Effects of TGF- β on Podocyte Growth and Disease Progression in Proliferative Podocytopathies

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Key Words

Cellular FSGS • Collapsing FSGS • Crescentic glomerulonephritis • Podocyte TGF- β • Ras/ERK • Smads

Abstract

Injured podocytes proliferate in cellular focal segmental glomerulosclerosis (FSGS), collapsing FSGS and crescentic glomerulonephritis, where TGF- β_1 is overexpressed in hyperplastic podocytes. Yet effects of podocyte TGF- β on podocyte growth and development of glomerulosclerosis have not been clearly defined. TGF- β activates Smads, Ras/extracellular signal-regulated kinase (ERK) and phosphatidylinositol-3-kinase (PI3K) pathways in podocytes, of which the major TGF- β /Smad signaling pathway appears to override the minor TGF- β -induced Ras/ERK/PI3K pathways. We provide evidence that increased TGF- β /Smad signaling activity by hyperplastic podocytes may lead to mesangial cell matrix overproduction and eventually to podocyte apoptosis and/or detachment, culminating in the development of glomerulosclerosis. In this regard, TGF- β , which is overexpressed by hyperplastic podocytes, may play an important role for the cellular and collapsing variants of FSGS to evolve into the classic FSGS pattern. In contrast, podocyte proliferation that is induced by Ras/ERK signaling activity in proliferative podocyte diseases seems to be mostly independent of TGF- β_1 ac-

tivity. Collectively, these data bring new insights into our understanding of the overexpression of TGF- β in hyperplastic podocytes in progressive glomerular diseases.

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Introduction

Podocytes are growth-arrested terminally differentiated cells that are unable to undergo cell division. Podocyte loss following glomerular injury may contribute to the development of focal segmental glomerulosclerosis (FSGS) [1–4], and yet podocytes proliferate in cellular FSGS [5, 6] and in collapsing FSGS [7–9]. Furthermore, proliferating podocytes contribute to the formation of crescent [10–14]. The podocytes that proliferate under these conditions are mostly dedifferentiated with loss of their differentiation markers, including WT1 and synaptopodin [7, 8, 10, 13–15]. In crescentic glomerulonephritis (GN), outside of true crescents, podocytes are damaged and participate in the formation of pseudocrescents, cellular or collapsing forms of FSGS, and true crescents [10]. Indeed, podocyte proliferation initiates crescent formation [11]. In this regard, cellular FSGS and crescentic GN may be anatomically and pathogenically linked [10, 12, 16].

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TGF- β is a multifunctional cytokine that plays an important role in glomerular diseases. Besides extracellular matrix (ECM) protein synthesis, TGF- β has effects on proliferation, hypertrophy, and apoptosis in renal cells. In the TGF- β_1 transgenic mice, an acute and massive increase in plasma levels of TGF- β_1 results in severe GN with occasional crescent formation [17, 18]. Indeed, TGF- β_1 is strongly expressed in the cellular crescents in the early stage of experimental crescentic GN [19]. Furthermore, podocytes covering the cellular lesion of FSGS exhibit increased expression of TGF- β_1 protein in human glomerular diseases [20, 21], and in Denys-Drash syndrome (DDS) mice [15]. So far, the effects of TGF- β in hyperplastic podocytes on podocyte growth and development of glomerulosclerosis have not been clearly defined. In this regard, this review will focus the discussion on the mechanisms whereby podocyte TGF- β would control podocyte growth and how it would contribute to FSGS formation in proliferative podocyte disease.

Cellular Lesion of FSGS versus Collapsing FSGS

The cellular lesion of FSGS comprises podocyte proliferation overlying the segmental scar, and is considered an initial lesion that leads to glomerular scarring in primary FSGS [5, 6]. Collapsing glomerulopathy is characterized by segmental or global wrinkling of the glomerular basement membrane with podocyte proliferation. It can be caused by various environmental insults, such as virus [human immunodeficiency virus-1 (HIV-1)] [22] and drugs (bisphosphonates) [23], or genetic factors [24]. Up to 46% of patients with primary FSGS show cellular or collapsing lesion [5]. In the renal allograft, recurrent FSGS often has cellular or collapsing features, indicating that the proliferative lesion of the podocytes is the first sign of recurrent disease [25, 26]. Indeed, evidence from repeat biopsies suggests that cellular and collapsing variants may evolve into the classic FSGS pattern in the course of disease progression [27].

The two terms, cellular lesion of FSGS and collapsing FSGS, had often been used interchangeably or synonymously, because the glomerular pathology of both lesions is basically identical [5, 6, 10]. However, some authors suggested that both lesions should be distinguished in view of the different morphologic, etiologic and prognostic implications [27–29]. A detailed discussion on both lesions is beyond the scope of this review, and we used the term cellular FSGS to encompass both lesions with podocyte proliferation.

Table 1. Diseases showing TGF- β overexpression in hyperplastic podocytes

Disease model	Results	Ref.
DDS mice	Strong TGF- β_1 expression in podocytes in cellular FSGS	15
Human FSGS	Strong TGF- β_1 expression in podocytes in cellular FSGS	21
Experimental crescentic GN	Strong TGF- β_1 expression in cellular crescents	19
Human IgA nephropathy	Strong TGF- β_1 expression in podocytes in cellular FSGS	20

DDS = Denys-Drash syndrome; FSGS = focal segmental glomerulosclerosis; GN = glomerulonephritis.

Occurrence of Cellular FSGS and Crescents in the Diseased Glomeruli

FSGS is a nonspecific heterogeneous form of renal injury [30]. FSGS following primary glomerular diseases has often been excluded in the category of secondary FSGS, because it was regarded as the nonspecific chronic scarred phase of the disease [27]. Nonetheless, cellular FSGS is present in IgA nephropathy (IgAN) [20], analogous to the existence of tip lesions in a variety of glomerular diseases [31].

Crescentic GN does not denote a specific etiologic form of GN, either. The occurrence of crescents has been reported even in nonproliferative podocyte diseases including primary FSGS [32], idiopathic membranous nephropathy [33, 34], and diabetic nephropathy [35, 36] with or without antineutrophilic cytoplasmic antibody seropositivity.

Altogether, damaged podocytes in varieties of the glomerular diseases can proliferate in the course of disease progression and exacerbation leading to the development of cellular FSGS and even crescents.

TGF- β Expression in Hyperplastic Podocytes in the Diseased Glomeruli

In human primary FSGS [21] and in DDS mice [15], where podocyte is the target of injury, strong expression of TGF- β_1 is observed in podocytes in the cellular lesion of FSGS. TGF- β_1 is also strongly expressed in the cellular crescents [19] (table 1).

In human IgAN, where mesangial cell is the target of injury, mesangial immunostaining for active TGF- β_1 is almost negligible, despite increased mesangial TGF- β_1 mRNA levels. Instead, podocytes covering the cellular lesion of FSGS exhibit strong expression of TGF- β_1 [20] (table 1). In chronic mesangial cell diseases, TGF- β_1 , which is secreted by mesangial cells and stored in mesangial matrix as latent complexes, seems to be localized to the podocyte surface and then activated [37].

Activated TGF- β_1 may bind to its receptor on podocytes, activating its downstream Smad or other signaling pathways to modulate the expression of its target genes.

TGF- β Signaling Intermediates Controlling Cell Growth

Smad Pathway

TGF- β causes growth arrest in late G1 of the cell cycle through Smad2 and Smad3. TGF- β receptor-mediated phosphorylation of these Smads induces their association with Smad4 followed by translocation in the nucleus in which these complexes activate transcription of specific genes, such as p15 and p21 [38]. Smad4-independent p21 induction by TGF- β through Smad2/3 signaling was also reported [39]. In podocytes, TGF- β_1 phosphorylates Smad2 [40, 41]. Expression levels of TGF- β_1 , TGF- β type II receptor and phosphorylated Smad2/Smad3 are increased in the podocytes covering the lesions of FSGS [21]. Thus, activated TGF- β /Smad signaling in injured podocytes may increase p15 and p21 (fig. 1).

TGF- β can also induce its downstream inhibitory Smad7, which in turn inhibits Smad2/3 phosphorylation via the negative feedback mechanisms [42]. Dominant negative Smads and the inhibitory Smad7 block p21 induction by TGF- β in a dose-dependent manner [43]. In TGF- β_1 transgenic mice, podocytes undergo apoptosis with overexpression of Smad7 protein [44]. TGF- β_1 induces Smad7 synthesis in cultured podocytes [45], in which TGF- β_1 and Smad7 each induce apoptosis [44] (fig. 1).

Ras/Mitogen-Activated Protein Kinase (MAPK) Pathway

TGF- β is able to signal via Ras protein, which plays an essential role in eukaryotic cell growth. Ras is required for TGF- β -mediated activation of extracellular signal-regulated kinase (ERK) [46]. It also inhibits TGF- β -in-

Fig. 1. Diagram showing the differential functions of transforming growth factor- β (TGF- β) in modulating podocyte proliferation, growth arrest and apoptosis. ERK = Extracellular signal-regulated kinase; p38 = p38 mitogen-activated protein kinase; PI3K = phosphatidylinositol-3-kinase; HIF = hypoxia-inducible factor.

duced nuclear accumulation of Smad2/Smad3 [47]. The Ras/MAPK kinase (MEK) pathway is required in part for TGF- β_1 -induced upregulation of p21, while p27 expression is unaffected by the TGF- β , MEK, or Ras [48]. ERK activation mediates primarily mitogenic and/or anti-apoptotic signaling [49], and attenuates the nuclear accumulation of the Smads [50].

In podocytes, TGF- β activates ERK [40, 41] and p38 MAPK [41, 44]. Activation of p38 MAPK [44] or p21 [51] is required for induction of apoptosis by TGF- β in podocytes (fig. 1).

Phosphatidylinositol-3-Kinase (PI3K) Pathway

TGF- β also rapidly activates anti-apoptotic mediator PI3K/AKT in podocytes, the kinetic profiles of which are similar to ERK [41] (fig. 1). Indeed, PI3K and ERK signals appear to be synergistically activated to mediate anti-apoptotic machinery [52]. In addition, TGF- β signaling through PI3K induces the podocyte expression of monocyte chemoattractant protein-1 [53].

Role of Ras/ERK Signaling in Podocyte Proliferation in Proliferative Podocytopathies

In cellular crescents, there is prominent TGF- β expression [19] and ERK activation [54]. TGF- β signaling appears to play a central role in the development of crescentic GN by inducing the activation of ERK [55].

ERK activation is shown in hyperplastic podocytes from the HIV-associated nephropathy and/or idiopathic collapsing FSGS patients [22]. Cyclin D1 is a key downstream target of ERKs [56]. In podocytes of HIV-transgenic mice, cyclin D1 protein is increased and the increase coincides with entry into the cell cycle [57], and yet previous studies failed to detect cyclin D1 protein in the cellular lesion of FSGS [58, 59] possibly associated with the defect of the antibody (Santa Cruz anti-cyclin D1 antibody), which cross-reacts with cyclin D2 and/or D3 [57].

Stabilization of hypoxia-inducible factor (HIF) in mice by selective deletion of the von Hippel-Lindau gene from podocytes, leads to the development of crescentic GN with expression of HIF target gene *Cxcr4* in podocytes [11]. *Cxcr4* is both required and sufficient for proliferation of podocytes *in vivo*. Overexpression of HIF-2 α is also shown in hyperplastic podocytes from the patients with HIV-associated nephropathy and HIV-1-transgenic mice [60]. Activation of HIF-1 occurs via PI3K and MAPK signaling pathways [61].

In sum, Ras/ERK and PI3K activation in podocytes could stimulate cyclin D1 expression and HIF activation resulting in podocyte proliferation in proliferative podocytopathies (fig. 1). In cultured podocytes, however, TGF- β_1 does not stimulate cell proliferation [62], although ERK is activated by TGF- β_1 [40, 41]. Thus, TGF- β_1 -induced ERK activation seems to play a minor role in podocyte proliferation in proliferative podocytopathies, while there are multiple other pathways to stimulate podocyte proliferation, independent of TGF- β_1 .

Significance of TGF- β Overexpression in Podocytes in Proliferative Podocyte Diseases

Activation of the TGF- β /Smad signaling in hyperplastic podocytes from the diseased glomeruli may lead to overproduction of ECM in the mesangial areas resulting in the formation of glomerulosclerosis [15, 21, 37]. In rats with reduced renal mass, TGF- β_1 mRNA overexpression in podocytes and TGF- β_1 -dependent activation of mesangial cells preceded the development of glomerulosclerosis [63].

TGF- β_1 induces Smad7 synthesis in cultured podocytes [45], in which TGF- β_1 and Smad7 each induce apoptosis [44]. Enhanced TGF- β activity may lead to podocyte apoptosis and/or detachment with podocytopenia [44, 64, 65]. The denuded glomerular basement membrane may adhere to Bowman's capsule, initiating the development of glomerulosclerosis [2, 3, 64, 65]. In this regard, TGF- β , which is overexpressed by hyperplastic podocytes, seems to play an important role for the cellular FSGS to evolve into the classic FSGS pattern in the course of disease progression.

TGF- β -induced Ras/ERK and PI3K signaling pathways in podocytes may promote the podocyte proliferation in diseased glomeruli. The major TGF- β /Smad signaling pathway, however, appears to override the minor TGF- β -induced Ras/ERK and PI3K pathways. In this regard, podocyte proliferation may be mostly independent of TGF- β activity in proliferative podocytopathies. Collectively, overexpression of TGF- β in hyperplastic podocytes seems to be the effect of the reparative process of fibrosis rather than the cause of podocyte proliferation.

Conclusions

Cellular FSGS and crescents are observed not infrequently in the diseased glomeruli, where TGF- β_1 is overexpressed in hyperplastic podocytes. In podocytes, TGF- β activates Smads, Ras/ERK and PI3K pathways, of which the major TGF- β /Smad pathway activity appears to override the minor TGF- β -induced Ras/ERK signaling activity. Enhanced TGF- β /Smad signaling activity in podocytes may lead to mesangial cell matrix overproduction and eventually to podocyte apoptosis and/or detachment, culminating in the development of glomerulosclerosis. In this regard, TGF- β , which is overexpressed by hyperplastic podocytes, seems to play an important role for the cellular FSGS to evolve into the classic FSGS pattern. In contrast, podocyte proliferation that is induced by Ras/ERK/PI3K signaling activity in proliferative podocyte diseases may be mostly independent of TGF- β_1 . Research on the podocyte TGF- β will further our comprehension of the podocyte growth and glomerulosclerosis and provide new therapeutic strategies to prevent the progression to renal failure in patients with proliferative podocyte disease.

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