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Actin-associated Proteins in the Pathogenesis of Podocyte Injury

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Abstract: Podocytes have a complex cellular architecture with interdigitating processes maintained by a precise organization of actin filaments. The actin-based foot processes of podocytes and the interposed slit diaphragm form the final barrier to proteinuria. The function of podocytes is largely based on the maintenance of the normal foot process structure with actin cytoskeleton. Cytoskeletal dynamics play important roles during normal podocyte development, in maintenance of the healthy glomerular filtration barrier, and in the pathogenesis of glomerular diseases. In this review, we focused on recent findings on the mechanisms of organization and reorganization of these actin-related molecules in the pathogenesis of podocyte injury and potential therapeutics targeting the regulation of actin cytoskeleton in podocytopathies.

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INTRODUCTION

The kidney glomerulus is a highly specialized structure that ensures the selective ultra filtration of plasma so that essential proteins are retained in the blood. The glomerulus consists of three layers: fenestrated endothelial cells that assemble the glomerular capillaries, a glomerular basement membrane (GBM) enriched in negatively charged glycosaminoglycans, a tight interdigitating network of foot processes to generate slit diaphragm (SD) and podocytes. Podocytes are terminally differentiated and highly specialized epithelial cells that cover the outer layer of GBM. They have an actin-based contractile apparatus, consisting of three morphologically and functionally different segments: a cell body, major processes and foot processes (FPs) with the filtration slits bridged by the slit membrane between them [1]. The complex architecture of FPs depends on their highly ordered parallel contractile actin filament bundles [2, 3] and on specific actin-associated proteins [4]. FPs are defined by three membrane domains: the apical membrane domain (AMD), the SD protein complex, and the basal membrane domain (BMD, also known as the sole plate, which is associated with the GBM) [1, 5]. All three domains are physically and functionally linked to the FP actin cytoskeleton, making it the common denominator in podocyte function and dysfunction [5, 6]. Interference with any of the three FP domains activates reorganization of actin filaments [7], resulting in FP effacement and proteinuria [5]. Hence, the actin cytoskeleton ultimately determines the structural maintenance of the filtration slits.

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Podocyte injury and loss have been observed in many human and experimental models of glomerular diseases, including minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous glomerulonephritis (MGN), diabetic nephropathy, hyperhomocysteinemia-associated glomerulosclerosis [8-12]. In the context of proteinuric kidney diseases, it is necessary to study the regulation of the actin cytoskeleton directly in podocytes because the dysregulation of actin cytoskeleton is closely associated with a disease phenotype. Under pathological conditions, podocytes exhibit various changes. Among these changes, FP effacement represents the most characteristic changes in cell shape of podocytes, which typically leads to the fusion of filtration slits and apical displacement of the SD [13-15]. This suggests an important role for actin and/or its associated proteins in the maintenance of the foot process organization [16].

FUNCTION OF ACTIN-ASSOCIATED PROTEINS IN PODOCYTOPATHIES

Actin cytoskeleton is an essential structural and functional element that controls cell shape, cell motility and adhesion. In order to fulfill its functions, the cytoskeleton must form highly organized dynamic structures, such as stress fibers, filopodia and lamellipodia. When extracellular environment is changed, these structures are disassembled and remodeled to meet the new requirements. In recent years, it has become clearer that the precise organization and regulation of the actin cytoskeleton in podocyte are essential for the maintenance of its normal structure and function. The assembly, maintenance and disassembly of actin cytoskeleton are mediated by a variety of actin-associated proteins with divergent molecular interactions and functional properties [17]. Therefore, proteins regulating or stabilizing the actin cytoskeleton are of critical importance for sustained function of glomerular filtration [18, 7, 19]. Mutations af-

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fecting these proteins lead to the rearrangement of the actin cytoskeleton and subsequent proteinuria [20]. Several human genetic diseases as well as transgenic mouse models revealed that mutations affecting several podocyte proteins, including α-actinin-4 [13], CD2-associated protein (CD2AP) [21], synaptopodin [22], myosin [23] and Rho GDIα [24], lead to renal disease owing to the disruption of filtration barrier and rearrangement of actin cytoskeleton [25, 26]. These results suggest that signaling from the SD modulates actin reorganization, and results in FP effacement.

1. ACTN4

ACTN4, encodes α-actinin-4, an actin-binding and cross-linking protein localized to podocytes in the renal glomerulus, predominantly in the foot processes [13]. It is thought to have important roles both in cross-linking of actin filaments into contractile bundles and in helping to form the anchoring complex for the ends of actin stress fibers. Mutations in α-actinin-4 result in increased affinity for actin, alter the mechanical properties of podocytes and cause a familial form of FSGS [13, 27, 28]. Knocking out or overexpressing α-actinin-4 in mice leads to proteinuria and FP effacement [29, 30]. Absence of α-actinin-4 could also has a direct effect on the actin cytoskeleton. Decreased affinity of α-actinin for actin has been shown to alter the mechanical properties of actin–α-actinin gels [28]. Thus, α-actinin is an important cytoskeleton-organizing protein, which acts as an adaptor in multi-protein complexes associated with actin filaments [31]. It may be a target of nephrotoxic drugs that induce process effacement and proteinuria [32].

2. CD2AP

CD2AP was originally identified as an adaptor molecule between adhesion receptors and the cytoskeleton of T cells. In podocytes, it is localized at the intracellular insertion site of the SD [33-35]. CD2AP functions as a scaffolding protein connecting the actin cytoskeleton to plasma membrane proteins, such as nephrin and polycystin-2 [21]. It binds directly to nephrin and podocin via a C-terminal domain [36, 33]. Meanwhile, CD2AP also interacts with actin [21], the actin-binding proteins CapZ [37], cortactin [38] and the α-actinin-modulating protein Synpo. CD2AP may thus have a role in the regulation of the actin cytoskeleton. Previous studies showed CD2AP knock-out mice died several weeks after birth of nephrotic syndrome [39], and podocyte-specific expression of CD2AP rescued lethality of CD2AP deficiency [40]. It has been demonstrated that the effacement of the podocyte foot processes in CD2AP-deficient mice was resulted from dysregulation of the podocyte actin cytoskeleton [41]. Our data also showed that CD2AP played an important role in albumin overload-induced podocyte injury via disruption of cytoskeleton [42-45]. Therefore, these studies indicated that CD2AP may play an important role in actin cytoskeleton dynamics and the maintenance of the SD.

3. Rho

The Rho small G protein family is an important mediator of actin cytoskeletal reorganization that results in changes in cell morphology, motility and adhesion [46]. Like other G proteins, small G proteins of the Rho family cycle between the two conformational forms: the GTP-bound active form and the GDP-bound inactive form. The Rho family proteins, including RhoA, Rac1 and Cdc42, are good candidates for the regulator of the cytoskeleton for branching cell morphology, which controls signal-transduction pathways that influence many aspects of cell behavior, such as cytoskeletal dynamics [47, 46]. RhoA regulates the assembly of contractile actin and myosin filaments, while Rac1 proteins regulate ruffling and lamellipodia formation, and Cdc42 regulates filopodium formation [48]. Recent studies reported that mice lacking Rho GDI-α, a negative regulator of Rho small G-proteins, developed massive proteinuria mimicking nephrotic syndrome, leading to death as a result of renal failure [24]. These data suggest that the signaling pathways of the Rho family regulated by Rho GDI-α play a critical role in maintaining the structure and physiological function of adult kidneys [24]. Activation of RhoA in podocytes leads to albuminuria accompanied by a range of histologic changes characteristic of minimal change disease and FSGS in humans [49]. Rac1 activation in podocytes has also been shown in several models of proteinuric kidney disease involving motile podocytes [50]. Cdc42-mutant mice exhibited protein-filled microcysts with hallmarks of collapsing glomerulopathy, as well as extensive effacement of podocyte foot processes with abnormal junctional complexes. Cdc42 deficiency impaired the polymerization of actin and developed congenital nephropathy [51]. These results suggest that Rho signaling networks may shed light on the mechanisms that regulate the formation of actin bundles and FP under physiological and pathological conditions.

4. Synaptopodin

The actin-binding protein, synaptopodin, is highly expressed in telencephalic dendrites and renal podocytes [52]. It is essential for the integrity of the podocyte actin cytoskeleton because synaptopodin-deficient mice displayed impaired recovery from protamine sulfate (PS)-induced FP effacement and lipopolysaccharide (LPS)-induced nephrotic syndrome [53, 22]. Bigenic heterozygosity for synaptopodin and CD2AP results in proteinuria and FSGS-like glomerular damage [54]. These data underscore the importance of synaptopodin and CD2AP for sustained kidney filter function.

Synaptopodin exists in three isoforms: neuronal Synpo-short, renal Synpo-long, and Synpo-T. All three isoforms specifically interact with α-actinin and elongate α-actinin-induced actin filaments [22]. These findings have helped to define a mechanistic role for synaptopodin as a regulator of the actin-bundling activity of α-actinin that may be important for the formation and dynamic reorganization of the actin cytoskeleton in podocyte FPs and the spine apparatus in the brain [22].

Moreover, recent studies have shown that synaptopodin may regulate the thick stress fiber and migration of cultured podocytes via regulation of RhoA signaling [55]. Mechanistically, synaptopodin induces stress fibers by stabilizing the GTPase RhoA [55] and suppresses filopodia by disrupting Cdc42–insulin receptor substrate p53– Mena signaling complexes [56]. Gene silencing of synaptopodin in podocytes caused the loss of stress fibres and the formation of aberrant non-polarized filopodia, and the impairment of cell migra-

tion. Therefore, synaptopodin is a regulator of RhoA signaling during the formation and dynamic reorganization of the actin cytoskeleton [55]. Similarly, synpo –/– podocytes showed impaired actin filament reformation *in vitro*. The expression of synaptopodin coincides with the formation of FP and the development of actin bundles in differentiated podocytes [57, 58].

Recent studies revealed that the onset of proteinuria represented a migratory event involving the activation of actin assembly-promoting signaling pathways [59]. Synaptopodin contributes to the stabilization the glomerular filter by shifting the plasticity of the podocyte actin cytoskeleton from a motile to a contractile phenotype [56]. Also, it has been shown that the expression of synaptopodin correlated well with the severity of the disease and was well preserved in benign forms of nephrotic syndrome such as MCD, and reduced in less benign forms such as FSGS. Taken together, these data indicate that synaptopodin is essential for the integrity of the podocyte actin cytoskeleton and for the regulation of podocyte migration [55].

5. Myosin

Nonmuscle myosin II, an actin-based motor protein, plays an essential role in actin cytoskeleton organization and cellular motility. It is composed of two heavy chains and two pairs of light chains. Myosin heavy chain II is another component of the FP actin cytoskeleton *in vivo* and stress fiber in cultured podocytes [2, 60]. Vertebrates have two nonmuscle myosin heavy chains, IIA and IIB, that are encoded by two distinct genes, MYH9 and MYH10, respectively [61]. Mutation of the MYH9 gene, which encodes the nonmuscular myosin heavy chain IIA, has been recently reported to be associated with the Fechtner syndrome (nephritis, deafness, congenital cataracts, macrothrombocytopenia, and characteristic leukocyte inclusions) [62, 23]. Podocyte specific deletion of MYH9 predisposed mice to glomerular disease in response to injury by doxorubicin hydrochloride [63]. Taken together, these data suggest that, in the kidney, nonmuscle heavy chain IIA is a major component of the actin-myosin contractile apparatus in the podocyte foot process. It could play a role in maintaining capillary wall integrity against hydraulic pressure in physiological conditions and/or contribute to the foot process retraction in pathological conditions [23]. This finding, as well as the demonstration of ACTN4 mutations in familial FSGS and the tight relationships between actin and the slit diaphragm through CD2AP, underlines the importance of the cytoskeleton regulation in the maintenance of podocyte function.

6. TRPC6

Transient receptor potential canonical channel 6 (TRPC6) is one of the important Ca^{2+} permeable ion channels in podocytes, which is a component of the glomerular SD [64]. Mutations in gene encoding TRPC6 have been shown to cause FSGS [65]. It is shown that TRPC6 is connected to podocyte actin cytoskeleton, which is rearranged upon overexpression of TRPC6 [66]. Podocytes overexpressing TRPC6 lead to higher intracellular Ca^{2+} ion concentration. This increase of intracellular Ca^{2+} downregulates synaptopodin in cytoskeleton and stimulates RhoA activity, which causes F-actin derangement and the decrease of foot processes [67]. Our previous study showed that inhibition of TRPC6 prevented the F-actin cytoskeleton disruption that was induced by albumin overload [68]. Overexpression of TRPC6 in podocytes is also found in acquired or inherited proteinuric kidney diseases, such as MCD, MGN and FSGS [66]. Under these disease conditions, alteration of TRPC6 function directly affects podocyte cytoskeletal dynamic and therefore plays a major role during podocyte injury and foot process effacement.

7. Integrins

Integrins link the GBM to the intracellular actin cytoskeleton through a set of integrin- and actin-associated proteins that include paxillin, talin, vinculin, α-actinin and filamin. Integrins not only serve as physical attachment sites for the actin filaments, but also regulate actin dynamics in response to extracellular stimuli by a way of 'outside-in' signaling through focal adhesion kinase, integrin-linked kinase (ILK) and the actin polymerization complex Arp2/3. Integrins can also alter their adhesive characteristics in response to cellular events, thereby providing 'inside-out' signaling [69]. In addition to integrins, the GBM is connected with the podocyte actin cytoskeleton through α- and β-dystroglycan and utrophin [5]. Both types of podocyte–matrix contacts are important to maintain FP structure and filtration barrier function. Collectively, these data indicate that the interaction among the actin cytoskeleton and integrins in podocyte FPs, and their correlation with GBM provides mechanical stability together with the capacity to undergo rapid changes.

8. Hsp27

Hsp27 is a low-molecular-weight heat shock protein that has been identified in podocytes [70]. Several studies have reported that Hsp27 is an actin-associated protein that inhibits actin polymerization *in vivo*, and its actin polymerization inhibiting activity is related to its state of phosphorylation [71]. In puromycin aminonucleoside (PAN)-induced nephrosis, high concentrations of Hsp27 in the podocytes as well as the phosphorylation during FP effacement have been identified [70]. Another study also found that Hsp27 was able to regulate morphological and actin cytoskeletal responses of podocytes to injury induced by PAN [19].

9. Nck

The Nck subfamily of adaptor proteins has been implicated in the regulation of actin dynamics. A recently discovered signaling pathway couples nephrin to the actin cytoskeleton through the adaptor protein Nck [72, 73]. After nephrin phosphorylation by Fyn, Nck binds to phosphonephrin and N-WASP [72, 73]. This in turn leads to the activation of the Arp2/3 complex, a major regulator of actin dynamics. They are analogous to the Nck-Pac and Nck-WASP complexes, which participate in Rac1 and Cdc42 signaling, respectively. These findings strongly linked nephrin tyrosine phosphorylation to the rearrangement of the actin cytoskeleton in FPs and indicate that the Nck adaptor proteins function as mediators of this connection [72, 73].

10. Palladin

The actin-associated protein palladin is expressed in different isoforms in a variety of embryonic and adult tissues as well as in different cell lines [74, 75]. Palladin is involved in dynamic processes of actin organization in podocytes. Ronty *et al.* [76] have demonstrated that palladin interacted with α-actinin through a novel α-actinin binding motif in the N-terminal. Colocalization studies in podocytes revealed that, in dense regions of stress fibers, palladin was found not only together with α-actinin-1 [74, 76], but also with α-actinin-4 and synaptopodin [52]. It directly binds to α-actinin and closely co-localizes with it in stress fiber dense regions and focal adhesions [74]. Downregulation of palladin expression leads to loss of stress fibers [77] and increased palladin expression is associated with cytoskeletal reorganization in dendritic cells, trophoblastic cells and astrocytes [74, 75, 78].

11. Other Actin-associated Proteins

Other cytoskeletal proteins may also take part in maintaining normal podocyte integrity and in mediating podocyte response to injury. Zonula occludens-1 (ZO-1) interacts with the actin-based cytoskeleton and may also participate in signaling events through tyrosine phosphorylation [79]. Recently, Sever *et al.* [80] showed that, in models of proteinuric kidney disease, induction of the cathepsin L protease leads to cleavage of the GTPase dynamin and consequent cytoskeletal reorganization. Podocalyxin is a type I membrane mucoprotein abundantly presented in podocytes. The integrity of its cytoplasmic domain is required for both cell adhesion and migration. Na^+/H^+-exchanger regulatory factor 2 (NHERF2) and ezrin, colocalize with podocalyxin along with the apical plasma membrane of podocytes, where they form a co-immunoprecipitable complex. The podocalyxin/ NHERF2 /ezrin complex interacts with the actin cytoskeleton, and this interaction is disrupted in pathologic conditions associated with changes in the foot processes, indicating its importance in maintaining the unique organization of podocytes [81]. There is also emerging evidence showing new molecules in mediating podcoyte cytoskeleton modulation, such as *IFN2*, which encodes inverted formin 2 [82]; *APOL1*, which encodes apolipoprotein A1 [83]; and *MYO1E*, which encodes myosin 1E [84, 85].

TARGETED THERAPIES FOR CYTOSKELETON IN PODOCYTOPATIES

1. Glucocorticoid

Recent studies have shown that glucocorticoid receptors are present in glomerular cells, including podocytes. These receptors have been shown to translocate into the nucleus of podocytes upon dexamethasone treatment [86]. Treatment of cultured murine podocytes with the glucocorticoid both protected and enhanced recovery from PAN-induced podocyte injury. Dexamethasone also increased total cellular polymerized actin, stabilized actin filaments against disruption by PAN, latrunculin, or cytochalasin, and induced a significant increase in the activity of the actin-regulating GTPase RhoA. These findings suggest that the anti-proteinuric effect of glucocorticoids is, at least in part, mediated by a direct effect on the podocyte actin cytoskeleton independent of immunosuppression [87].

2. Calcineurin inhibitor

The immunosuppressive action of the calcineurin inhibitor, CsA, stems from the inhibition of NFAT signaling in T cells. T-cell dysfunction is associated with some forms of proteinuria, including a subset of MCD in children. CsA can also induce a remission of proteinuria caused by primary glomerular diseases, such as MCD and FSGS. Podocytes are a direct target of CsA, independent of NFAT inhibition in T cells. It was found that CsA directly blocked calcineurin-mediated dephosphorylation of synaptopodin, thereby preserving the phosphorylation dependent synaptopodin-14-3-3b interaction. Preservation of this interaction, in turn, protects synaptopodin from CatL-mediated degradation and preserves a stable filtration barrier. The inducible expression of CatL-resistant synaptopodin in podocytes can prevent not only LPS-induced degradation of synaptopodin and proteinuria, but also the degradation of the other CatL target such as dynamin and ZO-1 [88]. Recent studies revealed that CsA exert an antiproteinuria effect in children with genetic forms of NS, such as mutation in the WT1, podocin and phospholipase C epsilon genes [89-92]. These data unveiled a calcineurin signaling pathway, which is operative in podocytes and contributes to the maintenance of kidney filter function. Altogether, the antiproteinuric effect of CsA results, at least in part, from the maintenance of synaptopodin protein levels in podocytes, which safeguard against proteinuria by maintaining podocyte phenotype. A recent study showed that most of the *MYO1E* mutated patients responded to cyclosporine which may act by stabilizing the cytoskeleton [84]. These results represent a new view of calcineurin signaling and shed further light on the treatment of proteinuric kidney diseases.

Tacrolimus (FK-506), a neutral macrolide with immunosuppressive properties, has highly similar biological properties as CsA. They both interfere with the TCR-mediated signaling pathway that results in the transcription of early T cell activation genes [93] and inhibits the nuclear factor of activated T cells signaling [94]. FK-506 reduces peptidyl-prolyl isomerase activity by binding to the immunophilin FKBP12 (FK506 binding protein). This FKBP12-FK506 complex interacts with and inhibits calcineurin thus inhibiting both T-lymphocyte signal transduction and IL-2 transcription [95]. Zhang *et al.* [96] found that FK-506 showed a rapid proteinuria remission in refractory IgAN patients. This suggests FK-506 may stabilize podocyte cytoskeleton through inhibition of calcineurin expression. Preliminary experiments reveal that FK-506 exerts a pharmacological action on the TRPC6 to diminish proteinuria. They have shown that FK-506 can inhibit channel activity in TRPC6 overexpressed cells and *in vivo* [97, 98]. This suggests a novel mechanism of action for FK-506.

3. Triptolide

Triptolide, a diterpene triepoxide, was identified as one of the major active components of Tripterygium wilfordii Hook F (TWHF). Pretreatment with triptolide could prevent disruption of actin filaments and synaptopodin induced by PAN which might attribute to the inhibition of ROS generation and subsequent p38 MAPK pathway, and restoration of RhoA activity [99]. This study suggests that triptolide could prevent the disruption of actin filaments in podocytes.

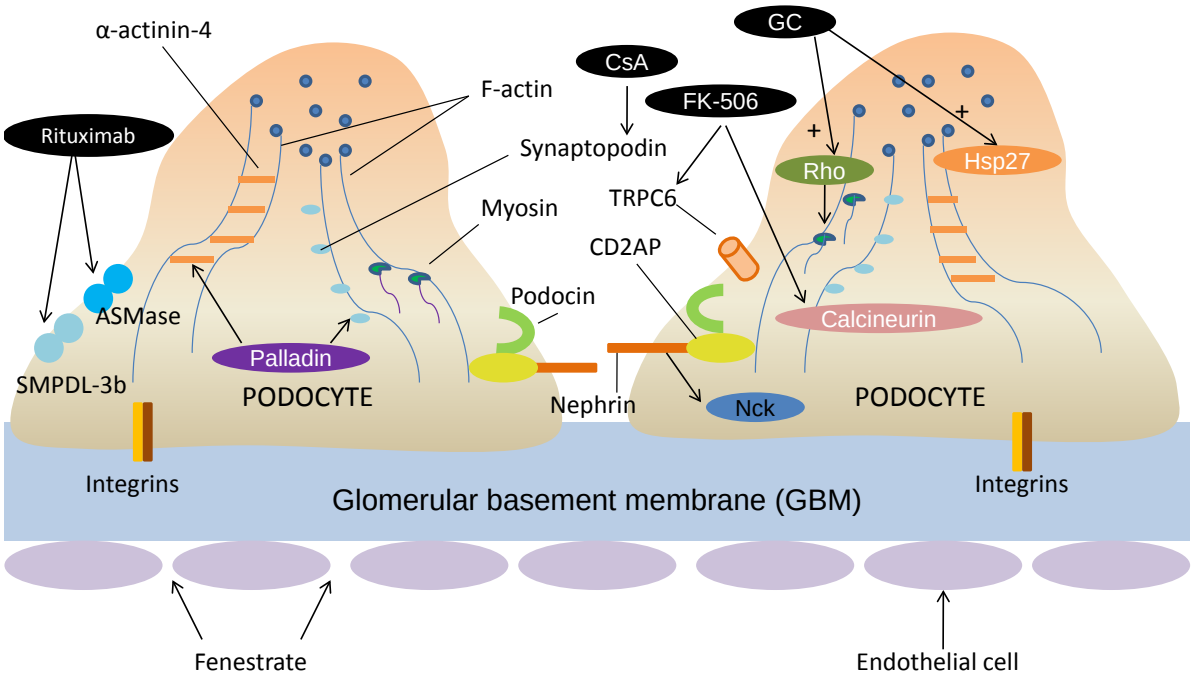

Fig. (1). Molecullar overview of glomerular filtration barrier. The glomerulus consists of three layers: podocyte, glomerular basement membrane and fenestrated endothelial cells. Molecules and pathways included are only those relevant to the function of cytokeleton. GC (glucocorticoid) increases total cellular polymerized actin and stabilizes actin filaments by increasing Hsp27 expression and Rho activity. Cyclosporin A (CsA) stabilizes the actin cytoskeleton and stress fibres by dephosphorylating synaptopodin; FK-506 may stabilize podocyte cytoskeleton through inhibition of calcineurin expression and inhibit TRPC6 activity; Rho regulates the assembly of contractile actin and myosin filaments; Palladin directly binds to α-actinin and closely co-localizes with snaptopodin; Rituximab could directly preserve SMPDL-3b expression and ASMase activity in podocytes.

4. Rituximab

Rituximab, an anti-CD20 monoclonal antibody with four transmembrane domains that is expressed on B-lineage cells and a small population of T cells [100]. Rather than modulation of B cells in FSGS pathogenesis, rituximab also has several applications in treating nephrological conditions, including acute allograft rejection and steroid-resistant nephrotic syndrome [101]. Recent studies demonstrated that rituximab treatment of high-risk FSGS patients is associated with lower incidence of posttransplant proteinuria and could directly preserve sphingomyelin phosphodiesterase, acid-like 3b (SMPDL-3b) expression, and acid sphingomyelinase (ASMase) activity in podocytes that have been exposed to sera from patients with recurrent FSGS [100, 102]. Rituximab might therefore act as a modulator of podocyte function.

These studies collectively point to the podocyte as an attractive target and, in particular, the actin cytoskeleton — the stabilization of which informs the complex morphologic features on which podocyte function depends. Research on podocyte-specific therapy could target podocyte protection drugs, which promote SD protein expression, localization or maintenance of function. Altogether these data show that proteins regulating the plasticity of the contractile apparatus of podocytes are of critical importance for the maintenance of glomerular filter function. Thus, podocyte-specific therapies may function through stabilization of the podocyte actin cytoskeleton.

CONCLUSIONS

This review highlights contributions from recent years that have enhanced our understanding of the pathophysiology mechnisms of cytoskeletal changes in podocytes, with emphasis on discoveries which may lead to the identification of therapeutic targets. The actin cytoskeleton serves as the "common final pathway" organizing FP effacement independent of the original underlying site or cause of podocyte damage. Development of drugs which protect the podocyte and in particular its actin cytoskeleton should be a therapeutic goal. In summary, the molecular mechanisms of podocyte cytoskeleton regulation are now better understood, as is their role in podcoyte injury. As more investigators continue to focus on podocytes, it is likely that future therapeutic targets will be identified, which will improve the renal survival of patients with podocyte diseases. Ongoing studies in many laboratories are aiming at an understanding of the dynamic relationship between SD proteins, the actin cytoskeleton, and the dynamics of FP structure in nephrotic syndrome and FSGS. These studies would be helpful to understand the biological mechanism underlying the podocyte response to injury, and shed much light on podcoyte-specific therapeutic interventions toward the regulation of cytoskeleton molecules.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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