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Cell Biology and Pathology of Podocytes

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Abstract

As an integral member of the filtration barrier in the kidney glomerulus, the podocyte is in a unique geographical position: It is exposed to chemical signals from the urinary space (Bowman's capsule), it receives and transmits chemical and mechanical signals to/from the glomerular basement membrane upon which it elaborates, and it receives chemical and mechanical signals from the vascular space with which it also communicates. As with every cell, the ability of the podocyte to receive signals from the surrounding environment and to translate them to the intracellular milieu is dependent largely on molecules residing on the cell membrane. These molecules are the first-line soldiers in the ongoing battle to sense the environment, to respond to friendly signals, and to defend against injurious foes. In this review, we take a membrane biologist's view of the podocyte, examining the many membrane receptors, channels, and other signaling molecules that have been implicated in podocyte biology. Although we attempt to be comprehensive, our goal is not to capture every membrane-mediated pathway but rather to emphasize that this approach may be fruitful in understanding the podocyte and its unique properties.

Keywords

glomerulus; foot process; slit diaphragm; actin cytoskeleton; calcium; angiotensin; synaptopodin; TRPC channels; Rac1; RhoA; integrins; mTOR; autophagy

INTRODUCTION

With every cardiac cycle, the kidney glomerulus filters blood into an ultrafiltrate that will ultimately become urine. Architecturally, the glomerulus or renal corpuscle consists of a glomerular tuft and Bowman's capsule. The basic unit of the glomerular tuft is a single capillary. The glomerular basement membrane (GBM) provides the primary structural scaffold for the glomerular tuft. Endothelial and smooth muscle–like mesangial cells providing capillary support are located inside the GBM, whereas podocytes are attached to the outer part of the GBM (Figure 1). There are therefore four resident cell types in the glomerulus: endothelial cells, mesangial cells, parietal epithelial cells of Bowman's capsule, and podocytes (Figure 1*a*). Podocytes are pericyte-like cells with a complex cellular organization consisting of a cell body, major processes, and foot processes (FPs). Podocyte

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FPs elaborate into a characteristic interdigitating pattern with FPs of neighboring podocytes, forming in between the filtration slits that are bridged by the glomerular slit diaphragm (SD) (Figure 1*b*, *c*). Podocyte FPs and the interposed SD cover the outer part of the GBM and play a major role in establishing the selective permeability of the glomerular filtration barrier, which explains why podocyte injury is typically associated with marked albuminuria. Podocytes are highly differentiated cells with limited capability to undergo cell division in the adult, and the loss of podocytes is a hallmark of progressive kidney disease. The function of podocytes is based largely on the dynamic regulation of their complex cell architecture, in particular the FP structure. Over the past decade, there has been a growing understanding of the role of membrane proteins in transducing extracellular cues into the podocyte intracellular milieu to effect critical architectural changes. Membrane proteins, e.g., ion channels, integrins, and growth factor receptors, are taking center stage in podocyte biology research. Here we provide insight into the physiological and pathophysiological pathways that regulate podocyte structure and function in health and disease.

PODOCYTE ARCHITECTURE: THE CENTRAL ROLE OF FOOT PROCESSES

Mature podocytes consist of three morphologically distinct segments: a cell body, major processes, and FPs (1). From the cell body, microtubule-rich major processes split into FPs (Figure 1*b*) containing an actin-based cytoskeleton (Figure 1*c*, *d*). Podocyte FPs elaborate into a highly branched interdigitating network with FPs of neighboring podocytes. The SD (Figure 1*c*) is a multiprotein complex similar to an adherens junction. The SD bridges the filtration slits between opposing podocyte FPs (1), thereby establishing the final barrier to urinary protein loss (2). FPs are characterized by a podosome-like cortical network of short, branched actin filaments and by the presence of highly ordered, parallel contractile actin filament bundles (Figure 1*d*) (3), which are thought to modulate the permeability of the filtration barrier through changes in FP morphology. The function of podocytes is based largely on their complex architecture, in particular on the maintenance of highly ordered, parallel, contractile actin filament bundles in FPs (3). In fact, similar to (pseudo)unipolar sensory neurons, the podocyte cell body and primary processes may simply play a trophic, supporting role to the critically important FPs. FPs are functionally defined by three membrane domains: the apical membrane domain, the SD, and the basal membrane domain associated with the GBM (Figure 1*d*). All three domains are physically and functionally linked to the FP actin cytoskeleton, thus lending a central role to actin for both podocyte function and dysfunction. Interference with any of the three FP domains changes the actin cytoskeleton from parallel, contractile bundles (3) into a dense network. The results are (*a*) FP effacement reflected by the simplification of the FP structure and loss of the normal interdigitating pattern (Figure 1*e*) and (*b*) proteinuria (4). FP effacement requires the active reorganization of actin filaments (5, 6), a process regulated at the molecular level by a multitude of signaling events involving, but not limited to, integrin activation, G protein–coupled receptor (GPCR) and growth factor receptor activity, and calcium (Ca^{2+}) influx pathways as upstream modulators of the actin cytoskeleton. In vivo studies have shown that FP effacement is always temporally related to the emergence of proteinuria (4, 7, 8) and thus suggest a causative link between these two events, which is a widely held belief in our field. However, the molecular steps leading to FP effacement are so hierarchical and precise that they lend credence to what remains a tantalizing hypothesis: FP effacement may be the podocyte's desperate attempt to adapt to toxic cues to prevent proteinuria (W. Kriz, personal communication).

PODOCYTE ORIGINS: PODOCYTE MATURATION IS ASSOCIATED WITH A PHYSIOLOGICAL EPITHELIAL-TO-MESENCHYMAL TRANSITION

During renal development, epithelial precursors give rise to mature podocytes, which are mesenchyme-like cells. Glomerular development proceeds in four stages: the renal vesicle stage, the S-shaped body stage, the capillary loop stage, and the maturing-glomeruli stage (9, 10). The transition from the S-shaped body stage to the capillary loop stage is critical for podocyte differentiation (1). During the early developmental stages, simple undifferentiated epithelial cells with apically located tight junctions form the immature podocyte precursor cell population (11). At this stage, the expression of podocalyxin (12) and of the tight junction protein ZO-1 (13) commences, and expression of the podocyte-specific transcription factor Wilm's tumor protein 1 (WT-1) is highest (14). As these immature podocytes enter the capillary loop stage, they lose their mitotic activity and begin to establish their characteristic complex cell architecture, including the appearance of FPs and the reorganization of cell-cell junctions into SDs (11), which are essentially modified adherens junctions (15). At this stage, ZO-1 migrates from its apical location down to the level of the slit membrane, where it is observed in a punctate pattern along the filtration slits (13). A similar apical-to-basal redistribution pattern occurs in the case of the atypical protein kinase C (aPKC), an important cell polarity protein (16–18). The phenotypic conversion occurring during the S-shaped body stage is accompanied by the expression of synaptopodin (19) and by the reappearance of vimentin (20), a phenotypic marker of mesenchymal cells.

The canonical developmental pathway leading to the formation of podocyte FPs is governed by the coordinated activities of numerous membrane or membrane-associated proteins or the transcription factors that regulate the expression of such membrane proteins. Mouse genetic studies have shown a central role for α3 integrin in the formation of mature FPs (21), a phenotype mirrored by the podocyte-specific deletion of β1 integrin (22, 23). The deletion of the nonclassical protocadherin FAT1 revealed the importance of this molecule for proper FP formation (24). Disruption of the cytoskeletal adaptor proteins Nck1/2, which are linked to the critical SD protein nephrin (25), resulted in complete failure to develop FPs (26), thereby phenocopying α3 integrin knockout mice. This result is not surprising, as Nck proteins are critically involved in integrin signaling (27). Furthermore, mice lacking podocalyxin also fail to form FPs and die soon after birth from renal failure (28). The podocyte transcription factor Kreisler (Table 1), whose targets include the SD proteins nephrin (25) and podocin (29), is also essential for FP formation (30). Moreover, disruption of Pod1 (also known as epicardin) leads to aberrant FP formation (Table 1) (31). These genetic studies reveal the importance of these molecules in podocyte development and, more specifically, in FP development. Table 1 summarizes transcription factors involved in the regulation of podocyte structure and function during development as well as under physiological and pathological conditions.

Podocyte development is relevant to mechanisms of podocyte injury in the adult. Mature podocytes are unable to undergo cell division in vivo. The evidence for this hypothesis comes from studies showing that the number of podocyte nuclei does not increase during postnatal and hypertrophic kidney growth (32–36). The transition of podocytes from the S-shaped body stage to the capillary loop stage and their branching into specialized cell architecture involve a neuron-like complex cell differentiation process, which is mutually exclusive with cell division. Interestingly, in response to certain stimuli [e.g., fibroblast growth factor (FGF)-2], podocytes may reenter the cell cycle and undergo nuclear division but cannot complete cell division (33). The only exception to this phenomenon is noted in HIV nephropathy, in which podocytes undergo a tumor-like proliferation, albeit without reparative capacity (37–40). Although the underlying molecular mechanisms leading to the

effective arrest of cytokinesis in vivo remain to be established, cyclins and cyclin-dependent kinases and their inhibitors have been implicated (reviewed in detail in Reference 41).

The inability of mature podocytes to undergo cell division and to replenish their population renders the glomerulus vulnerable to toxic cues, leading to significant podocyte loss, which is a hallmark in the development of chronic kidney failure (42). The search for a podocyte stem cell to replenish a diminishing podocyte pool has led to the notion that a population of cells in the parietal epithelium of the glomerulus may migrate into the glomerular tuft and differentiate into mature podocytes (43, 44). However, more recent evidence suggests that migrating parietal epithelial cells may be maladaptive for glomerular function (45). Further studies are likely to reveal if there is a population of cells capable of replenishing lost podocytes.

THE SLIT DIAPHRAGM: A MULTIPROTEIN SIGNALING HUB

The SD is a complex signal transduction unit with characteristics of a modified adherens junction that spans the 30–50-nm-wide filtration slits (15). The extracellular portion of the SD is made up of rod-like units that are thought to be composed of the extracellular domains of various transmembrane proteins such as nephrin and FAT (1). These rods are connected by a linear bar, forming a zipper-like pattern, which leaves pores the same size as or smaller than albumin (1). The SD's cytoplasmic portion contains a region of Triton-X-100-resistant (1), electron-dense material, which is reminiscent of the highly insoluble specialization of the submembranous actin cytoskeleton in neurons known as the postsynaptic density (PSD) (46). The PSD contains multiple receptors and ion channels linked through a multitude of adaptor proteins to the cytoskeletal core, forming a large protein network (47). Similarly, at the SD, ion channels such as TRPCs (where TRPC denotes transient receptor potential canonical) (48), receptors, integrins, and other membrane proteins (Figure 2) are connected to actin via a variety of adaptor and effector proteins.

The disruption of SD structure or function is a common theme in many kidney diseases arising at the podocyte level (49). The SD is thought to function as a key sensor for and as a regulator of adaptations in FP shape and length (7, 8). For example, the movement of each FP needs to be precisely coordinated with that of the FPs of neighboring podocytes to ensure the integrity of the filtration barrier. Such coordination is likely achieved through functional coupling of opposed FPs, which generates signaling cascades on both ends of the SD. This multiprotein network likely serves a far more complex role as a signaling platform and is not simply a physical sieve.

Nephrin is a well-known and widely studied SD membrane protein (8). Mutations in the *NPHS1* gene encoding for nephrin have been identified as the cause of congenital nephrotic syndrome of the Finnish type (25). Nephrin has a single transmembrane domain; a short intracellular tail; and a long, immunoglobulin-like extracellular moiety, which is thought to align parallel to the extracellular domains of neighboring nephrin molecules. Through its intracellular domain, nephrin is connected to the actin cytoskeleton by several adapter proteins and plays a pivotal part in the regulation of podocyte actin dynamics (7, 50). Among other pathways (reviewed in detail in Reference 7), a recently discovered signaling pathway couples nephrin to the actin cytoskeleton via the adapter protein Nck (26, 51). After nephrin phosphorylation by Fyn (52), Nck binds to phospho-nephrin and to N-WASP (26, 51), activating the Arp2/3 complex, a major regulator of actin dynamics (7, 26, 50, 51). Recent work has also shown that Fyn phosphorylation of nephrin promotes activation of phosphoinositide 3 kinase and Rac1 activity (53; please also see below).

Notably, a large body of human genetic, animal, and physiological studies, including micro-puncture experiments, over more than four decades had established the podocyte FPs with

the interposed SD as the final barrier to albumin (2, 8). However, some years ago, on the basis of live-imaging two-photon microscopy results, researchers developed the alternative hypothesis that nephrotic levels of albumin pass across the normal glomerulus filters and are subsequently retrieved by the proximal tubule (54). Through the use of the same two-photon microscopy approach, two recent independent studies convincingly refuted this leaky glomerular barrier hypothesis (55–57). Both Tanner (55) and Peti-Peterdi (57) directly confirmed the classical view that the glomerular filter is the primary barrier for albumin and that the glomerular sieving coefficient for albumin is extremely low (55–57), thereby corroborating the size and charge selectivity of the glomerular barrier (55, 58–60).

PODOCYTE INJURY IS THE HALLMARK OF PROTEINURIA AND GLOMERULAR DISEASE

Podocyte injury is the common denominator in many forms of human and experimental glomerular disease such as minimal change disease, focal segmental glomerulosclerosis (FSGS), membranous glomerulopathy, diabetic nephropathy (DN), and lupus nephritis (2, 4). The best-characterized pattern of injury involves a reorganization of the FP actin cytoskeleton that leads to FP effacement and to SD disruption (61, 62). The disruption of any of the three FP domains (Figure 1*d*), with the concomitant transformation of the actin cytoskeleton from parallel contractile bundles (3) into a dense network and loss of the normal interdigitating pattern (Figure 1*e*), leads to proteinuria (4).

There is a growing understanding of the sequence of events that mediates FP effacement and proteinuria (Figure 3*a*), followed over time by further phenotypic changes such as podocyte hypertrophy and ultimately podocyte detachment and loss (Figure 3*b*). The initial patterns of injury include (*a*) changes in SD structure or function (52, 63, 64), (*b*) interference with the GBM or the podocyte:GBM interaction (21, 65–70), (*c*) dysregulation of podocyte Ca^{2+} homeostasis (71, 72), (*d*) dysfunction of the podocyte actin cytoskeleton (50, 51, 62, 73–76), (*e*) modulation of the negative surface charge of podocytes (5, 77, 78), (*f*) activation of innate immunity pathways such as B7-1 signaling (79–81), (*g*) upregulation of CatL-mediated proteolysis (82–86), and (*h*) disturbances in the transcriptional regulation of podocyte function (87).

Importantly, a common theme to recent advances is the ever-growing significance of membrane proteins in the emergence of FP effacement and progressive podocyte injury (Figures 2 and 3). Growth factor receptors such as vascular endothelial growth factor (88, 89) and transforming growth factor β (90), GPCRs such as the angiotensin type 1 receptor (AT1R) (91–93), signaling through Notch (94, 95) or integrins (21–23, 96, 97), ion channels such as the TRPCs (48, 71, 98–100), and many other molecules (summarized in Figure 2) have been implicated in early podocyte injury. Although such molecules work in concert under physiological conditions, they become functionally uncoupled under disease conditions, leading to a disrupted cytoskeleton, the best known podocyte injury pattern to date.

Early podocyte injury is reversible if the actin cytoskeleton is repaired, allowing FPs to branch once again into their interdigitating pattern (Figure 3*a*). Sustained chronic podocyte injury can lead to the loss of glomerular and ultimately entire kidney function through three principal mechanisms: (*a*) the dysregulated pathway, (*b*) the inflammatory pathway, and (*c*) the degenerative pathway (101). First, in the dysregulated pathway, dedifferentiation of podocytes leads to podocyte proliferation within Bowman's space and the collapse of the glomerular tuft, with GBM wrinkling and capillary loss. Thus, so-called collapsing glomerulopathy occurs, for example, in HIV-associated nephropathy. Second, inflammatory mechanisms can lead to the fixation of podocytes to the parietal basement membrane

followed by the establishment of tuft adhesions to Bowman's capsule (101). Further proliferation of podocytes and parietal cells results in the formation of cellular crescents. When the lesion heals by fibrosis, segmental glomerulosclerosis occurs (101). Finally, in the degenerative form, which is most commonly observed, the persistence of podocyte injury can cause cell body attenuation, podocyte hypertrophy, detachment from the GBM, and podocyte death followed by the formation of synechiae by the attachment of parietal epithelial cells to nude GBM (Figure 3*b*). This attachment results in misdirected filtration toward the interstitium (101, 102). Through a series of ensuing changes (reviewed in detail in Reference 101), the loss of podocytes ultimately leads to glomerulosclerosis and to kidney failure (101). The contribution of proteinuria to the progression of kidney disease is a matter of debate (103). Some investigators believe that proteinuria can induce podocyte damage (104) or tubulointerstitial inflammation and progressive injury (105). However, severe experimental protein loss across the glomerular filter caused by repeated injection of rats with the antinephrin antibody 5-1-6 did not lead to progressive renal failure (106). The role of proteinuria in the progression of kidney failure may depend on the route of protein loss. Significant podocyte loss per glomerulus, and thus misdirected filtration into the periglomerular interstitium, leads to tubular destruction and the progression of kidney failure (101, 107). In contrast, protein loss across the filtration barrier without significant podocyte loss does not lead to disease progression and may be reversible (Figure 3*a*) (101, 108).

GENETIC CAUSES OF PODOCYTE INJURY

Human genetics have fueled our progress toward a molecular understanding of the SD and the modulators of FP architecture. In the past 15 years, human genetic studies revealed that mutations in the genes encoding nephrin (25), podocin (29), phospholipase C ε (109), and coenzyme Q10 biosynthesis mono-oxygenase 6 (110) give rise to early-onset proteinuria. Additionally, adult-onset proteinuria such as FSGS is associated with mutations in the genes encoding α-actinin 4 (62), CD2AP (111), INF2 (112), TRPC6 (48, 100, 113), and synaptopodin (114). Mutations in *LMX1B*, which encodes a transcription factor for collagen, result in podocyte abnormalities due to impaired cell adhesion to the abnormal GBM (115). Similarly, mutations in the gene encoding laminin β2, another component of the GBM, lead to podocyte injury and proteinuria (116). Finally, recent exome sequencing as well as a whole-genome linkage analysis revealed *MYO1E* mutations in childhood proteinuric disease and FSGS; *MYO1E* encodes a mutant form of nonmuscle class I myosin (117, 118). Table 2 summarizes human mutations affecting podocyte structure and function. Mouse genetic studies have revealed that additional proteins regulating the plasticity of the podocyte actin cytoskeleton such as Rho GDP dissociation inhibitor α (Rho GDIα) (119), podocalyxin (6), FAT1 (120), Nck1/2 (26, 51), synaptopodin (121, 122), and cofilin (123) are also of critical importance for sustained function of the glomerular filtration barrier. Taken together, human and mouse genetics have revealed that the dysregulation of the highly specialized podocyte actin cytoskeleton is closely associated with disease phenotypes. At present, the regulation of the actin cytoskeleton is probably the best understood aspect of podocyte function (see below).

With the advent of genomics, large population studies have revealed common variations in a number of genes that predispose or confer susceptibility to acquired proteinuric kidney disease. These gene polymorphisms are not directly linked to podocyte-specific defects, but this is an area of active research at this time. A large locus containing numerous genes was recently identified in African-American populations (124, 125). Initial studies revealed *MYH9* as a likely gene candidate in this locus. This was an attractive hypothesis, given previous work showing that *MYH9* is responsible for two genetic causes of proteinuria: Epstein and Fechtner syndromes (Table 2) (126). Interestingly, further work revealed that the likely candidate gene conferring risk for kidney disease is *APOL1.* This gene encodes

apolipoprotein L1, a molecule that is known for its trypanolytic properties and that confers an evolutionary advantage in African-Americans (127). Furthermore, common variations in *GPC5* (which encodes glypican 5) are also associated with acquired nephrotic syndrome (128).

MOLECULAR REGULATORS OF PODOCYTE FUNCTION IN HEALTH AND DISEASE

The past decade has brought significant new knowledge about membrane proteins that initiate signaling pathways important for podocyte structure and function (Figure 2). These molecules act as sensors of the podocyte's complex extracellular environment, receiving cues from the urinary (Bowman's) space, the vascular (capillary) space, and the GBM. These signals are subsequently transduced to the intracellular environment, modulating the actin cytoskeleton, gene transcription, and cellular metabolism pathways, among many others (Figures 2 and 3). Here we focus on a few signaling cascades that multiple scientists have investigated: Ca^{2+} signaling and TRPC channels, angiotensin signaling, integrins, and a mammalian target of rapamycin (mTOR) cascade intersecting with autophagy pathways. The roles of numerous other molecules are not clearly understood. Figure 2, a schematic of a podocyte plasma membrane decorated by membrane proteins, represents our concerted effort to be inclusive of the proteins studied in podocytes to date.

CALCIUM SIGNALING IN PODOCYTES

In conjunction with mounting evidence that proteinuria and podocyte FP effacement are mediated by rearrangements of the actin cytoskeleton (7, 51, 129), disrupted Ca^{2+} signaling and homeostasis were postulated as early events in podocyte injury (130). Complement C5b-9 complex–mediated podocyte damage is associated with an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (131). Protamine sulfate, which can cause FP effacement in vivo (132, 133), also increases $[Ca^{2+}]_i$ in vitro in both cultured cells (134) and isolated glomeruli (72). Bradykinin and angiotensin II (Ang II) application on differentiated mouse and rat podocytes, respectively, increases $[Ca^{2+}]_i$ (135, 136). A recent intriguing study also revealed that the Ca^{2+}-sensing receptor has prosurvival and actin-stabilizing effects in podocytes (137). Importantly, Ang II evokes a nonselective cationic current recorded from podocytes of isolated rat glomeruli (93). Once TRPC6 channel mutations were found in patients with familial FSGS (48, 100), TRP channels emerged as prime candidates for this as-yet-uncharacterized conductance. Recently, detailed electrophysiology unveiled TRPC5 and TRPC6 as the channels downstream of the Ang II–evoked nonselective cationic conductance (71), initially identified more than a decade ago (93).

Activation of the Ca^{2+}-dependent phosphatase calcineurin leads to cathepsin L–mediated cleavage of synaptopodin and to proteinuria (83). The calcineurin inhibitor cyclosporine A (CsA) prevents synaptopodin degradation in vitro, and mice resistant to cathepsin-mediated synaptopodin degradation are protected from proteinuria in vivo (83). Conversely, the activation of calcineurin in podocytes is sufficient to cause degradation of synaptopodin and proteinuria (83). Thus, the preservation of synaptopodin and the podocyte actin cytoskeleton provides an intriguing podocyte-specific, T cell– and NFAT-independent mechanism for the long-known antiproteinuric effect of calcineurin inhibition (83). However, NFAT-dependent mechanisms are also important in podocytes. Ca^{2+}-conducting and FSGS-causing TRPC6 mutations, but not wild-type TRPC6, induce constitutive activation of calcineurin- and NFAT-dependent gene transcription (138). Importantly, the activation of NFAT signaling in podocytes is sufficient to cause glomerulosclerosis in mice (139). A recent study supports the notion of a positive feedback loop, whereby NFAT-mediated increases in TRPC6 channel transcription lead to proteinuria (98). Although not experimentally addressed in this

study, the proposed mechanism of injury relates to excessive Ca^{2+} influx due to aberrantly high numbers of active TRPC6 channels on the podocyte plasma membrane. A similar feed forward cycle was demonstrated in a mouse model of cardiac hypertrophy: Calcineurin- and NFAT-mediated increases in TRPC6 transcription promote pathological cardiac remodeling (140). More recently, however, investigators showed that other TRPC channels mediate calcineurin-NFAT activation in cardiac myocytes (140–142), strongly suggesting that this pathway is not specific to TRPC6.

ANGIOTENSIN SIGNALING IN PODOCYTES

The podocyte-specific overexpression of the AT1R in rats is sufficient to cause proteinuria and FSGS-type lesions. This model may be one of the best rodent models of FSGS to date because the model mimics the human disease by exhibiting hyperlipidemia, hypoalbuminemia, and progressive kidney failure (91). At the cellular level, the activation of the AT1R transactivates the epidermal growth factor receptor (EGFR) in tubular epithelia (143) and in podocytes (144). Following an increase in cytosolic [Ca^{2+}], AT1R-EGFR interactions also activate downstream serine/threonine kinases such as the mitogen-activated protein kinase pathway (145). Ang II induces membrane ruffling and the loss of stress fibers (92), similar to the depletion of synaptopodin (83, 122) or of TRPC6 (71; see section below). Intriguingly, the AT1R signals to TRPC5 and TRPC6 channels in podocytes (71; see section below). The most compelling evidence to study the effects of angiotensin in podocytes comes from human trials in which the inhibition of AT1R signaling through angiotensin-converting enzyme inhibitors or angiotensin receptor blockers delayed disease progression in patients with diabetic kidney disease (146, 147).

CALCIUM, TRPC CHANNELS, RHO GTPASES, AND THE REGULATION OF THE ACTIN CYTOSKELETON

Previous studies in many cell types, including fibroblasts and neurons, had established an intimate association between Ca^{2+} influx and the activation of the Rho GTPases, which are cytoskeleton master regulators (148, 149). Given the central role played by Ca^{2+} and the actin cytoskeleton in podocyte biology, it is not surprising that the Rho GTPases have been the subject of numerous recent studies in podocytes. At the cellular level, a stationary podocyte phenotype, which suggests intact FPs, is thought to be due to the relative predominance of RhoA activity. In contrast, a predominance of Cdc42/Rac1 activity mediates a disease-associated motile phenotype, suggesting unstable or retracted FPs (7). Synaptopodin has emerged as an important regulator of Rho GTPases in podocytes. Synaptopodin promotes RhoA signaling by preventing its ubiquitination and proteasomal degradation. This results in the preservation of stress fibers in vitro and in safeguarding against proteinuria in vivo (83, 122). Synaptopodin-depleted podocytes, in which RhoA is ubiquitinated, display loss of stress fiber formation and aberrant filopodia (121). Synaptopodin also suppresses Cdc42 signaling through the inhibition of Cdc42:IRSp53:Mena complexes (150). Recent studies thus support the notion that synaptopodin stabilizes the kidney filter by promoting RhoA and inhibiting Cdc42, thereby preventing the reorganization of the podocyte FP cytoskeleton into a migratory phenotype (83). The promotility GTPase Rac1 was also implicated in podocyte biology: Rho GDIα–null mice develop heavy albuminuria (119) due to increased, constitutively active Rac1 signaling in podocytes (75). Rac1 promotes the accumulation of mineralocorticoid receptor into the podocyte nucleus through p21-activated kinase phosphorylation (75). Pharmacological intervention with a Rac1-specific small-molecule inhibitor (NSC23766) diminishes mineralocorticoid receptor hyperactivity and ameliorates proteinuria and renal damage in this mouse model of proteinuria (75). Taken together, these studies suggest an important role for the RhoGTPases in podocyte health and disease.

Since the discovery of gain-of-function *TRPC6* mutations in familial FSGS (48, 100), the molecular mechanisms involving TRPC channels have become a central question in podocyte biology. Recently, AT1R-activated TRPC5 and TRPC6 channels were unveiled as antagonistic regulators of actin dynamics and cell motility in podocytes through the regulation of Rac1 and RhoA, respectively (Figure 4) (71). The latter study showed that TRPC6 depletion results in the loss of stress fibers, Rac1 activation, and increased motility, all of which are rescued by constitutively active RhoA (71). Conversely, TRPC5 depletion leads to enhanced stress fiber formation, RhoA activation, and decreased motility, all of which are reversed by constitutively active Rac1 (Figure 4) (71). Two distinct signaling microdomains emerged: TRPC5 specifically interacts with and activates Rac1, whereas TRPC6 specifically interacts with and activates RhoA (Figure 4) (71). Consistent with previous studies (83), CsA restored synaptopodin expression in TRPC6-depleted cells (71). In contrast, synaptopodin expression was preserved in TRPC5-depleted podocytes (71). These results significantly extended our mechanistic understanding of TRPC channelopathies and provided a mechanistic link between AT1R activation, Ca^{2+} signaling, TRPC channels, and Rho GTPase activity in podocytes.

Mice overexpressing wild-type *TRPC6* and *TRPC6* gain-of-function mutants develop albuminuria and FSGS-type lesions (151). Although proteinuria in these mice was modest with low and variable penetrance and structural abnormalities were not observed before 6–8 months of age (151), these results are consistent with Ca^{2+} overload–mediated cellular injury and death, ultimately leading to FSGS. In keeping with cell culture data showing RhoA activation downstream of TRPC6 activity (71), recent work revealed that mice overexpressing constitutively active RhoA in a podocyte-specific manner also developed proteinuria and FSGS-type lesions (152). Taken together, these studies suggest that an unopposed or overactive TRPC6-RhoA pathway may cause irreversible injury, podocyte loss, and kidney failure. In keeping with this, a recent study reported that *TRPC6*-null mice were significantly protected from the proteinuric effects of Ang II (99). The observed protective role of *TRPC6* deletion is surprising, given that *TRPC6*-null mice were initially reported to be hypertensive at baseline due to (over)compensation by TRPC3 for the loss of TRPC6 (153). In the light of the antagonistic effects of TRPC5 and TRPC6 on podocyte actin dynamics (71), future studies will be needed to address possible compensatory or antagonistic relationships between TRPC channels in TRPC6 or other TRPC knockout mice. The development of podocyte-specific, inducible TRPC knockout mice is also likely to illuminate our understanding of in vivo TRPC channel function in podocytes.

INTEGRIN SIGNALING IN PODOCYTES

Integrins are heterodimeric cell adhesion receptors whose function is central to inflammation, immunity, tumor progression, development, and the maintenance of normal tissue architecture in mature organs (154, 155). Cell adhesion and spreading as well as remodeling of the extracellular matrix (ECM) involve bidirectional signaling and physical linkage between the ECM, integrins, and the cytoskeleton (154, 155). The actin cytoskeleton of podocyte FPs is linked to the GBM by α3β1 integrin (23, 96, 156), αvβ3 integrin (64, 157), and α-/β-dystroglycans (65, 158). Podocytes also express β4 integrin, and mutations in the gene encoding β4 integrin are associated with congenital FSGS and skin disease (159). Genetic inactivation of α3 (21) or β1 (22, 23) integrin causes podocyte FP effacement and kidney failure in newborn mice, thereby underscoring the critical role of α3β1 integrin in the development and maintenance of the glomerular filter. A recent study (160) suggested that soluble uPAR (suPAR) may be a factor responsible for the development of recurrent FSGS (161). According to this study, suPAR leads to recurrent FSGS through the activation of β3 integrin in podocytes (160). Of note, these data are in contrast with previous studies identifying β3 integrin as a mediator of protective osteopontin signaling in podocytes (157).

Future studies will be required to address the precise function of β3 integrin signaling in podocytes. In addition, the relative contribution of β3 integrin activation versus loss of sphingomyelin phosphodiesterase acid–like 3b protein expression (162) to the pathogenesis of recurrent FSGS remains to be resolved.

The β1 integrin–binding protein integrin-linked kinase (ILK) is another mediator of progressive podocyte damage (163). Podocyte-specific deletion of the ILK gene in mice causes progressive FSGS and renal failure (22, 164, 165). Activation of ILK in podocytes induces Wnt signaling, which in turn leads to the reduction of CD2AP and P-cadherin expression, podocyte detachment, and proliferation (166). Moreover, overexpression of ILK causes the rearrangement of the podocyte actin cytoskeleton, presumably via ILK-mediated phosphorylation of α-actinin (163). ILK serves as an adaptor that biochemically and functionally connects the GBM with the SD: It interacts with nephrin, α-actinin, PINCH, and α-parvin, playing a crucial role in podocyte adhesion, morphology, and survival (165, 167). A recent study also brought to light the importance of focal adhesion kinase (FAK) in podocytes, showing that its inhibition protects against proteinuria and FP effacement (168). This study concluded that podocytes isolated from conditional, podocyte-specific FAK knockout mice demonstrated reduced spreading and migration. How the FAK, the ILK, and other integrin-mediated pathways intersect to mediate adaptive or maladaptive podocyte adhesion to the GBM will undoubtedly be the subject of many future inquiries.

The role of B7-1 as a bidirectional regulator of T cell activation and tolerance is well established and involves the binding of B7-1 to its cognate receptors CD28, CTLA, or PD-L1 (169). Most interestingly, however, B7-1 is also an inducible mediator of podocyte injury and proteinuria (81). Initially identified due to its significant upregulation in α3 integrin knockout mice, it is also up-regulated in podocytes in human and experimental lupus nephritis and in nephrin knockout mice (81). The clinical significance of these results was underscored by the observation that podocyte expression of B7-1 correlates with the severity of human lupus nephritis. The latter study (81) also found that in vivo exposure to low-dose lipopolysaccharide (LPS) rapidly upregulates B7-1 in podocytes of wild-type and SCID mice, thereby leading to proteinuria. In contrast, mice lacking B7-1 are protected from LPS-induced proteinuria, demonstrating a functional link between podocyte B7-1 expression and proteinuria (81). LPS signaling through Toll-like receptor-4 reorganizes the podocyte actin cytoskeleton. Moreover, the activation of B7-1 in cultured podocytes leads to the reorganization of the vital SD proteins nephrin and CD2AP (81). Thus, the upregulation of B7-1 in podocytes by LPS contributes to the pathogenesis of proteinuria by disrupting the glomerular filter (81). Given the initial observation that B7-1 is upregulated in the absence of α3 integrin in vivo, future studies will be required to address how B7-1 might intersect with integrin-mediated signaling in podocytes.

PODOCYTE MAMMALIAN TARGET OF RAPAMYCIN SIGNALING AND AUTOPHAGY

The mTOR signaling cascade regulates a wide array of cellular processes, including cell growth, proliferation, and autophagy, in response to nutrients such as glucose, amino acids, and growth factors. mTOR is an evolutionarily conserved protein kinase and forms two functional complexes termed mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is a rapamycin-sensitive protein kinase that senses nutrient availability (170). mTOR signaling in podocytes has come to the forefront for two reasons. First, the mTOR inhibitor rapamycin, which is clinically used for immunosuppression after organ transplantation, can induce proteinuria, although the underlying molecular mechanisms are poorly understood. The idea that rapamycin can impair podocyte function is supported by the observation that its application on cultured podocytes decreases the protein abundance of nephrin, TRPC6,

and Nck and reduces cell adhesion and motility (171). Nephrin expression is also reduced in glomeruli of kidney-transplanted patients undergoing mTOR inhibition therapy (172). However, rapamycin can prevent the protamine sulfate–induced and Ca^{2+}-dependent disruption of the podocyte actin cytoskeleton, suggesting a potential beneficial effect of rapamycin on proteinuria (72).

In a second independent line of research, two recent studies revealed the importance of mTOR signaling in podocyte function and diabetic nephropathy in humans and mice. Genetic deletion of mTORC1 in mouse podocytes induced proteinuria and progressive glomerulosclerosis, whereas simultaneous deletion of both mTORC1 and mTORC2 from mouse podocytes aggravated the glomerular lesions (173). In contrast, increased mTOR activity accompanied human diabetic nephropathy, characterized by early glomerular hypertrophy and hyperfiltration. Curtailing mTORC1 signaling in mice by genetically reducing mTORC1 copy number in podocytes prevented glomerulosclerosis and ameliorated glomerular disease progression in diabetic nephropathy (173). Similarly, the activity of the mTOR complex was enhanced in podocytes of diabetic animals (174). Furthermore, podocyte-specific mTORC1 activation induced by ablation of an upstream negative regulator (PcKOTsc1) recapitulated many DN features, including podocyte loss, GBM thickening, mesangial expansion, and proteinuria, in nondiabetic young and adult mice (174). Abnormal mTORC1 activation caused mislocalization of SD proteins and endoplasmic reticulum (ER) stress in podocytes. Conversely, reduction of ER stress with a chemical chaperone significantly protected against both the podocyte phenotypic switch and podocyte loss in PcKOTsc1 mice. Finally, genetic reduction of podocyte-specific mTORC1 in diabetic animals suppressed the development of DN (174). Although protein synthesis and autophagic degradation are regulated in an opposite manner by mTOR (170), such regulation could be beneficial under certain conditions if these two events occurred in unison to handle rapid protein turnover. In keeping with this notion, a recent study revealed that spatial coupling of mTOR and autophagy augments the secretory phenotype of podocytes and macrophages (175). Narita and colleagues (175) observed a distinct cellular compartment at the *trans* side of the Golgi apparatus, the TOR-autophagy spatial coupling compartment (TASCC), where (auto)lysosomes and mTOR accumulated during Ras-induced senescence. mTOR recruitment to the TASCC was amino acid dependent and Rag guanosine triphosphatase dependent, and disruption of mTOR localization to the TASCC suppressed interleukin-6/8 synthesis. TASCC formation was observed during macrophage differentiation and in podocytes; both macrophages and podocytes displayed increased protein secretion. The spatial coupling of cells' catabolic and anabolic machinery could augment their respective functions and facilitate the mass synthesis of secretory proteins (175). Collectively, these data underscore the critical role of mTOR signaling in podocytes for glomerular function in health and disease. These results demonstrate the requirement for tightly balanced mTOR activity in podocyte homeostasis and suggest that mTOR inhibition may protect podocytes and prevent progressive diabetic nephropathy (173). Given the detrimental effect of prolonged rapamycin treatment on podocyte function, it will be important to test if reduction of podocyte mTOR activity can be harnessed as a potential therapeutic strategy to treat DN.

OUTLOOK AND FUTURE DIRECTIONS

In recent decades, the podocyte has been firmly established as the cell responsible for proteinuria and kidney damage. Yet much remains to be learned about this complex cell. In this review, we take a membrane biologist's view, examining membrane receptors, ion channels, and other signaling molecules implicated in podocyte biology. This approach may be fruitful in understanding the podocyte and its unique geographical properties because it brings a new perspective to the many challenges that lie ahead. Although we have made

significant inroads in identifying the molecular components of this complex cell, many central questions remain. One challenge is to gain a further understanding of how these molecules fit together and work in unison under physiological conditions and how the system is perturbed after a noxious insult. A membrane biology perspective may help in this regard if we consider the plasma membrane as the beginning of Ariadne's thread, which can help us navigate our way through what is truly a signaling labyrinth. Although disrupted cytoskeletal dynamics have emerged as a central mechanism for podocyte injury, another challenge is to go beyond the cytoskeleton in an effort to assign a molecular signature to pathways that may mediate the propagation of injury, such as metabolic dysregulation and autophagy. Indeed, little is known about the molecular determinants of pathological progression in podocytes. For example, does podocyte cell death precede detachment or vice versa? The future study of appropriate animal models of disease progression is also likely to be instrumental in this area. Finally, transmembrane receptors and ion channels have proven to be successful drug targets in other areas (176) and therefore should garner significant attention as putative drug targets for antiproteinuric therapies. The challenge will be to discern which of the multitude of podocyte membrane proteins, many of which we discuss here, will be the critical target(s) of choice.

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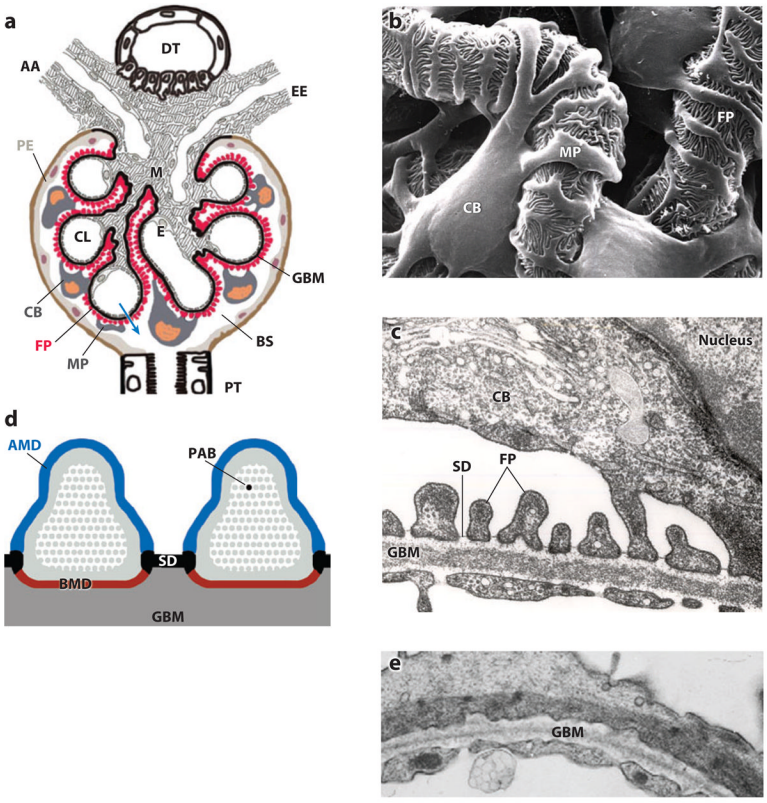

Figure 1.

The function of podocytes is based on their intricate cell architecture. (*a*) A glomerulus contains a capillary tuft that receives primary structural support from the glomerular basement membrane (GBM). Glomerular endothelial cells (E) lining the capillary lumen (CL) and mesangial cells (M) are located on the blood side of the GBM, whereas podocyte foot processes (FP) cover the outer part of the GBM. Podocyte cell bodies (CB) and major processes (MP) float in Bowman's space (BS) in primary urine. Along its route from the CL to BS (*blue arrow*), the plasma ultrafiltrate passes through the fenestrated endothelium, the GBM, and the filtration slits that are covered by the slit diaphragm (SD). AA, afferent arteriole; DT, distal tubule; EE, efferent arteriole; PE, parietal epithelium; PT, proximal tubule. (*b*) Scanning electron microscopy view from BS highlighting the intricate shape of podocytes. MP link the CB to FP, which interdigitate with FP of neighboring podocytes, thereby forming the filtration slits. (*c*) Transmission electron microscopy image of the filtration barrier consisting of fenestrated endothelium, GBM, and podocyte FP with the SD covering the filtration slits. (*d*) Podocyte FP are defined by three membrane domains: the apical membrane domain (AMD) (*blue*), the basal membrane domain (BMD) (*red*), and the SD (*black*). All three domains are connected to the underpinning actin cytoskeleton (*gray*) and with each other. PAB denotes a single parallel, contractile actin bundle containing α-actinin-4, myosin 9, and synaptopodin. (*e*) Under conditions of proteinuria, FP lose their normal interdigitating pattern and instead show effacement. A continuous sheet of cytoplasm filled with reorganized actin filaments is clearly visible.

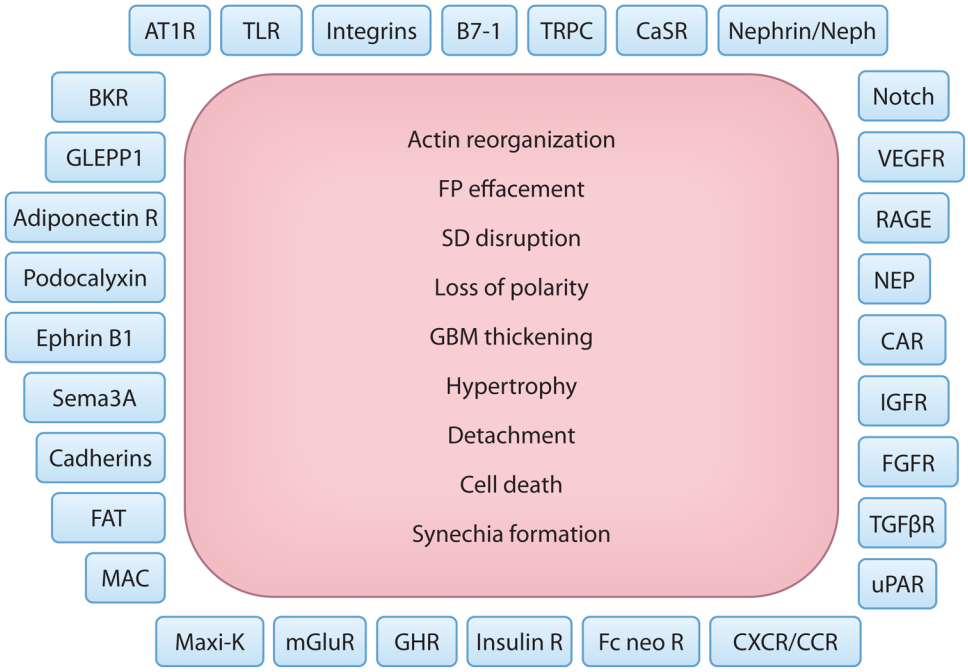

Figure 2.

Podocyte plasma membrane proteins and a canonical pattern of injury. Shown is an (incomplete) list of membrane proteins that have been implicated in the regulation of podocyte function in health and disease. Injured podocytes respond with a finite repertoire of changes, as depicted here. Our ability to repair the pathways initiated by the molecules on the podocyte plasma membrane to the precise cellular phenotypes listed here will provide not only novel insight but enormous opportunities for successful therapeutic interventions. Abbreviations: adiponectin R, adiponectin receptor; AT1R, angiotensin type 1 receptor; BKR, bradykinin receptor; CAR, Coxsackie and adenovirus receptor; CaSR, calcium-sensing receptor; CXCR/CCR, C-X-C/C-C chemokine receptor; FAT, protocadherin FAT1; Fc neo R, neonatal Fc receptor; FGFR, fibroblast growth factor receptor; FP, foot process; GBM, glomerular basement membrane; GHR, growth hormone receptor; GLEPP1, glomerular epithelial (podocyte) protein 1; IGFR, insulin growth factor receptor; insulin R, insulin receptor; MAC, membrane attack complex; Maxi-K, large-conductance calcium-activated potassium channel (also known as BK channel); mGluR, metabotropic glutamate receptor; NEP, neutral endopeptidase; RAGE, receptor for advanced glycation end products; SD, slit diaphragm; Sema3A, semaphorin-3A; TGF βR, transforming growth factor β receptor; TLR, Toll-like receptor; TRPC, transient receptor potential canonical; uPAR, urokinase receptor; VEGFR, vascular endothelial growth factor receptor.

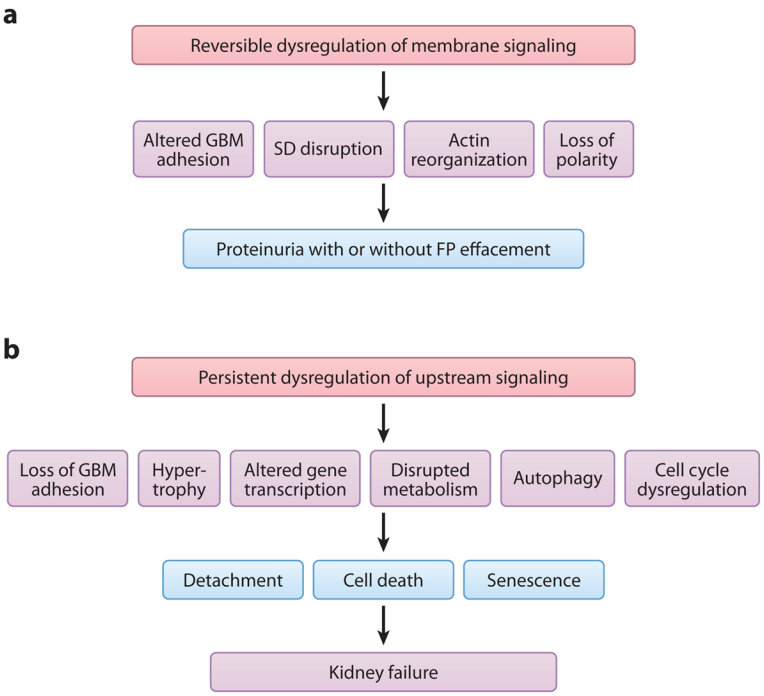

Figure 3.

Reversible and irreversible consequences of dysregulated podocyte signaling. (*a*) Dysregulated signaling at the plasma membrane may lead to reversible morphological changes. Therefore, proteinuria may arise, with or without foot process (FP) effacement (see text for details). However, if the upstream injurious signals are reversed, the cell morphology can revert back to physiological patterns. SD denotes slit diaphragm. (*b*) Persistence of podocyte injury is manifest in the activation of cellular processes that lead to irreversible changes such as loss of adhesion to the glomerular basement membrane (GBM), cell hypertrophy, transcriptional changes, disrupted metabolic pathways, autophagy, and cell cycle dysregulation. These irreversible changes can cause podocyte cell death or the detachment of podocytes from the GBM. Podocyte senescence may also be a manifestation of persistent injury, although less is known about this mechanism. The resulting loss of podocytes ultimately leads to irreversible glomerulosclerosis and kidney failure.

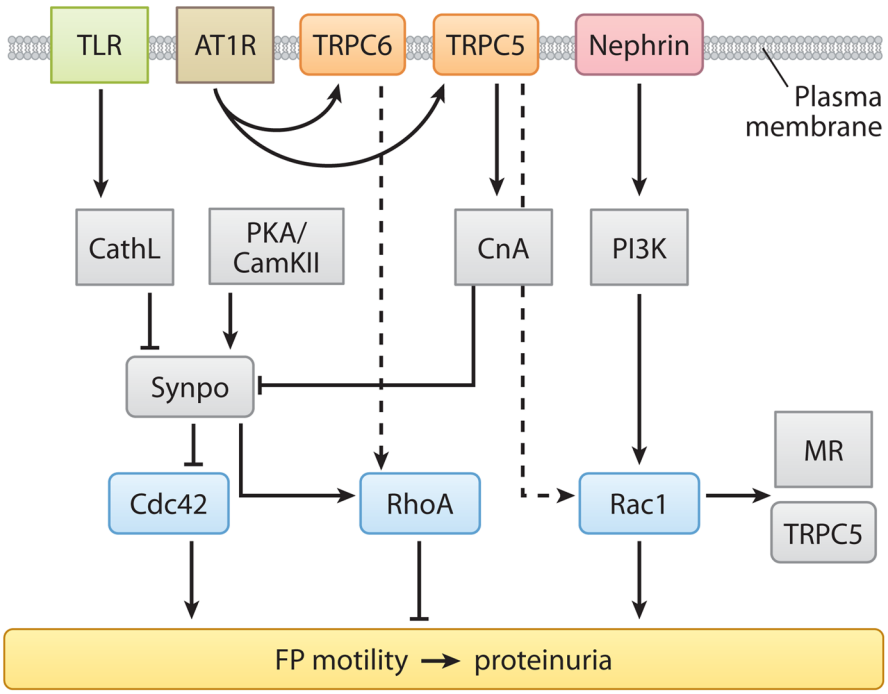

Figure 4.
An example of pathways at the intersection of AT1R and TRPC signaling at the plasma membrane as they converge on synaptopodin and Rho GTPase signaling in the cytosol to effect critical cytoskeletal remodeling. Dashed arrows indicate indirect effects between molecules. Abbreviations: TLR, Toll-like receptor; AT1R, angiotensin type 1 receptor; TRPC6/5, transient receptor potential canonical channels 6 and 5; CathL, cathepsin L; PKA/CamKII, protein kinase A/calcium-calmodulin-dependent protein kinase II; CnA, calcineurin; PI3K, phosphoinositide 3 kinase; Synpo, synaptopodin; MR, mineralocorticoid receptor; FP, foot process.

Table 1

Transcription factors in podocyte development, physiology, and pathology

Name	Putative target(s)	References
Wilm's tumor protein 1 (WT1)	Podxl (podocalyxin), Nphs1 (nephrin)	177–180
LMX1B	α3 and α4 collagens, Nphs2 (podocin)	115, 181, 182
FOXC2 (Mfh2)	Nphs2, α3 and α4 collagens, MafB	183
Kreisler (MafB)	Nphs1, Nphs2, CD2AP	30, 184
Pax2	WT1?	185, 186
ZHX1/ZHX2	WT1?	187, 188
Pod1 (epicardin)	Nphs2, α3 collagen, MafB?	31, 189
ZEB2	P/E cadherins	190
HIFα	CXCR4	191–193
Rbpj (Notch pathway)	?	95
NFAT	TRPC6	98, 138, 139
β-Catenin	?	194, 195

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Table 2

Human mutations affecting podocyte structure and function

Protein	Gene	Associated disease	Mode of inheritance	Clinical description	Reference
Nephrin	*NPHS1*	Congenital nephrotic syndrome of the Finnish type (CNF)	Autosomal recessive (AR)	Often massive proteinuria in utero and nephrotic syndrome postnatally; resistant to treatment	25
Podocin	*NPHS2*	Corticosteroid-resistant nephrotic syndrome (SRNS)	AR	Variable onset and severity of nephropathy; resistance to corticosteroid therapy	29
Coenzyme Q10 biosynthesis mono-oxygenase 6	*COQ6*	Corticosteroid-resistant nephrotic syndrome (SRNS)	AR	Early-onset SNRS with sensorineural deafness	110
PLCε1	*PLCE1*	Inherited nephrotic syndrome	AR	Nephrosis, diffuse mesangial sclerosis, and end-stage kidney disease (ESKD); may be reversible after early treatment	109
Laminin β2	*LAMB2*	Pierson's syndrome Occasionally oligosymptomatic disease variants	AR	Onset of nephrosis postnatally, mesangial sclerosis, microcoria	116
α-Actinin-4	*ACTN4*	Focal segmental glomerulosclerosis (FSGS)	Autosomal dominant (AD)	Mild proteinuria in adolescence, slow progression to FSGS and ESKD in adulthood	62
TRPC6	*TRPC6*	FSGS	AD	Proteinuria in adolescence and early adulthood; progression to FSGS and ESKD; pediatric/sporadic cases reported	48, 100
MYH9	*MYH9*	Epstein syndrome Fechtner syndrome	AD	Thrombocytopenia, hearing defects, and progressive proteinuria	126
LMX1B	*LMX1B*	Nail-patella syndrome	AD	Variable penetrance, nephrotic syndrome, and skeletal/nail abnormalities in children	115, 196
WT1	*WT1*	Denys-Drash syndrome (DDS) Frasier's syndrome (FS)	AD	Male pseudohermaphroditism with progressive nephropathy and ESKD; development of FSGS by 3 years (DDS) or later (FS)	197, 198
CD2AP	*CD2AP*	Sporadic FSGS	N/A	FSGS in African-American patients	111
Synaptopodin	*SYNPO*	Sporadic FSGS	N/A	FSGS in Chinese patients	114
Myosin 1E	*MYO1E*	Childhood FSGS	N/A	Progressive, steroid-resistant FSGS	117, 118
Apolipoprotein L-1	*APOL1*	Sporadic FSGS	N/A	Progressive proteinuria, FSGS, and ESKD in African-American patients	127
Glypican 5	*GPC5*	Acquired nephrotic syndrome	N/A	Progressive proteinuria and ESKD	128