Review

Pathogenic role of TGF-ß in the progression of podocyte diseases

Hyun Soon Lee

Department of Pathology, Seoul National University College of Medicine, Chongno-gu, Seoul, Korea

Summary. In patients with progressive podocyte diseases, such as focal segmental glomerulosclerosis and membranous nephropathy, there is enhanced expression of transforming growth factor (TGF-ß) in podocytes. Biomechanical strain in these diseases may cause overexpression of TGF-ß and angiotensin II (Ang II) by podocytes. Oxidative stress induced by Ang II may activate the latent TGF-ß. Increased TGF-ß activity by podocytes may induce not only the thickening of the glomerular basement membrane (GBM), but also podocyte apoptosis and/or detachment from the GBM, initiating the development of glomerulosclerosis. Furthermore, mesangial matrix expansion frequently occurs in podocyte diseases in association with the development of glomerulosclerosis. This review examines open questions on the pathogenic role of TGF-ß that links podocyte injury to GBM thickening, podocyte loss, mesangial matrix expansion and glomerulosclerosis in podocyte diseases. It also describes paracrine regulatory mechanisms of podocyte TGF-ß on mesangial cells leading to increased matrix synthesis.

Key words: Angiotensin II, Biomechanical strain, Glomerular basement membrane thickening, Mesangial matrix expansion, Oxidative stress, Podocyte apoptosis

Introduction

TGF-ß plays an important role in glomerular disease, and is mainly involved in extracellular matrix (ECM) protein synthesis of renal cells. Podocytes are the target of injury in most glomerular disease. In podocyte diseases such as focal segmental glomerulosclerosis (FSGS) (Kim et al., 2003), membranous nephropathy

(Shankland et al., 1996; Kim et al., 1999), Alport renal disease (Sayers et al., 1999), and Denys-Drash syndrome (DDS) (Patek et al., 2003), expression of TGF-ß mRNA and/or protein is increased in podocytes. TGF-ß may contribute to the thickening of the glomerular basement membrane (GBM) and abnormal deposition of ECM therein. Furthermore, enhanced TGF-ß activity may lead to podocyte apoptosis and/or detachment with podocytopenia, initiating the development of glomerulosclerosis (Schiffer et al., 2001; Wolf et al., 2005; Dessapt et al., 2009; Lee and Song, 2010). Glomerulosclerosis frequently complicates most renal diseases, and is characterized by capillary luminal collapse and accumulation of mesangial matrix. In progressive podocyte diseases, mesangial matrix expansion frequently occurs in association with glomerulosclerosis (Lee and Koh, 1993; Lee and Lim, 1995; Patek et al., 2003). The mechanisms whereby podocyte TGF-ß contributes to the progression of podocyte diseases are still poorly understood.

This review will focus the discussion on the pathogenic role of TGF-ß that links podocyte injury to GBM thickening, podocyte loss, mesangial matrix expansion and glomerulosclerosis in podocyte diseases.

Progressive podocyte diseases with TGF-ß overexpression in podocytes

FSGS

Intrarenal transcription of TGF-ß1 is enhanced in children with FSGS compared to those with minimal lesion, suggesting that TGF-ß1 gene transcription is indicative of progressive renal damage typical of FSGS (Strehlau et al., 2002). Expression of TGF-ß1 is increased in patients with primary FSGS, particularly in podocytes of sclerotic segments (Kim et al., 2003). Volume density of mesangial matrix is significantly greater in the FSGS patients than in minimal lesion cases. In patients with FSGS, the percent

glomerulosclerosis correlates directly with mesangial volume per glomerulus (Lee and Lim, 1995).

In rats with subtotal renal ablation, TGF-ß1 is upregulated by podocytes in response to enhanced transcapillary passage of plasma proteins, which precedes the development of glomerulosclerosis (Abbate et al., 2002).

Membranous nephropathy

Subepithelial immune deposition promotes injury to the glomerular filtration barrier, proteinuria, and eventual renal failure in patients with membranous nephropathy. Complement membrane attack complex (C5b-9) plays an important role in the development of podocyte injury and proteinuria in passive Heymann nephritis (PHN), an experimental model of human membranous nephropathy (Couser and Nangaku, 2006). Upregulation of TGF-ß1, α4(IV) and α1(IV) collagens, and laminin ß2 mRNAs by podocytes is shown in patients with membranous nephropathy (Kim et al., 1999). In addition, there are increased immunogold densities for polyclonal type IV collagen (in the distribution of the α1(IV) and α2(IV) collagen chains), α4(IV) collagen, laminin, and fibronectin in the subepithelial projections or spikes (Zhang and Lee, 1997). Expression of TGF-ß2 is also markedly increased in podocytes in experimental membranous nephropathy, together with upregulation of TGF-ß receptors (Shankland et al., 1996).

FSGS lesions are observed in 43% of the membranous nephropathy patients, in whom the degree of mesangial expansion and GBM thickening is significantly greater than the remaining cases without FSGS (Lee and Koh, 1993). In PHN, mesangial volume was also significantly elevated, together with GBM thickening (Remuzzi et al., 1999).

Diabetic nephropathy

Thickening of the GBM and expansion of the mesangial matrix are hallmarks of diabetic nephropathy. Podocytes are injured very early in the course of diabetic nephropathy. GBM thickening and expansion of the mesangial matrix occur even within a few years after the onset of type 1 diabetes (Drummond and Mauer, 2002). In insulin-dependent diabetes, the collagen α3(IV) through α5(IV) chains, collagen V, laminin, fibronectin, and serum proteins contribute to the thickened GBM (Miner, 1999).

In diabetic nodular glomerulosclerosis, podocytes covering the sclerotic segments show increased expression of TGF-ß1 mRNA and protein (Wahab et al., 2005). Enhanced expression of glomerular TGF-ß1 is observed mainly in podocytes of diabetic animals (Baba et al., 2005; Okada et al., 2006).

Alport renal disease

Alport syndrome is a primary genetic disease of the basement membrane. In the kidney, this disorder is characterized by an absence of collagen α3α4α5(IV) in the GBM, progressive thickening and multilamination of the GBM, proteinuria, and renal failure (Kalluri et al., 1997). Collagen α1/α2(IV), however, is retained throughout the GBM, together with the deposition of the laminin chains α1, α2 and ß1 (Kashtan et al., 2001; Abrahamson et al., 2003). In podocytes of α3(IV) collagen-knockout mice with Alport renal disease, mRNA expression of TGF-ß1, α1(IV) and α2(IV) collagen, fibronectin, and laminin ß1 chain is increased (Sayers et al., 1999). With disease progression, mesangial matrix and cells are increased, followed by the development of glomerulosclerosis (Kim et al., 1995; Gregory et al., 1996; Mazzucco et al., 2002).

DDS

Mutations of the Wilms' tumour suppressor gene, WT1, induce DDS, characterized by diffuse mesangial sclerosis. The development of glomerulosclerosis is preceded by de novo TGF-ß1 expression in DDS podocytes (Patek et al., 2003). A gene mutation in DDS podocytes may not be sufficient to cause TGF-ß overexpression (Jin et al., 1999), but in the presence of a second injury, such as intraglomerular hypertension, TGF-ß seems to be overexpressed by podocytes (Patek et al., 2003).

Altogether, TGF-ß is overexpressed by podocytes in progressive podocyte diseases, in which there are thickening of the GBM, mesangial matrix expansion and the eventual development of glomerulosclerosis (Table 1).

Induction of TGF-ß by glomerular hypertension or biomechanical strain in podocyte diseases

In progressive glomerular disease, increased intraglomerular pressure results in cellular strain and perpetuates further damage to the podocytes, eventually leading to glomerulosclerosis (Kriz et al., 1998). Glomerular hemodynamic adaptive changes, including hyperfiltration and hyperperfusion, seem to promote progressive glomerulosclerosis in patients with reduced

Table 1. Glomerular basement membrane (GBM) thickening and mesangial matrix expansion in podocyte diseases with TGF-ß overexpression.

Diseases	GBM thickening	Mesangial matrix expansion
FSGS	ND	yes
Membranous nephropathy	yes	yes
Diabetic nephropathy	yes	yes
Alport syndrome	yes	yes
Denys-Drash syndrome	ND	yes

TGF-ß: transforming growth factor-ß; FSGS: focal segmental glomerulosclerosis; ND: not determined.

nephron mass and diabetes (Ziyadeh and Wolf, 2008). The less cross-linked and possibly more elastic physical properties of the GBM in podocyte diseases may subject the podocytes to elevated biomechanical strain even under normal glomerular blood pressure. As the disease progresses and nephron mass is lost, glomerular hypertension develops, further exacerbating the biomechanical strain and the effector functions influenced by it (Meehan et al., 2009). In the remnant kidney model of glomerular capillary hypertension, TGF-ß1 (Abbate et al., 2002) and angiotensin II (Ang II) type 1 receptor (Durvasula et al., 2004) are upregulated by podocytes. In cultured podocytes, albumin load or mechanical strain increases the levels of TGF-ß1 and Ang II, as well as TGF-ß type I, II and III receptors (Abbate et al., 2002; Durvasula et al., 2004; Dessapt et al., 2009).

Together, an increase in glomerular capillary pressure may stimulate Ang II and TGF-ß1 expression in podocytes through mechanical force injury in progressive podocyte diseases (Fig. 1).

Effects of Ang II on TGF-ß signaling in podocyte diseases

The renin-angiotensin system (RAS) seems to be involved in podocyte injury through the induction of oxidative stress in experimental renal disease (Shibata et al., 2007; Whaley-Connel et al., 2008) and diabetic podocytopathy (Ziyadeh and Wolf, 2008). Ang II is a major active product of the RAS. NADPH oxidase produces reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, and is strongly expressed by podocytes (Tojo et al., 2007). Ang II may enhance the generation of ROS through the activation of NADPH oxidases in podocytes.

In podocyte diseases, expression levels of TGF-ß are increased in the podocytes (Shankland et al., 1996; Kim et al., 1999, 2003; Sayers et al., 1999; Patek et al., 2003; Wahab et al., 2005). The activity of TGF-ß is under strict control during developmental and pathological processes. TGF-ß is secreted as latent complexes. Several activation mechanisms for latent TGF-ß, such as ROS, proteolysis, some integrins, and thrombospondin-1, may exist in vivo. Ang II-induced ROS can activate the latent TGF-ß in injured podocytes (reviewed in Lee and Song, 2009). Unlike mesangial cells, podocytes do not overexpress TGF-ß1 in response to Ang II (Chen et al., 2005). Rather, Ang II stimulates the expression of the vascular endothelial growth factor (VEGF) (Chen et al., 2005), which, in turn, increases the expression of TGF-ß type II receptor and Smad2 phosphorylation (Chen et al., 2004). Although Ang II does not directly stimulate the expression of TGF-ß1 in podocytes, Ang II-induced oxidative stress in podocyte diseases may activate the latent TGF-ß and, subsequently, the TGF-ß signaling system in podocytes (Fig. 1).

Studies in animal models of chronic nephropathies have documented that RAS inhibitors significantly blunt the increased renal TGF-ß production. An angiotensin-converting enzyme (ACE) inhibitor prevents TGF-ß1 overexpression in podocytes and glomerulosclerosis in rats with reduced renal mass (Abbate et al., 2002). It also reduces the TGF-ß1, connective tissue growth factor (CTGF) and ECM protein overexpression in kidney and glomerulosclerosis in mice with Alport syndrome (Gross et al., 2003, 2004; Gross and Kashtan, 2009), and limits mesangial expansion in PHN (Remuzzi et al., 1999). Combined anti-TGF-ß and ACE inhibition therapy completely abrogates the glomerulosclerosis of overt diabetic nephropathy in the rat (Benigni et al., 2003). In addition, administration of Ang II type 1 (AT_1) receptor blocker to diabetic rats lowers glomerular expression of TGF-ß1 and VEGF (Vieitez et al., 2008).

Together, oxidative stress induced by Ang II may activate the latent TGF-ß in podocyte diseases and, subsequently, the TGF-ß signaling system in podocytes, eventually leading to glomerulosclerosis.

GBM thickening in relation to TGF-ß in podocyte diseases

Collagen type IV is the main component of the GBM, which includes six genetically distinct isoforms named α1(IV) to α6(IV). α3-α5(IV) chains originate
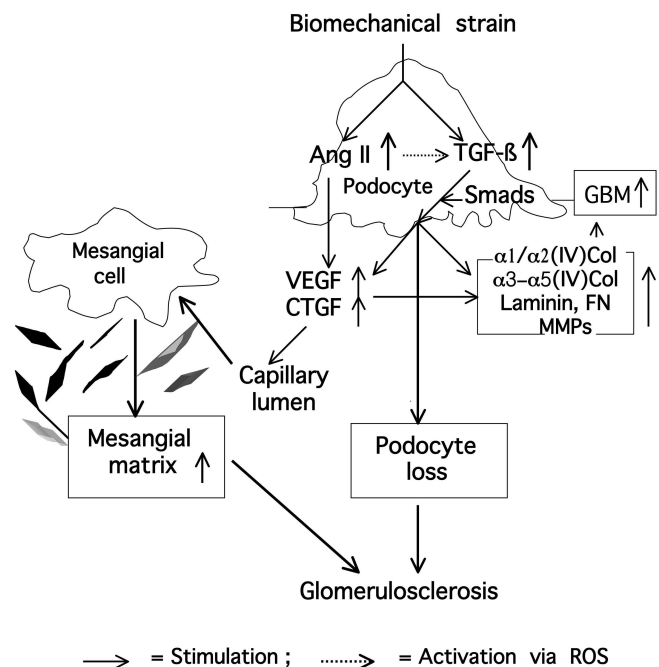

Fig. 1. Hypothetical pathway for glomerular basement membrane (GBM) thickening, podocyte loss and mesangial matrix expansion via activation of transforming growth factor-ß (TGF-ß)/Smad signaling in podocyte diseases. Ang II, angiotensin II; Col, collagen; CTGF, conntective tissue growth factor; FN, fibronectin; MMP, matrix metallo-proteinase; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.

solely from podocytes in both the developing and mature glomerulus (Abrahamson et al., 2009). In contrast, the α1/α2(IV) collagen network seems to originate mainly from glomerular endothelial cells (Lee et al., 1993) and is localized predominantly at the endothelial aspect of human GBM (Zhang and Lee, 1997). Laminin is the most ample glycoprotein in the GBM. Laminin-11 (α5ß2γ1) continues to be deposited in the GBM whereas the fetal laminin chains (α1, α2 and ß1) gradually disappear from the GBM (Miner, 2005).

In TGF-ß1 transgenic mice, the GBM is significantly thickened as compared with wild-type animals (Wogensen et al., 1999; Krag et al., 2007), where laminin ß2 chains and α4(IV) collagen are predominantly seen (Chai et al., 2003). In addition, aberrant deposition of fetal laminin α1, α2 and ß1 chains and α1/α2(IV) collagen appears in the GBM (Chai et al., 2003). Similar to the TGF-ß1 transgenic mice, there is aberrant expression of collagen α1/α2(IV), and laminin α1, α2 and ß1 in the thickened GBM in cases with membranous nephropathy and Alport's syndrome (Zhang and Lee, 1997; Kashtan et al., 2001; Fischer et al., 2000; Cosgrove et al., 2000; Abrahamson et al., 2003). TGF-ß1 increased α3(IV) collagen expression in cultured mouse podocytes, although it decreased the levels of α1(IV) and α5(IV) mRNA and/or protein (Iglesias-de la Cruz et al., 2002). Collectively, GBM thickening by abnormal deposition of ECM in podocytopathies could be due to the enhanced TGF-ß1 levels in podocytes.

A further potential mechanism for GBM destruction and thickening involves the action of proteolytic enzymes, such as matrix metalloproteinases (MMPs). MMP-9 expression is increased in podocytes in experimental membranous nephropathy (McMillan et al., 1996). Levels of MMPs are increased in the glomeruli of Alport mice and kidneys of patients with Alport syndrome (Zeisberg et al., 2006). The aberrant collagen α1/α2(IV) network deposited in the GBM contains fewer interchain crosslinks than wild-type GBM, and is more susceptible to proteolytic degradation by endogenously expressed MMPs (Kalluri et al., 1997; Zeisberg et al., 2006). Blocking the activity of specific MMPs has been shown to ameliorate the progression of glomerular pathology (Zeisberg et al., 2006). In cultured podocytes, TGF-ß1 stimulates the production of MMP-9 (Liu et al., 2005; Li et al., 2008), and many of the MMPs (MMP-3, -9, -10, and -14) are induced by mechanical strain (Meehan et al., 2009). Together, increased TGF-ß1 levels in podocytes may induce MMPs, resulting in proteolytic damage and thickening of the GBM in progressive podocyte diseases.

GBM thickening in diabetic mice is prevented by Smad3 deficiency (Wang et al., 2007) or administration of anti-TGF-ß antibody (Chen et al., 2003). Inhibition of TGF-ß signaling activity, by injecting a soluble TGF-ß1 type II receptor as a competitive inhibitor, prevents irregular thickening of the GBM in Alport mice (Cosgrove et al., 2000).

In summary, enhanced TGF-ß/Smad signaling in podocytes seems to play an important role in GBM thickening by overproduction of abnormal ECM proteins and by impaired GBM degradation in podocyte diseases (Fig. 1) (Table 2).

Pathogenic role of TGF-ß in the development of glomerulosclerosis in podocyte diseases

Podocyte loss in relation to TGF-ß: the link to glomerulosclerosis

In podocyte diseases, enhanced TGF-ß activity in podocytes may lead to podocyte apoptosis and/or detachment with podocytopenia (Schiffer et al., 2001; Wolf et al., 2005; Dessapt et al., 2009; Lee and Song, 2010). C5b-9 can induce apoptosis of podocytes in membranous nephropathy (Mundel and Shankland, 2002), a process that may involve TGF-ß. Apoptosis is also observed in the crescentic lesion of DDS kidneys (Yang et al., 2004), where TGF-ß is overexpressed in hyperplastic podocytes (Lee and Song, 2010). TGF-ß1 phosphorylates Smad2 in podocytes (Schiffer et al., 2004; Liu et al., 2005). Activated TGF-ß/Smad signaling in injured podocytes may increase p15 and p21, resulting in growth arrest (Lee and Song, 2010). In TGF-ß1 transgenic mice, podocytes undergo apoptosis at an early stage of glomerulosclerosis with overexpression of Smad7 (Schiffer et al., 2001). In CD2-associated protein deficient mice, TGF-ß-induced podocyte apoptosis is an early pathomechanism developing FSGS (Schiffer et al., 2004).

Another mechanism of podocyte loss in podocyte diseases may relate to the detachment of podocytes from the GBM. Integrins attach cells to ECM. α3ß1 integrin is an adhesion receptor for laminins and type IV collagen and is located in the basal plasma membrane of podocytes (Kreidberg and Symons, 2000). Downregulation of α3ß1 integrin is observed in the podocytes of patients with primary FSGS (Chen et al., 2006) and diabetes (Chen et al., 2000) associated with podocytopenia. TGF-ß1 suppresses the glomerular expression of α3 integrin in nephrotic rats (Kagami et

Table 2. Evidence to support the hypothesis that TGF-ß/Smad signaling induces GBM thickening.

Disease model	Results	1st author
TGF-ß1 transgenic mice	Thickening of the GBM	Wogensen (1999) Krag (2007)
Smad3-knockout diabetic mice	Prevents GBM thickening	Wang (2007)
Anti-TGF-ß treatment in diabetic mice	Prevents GBM thickening	Chen (2003)
Soluble TGF-ß type II receptor treatment in Alport mice	Prevents GBM thickening	Cosgrove (2000)

TGF-ß: transforming growth factor-ß; GBM: glomerular basement membrane.

al., 1993). In cultured podocytes, TGF-ß1 and mechanical stretch significantly reduce the α3ß1 integrin expression linked to decreased podocyte adhesion and increased apoptosis (Dessapt et al., 2009). Thus, TGF-ß1 may reduce podocyte adhesion to the GBM via downregulation of α3ß1 integrin, resulting in podocyte depletion in podocyte diseases.

In patients with FSGS and membranous nephropathy, nephrin mRNA expression by podocytes is significantly decreased as compared with minimal lesion cases (Kim et al., 2002). Recent studies have shown that TGF-ß1 suppresses the slit diaphragm-associated protein P-cadherin, zonula occludens-1, and nephrin in cultured podocytes (Li et al., 2008). These observations also support the notion that more severe and/or longer podocyte injury induced by TGF-ß may lead to podocyte detachment from GBM and/or apoptosis in podocyte diseases (Li et al., 2008; Liu, 2010).

Podocytes are growth-arrested terminally differentiated cells, and are incapable of replication following their loss. Accordingly, any significant damage to the podocyte must be viewed as a potential starting point for irreversible glomerular damage (Kriz et al., 1998; Mundel and Shankland, 2002; Kriz and LeHir, 2005; Wolf et al., 2005; Ziyadeh and Wolf, 2008). After detachment of podocytes from the GBM with loss of the entire cell into urinary space, the denuded GBM may adhere to the Bowman's capsule with synechiae formation, initiating the development of FSGS (Kriz et al., 1998; Kriz and LeHir, 2005).

In summary, TGF-ß may induce podocyte apoptosis and detachment from the GBM in podocyte diseases leading to the development of glomerulosclerosis (Fig. 1).

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

In patients with podocyte diseases, such as FSGS and membranous nephropathy, mesangial matrix expansion is frequently observed in association with the development of glomerulosclerosis (Lee and Koh, 1993; Lee and Lim, 1995). Podocyte-specific injury in transgenic mice induced mesangial expansion and glomerulosclerosis (Matsusaka et al., 2005). Increased intraglomerular pressure has been linked to podocyte injury, mesangial cell matrix overproduction, thickening of the GBM and the eventual development of glomerulosclerosis (Ziyadeh and Wolf, 2008). Conditioned medium of albumin-stimulated podocytes, like TGF-ß1 itself, induced expression of α-smooth muscle actin, a sclerosing phenotype, in cultured mesangial cells, an effect blocked by anti-TGF-ß1 (Abbate et al., 2002).

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). Even though mesangial cells secret TGF-ß in cases with diabetic nephropathy, it is in latent form, which may be localized to the podocyte surface to be activated (Lee and Song, 2009). Collectively, TGF-ß/Smad signaling in podocytes seems to play a crucial role in mesangial matrix expansion in podocyte diseases.

Paracrine effector mechanism of CTGF and VEGF for TGF-ß to act on mesangial cells

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. Instead, some TGF-ß-induced humoral factors produced by podocytes seem to have fibrogenic effects on mesangial cells (Lee and Song, 2009).

CTGF is a major autocrine growth factor induced by TGF-ß. TGF-ß1 induces CTGF mRNA and protein expression in podocytes (Ito et al., 2001). 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). It is increased particularly in the glomeruli of patients with mesangial matrix expansion (Suzuki et al., 2003). 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).

VEGF is a potent angiogenic molecule and is detected predominantly in podocytes (Bailey et al., 1999; Wendt et al., 2003). And yet, glomeruli are not sites of angiogenesis, possibly because podocytes mainly express $VEGF_{165}b$ protein, which inhibits $VEGF_{165}$-mediated angiogenesis (Cui et al., 2004). VEGF may play an important role in TGF-ß1-induced glomerular fibrosis (Chen et al., 2004, 2005). TGF-ß1 stimulates VEGF expression in podocytes (Iglesias-de la Cruz et al., 2002). Anti-VEGF attenuates mesangial matrix expansion in diabetic mice (Flyvbjerg et al., 2002).

Damage to podocytes in various glomerular diseases has the potential for releasing large amounts of VEGF locally (Shulman et al., 1996). In patients with membranous nephropathy, VEGF expression in podocytes is either increased (Shulman et al., 1996) or decreased (Honkanen et al., 2003), or shows no change (Bailey et al., 1999; Siviridis et al., 2003), while it is increased in the mesangium (Honkanen et al., 2003). 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), whereas VEGF mRNA-positive cells are reduced in patients with diabetic nephropathy (Bailey et al., 1999).

Contrary to the general perception that solutes cannot move against the flow of glomerular filtration, about one third of VEGF secreted from podocytes could reach the capillary lumen and accumulate there (Katavetin and Katavetin, 2008). Although it is not clear whether this is also true for CTGF, the experiments performed by Yokoi et al. (2008) support that possibility.

In summary, TGF-ß-induced CTGF and VEGF secretion by podocytes may act as an effector mechanism, necessary for mesangial matrix accumulation in podocyte diseases, culminating in the development of glomerulosclerosis (Fig. 1).

Therapeutic strategies to prevent the progression of podocyte diseases

Inhibitors of RAS

An ACE inhibitor prevents renal or podocyte TGF-ß1 overexpression and glomerulosclerosis in rats with reduced renal mass (Abbate et al., 2002) and in Alport mice (Gross et al., 2003, 2004; Gross and Kashtan, 2009). AT_1 receptor blocker also reduces the glomerular expression of TGF-ß1 in diabetic rats (Vieitez et al., 2008). Combined ACE inhibition and anti-TGF-ß therapy completely abrogates glomerulosclerosis in experimental diabetic nephropathy (Benigni et al., 2003). The recently discovered ACE2 can form a vasodilatory compound, angiotensin-(1-7), from Ang II. Chronic treatment with angiotensin-(1-7) alleviates NADPH oxidase-mediated oxidative stress and renal vascular dysfunction in diabetic hypertensive rats (Benter et al., 2008). Thus, drugs that suppress Ang II activity may have the potential for impeding the process of TGF-ß-mediated glomerulosclerosis via a decrease in NADPH oxidase in podocyte diseases.

TGF-ß signaling antagonists

Inhibitors of TGF-ß/receptor action

Anti-TGF-ß antibody inhibits mesangial matrix expansion and/or GBM thickening in diabetic mice (Ziyadeh et al., 2000; Chen et al., 2003). Antisense TGF-ß oligonucleotides also reduce the expression of renal matrix components in diabetic mice (Han et al., 2000). Injecting a soluble TGF-ß1 type II receptor into Alport mice (Cosgrove et al., 2000) and diabetic rats (Russo et al., 2007) prevents the thickening of the GBM and renal cortical fibrosis, respectively. Oral administration of GW788388, an inhibitor of TGF-ß type I and II receptor kinases, reduces renal fibrosis in diabetic mice (Petersen et al., 2008). GBM thickening and mesangial matrix expansion are also reduced in Smad3-knockout diabetic mice (Wang et al., 2007).

TGF-ß is an anti-inflammatory cytokine and immunosuppressant, and, therefore, complete disruption of TGF-ß signaling could have serious adverse consequences (Yaswen et al., 1996). Consequently, downstream pathways of TGF-ß signaling may provide possibilities for more specific treatment targets as described below.

Inhibitors of downstream pathways of TGF-ß signaling

Overexpression of Smad7, an inhibitory factor in TGF-ß signaling, reduces renal fibrosis in animals with subtotal nephrectomy (Hou et al., 2005).

Bone morphogenic protein (BMP)-7 is a growth factor of the TGF-ß superfamily that counteracts the fibrogenic action of TGF-ß (Wang and Hirschberg, 2004; Zeisberg 2006). Maintenance of BMP-7 reduces podocyte dropout and renal fibrosis in BMP-7 transgenic mice with diabetic nephropathy (Wang et al., 2006). BMP-7 may exert its antifibrotic action by inhibiting fibrogenic Smad signaling (Wang and Hirschberg, 2004; Hirschberg, 2005) and by inducing the expression of active MMP-2 (Zeisberg et al., 2003).

Hepatocyte growth factor (HGF) gene therapy inhibited mesangial expansion and glomerulosclerosis in rats with advanced diabetic nephropathy associated with suppression of renal TGF-ß1 and mesangial CTGF upregulation (Cruzado et al., 2004). HGF antagonizes TGF-ß/Smad signaling in diverse types of kidney cells by blocking the nuclear translocation of activated Smad (Liu, 2004) and activation of Smad transcriptional corepressors, TGIF (Dai and Liu, 2004) and SnoN (Yang et al., 2003). SnoN and Ski are diminished in the fibrotic kidney, suggesting that the loss of Smad antagonists is an important mechanism that amplifies the TGF-ß signal (Yang et al., 2003; Fukasawa et al., 2006).

Conclusions

Mechanical pressure or biomechanical strain in progressive podocyte diseases may upregulate Ang II and TGF-ß expression in podocytes. Oxidative stress induced by Ang II may activate the latent TGF-ß in podocyte diseases. Enhanced TGF-ß activity by podocytes may induce GBM thickening by overproduction of abnormal ECM proteins and by impaired GBM degradation in podocyte diseases. It may also lead to podocyte apoptosis and detachment from the GBM, initiating the development of glomerulosclerosis. Furthermore, activated TGF-ß/Smad signaling by podocytes may induce CTGF and VEGF overexpression, which may act as a paracrine effector mechanism on mesangial cells to stimulate mesangial matrix synthesis. Research on the activation of TGF-ß signaling by podocytes and its downstream effectors, CTGF and VEGF, will further our comprehension of the cellular and molecular mechanisms of disease progression in podocytopathies and provide new therapeutic strategies for these common glomerular diseases.

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