has a very short half-life in plasma (2-3 min) (Coffey et al., 1987), in contrast to the latent TGF-ß complex with a significantly longer half-life (>100 min) (Wakefield et al., 1990). Thus, latent TGF-ß, rather than active TGF-ß, may be localized to the podocyte surface after its release from the mesangial matrix.

**Activation of latent TGF-ß by podocytes**

The renin-angiotensin-aldosterone system (RAAS) seems to be involved in podocyte injury through the induction of oxidative stress in experimental renal disease (Chiba et al., 2002; Nagase et al., 2006; Whaley-Connell et al., 2008; Shibata et al., 2007). Diabetic podocytopathy is also mediated by Ang II (Ziyadeh and Wolf, 2008). Furthermore, podocyte-specific overexpression of the antioxidant metallothionein reduces the glomerular injury in diabetic mice, suggesting that oxidative damage to the podocyte plays a pathogenetic role in diabetic nephropathy (Zheng et al., 2008). Salt loading in spontaneously hypertensive stroke-prone rats induces renal inflammation/fibrosis, podocyte injury, and an increase in plasmin and MMP-2 activity (Gianella et al., 2007). Collectively, injured podocytes mediated by RAAS seem to overproduce ROS, which can activate the latent TGF-ß localized to their surface, either directly or indirectly, through the activation of proteolytic enzymes in chronic renal disease.

Control renal biopsies exhibit expression of urokinase-type PA (uPA) mRNA confined to the podocytes. Strong expression of uPA protein and mRNA is sometimes observed in podocytes within crescents, suggesting that damaged podocytes can release plasmin (Lee et al., 2001). In human FSGS lesions, the expression levels of TGF-ß1, TSP-1 and TGF-ß type II receptor mRNAs and proteins and phosphorylated Smad2/Smad3 are increased in podocytes (Kim et al., 2003). These observations suggest that damage to podocytes may stimulate the TGF-ß/Smad signaling pathway, and that TSP-1 may activate TGF-ß1 in podocytes in chronic glomerular disease.

Altogether, podocyte-derived ROS, plasmin and TSP-1 may release mature TGF-ß from LAP localized to the podocytes in the diseased glomeruli. The activated TGF-ß may bind to its receptor on podocytes, activating the TGF-ß/Smad signaling pathway to induce the overexpression of its target genes, such as CTGF and VEGF.

**Paracrine interaction of growth factors between podocytes and mesangial cells in the diseased glomeruli**

Podocyte-specific injury in adult mice leads to mesangial expansion and glomerulosclerosis (Matsusaka et al., 2005), suggesting that some humoral factors produced by podocytes seem to have fibrogenic effects on mesangial cells (Table 2). In support of this hypothesis, CTGF mRNA expression occurred only in the podocytes and glomerular parietal epithelial cells during the early stage of the anti-Thy-1.1 nephritis, a model of mesangiopathy (Ito et al., 2001). Furthermore, induction of diabetes in podocyte-specific CTGF-transgenic mice results in an increased mesangial CTGF expression with more severe mesangial expansion than diabetic wild-type mice (Yokoi et al., 2008) (Table 2). Although the glomerular expression of VEGF seems to be confined to the podocytes (Wendt et al., 2003), anti-

### Table 2. Animal experiments to support the hypothesis that podocyte-derived growth factors induce mesangial matrix accumulation.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Results</th>
<th>1st author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Podocyte-specific injury in mice</td>
<td>Mesangial expansion</td>
<td>Matsusaka (2005)</td>
</tr>
<tr>
<td>Anti-Thy-1.1 nephritis</td>
<td>CTGF mRNA expression confined to podocytes</td>
<td>Ito (2001)</td>
</tr>
<tr>
<td>Diabetes in podocyte-specific CTGF-transgenic mice</td>
<td>Increased mesangial CTGF expression and more severe mesangial expansion than wild-type mice</td>
<td>Yokoi (2008)</td>
</tr>
<tr>
<td>Anti-VEGF administration into diabetic mice</td>
<td>Decreased mesangial matrix expansion</td>
<td>Flyvbjerg (2002)</td>
</tr>
<tr>
<td>Diabetes in podocyte-specific BMP-7-transgenic mice</td>
<td>Prevents glomerular fibrosis without change in TGF-ß levels</td>
<td>Wang (2006)</td>
</tr>
</tbody>
</table>

TGF-ß, transforming growth factor-ß; CTGF, connective tissue growth factor; VEGF, vascular endothelial growth factor; BMP-7, bone morphogenic protein-7.
VEGF attenuates the mesangial matrix expansion in diabetic mice (Flyvbjer g et al., 2002). BMP-7 counteracts the fibrogenic action of TGF-β, possibly by inhibiting the fibrogenic Smad signaling. Transgenic expression of BMP-7 in podocytes of diabetic mice prevents glomerular fibrosis without changing renal TGF-ß levels (Wang et al., 2006), suggesting that the activated TGF-ß/Smad signaling in podocytes may lead to mesangial matrix accumulation.

Contrary to the general perception that solutes cannot move against the flow of glomerular filtration, about one third of VEGF secreted from podocytes would reach the capillary lumen and accumulate there (Katavetin and Katavetin, 2008). Indeed, diffusion dominates the transport of most solute in the glomerular basement membrane (Deen et al., 2001). Although it is not clear whether this is also the case for CTGF, the experiments performed by Yokoi et al. (2008) support that possibility.

To sum up, latent TGF-ß1 complexes secreted by mesangial cells seem to be stored in the mesangial matrix in chronic glomerular disease. Soluble forms of latent TGF-ß released from the mesangial matrix may be localized to the podocyte surface, where ROS, plasmin and TSP-1 seem to activate the latent TGF-ß in podocytes. TGF-ß-induced CTGF and VEGF secretion by podocytes may act as a paracrine regulatory mechanism, necessary for the mesangial matrix accumulation (Fig. 1).

**Fig. 1.** Hypothetical pathway for mesangial matrix accumulation via activation of latent TGF-ß by podocytes in chronic glomerular disease. CTGF, connective tissue growth factor; VEGF, vascular endothelial growth factor; TGF-ß, transforming growth factor-ß; TSP-1, thrombospondin-1; ROS, reactive oxygen species.

**Conclusions**

Experimental and clinical data suggest that latent TGF-ß complexes secreted by mesangial cells are stored in the mesangial matrix, from which soluble forms of latent TGF-ß may be released and localized to the podocyte surface in chronic glomerular disease. Podocyte-derived ROS, plasmin and TSP-1, particularly RAA5-induced oxidative stress, seem to be involved in TGF-ß activation. Active TGF-ß may induce CTGF and VEGF overexpression in podocytes, which may act as a paracrine regulatory mechanism on mesangial cells to stimulate mesangial matrix synthesis. Research on the glomerular production and activation of TGF-ß and its downstream mediators, CTGF and VEGF, will further our comprehension of the mesangial matrix accumulation and provide new therapeutic strategies to prevent the development of glomerulosclerosis in patients with chronic kidney disease.

**References**


with increased plasma levels of TGF-ß1 develop progressive renal disease. Lab. Invest. 74, 991-1003.


TGF-β and glomerulosclerosis


Accepted February 23, 2009