TGF-β1 Regulates the PINCH-1–Integrin-Linked Kinase–α-Parvin Complex in Glomerular Cells

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Glomerular damage is a major cause of renal failure. Recent studies suggest that a ternary protein complex that consists of PINCH-1, integrin-linked kinase, and α-parvin, cytoplasmic components of cell–extracellular matrix adhesions, plays pivotal roles in regulation of glomerular cell behavior. It is reported here that TGF-β1, a key factor in the progression of glomerular failure, regulates the PINCH-1–integrin-linked kinase–α-parvin (PIP) complex formation in glomerular podocytes and mesangial cells. Treatment of podocytes with TGF-β1 inhibited the PIP complex formation. Forced disruption of the PIP complex in podocytes activated p38 mitogen-activated protein kinase and promoted apoptosis. Importantly, inhibition of p38 mitogen-activated protein kinase, either with a chemical p38 inhibitor (SB202190) or with a dominant negative form of p38α, alleviates podocyte apoptosis that is induced by the disruption of the PIP complex. In contrast to an inhibitory role in podocytes, TGF-β1 promotes the PIP complex formation in mesangial cells. Thus, TGF-β1 regulates the PIP complex in a cell type–dependent manner. Because the PIP complex promotes glomerular mesangial matrix deposition and protects podocytes from apoptosis, the TGF-β1–induced up- and downregulation of the PIP complex likely contribute to the pleiotropic effects of TGF-β1 on different glomerular cell types and hence the progression of glomerular failure.

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G lomerulus is a specialized filtration unit that is essential for kidney function. It consists of three major cell types (podocytes, mesangial cells, and endothelial cells) that are intimately associated with glomerular extracellular matrix (ECM; e.g., mesangial matrix or glomerular basement membrane). Alterations of glomerular cells are causal factors in many kidney diseases, including diabetic nephropathy, the major cause of end-stage renal failure. Mesangial matrix expansion correlates closely with deterioration of renal function and therefore long has been considered an important factor in progressive glomerular failure (1,2). It is clear now that alterations of podocytes, including an elevation of apoptosis, also are crucially involved in the development of proteinuria and glomerulosclerosis (3–10).

Cell–ECM adhesion is a key determinant of glomerular cell behavior. It provides a physical connection between ECM and intracellular compartment and transmits chemical signals between them. At the molecular level, cell–ECM adhesion is mediated by a network of transmembrane adhesion receptors (e.g., integrins) and a selective group of integrin-proximal cytoplasmic proteins, which link the integrins to the actin cytoskeleton and signaling proteins. PINCH-1, integrin-linked kinase (ILK), and α-parvin are cytoplasmic components of this "integrin–actin" network (reviewed in references [11–13]). Through two direct interactions, one mediated by the PINCH-1 N-terminal LIM1 domain and the ILK N-terminal ankyrin repeat (ANK) domain and the other mediated by the ILK C-terminal domain and the α-parvin calponin homology 2 domain, they form a ternary protein complex (14–16). The PINCH-1–ILK–α-parvin (PIP) complex has been found in a wide variety of cell types, including glomerular mesangial cells (17) and podocytes (18). Recent molecular, cell biological, and genetic studies have suggested that it is a key part of the cellular machinery that regulates the behavior of renal cells, including glomerular mesangial cells and podocytes (17–26). The specific functions of the PIP complex, however, seem to be cell type dependent. For example, ILK expression in mesangial cells is increased significantly in patients with diabetic nephropathy (19). Forced disruption of the PIP complex in mesangial cells significantly reduced fibronectin matrix deposition (17), suggesting a positive role of the PIP complex in this process. In podocytes, the PIP complex is involved in regulation of cell adhesion, cytoarchitecture, and apoptosis (18). The importance of the PIP complex for maintenance of normal glomerular functions is manifested by recent studies in podocyte-specific ILK knockout mice. Ablation of ILK from podocytes resulted in heavy albuminuria, progressive FSGS, and kidney failure, which ultimately led to animal death (25,27).

Given the pivotal roles of the PIP complex in regulation of glomerular cell behavior, alterations of the PIP complex formation under stress conditions likely contribute to the progression of glomerular failure.

Previous studies have demonstrated that TGF-β1 plays cru-
differential roles in the progression of glomerular failure. It elicits diverse effects on glomerular cells, including stimulation of mesangial matrix expansion and promotion of podocyte shape change, detachment, and apoptosis (9,28–34). These studies prompted us to investigate whether TGF-β1 regulates the PIP complex formation in glomerular cells. We report here the findings. Our results provide new information on the upstream regulators as well as downstream signaling effectors of the PIP complex in its regulation of glomerular cell behavior. Furthermore, they suggest a mechanism through which TGF-β1 exerts different effects on glomerular podocytes and mesangial cells.

Materials and Methods

Antibodies, Cells, and Other Reagents

Mouse monoclonal anti–ILK antibody (clone 65.1), anti–α-parvin antibodies (clones 1D4 and 3B5), and rabbit polyclonal anti–PINCH-1 antibody were described previously (14,15). Mouse monoclonal anti–80K-H antibody (clone 3C3) was generated using GST fusion protein that contained human 80K-H residues 438 to 527 as an antigen following a protocol that we previously described (15). The monoclonal anti–80K-H antibody specifically recognizes GFP-tagged recombinant 80K-H protein in Western blotting (data not shown), confirming the specificity of the mAb. Mouse monoclonal anti–phospho-serine/threonine-Pro antibody (MPM2) was purchased from Upstate Biotechnology (Lake Placid, NY). Mouse monoclonal anti–FLAG antibody (M5) was from Sigma (St. Louis, MO). Antibodies that recognize p38, phospho-p38 (Thr180/Tyr182), Akt, and phospho-Akt (Ser473) were purchased from Cell Signaling Technology (Beverly, MA). Antibodies that recognize p38, phospho-serine/threonine-Pro antibody, or mouse monoclonal anti–phospho-serine/threonine-Pro antibody MPM2.

Adenoviral Expression Vectors and Infection

Adenoviral-expressing vectors that encoded the FLAG-tagged PINCH-1–binding ANK fragment of ILK (residues 1 to 230) or the PINCH-1–binding defective ANK fragment (residues 1 to 230), in which D31 was substituted with Ala (D31A), were generated as we described previously (18,19). The adenoviral vector that encoded a FLAG-tagged dominant negative kinase-deficient mutant p38α mitogen-activated protein kinase (MAPK) [Flag-p38(AGF)] (36), in which the activating phosphorylation sites (T180Y182) were substituted with Ala and Phe, was provided by Drs. Tong-Chuan He and Bert Vogelstein (Howard Hughes Medical Institute, Johns Hopkins Oncology Center, Baltimore, MD).

For adenoviral infection experiments, podocytes were cultured under nonpermissive condition for 9 d. The cells were infected with adenoviral vectors that encoded FLAG-ANK, FLAG-D31A, or β-galactosidase. In some experiments (as specified in each experiment), podocytes in which the PIP complex was disrupted were treated with chemical or dominant negative inhibitors of p38α MAPK. For pharmacologic inhibition of p38α, SB203580 (5 μM) was added to the cultured medium 1 d after the cells were infected with FLAG-ANK. For dominant negative inhibition of p38α, podocytes were co-infected with adenoviruses that encoded FLAG-ANK and FLAG-tagged kinase-deficient p38α (AGF) mutant. The infection efficiency was monitored by the expression of GFP encoded by the viral vectors, which typically reached 80 to 90% within 3 d. Three days after the adenoviral infection, the cells were harvested and the expression of FLAG-ANK, FLAG-D31A, and Flag-p38(AGF) was confirmed by Western blotting. The formation of the PIP complex, caspase-3 activity, activating phosphorylation of p38, and Akt were analyzed as specified in each experiment.

Caspase-3 Assay

Caspase-3 activity was measured using fluorescent caspase-3 substrate VII (Ac-DEVD-afc; Calbiochem, San Diego, CA) following the manufacturer’s protocol. Briefly, podocytes were cultured under nonpermissive condition and infected with adenoviral vectors as described previously. Three days after the adenoviral infection, the cells were lysed with the lysis buffer (1% Triton X-100 in 50 mM Tris-HCl [pH 7.4] that contained 150 mM NaCl, 5 mM EDTA, 2 mM Na,VO₄, 2.5 mM NaPO₄, 100 mM NaF, 200 mM microcystin-LR, and protease inhibitors). The cell lysates (500 μg) were mixed with 10 μg of mouse monoclonal anti–α-parvin antibody 1D4. The samples were incubated for 3 h, mixed with UltraLink Immobilized Protein A/G (Pierce, Rockford, IL), and then incubated for and additional 2 h. The beads were washed four times, and the proteins bound were released from the beads by boiling in SDS-PAGE sample buffer for 5 min. The samples were analyzed by Western blotting with mouse monoclonal anti–α-parvin antibody 3B5, mouse monoclonal anti–ILK antibody 65.1, rabbit polyclonal anti–PINCH-1 antibody, or mouse monoclonal anti–phospho-serine/threonine-Pro antibody MPM2.

Immunoprecipitation and Western Blot

The formation of the PIP complex in mesangial cells and podocytes was analyzed by immunoprecipitation and Western blotting as we described previously (15,16,18). Briefly, the cells were lysed with the lysis buffer (1% Triton X-100 in 50 mM Tris-HCl [pH 7.4] that contained 150 mM NaCl, 5 mM EDTA, 2 mM Na,VO₄, 2.5 mM NaPO₄, 100 mM NaF, 200 mM microcystin-LR, and protease inhibitors). The cell lysates (500 μg) were mixed with 10 μg of mouse monoclonal anti–α-parvin antibody 1D4. The samples were incubated for 3 h, mixed with UltraLink Immobilized Protein A/G (Pierce, Rockford, IL), and then incubated for and additional 2 h. The beads were washed four times, and the proteins bound were released from the beads by boiling in SDS-PAGE sample buffer for 5 min. The samples were analyzed by Western blotting with mouse monoclonal anti–α-parvin antibody 3B5, mouse monoclonal anti–ILK antibody 65.1, rabbit polyclonal anti–PINCH-1 antibody, or mouse monoclonal anti–phospho-serine/threonine-Pro antibody MPM2.
Results
TGF-β1 Inhibits the Formation of the PIP Complex in Glomerular Podocytes

To test whether TGF-β1 plays a role in regulation of the PIP complex formation, we immunoprecipitated α-parvin from podocytes that were treated with or without TGF-β1. Western blotting analysis with an anti-α-parvin mAb showed that a similar amount of α-parvin was immunoprecipitated from the TGF-β1–treated podocytes (Figure 1A, lane 4) as well as the TGF-β1–untreated control podocytes (Figure 1A, lane 3). Probing the same samples with an anti-ILK antibody showed that the amount of ILK in the α-parvin immunoprecipitates that were derived from the TGF-β1–treated podocytes was reduced (Figure 1B, compare lanes 3 and 4). Consistent with this, the amount of PINCH-1 that was co-immunoprecipitated with α-parvin also was reduced substantially in response to TGF-β1 (Figure 1C, compare lanes 3 and 4). Thus, treatment of podocytes with TGF-β1 reduces the complex formation of ILK and PINCH-1 with α-parvin. In control experiments, consistent with previous studies showing that TGF-β1 promotes podocyte apoptosis (37), treatment of podocytes with TGF-β1 significantly increased the caspase-3 activity (Figure 1D).

The N-terminus of α-parvin consists of several proline-directed serine/threonine phosphorylation sites (38). We previously showed that the proline-directed serine/threonine phosphorylation of α-parvin influences its complex formation with ILK and PINCH-1 (18). To gain insight into the mechanism whereby TGF-β1 inhibits the complex formation of ILK and PINCH-1 with α-parvin, we probed the anti-α-parvin immunoprecipitates with a monoclonal anti–phospho-serine/threonine antibody (MPM2) that preferentially recognizes proline-containing phospho-serine/threonine epitopes (39). The result showed that, despite the presence of a similar amount of α-parvin protein in immunoprecipitates that were derived from the TGF-β1–treated and –untreated podocytes (Figure 2A, lanes 1 and 2), the amount of serine/threonine-phosphorylated α-parvin is reduced substantially in TGF-β1–treated podocytes (Figure 2B, compare lanes 1 and 2). Thus, TGF-β1 likely inhibits the

![Image](image1.png)

Figure 1. TGF-β1 inhibits the formation of the PINCH-1–intergrin-linked kinase (ILK)–α-parvin (PIP) complex in podocytes. Mouse podocytes were cultured under nonpermissive condition and treated with (lanes 2 and 4) or without (lanes 1 and 3) TGF-β1 (2 ng/ml) for 24 h. Anti–α-parvin immunoprecipitates were prepared as described in Materials and Methods. The cell lysates (lanes 1 and 2) and anti–α-parvin immunoprecipitates (lanes 3 and 4) were analyzed by Western blotting with antibodies that recognize α-parvin (A), ILK (B), and PINCH-1 (C), respectively. Note that the amount of ILK and PINCH-1 that was associated with α-parvin was reduced in response to TGF-β1. Asterisks in A and B indicate the positions of mouse anti-α-parvin Ig heavy chains that were derived form the immunoprecipitates. (D) Mouse podocytes were treated with TGF-β1 (2 ng/ml) as described in A through C. The caspase-3 activity of the TGF-β1–treated podocytes was compared with that of untreated podocytes. Bars represent mean values of the fold increase ± SD from two independent experiments.

![Image](image2.png)

Figure 2. TGF-β1 inhibits proline-directed phosphorylation of α-parvin in podocytes. Mouse podocytes were cultured under nonpermissive condition and were treated with (lane 2) or without (lane 1) 2 ng/ml TGF-β1 for 24 h. α-Parvin was immunoprecipitated from the cells with mouse monoclonal anti–α-parvin antibody 1D4. The immunoprecipitates were analyzed by Western blotting with mouse monoclonal anti–α-parvin antibody 3B5 (A) or mouse monoclonal anti–phospho-serine/threonine-Pro antibody MPM2 (B). Note that despite the presence of a similar amount of α-parvin protein (A, compare lanes 1 and 2), much less phospho–α-parvin was detected in the sample that was derived from TGF-β1–treated podocytes (B, compare lane 2 with lane 1). Lane 3 in B was loaded with 1D4 IgG control, which was prepared as those in lanes 1 and 2 except that cell lysates were omitted (to show the 1D4 IgG bands).
complex formation of ILK and PINCH-1 with α-parvin through, at least in part, downregulation of the proline-directed serine phosphorylation of α-parvin.

**Disruption of the PIP Complex Activates p38 MAPK but Does Not Affect Akt Activation in Podocytes**

Our recent studies suggest that disruption of the PIP complex promotes podocyte apoptosis (18). The downstream signaling effectors that are involved in this process, however, had not been determined. One attractive candidate is p38 MAPK, which has been shown to be involved in TGF-β1–induced podocyte apoptosis (37). Alternatively, the PIP complex could regulate apoptosis through Akt, a signaling intermediate that is crucially involved in survival signaling. Loss or inhibition of PINCH-1, ILK, or α-parvin in many types of cells, particularly those that are derived from cancers, reduces Akt activation (reviewed in references [11,13,40]). To test these possibilities experimentally, we infected the cells with adenoviral expression vectors that encoded β-galactosidase (as a negative control), a FLAG-tagged ILK fragment (residues 1 to 230) that contained the PINCH-1–binding ANK domain (FLAG-ANK), or a FLAG-tagged PINCH-1–binding defective ILK fragment (residues 1 to 230) in which D31 is substituted with Ala (FLAG-D31A) (18). The expression of FLAG-ANK (Figure 3A, lane 2) and FLAG-D31A (Figure 3A, lane 3) in the corresponding infectants was confirmed by Western blotting with a monoclonal anti-FLAG antibody. As expected, a similar amount of ILK (Figure 3C) was co-immunoprecipitated with α-parvin (Figure 3B) from the β-gal control cells as well as the cells that overexpressed FLAG-ANK or FLAG-D31A. Western blotting analysis of the same samples with an anti–PINCH-1 antibody showed that the amount of PINCH-1 that was co-immunoprecipitated with ILK and α-parvin was markedly reduced in cells that overexpressed ANK (Figure 3D, lane 2) but not in those that overexpressed D31A (Figure 3D, lane 3). Thus, overexpression of ANK but not that of D31A effectively disrupted the PINCH-1–ILK interaction and hence the formation of the PIP complex.

Next, we assessed the effect of disruption of the PIP complex on activating phosphorylation of p38 MAPK. Overexpression of FLAG-ANK, which inhibited the PIP complex formation (Figure 3, A through D, lane 2), increased the activating phosphorylation (Figure 3, E and H, lane 2) but not the protein level (Figure 3, F and I, lane 2) of p38 MAPK. By contrast, overexpression of FLAG-D31A, which did not inhibit the PIP complex formation (Figure 3, A through D, lane 3), failed to increase the activating phosphorylation of p38 MAPK (Figure 3, E and H, lane 3). These results suggest that the PIP complex plays an important role in suppression of the activating phosphorylation of p38 MAPK. Consistent with previous studies (37), treatment of podocytes with TGF-β1 increased the activating phosphorylation of p38 MAPK (Figure 4). Collectively, these results suggest that the TGF-β1–mediated downregulation of the PIP complex likely contributes to the increase of the activating phosphorylation of p38 MAPK in podocytes.

In parallel experiments, we analyzed the effect of disruption of the PIP complex on Akt. The results showed that the activating phosphorylation of Akt (Figure 5), unlike that of p38,
was not altered by overexpression of FLAG-ANK. Thus, unlike in many other cell types in which the PIP complex is involved in the activation of Akt, disruption of the PIP complex does not alter the activating phosphorylation of Akt in podocytes.

**Inhibition of p38 Alleviates Podocyte Apoptosis Induced by the Disruption of the PIP Complex**

To determine the functional significance of p38 activation that is induced by the disruption of the PIP complex, we disrupted the PIP complex in podocytes either in the presence or in the absence of SB202190, a chemical inhibitor of p38. As expected, overexpression of FLAG-ANK but not that of FLAG-D31A significantly increased caspase-3 activity in podocytes (Figure 6A). Treatment of the podocytes with SB202190 completely blocked the increase of the caspase-3 activity that was induced by the disruption of the PIP complex (Figure 6A), suggesting that p38 activation is critically involved in the podocyte apoptosis that is induced by the disruption of the PIP complex.

To test this further, we expressed a dominant negative form of p38α (AGF), in which the activating phosphorylation sites (T180/Y182) on p38α are mutated (36), in FLAG-ANK–expressing podocytes (Figure 6B, lane 3). As expected, expression of p38α (AGF) in the podocytes inhibited the increase of caspase-3 activity that was induced by the disruption of the PIP complex (Figure 6B, lane 3). This finding further confirms that p38 is a critical regulator of podocyte apoptosis induced by the disruption of the PIP complex.

**Figure 4.** TGF-β1 promotes the activating phosphorylation of p38. Mouse podocytes were cultured in the presence (lane 2) or absence (lane 1) of TGF-β1 (2 ng/ml) for 24 h as described in Materials and Methods. The cell lysates (15 µg/lane) were analyzed by Western blotting with antibodies that recognize Thr180/Tyr182-phospho-p38 (A) or total p38 protein (B). (C) The membrane that was used in B was reprobed with an anti-actin antibody (as a loading control).

**Figure 5.** Inhibition of the PIP complex formation in podocytes does not alter activating phosphorylation of Akt. Mouse podocytes were cultured under nonpermissive condition and infected with adenoviral vectors that encoded β-galactosidase (lane 1), FLAG-tagged PINCH-1–binding ANK fragment of ILK (lane 2), or FLAG-tagged PINCH-1–binding defective ANK fragment that bears the D31A mutation (lane 3) as described in Figure 3. The cell lysates (15 µg/lane) were analyzed by Western blotting with antibodies that recognize Akt (A) or phospho-Akt (Ser473; B).

**Figure 6.** Inhibition of p38 alleviates podocyte apoptosis that was induced by the disruption of the PIP complex. (A) Mouse podocytes were cultured under nonpermissive condition and infected with the β-gal, FLAG-ANK, or FLAG-D31A adenoviruses either in the absence (β-gal, ANK, or D31A) or in the presence of SB202190 (ANK + SB) as described in Materials and Methods. Caspase-3 activities from cells that expressed FLAG-ANK, FLAG-D31A, or FLAG-ANK in the presence of SB202190 were compared with that of the β-gal control cells. Bars represent mean values of the fold increase ± SD from two independent experiments. (B through D) Mouse podocytes were cultured under nonpermissive condition and infected with the β-gal, FLAG-ANK, or FLAG-D31A adenoviruses that encoded β-gal (lane 1), FLAG-ANK (lane 2), or FLAG-ANK and the dominant negative FLAG-p38α (AGF) mutant (lane 3). Three days after the adenoviral infection, the cells were lysed and analyzed by Western blotting with antibodies that recognize p38 (B) or phospho-p38 (Thr180/Tyr182) (C). (D) Caspase-3 activities from cells that expressed FLAG-ANK or FLAG-ANK and the dominant negative p38α were compared with that of the β-gal control cells. Bars represent mean values of the fold increase ± SD from two independent experiments.
FLAG-ANK in podocytes increased the activating phosphorylation of p38 (Figure 6C, compare lanes 1 and 2). Expression of the dominant negative form of p38α effectively prevented the FLAG-ANK–induced activating phosphorylation of p38 (Figure 6C, lane 3). Importantly, inhibition of p38 activation with the dominant negative inhibitor, like that with the chemical p38 inhibitor (Figure 6A), prevented the increase of caspase-3 activity that was induced by the disruption of the PIP complex (Figure 6D). Thus, p38 MAPK functions as a key downstream effector in the PIP complex-mediated apoptosis signaling in podocytes.

TGF-β1 Promotes the Formation of the PIP Complex in Mesangial Cells

TGF-β1 plays a prominent role in stimulation of mesangial matrix deposition, a process that the PIP complex promotes (17). To test whether TGF-β1 plays a role in regulation of the PIP complex in mesangial cells, we immunoprecipitated α-parvin from TGF-β1-treated and –untreated mesangial cells, respectively. Western blotting analyses showed that a similar amount of α-parvin was immunoprecipitated from the TGF-β1–treated (Figure 7A, lane 4) as well as the TGF-β1–untreated cells (Figure 7A, lane 3). Probing the same samples showed that the amounts of ILK (Figure 7B, lane 4) and PINCH-1 (Figure 7C, lane 4) that were associated with α-parvin increased in response to TGF-β1. Thus, in mesangial cells, unlike in podocytes, TGF-β1 promotes the formation of the PIP complex.

![Figure 7](image)

**Figure 7.** TGF-β1 promotes the PIP complex formation in mesangial cells. Rat mesangial cells were treated with (lanes 2 and 4) or without (lanes 1 and 3) 2 ng/ml TGF-β1 for 24 h. Anti–α-parvin immunoprecipitates were prepared as described in the Materials and Methods section. The cell lysates (lanes 1 and 2) and immunoprecipitates (lanes 3 and 4) were analyzed by Western blotting with antibodies that recognize α-parvin (A), ILK (B), and PINCH-1 (C), respectively. Note that the amounts of ILK and PINCH-1 that were associated with α-parvin were increased in response to TGF-β1.

**Discussion**

Alterations of glomerular cell-matrix adhesion and signaling are crucially involved in the progression of glomerular failure. The results presented in this article demonstrate that TGF-β1, whose level often is increased in injured glomeruli, regulates the formation of the PIP complex in glomerular cells. Because the formation of the PIP complex is crucial for control of the behavior of glomerular cells (e.g., podocytes), this study suggests a novel regulatory pathway through which TGF-β1 could influence glomerular cell behavior and thereby contribute to progressive glomerular failure.

Several lines of evidence suggest that the “TGF-β1–PIP complex” pathway likely plays a role in regulation of podocyte apoptosis. First, treatment of podocytes with TGF-β1 inhibits the PIP complex formation (Figure 1), suggesting that TGF-β1 is an upstream regulator of the PIP complex. Second, dominant negative inhibition of the PIP complex, like treatment with TGF-β1 (37), promotes podocyte apoptosis. Thus, the TGF-β1–induced inhibition of the PIP complex likely contributes to its induction of apoptosis in podocytes. Third, Schiffer et al. (37) demonstrated that treatment of podocytes with TGF-β1 increases the activating phosphorylation of p38, a feature that is not shared by podocyte apoptosis that is induced by some other factors such as Smad 7. It is interesting that the studies that are presented in this article show that disruption of the PIP complex in podocytes also promoted the activating phosphorylation of p38 (Figure 3). Thus, podocyte apoptosis that is induced by the disruption of the PIP complex shares this signaling “signature” of the TGF-β1–induced podocyte apoptosis. Finally, podocyte apoptosis that was induced by the disruption of the PIP complex, like that induced by TGF-β1 (37), was completely blocked by the inhibition of the p38 (Figure 6). Collectively, these results provide strong evidence for the “TGF-β1–PIP complex-p38” signaling pathway in regulation of podocyte apoptosis.

How does TGF-β1 inhibit the formation of the PIP complex in podocytes? We have found that treatment of podocytes with TGF-β1 reduces proline-directed serine/threonine phosphorylation of α-parvin (Figure 2). Because the proline-directed serine/threonine phosphorylation of α-parvin is known to regulate the PIP complex formation (18), a plausible mechanism through which TGF-β1 inhibits the PIP complex formation is by modulation of α-parvin phosphorylation. TGF-β1 transmits signals through a complex cascade of protein kinases and phosphatases, which often are regulated in a cell type– and context-dependent manner (31,41). Identification of the specific protein kinases or phosphatases that are responsible for the altered α-parvin phosphorylation in response to TGF-β1 signaling will be an important area of future research.

Akt is an important survival signaling intermediate. Loss of ILK, PINCH-1, or α-parvin compromises Akt activation in many but not all cell types (reviewed in references [11–13]). The involvement of the PIP complex in Akt activation seems to be particularly prominent in cancer cells, suggesting that this mode of regulation is associated with malignant transformation. These studies raised an interesting question as to whether the PIP complex plays a role in the survival of cells in which
Akt activation is independent of the PIP complex. The findings that disruption of the PIP complex in podocytes did not impair Akt activation but promoted apoptosis suggest a positive answer. Furthermore, we have demonstrated that under this circumstance, the PIP complex protects cells from apoptosis through suppression of the p38 activation. p38 activation is crucially involved in podocyte injury (37,42–50). Our findings, therefore, reveal an important Akt-independent signaling pathway through which the PIP complex protects podocytes from apoptosis.

The finding that TGF-β1 promotes rather than inhibits the PIP complex formation in mesangial cells suggests that the effects of TGF-β1 on the PIP complex are cell type dependent. In mesangial cells, the PIP complex promotes fibronectin matrix deposition (17), a process in which the connection between integrins and the actin cytoskeleton is indispensable (51). Disruption of the PIP complex, which presumably impairs the integrin-actin cytoskeleton interaction, reduces mesangial cell deposition (17). Because TGF-β1 functions in the progression of glomerular failure via exerting diverse effects on glomerular cells, including both stimulation of mesangial matrix expansion and promotion of podocyte apoptosis (9,28–34), the TGF-β1–induced up- and down-regulation of the PIP complex in mesangial cells and podocytes likely contribute to the pleiotropic effects of TGF-β1 during glomerular injury.

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Disclosures

None.

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