Review

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Podocytes: recent biomolecular developments

Abstract: Podocytes are postmitotic renal glomerular cells with multiple ramifications that extend from the cell body. Processes departing from a podocyte interdigitate with corresponding projections from neighboring cells and form an intricate web that enwraps the glomerular capillary completely. Podocyte processes are interconnected by the slit diaphragm, an adhesion junction mostly formed by Ig-like molecules, cadherins/protocadherins, ephrin/eph, and neurexin molecules organized in an assembly that resembles synaptic junctions. Podocyte failure is primarily or secondarily implicated in all forms of proteinuric glomerular diseases, as confirmed by the morphological changes of their elaborate cell architecture detectable by electron microscopy. Importantly, mutations of podocyte proteins are responsible for the most severe forms of congenital nephrotic syndrome. In the last 15 years, progressive technological advances have aided the study of podocyte biology and pathology, confirming the relevance of podocyte molecules and signaling pathways for the function of the glomerular filter. This review will examine the most important and newest discoveries in the field, which is rapidly evolving, hopefully leading to a detailed knowledge of this fascinating cell and to the development of specific therapeutic options for proteinuric diseases.

Keywords: actin cytoskeleton; foot processes; nephrin; podocyte; proteinuria; slit diaphragm.

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Introduction

Proteinuria, i.e., the presence of proteins in the urine, is a common symptom of kidney diseases and is caused by leakage of proteins from the glomerulus. When the protein level is >3 g/day, the proteinuria is defined as nephrotic. Histologically, nephrotic proteinuria in primary glomerular diseases can present with multiple features, from minimal changes to mesangial expansion; however, the most common picture is that of focal segmental glomerulosclerosis (FSGS), i.e., the presence of a segmental condensation of the glomerular tuft.

The glomerular filtration barrier is composed of a convoluted capillary sustained by the mesangium. The capillary possesses a fenestrated endothelium that lies on the glomerular basement membrane and is externally covered by specialized cells called podocytes. Podocytes are extremely ramified cells; the cell body gives rise to multiple primary, secondary and tertiary (foot) processes that intertwine among themselves and those departing from neighboring cells (Figure 1). By this organization, podocyte ramifications form a close net that completely enwraps the glomerular capillary, with the primary function of avoiding loss of nutrients and permitting the elimination of toxins produced by the body daily.

Work from the last 15 years has progressively emphasized the role of podocytes in the maintenance of a healthy glomerulus, owing to the discovery that mutations of single podocyte proteins are sufficient to cause the most severe forms of proteinuria (1). The critical role of these proteins has been confirmed by the appearance of proteinuria and glomerular damage in mice carrying podocyte-specific deletion of these molecules, and cell-based approaches have further delineated their functional aspects.

While a full understanding of the podocyte remains to be achieved, research is demonstrating that podocytes

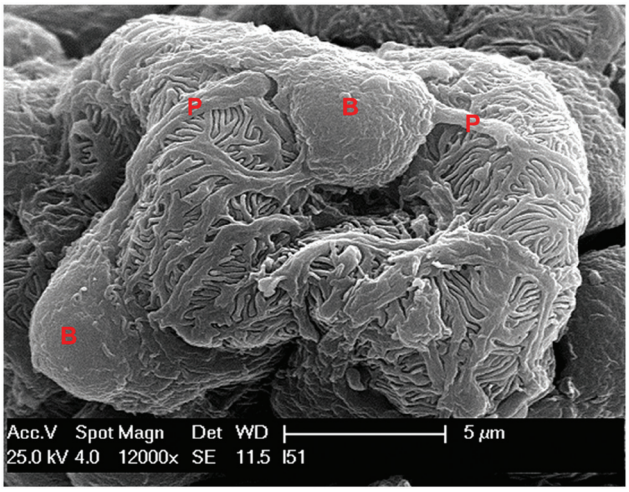

Figure 1 Podocyte structure.
Scanning electron micrograph illustrating the complex ramifications of podocytes completely covering the external side of the glomerular capillary. From the cell body (B) depart primary processes (P) further dividing into tiny ramifications that intertwine with processes coming from neighboring cells.

govern glomerular intercellular signaling and have a key function in renal biology; thus, the term 'podocytopathy' has been coined to better specify glomerular diseases with prominent podocyte involvement (2).

Technological advances

The development of novel technologies has been rapidly advancing the field of podocyte biology, overcoming several difficulties related to the convoluted structure of the glomerulus and its profound location in the body. Among these techniques, confocal microscopy and multiphoton microscopy coupled to the use of transgenic animals specifically expressing fluorescent markers in the podocytes are not only generating high-quality images of podocyte details but are also providing novel information on podocyte behavior in healthy and disease states. For instance, confocal microscopy applied to preparations of freshly isolated glomeruli taken from mice with green fluorescent protein (GFP)-expressing podocytes allowed Höhne et al. (3) to detect and analyze periodic contractions of the glomerular structure.

A stochastic multicolor Cre-reporter mouse, randomly encoding four different fluorescent proteins in podocytes, was generated and analyzed by Tao et al. (4). The method enabled to show the structure of healthy podocytes and compare them with those altered by Adriamycin injection, a largely adopted mouse model of human FSGS. In the disease model, the authors confirmed a generalized alteration of foot process morphology, but observed a high degree of heterogeneity between podocytes, with only some of them of enlarged size while others maintained a normal dimension. This heterogeneity may well be the basis for explaining the focal and segmental nature of the lesion that characterizes the disease in humans.

The application of multiphoton microscopy is by far the best method to obtain *in vivo* images of the kidney glomerulus in living animals, with exceptional spatial and temporal resolution (5). By using a mouse expressing a fluorescent calcium sensor exclusively in podocytes, Burford et al. (6) were recently able to show intracellular calcium ($[Ca^{2+}]_i$) changes specifically in these cells. The authors compared steady-state conditions with stimulation by angiotensin 2 and models of focal podocyte damage. They showed that podocyte injury induced in a few podocytes triggered a robust and sustained elevation of podocyte $[Ca^{2+}]_i$ around the injury site and promoted cell-to-cell propagating podocyte $[Ca^{2+}]_i$ waves along the capillary loops, which resulted in contraction of the glomerular tuft and increased albumin permeability.

The zebrafish (*Danio rerio*) pronephros is more and more used by the nephrology community because it constitutes a simple and accessible system to study *in vivo* the numerous aspects of renal cell biology. The major advantages are the transparency of zebrafish larvae; the rapid maturation of the pronephros; the similarity of the glomerular structure to the mammalian glomerulus (7); and the conserved expression of molecules such as nephrin, podocin, and Wilms tumor 1 (wt1), which are relevant to podocyte health in mammals (8, 9).

As an example, translucent zebrafish larvae (casper) expressing enhanced GFP (eGFP) specifically in podocytes (wt1a:eGFP larvae) were used by Endlich et al. (10) and observed during a period of up to 23 h by intravital two-photon microscopy. The authors were able to show that podocytes do not seem to migrate or modify the branching pattern of their major processes during the observation period, suggesting that movement is not a common feature of mature, healthy podocytes.

Our group recently developed a model of zebrafish podocyte damage (11) by the addition of low doses (10–20 μM) of Adriamycin to 9 hpf (hours postfertilization) old embryos for 48 h. This was enough to cause profound podocyte changes with loss of glomerular permeability, without inducing systemic or cardiac damage. Being the selective podocyte changes obtained in the embryo, this simple model can be instrumental for developmental studies and for drug-screening purposes.

An additional, as yet underdeveloped, novel technique is the derivation of podocytes from induced pluripotent stem cells (iPS) (12). The technology holds interesting and multiple research and therapeutic implications; iPS can be obtained from patients' accessible cells, such as fibroblasts or cells from the urinary sediment, and studied to elucidate disease mechanisms. The therapeutic potential of iPS, as well as of stem/precursor cells, has enormously increased with the first successful repopulations of organ scaffolds, including the kidney (13).

The contribution of high-throughput techniques, such as expression microarrays and proteomics, has been essential in discovering novel molecules expressed by developing and mature podocytes (14), and to compare healthy and diseased cells to identify the molecular pathways activated during disease processes. Nonetheless, major advances in podocyte biology have been driven thus far by genetic discoveries. In this field, technology is rapidly boosting the detection of molecules that are mutated in glomerular diseases, and the use of DNA microarrays and next-generation sequencing are revolutionizing the diagnosis of podocytopathies and are revealing the complex podocyte molecular network critical for cell development and behavior in healthy and disease states.

Essential role of nephrin

The first podocyte molecule uncovered by genetic analysis was nephrin, encoded by the gene *NPHS1* and mutated in the most severe form of proteinuric disease, i.e., congenital nephrotic syndrome of the Finnish type (15). Nephrin is a transmembrane protein of the immunoglobulin superfamily, formed by an N-terminal signal peptide, an extracellular domain containing eight Ig-like modules and one fibronectin type III-like module, a single transmembrane domain, and an intracellular C-terminal domain.

Nephrin expression in podocytes is regulated by several transcription factors (16), including WT1, which is a demonstrated key factor in renal development and in the adult glomerulus (17). Starting from the capillary stage of glomerular maturation, WT1 expression becomes restricted to podocytes and activates the nephrin gene by binding to a conserved region of the human nephrin promoter (18). In the mouse, the WT1 binding region is approximately 600 bp more upstream than the homologous site in humans (19).

Nephrin synthesis is further regulated by epigenetic mechanisms. Direct regulation by DNA methylation was uncovered by Ristola et al. (20), who identified three CpG islands within the intergenic region between the nephrin and *Neph3* genes and their coding regions. More recently, the transcription factor KLF4 was described as a regulator of nephrin DNA methylation (21); overexpression of KLF4 was able to demethylate nephrin DNA and induce nephrin transcription.

Furthermore, indirect posttranscriptional regulation, through the suppression of transcription of WT1, can be ascribed to miRNA193a, as recently described by Gebeshuber and colleagues (22). The authors observed overexpression of miR-193a in patients with FSGS and demonstrated that WT1 is the main target of this microRNA (miRNA). miRNA binding was found to reduce WT1 translation, with important consequences on the expression of nephrin that result in podocyte damage.

An additional level of nephrin regulation is achieved by SUMOylation. SUMO (small ubiquitin-like modifier) is an ubiquitin-like protein. SUMO modification of the lysine residues of target proteins blocks ubiquitination, ensuring protein stabilization (23). SUMO seems to target lysines 1114 and 1224 of the intracellular domain of murine nephrin and lysine 1100 of human nephrin, contributing to the fine tuning of nephrin turnover (24).

Nephrin appears to be part of a raft domain of the slit diaphragm, where it colocalizes with other proteins, including podocin and the calcium channel TRPC6 (transient receptor potential calcium channel 6) (25, 26). In this raft domain, nephrin acts as a signaling platform, sending messages from outside the cell to the actin cytoskeleton (27). Signals are conveyed through tyrosine phosphorylation of the intracellular domain of nephrin, which is mainly due to the activity of the Src family kinase Fyn (28).

Phosphorylation is important for raft-mediated nephrin internalization (29) and is an event needed for podocyte foot process development and maintenance, as demonstrated by the finding that phosphorylated nephrin recruits adaptor proteins such as Nck1/2, Grb2, and Crk1/2, resulting in the assembly of protein complexes that regulate actin polymerization (30). Additionally, Jin and coworkers (31) recently discovered that nephrin phosphorylation can be triggered by the binding of sFlt1, a soluble form of the VEGF receptor fms-related tyrosine kinase 1 (Flt1) produced by podocytes and acting in a paracrine fashion to regulate podocyte intracellular signaling.

Either increased or decreased nephrin phosphorylation has been alternatively found in rodent models of podocyte damage and in human glomerular disease (32–34). *In vitro*, clustering of nephrin and its tyrosine phosphorylation were shown to induce lamellipodia formation, contributing to a motile podocyte phenotype that has been linked to pathological states (35). Collectively,

these data confirm the importance of a tight balance of nephrin phosphorylation, which constitutes a powerful example of the highly regulated equilibrium needed by podocytes to preserve their function.

The extracellular domain of nephrin is suspected to be highly glycosylated; it contains binding sites for heparan sulfate and free cysteines that serve to form disulfide bonds with adjacent molecules. *Cis* and *trans* homophilic and heterophilic interactions of nephrin with itself and with Neph family proteins (Neph1, Neph2, and Neph3) are required to provide stability to the slit diaphragm and maintain the filtering function of the glomerular barrier (36–38).

Among the proteins that constitute the slit diaphragm, the prominent role of nephrin is confirmed by the lethal phenotype of nephrin-null mice and by the constant reduction in nephrin expression that occurs in both experimental and human glomerular diseases. Nephrin appears to be altered or downregulated at the very first stages of podocyte damage, preceding morphological alterations detectable by electron microscopy and largely preceding the development of proteinuria (39).

Nephrin is an expression-restricted protein and, besides glomerular podocytes, is expressed by a few other mammalian cell types, such as neuronal cells, lymphocytes, testis cells, pancreatic β cells, and developing epicardium and coronary vessels (40–44). The expression of nephrin in neuronal cells is of particular interest because the nephrin orthologues in *Caenorhabditis elegans* (Syg-2) and *Drosophila melanogaster* (Hibris) are crucial players in synapse targeting and positioning (45, 46), suggesting that, evolutionarily speaking, the original function of nephrin is that of a synaptic adhesion molecule.

Since its discovery, the neuronal expression of nephrin has been repetitively acknowledged (15, 40, 47). During development, nephrin mRNA was observed in the hindbrain and spinal cord. From embryonic day 13 (E13) to E17, nephrin mRNA was detected in the neuroepithelium of the cerebellar primordium at the roof of the fourth ventricle. In newborn mice, β-galactosidase expression driven by the nephrin promoter (40) was observed in the cerebellum, along the midline of the mesencephalon, and in some glomeruli of the main olfactory bulb; at postnatal day 16, it was visible in some of the glomeruli of the main olfactory bulb and in the dentate gyrus molecular layer of the hippocampus.

In the adult rodent central nervous system (48), the expression of endogenous nephrin was diffusely identified in the motor cortex, whereas the somatosensory cortex appeared completely negative. Sparse positive cells were present in the corpus callosum, and a diffuse expression was found in the choroid plexus. Stronger nephrin staining was detected in the pons, medulla oblongata, and olfactory bulb. Expression was also observed in the dorsal striatum (caudate nucleus and putamen) and thalamus. Within the hippocampus, nephrin was expressed by some pyramidal neurons of the CA3 region. A mild scattered immunostaining was found in the CA1 region, whereas the 'ilo' was completely negative. In the cerebellum, nephrin was expressed by Purkinje cells and by granule cells of the nuclear layer.

The presence of nephrin in basal ganglia and motor cortex, and the complete negativity of the sensory cortex, suggest the involvement of nephrin in distinct brain networks related to movement. The association of nephrin with movement activities is further confirmed by its presence in the Purkinjie cells of the cerebellum (48), and helps explain the ataxic symptoms of nephrin-deficient mice, when their survival is prolonged by reexpressing nephrin only in the kidney (49).

Neuronal-like signaling

The presence of nephrin in the central nervous system is one of the multiple molecular links between podocytes and neuronal cells. Interestingly, a distinctly neuronal character has been repetitively detected in mRNA and protein expression studies of freshly isolated podocytes, during both development and adulthood (14, 50, 51).

Although a number of renal progenitors possess neuronal features (52) or can differentiate into cells expressing neuronal markers (53–55), the kidney and the nervous system do not derive from the same embryonic leaflets; the kidney develops from the mesoderm (56), whereas the nervous system has an ectodermal origin (57). Nonetheless, during developmental phases, factors involved in neuronal pathfinding, such as GDNF (58), slit and ROBO (59), plexins and semaphorins (60), have been shown to guide renal and podocyte maturation. Furthermore, axon guidance molecules such as ROBO and neuritin were found highly enriched in a glomerular gene expression dataset (61), supporting a regulatory function during adulthood.

Podocytes and neuronal cells are both postmitotic, polarized, and highly ramified. They display the same cytoskeletal organization (62), with actin and some expression-restricted actin-binding molecules, such as synaptopodin (63), densin (64), and drebrin (65), respectively localized in podocyte foot processes and neuronal dendritic spines, where actin dynamics are essential for

maintaining and regulating shape and function (66, 67). In addition, the very same protein network – essentially based on dynamin, synaptojanin, and endophilin – that operates at the interface between endocytosis and actin at neuronal synapses, appears critical in regulating the process of endocytosis in podocytes and seems relevant to both the formation and maintenance of the glomerular filtration barrier (68).

The most relevant example of analogies between podocytes and neurons are the highly specialized cell-cell contacts, the slit diaphragm of podocytes, and the synaptic cleft of neuronal cells. These are narrow extracellular spaces characterized by electron-dense material that tightly links opposing membranes, and is composed by extracellular matrix components and the extracellular domains of adhesion molecules (66).

Notably, adhesion molecules at the slit diaphragm and the synaptic cleft belong to the same families, such as cadherins/protocadherins (69, 70), Ig-like proteins (71, 72), and neurexin (73). Podocalyxin, which was first described as a protein specifically expressed at the podocyte surface and is indispensable for foot process formation (74), was then identified as an adhesion molecule involved in neurite growth, branching, and axonal fasciculation (75). Furthermore, podocytes have been shown to express ephrin-eph family members. Among them, Efnb1 localizes at the slit diaphragm of mature podocytes, and its expression has been found decreased in a rat model of glomerulonephritis (76).

Loss of Fat1, which encodes a giant protocadherin, leads to foot-process effacement, massive proteinuria, and early perinatal lethality (77). Fat1 indeed seems to be important for planar cell polarity and actin-dependent cell motility (78), making this molecule relevant in setting and maintaining the structural organization of the slit diaphragm. As for their function, apart from promoting the stability of synapses, many data support the role of synaptic adhesion molecules in target recognition, to help in choosing the right partners from a network of processes (79). In this respect, the highly conserved Ig-like molecules nephrin and Neph1 are crucial for both the assembly of functional neuronal circuits (72) and the formation of the podocyte slit diaphragm (80).

Podocytes are exposed to a wide variety of chemical and mechanical stimuli, both from the bloodstream and the urinary space. To constantly maintain proper glomerular filtration, they need to communicate among themselves and with the other glomerular cells to rapidly identify and properly discriminate between physiological and pathological changes. To this purpose, they appear to use a rapid, highly informative, neuron-like system of communication. Indeed, podocytes express a wide variety of neurotransmitter receptors and contain synaptic-like vesicles that undergo cycles of spontaneous and highly regulated endocytosis/exocytosis, with release of neurotransmitters, including glutamate (81).

Imbalances of glutamate receptor activity, either the blockade or an excessive activation, are harmful to neuronal cells (82, 83). In podocytes, sustained activation of the NMDAR results in oxidative stress leading to apoptotic cell death (84), and NMDAR antagonists cause profound remodeling of the actin-myosin podocyte cytoskeleton and disappearance of nephrin from podocyte cell processes (85). Similarly, mice lacking the metabotropic glutamate receptor 1 (Grm1) display proteinuria and podocyte changes, with major reduction of nephrin expression and actin remodeling (86). Concordantly, the metabotropic glutamate group 1 receptor agonist dihydroxyphenylglycine (DHPG) was able to attenuate proteinuria in rodent models of podocyte damage (87).

Interestingly, the glutamate receptors NMDAR and Grm1 were shown to coimmunoprecipitate with nephrin, the kinase Fyn, and the scaffolding molecule PSD95 in both podocytes and neuronal cells (48). These findings point to a common function for nephrin that seems to behave as a synaptic adhesion molecule in both cell types, by recruiting and positioning glutamate receptors, and linking them to intracellular signaling pathways that ultimately act on the actin cytoskeleton (Figure 2).

Podocyte signaling pathways ultimately converge on the actin cytoskeleton

All cells contain actin, a globular protein that polymerizes into filaments with different types of organization: branched and cross-linked networks, parallel bundles, and antiparallel contractile structures, which are differentially located in various subcellular compartments. The final assembly of these structures is responsible for the cell shape, which constitutes the basis for specific cell functions.

The actin cytoskeleton is not immobile; on the contrary, it is highly dynamic owing to the constant assembly and disassembly of actin monomers as a consequence of the information flow received through intracellular signaling pathways (88). These processes are relevant to the behavior of every cell, but become especially important in cells with complex morphology, i.e., ramified cells. Cells with ramifications, such as neuronal cells, osteocytes,

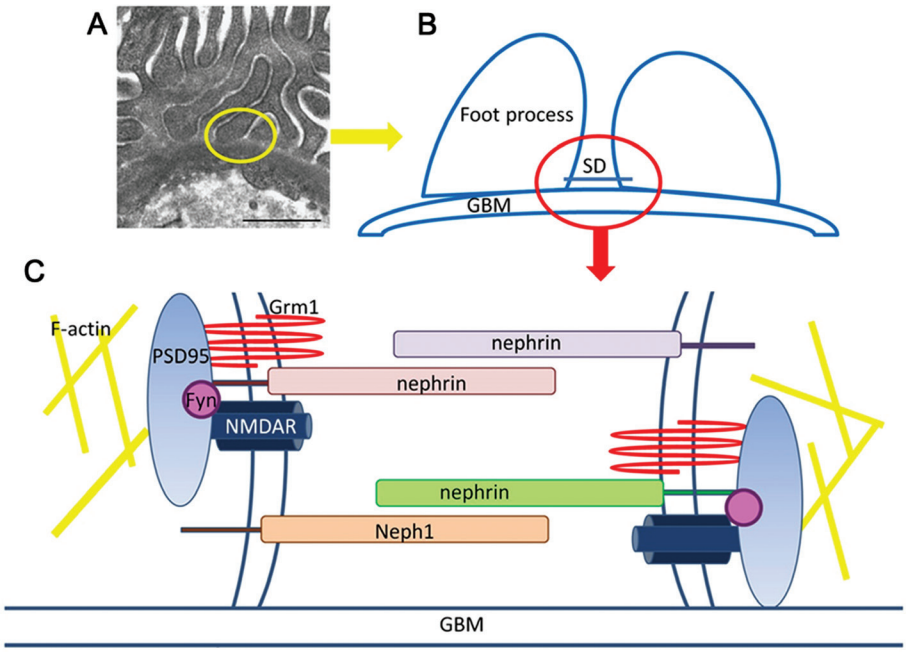

Figure 2 Schematic overview of the slit diaphragm.
(A) Transmission electron micrograph of podocyte foot processes. Scale bar, 1 μm. (B) Schematic illustration of podocyte processes, lying on the glomerular basement membrane (GBM) and laterally connected by the slit diaphragm (SD). (C) According to our data (48), nephrin behaves in podocytes as a synaptic adhesion molecule that clusters glutamate receptors and connect them through scaffolding molecules, such as PSD95, to the actin cytoskeleton of the foot processes.

and podocytes, share a common cytoskeletal organization, with actin mainly concentrated in the arborized cell processes. Furthermore, ramifications are also the location of specialized intercellular junctions, i.e., synaptic junctions in neuronal cells, gap junctions in osteocytes, and slit diaphragms in podocytes, which are also signaling centers that transfer intercellular information and convey messages from outside the cell.

Disease states are characterized by morphological changes of these junctional contacts, as well as cytoskeletal changes of the ramified processes, confirming that the preservation of the arborized cytoskeletal organization and specific intercellular contacts means preservation of the function of these cell types. In neurological diseases, such as Alzheimer's disease, the number and shape of synapses are profoundly modified (89). In bone diseases, such as osteoarthritis, osteocyte morphology is altered, with fewer and disorganized dendrites (90). Similarly, in proteinuric diseases, podocytes lose the periodic assembly of their processes and the slit diaphragms disappear or are apically relocated in a phenomenon termed foot process effacement (91). Therefore, it is not surprising that mutations of actin-binding and actin-related molecules are causative of nephrotic syndrome in humans.

One of the first actin-related molecules to be uncovered by genetic studies was α-actinin4, an actin-bundling protein required for normal podocyte adhesion (92). α-Actinin4 is essential for maintaining cell spreading, motility, and contractility. Mutations reported in patients with FSGS involve the actin-binding domain of the protein, resulting in increased binding affinity for actin, which disrupts the equilibrium of actin dynamics (93). Furthermore, the molecule can shuttle between the nucleus and the cytoplasm, suggesting a potential involvement in the regulation of transcription (94).

More recently, mutations of *ARHGDIA* and anillin were found in families of subjects with FSGS. *ARHGDIA* is the gene encoding the Rho GDP dissociation inhibitor α, a regulator of the Rho family of small GTPases known to control the dynamics of actin remodeling. ARHGDIA and the Rho small GTPases RhoA, Rac1, and Cdc42 form an interactive complex that is disrupted by mutations, with the final result of increasing Rac1 and Cdc42 activity (95). Anillin, an actin-binding molecule important for cytokinesis and cell growth, was shown to interact with Rho GTPase, F-actin, and myosin II, all of which regulate podocyte structure and function. When anillin is knocked down, active Rho (Rho-GTP), F-actin, and myosin II are consequently altered at the intercellular junctions (96).

It is presently believed that the stability of foot processes is represented by a stationary phenotype, whereas their instability corresponds to a motile phenotype. Confirmatory evidences come from studies in which proteinuria is present in animals null for *Cdc42* or bearing a dominant-negative *Rho-a* mutant (97, 98), or a constitutively active *Rac1* (99), all of which lead toward motile podocytes. However, imbalances in both senses are harmful, as a decreased podocyte migratory ability is the consequence of mutations of the non-muscle class I myosin *MYO1E* causative of FSGS in humans (100).

Members of the formin family of proteins have essential roles in coordinating both actin and microtubule assembly and dynamics (101). Among the formins, inverted formin 2 (*INF2*) has been found mutated in autosomal dominant forms of FSGS (102) and in FSGS occurring in Charcot-Marie-Tooth disease (103). Mutations associated with both syndromic and non-syndromic forms are clustered in exons encoding the diaphanous inhibitory domain (DID) of *INF2*. Most syndromic mutations are localized between two putative DID-binding pockets, affecting DID function more severely than mutations related to the non-syndromic disease. The DID mediates the autoinhibition of INF2 through its interaction with the C-terminal diaphanous autoregulatory domain, and allows INF2 to accelerate actin polymerization/depolymerization and to regulate protein targeting to the plasma membrane by forming complexes with Rho-GTPases, CDC42, and myelin and lymphocyte (MAL) protein, or MAL2 in podocytes and Schwann cells (104, 105).

Signaling to the cytoskeleton, as well as most intracellular signaling, is conveyed by calcium. This has been known for years, although interest in calcium signaling in podocytes increased after gain-of-function mutations of *TRPC6* were found in patients with familial FSGS (106, 107) and increased glomerular expression of TRPC6 was observed in renal biopsies from patients with acquired forms of proteinuric diseases (108), sustaining the hypothesis that increased activity of the channel, by increasing intracellular calcium, damaged podocytes and caused proteinuria. Novel findings are now emerging showing that mice with podocyte-specific overexpression of TRPC6 seem less susceptible to nephrotoxic serum nephritis, and the opposite occurs in mice null for the channel (109), therefore confirming the need for a tight balanced control in channel regulation.

Podocytes possess at least another channel of the same family, TRPC5, which is highly expressed in the brain, where its loss is responsible for deficits in gait and motor coordination (110). In podocytes, TRPC5 seems to activate Rac1 and induce a pathologically motile phenotype (111), and additional work has found that TRPC5 is a prominent mediator of acute events leading to proteinuria (112).

Expert opinion and outlook

Development of targeted therapy

Increased knowledge of podocyte biology and of mechanisms leading to podocyte dysfunction has the potential to lead to better drugs, directed against relevant molecular targets. Importantly, in the last years, several evidences are supporting a direct action of immunosuppressive drugs on podocytes.

Podocytes have been shown to express glucocorticoid receptors; corticosteroids, which are widely used in glomerular diseases, are able to directly induce transcriptional and posttranscriptional events in murine podocytes (113). Dexamethasone has been shown to increase nephrin phosphorylation, which can be blocked by a glucocorticoid receptor antagonist but not by a mineralocorticoid receptor antagonist (114). Similarly, cyclosporine A seems to directly block calcineurin in podocytes, resulting in protection of synaptopodin and dynamin from the enzyme cathepsin-L (115), with the effect of stabilizing the actin cytoskeleton. More recently, a molecular target for rituximab (anti-CD20) has been found in podocytes, supporting a possible direct activity of the antibody. Binding of rituximab to sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) causes acid sphingomyelinase (ASMase) translocation from the intracellular compartment to the external leaflet of the plasma membrane. The change induces ASMase activation and increase in ceramide content in membrane raft microdomains, with a stabilizing effect on the cell (116).

Importantly, abatacept has been shown to reduce proteinuria in subjects with recurrent or primary FSGS by acting on the costimulatory molecule B7-1, which is *de novo* expressed on the podocyte surface during disease (117). B7-1 blockade has profound effects on integrin β1 activation, with amelioration of podocyte adhesion and function. In addition, integrin β1 activation can result from activated Rap1, a small G protein with 53% amino acid identity to Ras (118). From recent results, it appears that podocytes need integrin β1 activation to remain attached to the basement membrane after injury. Reduced Rap1 activation, as it occurs during human and experimental podocyte damage, can be due to increased activity of Rap1GAP, a GTPase-activating protein that accelerates the hydrolysis of bound GTP to GDP, blocking the activity of small G proteins (119).

Another emerging possibility of directly addressing the podocyte with therapy is by acting on the Notch pathway, either by blocking Notch1, as recently reviewed by Kato and Susztak (120), or by activating Notch2, as recently described by Tanaka and coworkers (121). Several compounds have been produced that are influencing the Notch pathways, and human trials are already being conducted in the oncology field (https://clinicaltrials.gov). However, these interventions have to be considered with caution in kidney diseases, because of potentially concomitant but different expression and pathway activation in glomeruli as compared with the tubulointerstitial compartment (122).

Nonetheless, these studies support the notion that podocytes can be directly targeted by drugs, suggesting that future therapies will be personalized, on the basis of molecular findings detected in the single subject.

Highlights

- Podocytes are highly ramified cells mainly responsible for glomerular filtration.
- Among the molecules expressed specifically by podocytes, nephrin has a prominent role in establishing and maintaining podocyte function.
- By using signaling modules similar to those of neuronal cells, podocytes direct intercellular communication in the glomerulus.
- Intracellular signals ultimately converge on the actin cytoskeleton. Actin dynamics regulate cell shape and function.
- Better knowledge of podocyte pathophysiology is leading to the development of novel drugs directly targeting molecular pathways.

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