A Review of Podocyte Biology

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Keywords

Renal glomerulus · Podocytes · Actin · Nephrin · Adhesion · Signaling · Proteinuria · Nephrotic syndrome · Therapeutic targets

Abstract

Background: Podocyte biology is a developing science that promises to help improve understanding of the mechanistic nature of multiple diseases associated with proteinuria. Proteinuria in nephrotic syndrome has been linked to mechanistic dysfunctions in the renal glomerulus involving the function of podocyte epithelial cells, including podocyte foot process effacement. **Summary:** Developments in imaging technology are improving knowledge of the detailed structure of the human renal glomerulus and cortex. Podocyte foot processes attach themselves to the glomerular capillaries at the glomerular basement membrane (GBM) forming intercellular junctions that form slit diaphragm filtration barriers that help maintain normal renal function. Damage in this area has been implicated in glomerular disease. Injured podocytes undergo effacement whereby they lose their structure and spread out, leading to a reduction in filtration barrier function. Effacement is typically associated with the presence of proteinuria in focal segmental glomerulosclerosis, minimal change disease, and diabetes. It is thought to be due to a breakdown in the actin cytoskeleton

of the foot processes, complex contractile apparatuses that allow podocytes to dynamically reorganize according to changes in filtration requirements. The process of podocyte depletion correlates with the development of glomerular sclerosis and chronic kidney disease. Focal adhesion complexes that interact with the underlying GBM bind the podocytes within the glomerular structure and prevent their detachment. **Key Messages:** Knowledge of glomerular podocyte biology is helping to advance our understanding of the science and mechanics of the glomerular filtering process, opening the way to a variety of new potential applications for clinical targeting.

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Introduction

The pathogenesis of proteinuria in nephrotic syndrome has been linked to mechanistic pathways in the renal glomerulus that involve the function of the podocyte epithelial cells [1]. Patients with nephrotic syndrome demonstrate a distinct disease-phenotype relationship that involves foot process effacement or the spreading of podocyte foot processes; however, the details of the association remain unclear. Podocyte biology is a developing science that promises to help improve our understanding of the mechanistic nature of multiple diseases associated

Fig. 1. High-magnification helium ion microscopy imaging of glomerular structures [3]. **a** Detail of a glomerular podocyte showing a secondary projection and interdigitating foot processes. White arrows: Tubular projections with bulbous ends emerging from the podocyte membrane. Black arrows: Small irregularities of unknown nature on the external surface of the podocyte membrane. Scale bars, 120 nm. **b** Detail of 4 “filtration” regions (slit dia-

phragms) between 5 adjacent podocyte foot processes. Small arrows: Numerous cross-bridging filaments extend at regular intervals across the space between adjacent foot processes. Large arrows: In some regions, the delicate structures appear damaged, revealing another structure below, which may represent the glomerular basement membrane. Scale bars, 100 nm.

with proteinuria in nephrotic syndrome. Here, we provide an overview of podocyte biology, highlighting some recent developments and identifying areas for future study. It is the first of a series of articles summarizing presentations and discussions from a roundtable discussion focused on the management of proteinuria in nephrotic syndrome [2].

Glomerulus Structure

Within the nephron, the glomerular filtration barrier is responsible for the selective filtration of blood from the afferent arteriole through to the Bowman’s space. The filtration barrier includes 3 layers: glomerular epithelium, the basement membrane, and the slit diaphragms, which are formed by the foot processes of the podocytes. The slit diaphragm is the final barrier preventing passage of proteins into the urinary filtrate. The filtrate that passes into the Bowman’s space continues into the proximal tubule and loop of Henle for further processing.

Developments in imaging technology are allowing elucidation of glomerular features at a higher magnification than was previously possible. Helium ion microscopy, a novel imaging technology that uses a scanning beam of

He⁺ ions to produce high-quality subnanometer-resolution images of uncoated biological tissues, has enabled the imaging of the kidney to be explored in depth for the first time using rat tissue [3]. Using helium ion microscopy, fine details such as membrane texture and membranous nanoprojections on the glomerular podocytes have been visualized for the first time, while pores within the filtration slit diaphragm have been seen in much greater detail than with conventional scanning electron microscopy (Fig. 1) [3].

The branching processes of podocytes surround and envelop the glomerular capillaries within the Bowman capsules [3]. The podocyte foot processes form complex interdigitations with adjacent foot processes from neighboring podocytes. Numerous thread-like nanoprotrusions with bulbous ends originate from the major and minor processes and project into the urinary space [3]. These protrusions are thought to be an artifact of tissue processing but are also seen in diseases. It has been referred to as microvillous transformation. The surface of the podocyte plasma membrane contains depressions 20–30 nm in size that are reminiscent of intramembrane particles; their nature is currently unknown. The podocyte filtration barrier appears as a ladder-like structure at the interface between adjacent foot processes with “slit diaphragm” structures overlaying the basal lamina [3].

Fig. 2. Podocyte maturation and foot process assembly (theoretical model) [4]. **a** Podocytes begin as discs of columnar epithelial cells attached along their entire lateral membranes. **b** Podocytes lose their lateral cell attachments, except at their base, and begin to interdigitate along the basal aspect of the lateral membrane.

c Podocyte cell bodies become independent of each other, but remain attached through interdigitated foot processes. Adapted with permission of The Company of Biologists, from Quaggin and Kreidberg [4]; permission conveyed through Copyright Clearance Center, Inc.

Podocyte Development and Injury

Podocytes are atypical epithelial cells that transform into 3 separate structural and functional elements: a large cell body, major extending processes, and foot processes [4]. During normal podocyte development, the cuboidal epithelial cell sends out several large extensions that branch into major primary and secondary processes and, finally, into tertiary foot processes, taking on an octopus-like structure as they mature (Fig. 2) [4]. The foot processes are involved in a complex series of interdigitations with foot processes from other cells before they attach themselves to the glomerular capillaries [4]. This is a highly polarized mechanism in which the foot processes are able to detect and avoid interlinking with their own cellular protrusions in a manner that remains unclear.

During this period of transformation, the cell junction between the apical and basolateral surfaces appears to migrate downward and becomes the intercellular junction where the foot processes adhere to the glomerular basement membrane (GBM). This forms a cell-cell junction, called the slit diaphragm, which is an important structure that helps connect adjacent foot processes and forms a filtration barrier involved in the maintenance of normal renal function. Damage in this area has been implicated in the initiation and progression of glomerular disease [4]. Primitive evolutionary models of the slit diaphragm, including nephrocyte filtration barriers in the fly kidney and pericardium, and in the zebra fish, may help to shed more light on this process [5, 6]. Both models contain analogs of nephrin and other specialist constituent proteins of the slit diaphragm (all highly con-

served proteins); in mutant models lacking these proteins, the filtration barrier function is dramatically affected [5].

The process by which glomerular podocyte differentiation occurs remains unclear and is a subject for further research. While neurons are known to develop in a similar fashion, the number of isoforms of proteins involved in axonal or dendritic development limits extrapolation from mice models, while the lack of an *in vitro* model that displays interdigitating foot processes precludes the direct study of foot process assembly [4].

When podocytes become injured they undergo a process of effacement in which they lose their structure and become diffuse, spreading out and leading to a reduction in filtration barrier function. Effacement is thought to be due to a breakdown in the actin cytoskeleton of the foot processes, which is a complex contractile apparatus that allows podocytes to be dynamic in nature and reorganize themselves rapidly according to changes in filtration requirements [7, 8]. Effacement is typically associated with the presence of proteinuria (especially in diseases such as focal segmental glomerulosclerosis [FSGS], minimal change disease, and diabetes). However, not all cases of proteinuria show podocyte effacement, and the structure-function relationship is still not well understood. Contrary to prior belief, effacement is not passive but is a process highly based on signaling, suggesting that it may be possible to exploit for therapeutic targeting. Podocyte depletion can also occur and has been found to correlate with the development of glomerular sclerosis and chronic kidney disease [9].

Table 1. Examples of mutations associated with development of FSGS and nephrotic diseases in humans [48]

Gene/protein	Disease/phenotype	Age of onset
Podocytes		
<i>NPHS1</i> /nephrin	FP effacement/proteinuria	Birth
<i>NPHS2</i> /podocin	FP effacement/proteinuria	Late
<i>PLC epsilon 1</i>	DMS	Early
<i>TRPC6</i>	FSGS	Variable
<i>ACTN4</i> /alpha-actinin-4	FSGS	Late
<i>COQ2/coenzyme Q10</i>	FSGS	Late
<i>PDSS2</i> /prenyl diphosphate synthase subunit 2	FP effacement/proteinuria	Late
<i>INF2</i>	FSGS	Late
Glomerular basement membrane		
<i>Lamb2</i> /laminin β 2	DMS/FSGS (Pierson syndrome)	Perinatal lethality
Transcription factors		
<i>WT1</i>	Denys-Drash and Frasier syndrome	Early
<i>LMX1B</i> /LIM homeobox transcription factor	Nail patella syndrome	Early
<i>SMARCA1</i>	FSGS/steroid-resistant NS	Late
DMS, Diffuse mesangial sclerosis; FSGS, focal segmental glomerulosclerosis; NS, nephrotic syndrome.		

Gene Discovery in Hereditary Nephrotic Syndrome

Since the identification of the *NPHS1* gene for the first specialized nephrotic protein, nephrin, approximately 40 genes have been implicated in the development of FSGS and related nephrotic diseases [10, 11]. Most can be divided into 2 groups: those that are associated with actin dynamics and largely affect the structural integrity of the foot processes, and those that are related to adhesion to the GBM and may contribute to the loss of podocyte density as podocytes detach. There are also those such as *INF2* that appear to be more ubiquitous in nature, and their specific role in podocyte function remains unclear (Table 1).

Patients with mutations in these genes often develop nephrotic syndromes of genetic origin; however, not all of these patients present in childhood. There is a broad age spectrum through to late adulthood, including many patients with nephrin mutations whose symptoms manifest in their 20s and 30s [12]. Thus, it is possible that there is a multifactorial influence, which could include genetic susceptibility, environmental factors, and epigenetic changes, as well as other health triggers such as obesity, ischemia, heart failure, or sleep apnea [13]. This would also help to explain variations in response to steroids and immunosuppression. Diseases such as FSGS, which used to be thought of as a single disease, now are thought to be more individualized, with outcomes dependent not just on their histopathology but also on the underlying mechanisms of disease [9].

Podocytes: The Role of Actin

Actin is the key component of the foot processes of podocytes in comparison with the primary and secondary processes, which are microtubular-based. This difference may explain why only the foot processes are implicated in glomerular disease.

Actin proteins form the main cytoskeleton of the foot process, but are also involved in anchoring the podocyte to its environment [14]. Beneath the plasma membrane of the foot process, the cortical actin network binds with specialist proteins of the slit diaphragm (especially nephrin, podocin, and NEPH1) at unique tethering points. In addition, the focal adhesion complexes interact with the underlying GBM to bind the podocytes within the glomerular structure and prevent their detachment [15].

Wiggins [9] proposed a podocyte depletion hypothesis that states that, regardless of the form of initial insult to the glomerulus (immune, toxic, infectious, ischemic, etc.), the outcome depends on whether the number of normal working mature podocytes becomes depleted. An elegant human diphtheria toxin receptor transgene model was used to deplete podocytes in a dose-related manner through toxin-induced injury [16]. A critical percentage of podocyte depletion of >40% was found in a glomerulus, at which point the exposed GBM is thought to begin adhering to the Bowman capsule, starting the sclerotic process that progresses over time to end-stage kidney disease. Levels of damage below this threshold appear to be com-

pensated for by the remaining podocytes spreading out to cover the deficit, although glomerular scarring may still occur [16]. Further support of the hypothesis of podocyte hypertrophy following injury is the mammalian target of rapamycin-target 4E-BP1-deletion rat model. In the absence of 4E-BP1, podocytes are unable to undergo hypertrophy, which results in progressive scarring and the development of FSGS [17].

During development and after injury, foot processes exhibit different actin-based structures, in particular, broad membrane protrusions called lamellipodia and long, thin, sharp structures called filopodia. At the same time, the focal adhesion complex on the underside of the cells is involved in movement as well as tethering the foot processes to the GBM. The foot processes use opposing forces that pull on the cell and push the protrusions to extend. Each movement involves breaking down an existing adhesion point and re-instating it in a new location once the lamellipodia move forward (Fig. 3) [18]. The lack of knowledge about the degree to which normal podocytes move may be due to the limitations of current scanning technology, but they are thought to be largely stable, static structures in normal, healthy glomeruli.

Actin also has a role in intracellular transport, with actin-based motor proteins used to carry material across the length of the podocyte foot process; one example is the motor protein, Myo1c, a podocyte protein that facilitates the transport of slit diaphragm protein NEPH1 to the podocyte membrane [19]. The presence of Golgi outposts, as seen in the dendrites of neuronal networks, has not been shown in podocytes. It would appear that in podocytes, newly manufactured proteins travel a fairly long distance from the nucleus to the foot processes or intercellular junction.

The Role of Nephrin in Actin Dynamics

Nephrin is a specialist glomerular adhesion protein that is expressed as the podocyte matures and begins to differentiate and form protrusions. In addition to its structural role, nephrin is also involved in podocyte signaling. It belongs to the immunoglobulin superfamily and consists of 8 extracellular immunoglobulin-like modules, a fibronectin type III-like domain, and a cytosolic C-terminal tail. It is a single-pass, transmembrane protein that interacts with other nephrin proteins (both trans and cis) within and outside the cell [20, 21].

Abnormalities of nephrin caused by mutations in the *NPHS1* gene have been implicated in the autosomal recessive congenital nephrotic syndrome of the Finnish

type, first identified in 1998 [11]. The single gene mutations are responsible for the developmental failure of the podocyte foot processes and slit diaphragms, with extensive proteinuria present in utero. Children affected with this condition are nephrotic at birth [22]. The majority of the *NPHS1* mutations are seen within the extracellular domain, with one recorded in the cytoplasmic domain, and only a few within the fibronectin domain [20].

Mice models of nephrin deficiency (nephrin null) clearly show abnormalities in the tertiary foot processes, while the primary and secondary processes remain minimally changed. Changes include shorter tertiary processes, loss of normal polarity, and abnormalities of intercellular junction formation. The abnormalities are associated with changes in normal foot process morphogenesis brought about by a lack of the nephrin signaling complex that controls the podocyte morphology and filter integrity [23]. This signaling process is thought to be phosphorylation-mediated, owing to the transient appearance of phosphorylated tyrosines in nephrin cytoplasmic domains in the developing newborn mice kidneys, which are no longer apparent in adult mice. These phosphorylated tyrosines are able to bind to the SH2 domain and thus interact with Nck, leading to the nucleation and polymerization of actin filaments. Once the mature intercellular junctions have been formed, phosphorylation ceases and nephrin signaling stops, confirming a functional relationship between podocyte intercellular junction-associated proteins and actin cytoskeleton dynamics [23, 24].

Nephrin can also become tyrosine phosphorylated in cases of podocyte injury (e.g., protamine sulfate exposure), allowing the podocyte foot processes to remodel their actin structure to some extent [24, 25]. While it is known that there is an upregulation of tyrosine phosphorylation in glomerular injury, the identity of the other tyrosine kinases in addition to Src kinases that might be involved remains unclear.

The process by which the nephrin signaling events affect the assembly and regulation of actin polymerization complexes at the cell membrane also remains unclear. They have been studied in an artificial signaling system in which a CD16 extracellular domain and a CD7 transmembrane domain were attached to the nephrin cytoplasmic domain in association with tyrosine kinases in a model that enables development of CD16 clusters and phosphorylation using an anti-CD16 antibody [21, 26]. This activation of the Nephrin-NEPH1 complex is sufficient to induce actin polymerization, with actin tails visible by confocal microscopy underneath the clustered CD16 nephrin, as well as recruiting Nck, a classic actin nucleation mole-

Fig. 3. Actin-based structures responsible for movement and extension of podocyte foot processes. **a** Reproduced with permission from Koch Institute at MIT from "Lamellipodia Protrusion and Cell Migration 2" by Guillaume Carmona and Frank Gertler, 2016. **b** Republished with permission of Rockefeller University

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cule, and Src kinase [21, 23]. It has also been shown to be possible to reverse the effect by depolymerizing actin using cofilin, an actin-depolymerizing protein that has a natural role within podocytes in the recycling of actin. Nephrin has also been found to be a regulator of this negative process through a system of dephosphorylation involving a variety of signaling mediators [27].

Nephrin phosphorylation is thought to have a further role in actin dynamics through the regulation of lamellipodia formation, utilizing its ability to assemble protein complexes that can bind actin together into broad 3-dimensional structures that resemble lamellipodia. In vitro experiments have shown that it is possible to crosslink actin monomers using proteins such as SHIP2 (SH2 domain containing 5' inositol phosphatase), filamin, and lamellipodin, which are recruited and regulated by nephrin [28].

Small GTPases and Actin Dynamics

A number of human FSGS mutations have been shown to involve proteins or protein-protein interaction complexes that regulate podocyte structure. These include laminin/integrin receptors that regulate focal adhesions, glomerular slit diaphragm proteins, actin-binding proteins, and actin-regulatory proteins like small GTPases of the Rho/Rac/Cdc42 family of proteins [29].

Small GTPases have been identified as important regulators of actin dynamics in podocytes. The GTPase-activating protein (GAP) Rho-GAP 24 (*Arhgap24*) was found to be upregulated during podocyte differentiation; conversely, a loss of function mutation in the *Arhgap24* gene was identified in a family with inherited kidney disease [30]. Synaptopodin is essential for the integrity of the podocyte actin cytoskeleton and for the regulation of podocyte cell migration and regulates podocyte actin dynamics and migration by modulating RhoA signaling [31]. Epidermal growth factor receptor/Src-mediated phosphorylation of synaptopodin in podocytes stimulates binding to calcineurin, which results in synaptopodin degradation and enhanced Rac1 signaling, leading to the loss of actin stress fibers [32]. Rac1-GAP, which is associated with proteinuric kidney disease, increases when RhoA levels decrease. RhoA signaling is also impacted by synaptopodin levels, such that when synaptopodin levels are reduced, RhoA levels and activity are decreased [30]. Mutations in the gene coding for ARHGDI1, which forms a complex with Rho-GTPases in podocytes, have also been demonstrated to interfere with Rho-GTPase signaling, thereby enhancing migration of cultured podocytes [33].

Further support for the importance of small GTPases in podocyte actin dynamics comes from studies in which mice lacking Cdc42, a Rho-GTPase in the same family as Rac1 and RhoA, universally developed congenital nephropathy and died of renal failure within 2 weeks of birth [34]. The Cdc42-knockout kidneys exhibited signs of collapsing glomerulopathy, extensive podocyte foot process effacement, and abnormal expression of podocyte markers and cell polarity proteins, highlighting the role of Cdc42 in formation of the podocyte, actin polymerization involved in slit diaphragm organization, and glomerular function [34].

Inappropriate activation of Rac1 or RhoA can also be detrimental. Mice with doxycycline-induced constitutively active Rac1 (Rac1Q61L) experienced changes in the podocyte actin cytoskeleton, including induction of lamellipodium formation, focal foot process effacement, and increased podocyte membrane motility; those with activated RhoA demonstrated stabilization of the cytoskeleton and suppression of membrane motility [35].

The Podocyte Effacement Process

Podocyte effacement can be thought of as lamellipodia formation. It is a very dynamic, highly adenosine, triphosphate-consuming, phosphorylation-driven, signaling-based process that involves the rearrangement of the actin cytoskeleton and invokes integrin-focal adhesion dynamics.

By preventing or reducing some of the significant events that increase podocyte mobility (e.g., focal adhesion turnover or lamellipodia formation), it may be possible to prevent podocyte effacement and thus limit glomerular injury. Efforts to identify a process to downregulate nephrin signaling during podocyte injury have focused on SHP-2, a phosphatase that is found within the cytoplasm, and is known to be associated with phosphorylated nephrin during podocyte development and injury. However, when nephrin and SHP-2 were put together in vitro, they were found to upregulate nephrin phosphorylation rather than decrease it, suggesting a role in the activation of Src kinases [25]. Furthermore, mice models with SHP-2 deleted from the podocytes were protected from injury with protamine sulfate [25]. Functional integrity of the glomerular filter cannot be assessed with the protamine sulfate model of podocyte injury, as the animals are sacrificed following the experiment. SHP-2-deleted mouse podocytes subjected to the nephrotoxic serum demonstrated that proteinuria is reduced in addition to the absence of foot process

effacement [25]. Similar observations have been made when focal adhesion regulatory proteins, such as focal adhesion kinase (FAK), Crk, and uPAR, are deleted in the mouse [24, 36, 37]. Deletion of non-receptor phosphatase PTP1B in podocytes conferred protection from injury, as overexpression of PTP1B resulted in increased FAK phosphorylation and activity of Src kinases [38].

Podocyte: Links to Glomerular Basement Membrane

A key interaction is the interface of podocytes with the GBM, where attachments are made through a series of focal adhesion complexes and cell surface receptors. The GBM is a dense matrix of a number of extracellular components including collagen IV and laminin. It provides a scaffold for interconnecting endothelial cells and podocytes to form an effective glomerular filtration barrier. Integrity and function depend on both cell-to-cell and cell-to-matrix adhesion. Podocyte adhesion occurs through a number of receptors, including integrins, syndecans, and dystroglycan, all of which interact with actin to ensure barrier integrity. The most important of these is the integrin heterodimer $\alpha3\beta1$ [39]. The GBM interface is also an important signaling environment involving signaling networks of integrin and adhesion molecules [39].

Phospho-FAK and phospho-Cas are important kinases involved in the focal adhesion process, although the degree of phosphorylation varies across different diseases. Both are seen markedly less in FSGS compared with membranous nephropathy and minimal change disease, suggesting that there may be important differences in their signaling processes [24]. Lower levels of phospho-SHP-2 staining were observed in the glomerulus from biopsy samples from FSGS patients compared with those from patients with membranous and minimal change disease [25]. The discrepancy could be explained by a decrease in the number of podocytes in FSGS at the time of biopsy; alternatively, there could be distinct signaling pathways involved in the different proteinuric glomerular diseases.

While the nephrin phosphorylation signaling process is important at the GBM, as demonstrated by the protamine sulfate/heparan sulfate knockout mouse models, the focal adhesion complex also plays a key role. Deletion of FAK or Crk in mouse models also prevented foot process effacement [24, 36].

Integrin-activation-based signaling is another important process at the podocyte-GBM junction and may play a role in nephrin tyrosine phosphorylation that is separate from proposed ligand binding. Integrins are trans-

membrane heterodimeric receptors to various extracellular membrane components, including laminin, collagen, and fibronectin, based on their specific binding targets. They are involved in activating kinases that cause phosphorylation and mediate interactions between cells as well as between cells and the extracellular matrix [40]. In vitro models have shown that it is possible to prevent phosphorylation of nephrin through inhibition of the activation of the integrin complex using anti-integrin $\beta1$ monoclonal antibodies in cultured podocytes [40].

Other molecules that form part of the GBM include the transmembranous protein syndecan, which is an important signaling and adhesion molecule. Syndecan interacts with integrins, moving them away so as to allow the plasma membrane to conform and a focal adhesion complex to form. Syndecans are also involved in the polymerization of actin and the protrusion of the membrane [41].

The T-cell co-stimulatory molecule B7-1 (CD80) is induced in podocytes in various animal models of proteinuria and is found in patients with certain glomerular diseases. Podocyte B7-1 expression is not evident in the normal human kidney. B7-1 promotes disease-associated podocyte migration through inactivation of $\beta1$ integrin [42]. The expression of integrin $\alpha3\beta1$ is important to the normal formation and integrity of the GBM and prevents stress-mediated injury in the glomerulus. Loss and/or mutations of podocyte integrin $\alpha3\beta1$ cause detachment and loss of podocytes resulting in damage to the glomerulus [43].

Podocyte-Endothelial Cell Link and Vascular Endothelial Growth Factor Signaling

Podocyte dysfunction and depletion in glomerulosclerosis has been found to be dependent on a reciprocal crosstalk between glomerular podocytes and endothelial cells. One such reported signaling crosstalk is mediated by endothelin-1 (EDN1)/endothelin receptor type A (EDNRA)-dependent mitochondrial dysfunction, as demonstrated by in vitro results from both direct co-culture and media transfer studies of podocyte injury. Podocyte-generated EDN1 is associated with increased endothelial EDNRA expression, leading to endothelial mitochondrial oxidative stress and endothelial cell dysfunction via EDNRA, damaging factors responsible for foot process effacement, apoptosis, and/or detachment of podocytes, and, subsequently, podocyte depletion and glomerulosclerosis [44].

Vascular endothelial growth factor A (VEGF-A) is essential for the development and maintenance of the glomerular filtration barrier. Dysregulation of VEGF expres-

sion within the glomerulus has been demonstrated in a wide range of nephrotic diseases, although the significance of these changes is unknown. Pre-eclampsia/eclampsia due to VEGF overexpression is a classic example of endothelial dysfunction resulting in podocyte foot process effacement and can act as a good model of proteinuria that is endothelium-based.

VEGF-A is highly expressed in podocytes, and the maintenance of stable VEGF levels is important for podocyte health. VEGF needs to be precisely regulated, as both overexpression and underexpression can lead to injury. In heterozygous mice models, podocyte-specific overexpression of VEGF-A produced a collapsing glomerular phenotype by the age of 2.5 weeks; in a separate experiment, transgenic mice models expressing human VEGF were protected from puromycin-induced injury, which could potentially be a dose-related effect [45, 46].

The role of podocyte VEGF-A in regulating $\alpha_v\beta_3$ integrin signaling in the mouse GBM has been shown in vivo [47]. $\alpha_v\beta_3$ integrin plays an important role in angiogenesis and in hypertension-induced vascular remodeling in the kidney. Interruption to this signaling process has been shown in vivo to damage the glomerular filtration barrier, resulting in proteinuria and acute renal failure [47]. Podocyte VEGF knockdown in cultured mice podocytes also has been found to disrupt $\alpha_v\beta_3$ integrin activity through decreasing VEGF receptor 2 signaling activity [47]. Mediation of the VEGF signaling process may be a future potential target for therapeutic intervention.

Future Lines of Investigation

Suggested areas for future study include a better understanding of nephrin focal adhesion complex proteins, especially their post-translation modifications under dif-

ferent conditions; of VEGF signaling, including a better understanding of the fine-tuning of VEGF levels; of podocyte-endothelial crosstalk; of integrin signaling and its activation effects; and of the process and implications of podocyte loss. Increased knowledge of podocyte biology is helping to advance our knowledge of the science and mechanics of the glomerular filtering process, pointing the way to a variety of interesting potential applications for clinical targeting. Further investigations into the genetic mutations responsible for podocytopathies are necessary to guide our understanding of potential therapeutic targets that may address the unmet needs of patients with treatment-resistant nephrotic syndrome.

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