Podocyte Biology and the Emerging Understanding of Podocyte Diseases

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Key Words

Focal segmental glomerulosclerosis · Minimal-change disease · Slit diaphragm

Abstract

The understanding of the unique molecular apparatus of the podocyte has increased dramatically in recent years. This new knowledge has improved the diagnosis and classification of the diseases that have been termed podocytopathies. Podocyte injury frequently leads to reorganization of the slit diaphragm and reorganization of the foot process structure. Four major causes of foot process effacement can be identified, with some due to genetic mutations and others due to acquired conditions: (1) impaired formation of the slit diaphragm complex; (2) abnormalities of the glomerular basement membrane or the adhesion of podocytes to the glomerular basement membrane; (3) abnormalities of the actin cytoskeleton and associated proteins, and (4) alterations in the apical membrane domain of the podocyte. The major podocytopathies can also be organized into four categories, including those with a normal glomerular histology, diffuse mesangial sclerosis, focal segmental glomerulosclerosis, and collapsing glomerulopathy.

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Introduction

Podocytes are highly differentiated cells with a complex cellular morphology. They are located inside the kidney glomerulus, a twisted globe of capillaries through which the blood is filtered hydrostatically through a high-volume/high-discrimination filter [1–3]. Podocyte foot processes are anchored to the glomerular basement membrane (GBM) via $\alpha_3\beta_1$ -integrin [4] and α - and β -dystroglycans [5, 6]. Neighboring foot processes are connected by a specialized cell-cell junction, the glomerular slit diaphragm, which represents the main size-selective filter barrier in the kidney [1–3]. The slit diaphragm is thought to be a modified adherens junction [7]. Our understanding of its molecular components continues to grow; the slit diaphragm is known to include P-cadherin [7], nephrin [8–10], the giant protocadherin mFATq [11, 12], podocin [13], and Neph1 [14–16].

Podocytes are injured in many forms of human and experimental glomerular disease, including minimal-change disease (MCD), focal segmental glomerulosclerosis (FSGS), collapsing glomerulopathy, diabetic nephropathy, membranous glomerulopathy, crescentic glomerulonephritis, and lupus nephritis [1, 3, 17]. Independent of the underlying disease, the early events are characterized by molecular alterations of the slit diaphragm without visible morphological changes or, more obviously, by a reorganization of the foot process structure with fusion of filtration slits and apical displacement of the slit diaphragm

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Fig. 1. Simplified schematic showing the molecular anatomy of the podocyte foot processes [modified from ref. 83]. The four boxes 1–4 in the right half define the four domains of the foot processes: SD complex (1), basal domain and GBM (2), actin cytoskeleton (3), and apical plasma domain with glycocalyx. Of note, interference with any of these four domains can cause foot process effacement and nephrotic syndrome.

[1, 18, 19]. Based on recent progress in the molecular pathology of podocytes, four causes of foot process effacement and proteinuria can be identified (fig. 1): (1) impaired formation of the slit diaphragm complex and its associated lipid rafts; (2) abnormalities in the GBM or the adhesive interaction between the podocyte and the GBM [5, 6, 20–25]; (3) abnormalities of the actin cytoskeleton [26, 27], and (4) alterations in the apical membrane domain of podocytes [28–31].

Slit Diaphragm in Normal and Diseased Glomeruli

The slit diaphragm is a complex of proteins located in the extracellular space, bridging adjacent foot processes and measuring 30–40 nm in length [1–3]. The recent discovery of several novel podocyte proteins and their mutation analysis, including mFAt1 [12], the nephrin homologue Nep1 [14], nephrin [32], CD2-associated protein (CD2AP) [33, 34], and podocin [35], have emphasized the critical role of the slit diaphragm in maintaining the normal function of the glomerular filtration barrier. However, the mechanisms regulating the structural changes that occur during foot process fusion are still largely unknown. A fuller understanding of the molecular basis of glomerular kidney disease requires elucidation of the relationship between slit diaphragm proteins and the maintenance of foot process structure. A simplified schematic of the foot processes and the molecular makeup of the slit diaphragm complex is shown in figure 1.

The identification of the nephrin gene *NPHS1* by positional cloning elucidated the genetic defect underlying the

congenital nephrotic syndrome of Finnish type. Nephrin is a large transmembrane molecule with immunoglobulin-like domains. The role of nephrin in the pathogenesis of foot process effacement has been also confirmed by injection of antinephrin antibody in animals which resulted in foot process effacement [36]. Similarly, inactivation of *NPHS1* in mice resulted in proteinuria and partial effacement of the foot processes [37, 38]. In a rat model of antibody-induced foot process effacement, it has been shown that nephrin oligomers associate within signaling microdomains of the plasma membrane, also known as lipid rafts, and that these localize to the slit diaphragm [39]. The in vivo injection of an antibody against a podocyte-specific ganglioside leads to morphological changes of the filtration slits resembling foot process effacement [40]. In this model of foot process effacement, nephrin dislocates to the apical pole of the narrowed filtration slits and undergoes tyrosine phosphorylation [39]. These data suggest that lipid rafts are important in the spatial organization of the slit diaphragm under both physiological and pathological conditions [39] and that tyrosine phosphorylation of slit diaphragm proteins may regulate their subcellular redistribution. Redistribution of nephrin on the luminal side of podocytes has also been described in human nephrotic syndrome [41].

NPHS2 is the genetic cause of autosomal recessive, steroid-resistant nephrotic syndrome in some patients [35, 42, 43] and may also cause sporadic FSGS [44]. The *NPHS2* gene product, podocin, is a new member of the stomatin family of hairpin-like integral membrane proteins with intracellular N and C termini. Stomatin is present as high-order oligomer in erythrocyte lipid rafts, where it may serve a scaffolding function [45]. Podocin

localizes to the podocyte foot process membrane, at the insertion site of the slit diaphragm [13]. Like nephrin, podocin oligomers form in lipid raft. Moreover, glutathione S-transferase pull down experiments revealed that podocin associates via its C terminus with CD2AP and nephrin. Co-immunoprecipitation of proteins from glomerular extracts demonstrates that podocin interacts with CD2AP and nephrin. Furthermore, *in vitro* studies revealed a direct interaction of podocin and CD2AP [13]. Hence, similar to the role of stomatin in erythrocytes, podocin may act as a scaffolding protein, serving in the structural organization of the slit diaphragm and the regulation of its filtration function [12, 20].

mFAT1 is a nonclassical giant protocadherin which was previously localized to the slit diaphragm [11]. Mice lacking mFAT1 exhibit perinatal lethality, most probably caused by loss of the renal glomerular slit junctions and fusion of podocyte foot processes [12]. These data confirm a necessary role for FAT1 in the development of the slit diaphragm, a modified adhesion junction of podocytes [12].

Although podocytes lack the junctional complexes typical of polarized epithelial cells, the podocyte slit diaphragms have been reported to share some proteins with tight junctions, such as zona occludens (ZO) 1. ZO-1 is located on foot process at the point of insertion of slit diaphragms [46]. ZO-1 interacts with the actin-based cytoskeleton and may also participate in signaling events through tyrosine phosphorylation [47]. ZO-1 may have an important role in regulating the function of the slit diaphragm [7]. In support of a functional role for ZO-1, spontaneously proteinuric Munich Wistar Frömter rats have normal podocyte foot processes and slit diaphragms, with the only apparent defect presenting as a redistribution of ZO-1 in the cytoplasm and on the cytoplasmic surface of the cell membrane [48].

Podocyte Adhesion to the GBM

The glomerular filtration barrier is composed of fenestrated endothelium, GBM, and the podocyte foot processes and slit diaphragms. An intact glomerular filtration barrier is a critical element of the normal kidney function, and failure of this filtration complex leads to proteinuria. Proteinuria is invariably associated with foot process effacement, flattening, and retraction. To maintain the highly ordered foot process architecture, the adhesion of the podocyte to the underlying GBM is tightly controlled by the expression of particular proteins.

$\alpha_3\beta_1$ -integrin binds to several components of the GBM such as collagen IV, fibronectin, laminin, and endactin/nidogen. Ligand binding induces clustering of integrins to form focal adhesions and recruitment of intracellular cytoskeletal proteins [49]. These findings suggest that the integrin-binding status in podocytes might influence the cytoskeletal organization and thereby contribute to determining the foot process shape [49]. Blockade of the β_1 -integrin-binding domain by specific antibodies leads to foot process effacement, proteinuria, and detachment of the podocytes from the GBM [4, 50–52]. In addition, podocytes can express β_4 -integrin which may affect the glomerular permeability in FSGS [53].

Dystroglycans are heterodimeric proteins composed of a transmembrane component, the beta subunit, and an extracellular component, the alpha subunit. Dystroglycans are connected to the actin-based cytoskeleton through dystrophin (in skeletal muscle) and urotrophin (in the podocyte). Both integrins and dystroglycans are coupled to the podocyte actin cytoskeleton. Interestingly, the dystroglycan expression is lost only in MCD [5, 6]. Moreover, in MCD, the dystroglycan expression is negatively correlated with disease activity: when proteinuria occurs, the dystroglycan expression is markedly reduced, and foot processes are effaced, but, once the foot processes are restored, dystroglycan expression returns to normal. This phenomenon appears to be specific for MCD. In FSGS, the expression of dystroglycans remains unchanged, suggesting that other mechanisms must be responsible for podocyte detachment.

Podocyte detachment from the GBM, whether from apoptosis, necrosis, or loss of adhesive interaction, may play a central role in the pathogenesis of FSGS, particularly in hyperfiltration FSGS, as proposed by Kriz et al. [54]. Podocytes undergo morphological changes, including foot process effacement, reorganization of the cytoskeleton, pseudocyst formation within the podocyte cytoplasm, and detachment from the GBM, leaving the GBM denuded [54–56]. Podocytes lack proliferative capacity under normal circumstances and cannot repopulate denuded areas. Instead, parietal epithelial cells contact the denuded GBM to form a synechia and ultimately a scar.

Foot Process Cytoskeleton

Foot processes contain an elaborate and dynamic actin-based cytoskeleton. This structure maintains the normal architecture of the foot processes, including the proper positioning of transmembrane proteins and the slit dia-

phragm. The major molecular components of the cytoskeleton are actin, α -actinin [57, 58], and synaptopodin [59, 60]. In nephrotic syndromes, the actin-based cytoskeleton is reorganized and is condensed against the sole of the podocyte foot process. Of note, the reorganization of the actin cytoskeleton is preceded by upregulation of α -actinin [26]. α -Actinin-4 is an actin filament cross-linker, shown to be involved in certain forms of FSGS [27, 61]. Also, it has been shown that, among the spectrum of diseases with nephrotic syndrome and podocyte injury, the synaptopodin expression correlates with the severity of the disease and is well preserved in benign forms of nephrotic syndrome, such as MCD, and reduced in less benign forms such as FSGS [62–64]. Several recent studies reveal a direct interaction between podocin, nephrin, and CD2AP; moreover, CD2AP interacts with actin, indicating a close relationship between the cytoskeletal structure and the configuration of the slit diaphragm [65–67].

Negative Surface Charge on the Luminal Cell Membrane

The podocyte surface is divided into two parts by the slit diaphragm: the luminal side, facing the urinary space, and the abluminal side which corresponds to the sole of the foot processes and is located against the GBM. On the luminal side, podocytes are equipped with a well-developed glycocalyx, containing heavily sialylated glycoconjugates and sulfated molecules. This surface coat contributes to the negative charge of the filtration barrier and is mostly made of podocalyxin [68]. Podocalyxin is synthesized by glomerular endothelial cells and by podocytes and is inserted in the luminal side of the membrane during development, when foot processes and slit diaphragms are formed [69]. Podocalyxin is of critical importance for the formation and preservation of the characteristic cellular architecture of podocytes [28, 29, 70] and possibly for preventing parietal cell adherence to podocytes. Podocalyxin knockout mice have immature glomeruli with flattened podocytes, supporting the theory that podocalyxin contributes directly to the stability of foot processes [31]. Another important molecule on the luminal membrane is GLEPP-1, a transmembrane protein tyrosine phosphatase with possible receptor function [71]. Its precise function in podocytes is not yet clear, but it has been proposed that GLEPP-1 plays a role in regulating the glomerular pressure/filtration rate relationship through an effect on podocyte structure and function [72].

The expression of podocalyxin and GLEPP-1 has been studied in nephrotic syndrome. Whereas in MCD, the expression of these molecules is well preserved, in FSGS and collapsing glomerulopathy, the expression of GLEPP-1 and podocalyxin is reduced [62].

Podocyte Transcription Factors

The Wilms' tumor (WT) 1 expression is highly characteristic of podocytes, at any developmental stage. In the fetal kidney, WT1 is expressed in metanephric blastema, condensing mesenchyme, renal vesicles, and developing podocytes. In adult life, the WT1 expression is restricted to podocytes [73]. The *PAX2* gene encodes a transcription factor expressed early during development and is a WT1 target gene. The *PAX2* expression is downregulated when the WT1 expression increases in maturing glomeruli. Both WT1 and *PAX2* knockout mice lack kidneys, testifying to the critical importance of these transcription factors in metanephric development. Overexpression of *PAX2* generates multiple kidney abnormalities [74].

The WT1 expression is altered in certain congenital and acquired human diseases. In particular, the WT1 expression is lost in podocytes of both collapsed glomeruli and normal-appearing glomeruli in collapsing glomerulopathy, indicating that the podocyte phenotype is dysregulated [62]. The Denys-Drash syndrome is a rare disorder of urogenital development, characterized by the association of early-onset nephrotic syndrome, male pseudohermaphroditism, and nephroblastoma. Patients develop renal insufficiency by the age of 5 years. The glomerular lesions are characterized by diffuse mesangial sclerosis. Interestingly, in these patients, the WT1 expression is abnormal and is associated with increased expression of *PAX2*. These findings are also associated with podocyte hyperplasia and expression of proliferating cell nuclear antigen, indicating that the podocytes have reentered the cell cycle [75]. The podocyte proliferation is also characteristic of collapsing glomerulopathy [76, 77].

Pod1 is a basic-helix-loop-helix transcription factor that is highly expressed in the mesenchyme of developing organs that include heart, lung, gut, and podocytes [78]. Mice lacking *Pod1* fail to develop mature glomeruli, and the podocyte development appears to be arrested at the S-shaped body stage [79].

Mutations in the LIM homeodomain transcription factor *LMX1B* cause nail-patella syndrome, an autosomal dominant disease with skeletal abnormalities, nail hypoplasia, and nephropathy [24]. *LMX1B*^{-/-} podocytes have

reduced numbers of foot processes, are dysplastic, and lack typical slit diaphragms, indicating an arrest in development, but they express near-normal levels of nephrin, synaptopodin, ZO-1, α_3 -integrin, and GBM laminins [38, 80, 81]. Several *LMX1B*-binding sites in the putative regulatory regions of both the genes encoding for CD2AP and podocin have been identified. Moreover, in vitro experiments demonstrated that *LMX1B* binds to these sequences and can activate transcription through them in cotransfection assays. Thus, *LMX1B* regulates the expression of multiple podocyte genes critical for podocyte differentiation and function. These data indicate that reduced levels of specific podocyte proteins may contribute to nail-patella syndrome associated nephropathy [38, 80, 81].

Most recently it was shown that the mouse *kreisler* (*Krml1/MafB*) segmentation gene is required for differentiation of podocytes [82]. In contrast to *LMXB1* and *Pod1*, *kreisler* appears to be involved in the terminal steps of podocyte differentiation, including the formation of the normal elaborate foot process architecture [82]. Based on these findings, it will be very interesting to determine whether reduced *kreisler* expression leads to foot process effacement.

Podocyte Response to Injury

Podocyte injury, if not repaired, leads to severe progressive glomerular disease [1, 83]. Early podocyte manifestations of injury include vacuolization, pseudocyst formation, and detachment from the GBM, resulting in podocyte depletion. These events underlie the formation of synechiae via attachment of parietal epithelial cells of Bowman's capsule to denuded GBM areas [54, 84]. These changes are irreversible and ultimately lead to the development of glomerulosclerosis and end-stage renal failure [54, 85]. Studies in human diabetic nephropathy [86–88], the chronic puromycin model of glomerulosclerosis [89], and transforming growth factor beta 1 (TGF- β 1) transgenic mice [90] collectively provided convincing evidence for a correlation between the loss of podocytes and the progression of glomerular diseases. For a comprehensive review see the recent paper by Böttinger and Bitzer [91].

The TGF- β pathway controls an array of cellular responses to various forms of chronic glomerular injury, including extracellular matrix accumulation, proliferation, hypertrophy, and podocyte apoptosis [90, 92–96]. Increased expression of TGF- β isoforms and components of the intracellular signaling system has been described in

most animal models of glomerular disease and in progressive human disease, irrespective of the underlying etiology [97]. Together, these studies implicate the TGF- β pathway as a key molecular signaling cascade involved in mediating cellular pathomechanisms resulting in glomerulosclerosis and interstitial fibrosis [95]. As described below, primary and secondary forms of FSGS are characterized by depletion of podocytes and constitute a central manifestation of chronic progressive glomerular diseases [98]. Podocytes undergo apoptosis at early stages during the course of progressive glomerulosclerosis in TGF- β 1-transgenic mice [90]. Apoptosis is associated with progressive depletion of podocytes and precedes mesangial expansion. Smad7 protein expression was strongly induced specifically in damaged podocytes of transgenic mice and in cultured murine podocytes treated with TGF- β . TGF- β 1 and Smad7 each induced apoptosis in podocytes, and their coexpression had an additive effect [90]. Activation of p38 mitogen-activated protein kinase and caspase 3 was required for TGF- β -mediated apoptosis, but not for apoptosis induced by Smad7. Unlike TGF- β , Smad7 inhibited nuclear translocation and transcriptional activity of the cell survival factor nuclear factor kappa B [90]. These results suggest a novel functional role for Smad7 as amplifier of TGF- β -induced apoptosis in podocytes and a new pathomechanism for podocyte depletion in progressive glomerulosclerosis [90].

Classification of Podocytopathies

Podocytes are injured in many forms of human and experimental glomerular diseases, including MCD, FSGS, collapsing glomerulopathy, membranous nephropathy, and diabetic nephropathy. Podocyte damage plays a central role in the pathomechanism of nephrotic syndrome. It has been demonstrated that numerous distinct molecular events, occurring in podocyte cell body, primary processes, nucleus, foot processes, and, in particular, in the slit diaphragm, can lead to foot process effacement and proteinuria. In line with these new discoveries in podocyte biology, we propose a new classification of podocyte diseases (table 1).

First are podocyte diseases with a normal glomerular histology. In MCD, a defect in the expression of dystroglycans seems to be the underlying molecular defect. In this case, nephrotic syndrome and podocyte damage are reversible. A more severe form of nephrotic syndrome is the congenital nephrotic syndrome of the Finnish type which rapidly progresses to renal failure. Recurrent FSGS fol-

Table 1. Classification of podocyte diseases

Histology	Pathologic variant	Critical pathogenic factors
Normal	minimal-change disease congenital nephrotic syndrome	dystroglycan nephrin
Diffuse mesangial sclerosis	Denys-Drash syndrome Frasier syndrome	WT1 WT1
Focal segmental glomerulosclerosis	idiopathic genetic hyperfiltration	unknown podocin, α -actinin-4, β_1 -integrin, CD2AP, <i>LMX1B</i> mechanical stress
Collapsing glomerulopathy	idiopathic viral	unknown HIV-1, Parvovirus B19, SV40?

lowing renal transplantation presents initially with a normal renal histology, later evolving into glomerulosclerosis, and has been associated with a plasma protein factor. Second are the syndromes with diffuse mesangial sclerosis, including Denys-Drash and Frasier syndromes, where the defect is WT1 mutation [99]. The third group of podocyte diseases is represented by FSGS. The damage can be idiopathic, related to molecular defects at the level of the slit diaphragm [35, 42], transcriptional factors, or actin cytoskeleton, or secondary to mechanical stress such as

that driven by glomerular hyperfiltration [54–56]. The fourth category of podocyte diseases includes those characterized by collapse of the capillary loop. These include idiopathic collapsing glomerulopathy and HIV-1-associated collapsing glomerulopathy and are characterized by dedifferentiation, reflected by loss of maturity markers, dysregulation, reflected by loss of WT1 expression [62, 100], and de novo expression of other cell line characteristic markers, such as macrophage-specific proteins [44].

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