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Podocyte Biology for the Bedside

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Abstract

The explosion of podocyte biology over the last decade has radically altered our views on the pathophysiology of proteinuria, glomerular disease, and progressive kidney disease. In this review, we highlight some of these landmark findings, but focus on recent advances in the field and the implications for translating this biology into therapy for podocyte diseases.

Keywords

podocyte; proteinuria; glomerulus; glomerular disease; focal segmental glomerulosclerosis; minimal change disease; membranous nephropathy

BACKGROUND

Human kidneys contain approximately one million nephrons, each consisting of a glomerulus and renal tubule. The glomerulus is a tuft of capillary loops supported by the mesangium, and enclosed in a pouch like extension of the renal tubule known as Bowman capsule. It consists of four resident cell types: the mesangial cell, glomerular endothelial cell, visceral epithelial cell (herein referred to as the podocyte), and the parietal epithelial cell which lines Bowman basement membrane (Figure 1). Each glomerular cell has been well discussed in recent review articles¹⁻³. In this review we will discuss the function of the podocyte, and highlight recent advances in podocyte biology that pertain to the clinical diagnosis and treatment of the glomerular disease highlighted in the case vignette.

CASE VIGNETTE

A 30 year old white male presents with a four week history of nephrotic syndrome (proteinuria 8.4g per day with serum albumin 2.6g/dl [26g/L]), normal kidney function (serum creatinine, 1.0 mg/dl [88 μmol/L]; eGFR, 101 mls/min/1.73m² [1.7 mL/s/1.73m²] using the CKD-EPI equation) and new onset hypertension. He undergoes a kidney biopsy

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which demonstrates focal segmental glomerulosclerosis (FSGS; not otherwise specified variant). No secondary causes are identified and he is treated with a 4 month course of high dose steroids. He proves to be steroid resistant. Despite further therapy with cyclosporin, his kidney function progressively deteriorates, and 24 months after first presentation he commences peritoneal dialysis.

This is a sadly familiar course for a disease of the podocyte, with many different clinical variants and diverse pathophysiology. The following discussion of podocyte biology and treatment will help to frame podocyte diseases in the current clinical context, and offer some hope for future patients with podocytopathies.

PATHOGENESIS

Normal Structure of the Podocyte

To understand the recent advances in disease pathogenesis, we begin with an understanding of the normal structure and function of podocytes, which are highly differentiated epithelial cells. The podocyte sits on the outside of the glomerular capillary loop, consists of a large cell body in the urinary space, and connects to the underlying glomerular basement membrane (GBM) via major processes with long interdigitating foot processes that extend around the capillary loop covering the GBM (Figures 1 & 2). Integrins (predominantly $\alpha\beta1$ integrin) and α - and β -dystroglycans bind podocyte foot processes to the GBM (Figure 3). Between the foot processes, the filtration slit is bridged by a 40nm wide slit diaphragm that is highly permeable to water and small solutes; however, the small size of the pore (5-15nm) limits the passage of larger proteins including albumin. The slit diaphragm comprises nephrin and other related structural proteins which are intimately connected to the actin cytoskeleton. Signaling pathways from these slit diaphragm molecules regulate actin dynamics and allow podocytes, especially the podocyte foot processes, to actively alter their shape. The mechanical stability of podocytes may be further strengthened during injury by the upregulation of intermediate filament proteins such as nestin and vimentin. As will be discussed below, hereditary and acquired abnormalities in slit diaphragm components and/or actin cytoskeleton result in marked proteinuria.

Major Functions of the Podocyte

In order to understand the clinical features and consequences of podocyte diseases, an understanding of their normal functions is required (Box 1). These include the following:

1. **Structural support of the capillary loop:** The podocyte has an extensive actin cytoskeleton displaying an intermediate phenotype between epithelial and mesenchymal cells with features of contractile smooth muscle cells. These mesenchymal features allow contraction and support of the glomerular capillary, counteracting the glomerular capillary hydrostatic pressure (~60 mmHg), which is much greater than other capillary beds. Recent studies suggest that molecules in the slit diaphragm such as podocin serve a mechano-sensing function.
2. **Glomerular filtration barrier:** the filtration barrier to macromolecules is composed of three layers: the innermost glomerular endothelial cell and overlying glycocalyx, the GBM, and the outermost podocyte. Multiple examples of both inherited and acquired injury to the podocyte, especially the slit diaphragm domain, have shown the critical role of the podocyte in the prevention of proteinuria (reviewed in^{4, 5}). The podocyte also maintains the glomerular filtration barrier by removing protein and immunoglobulins that may clog the filter.
3. **Synthesis and possible repair of the GBM:** The major component of the GBM is type IV collagen. The initial $\alpha1\alpha2\alpha1$ network is secreted by the glomerular

endothelial cell during fetal development, but in early life, this is replaced by the more robust $\alpha3\alpha4\alpha5$ network secreted by the podocyte. Failure to secrete this network results in a range of hereditary nephropathies (Alport syndrome, nail patella syndrome, thin basement membrane disease), the so-called type IV collagenopathies, all of which may be considered podocyte disorders. The podocyte also produces other GBM components, and the balance of pro- and anti-fibrotic factors from the podocyte controls GBM turnover and maintenance.

4. Cross talk with other glomerular cells for their function: The podocyte does not function in isolation, and despite being separated from other glomerular tuft cells by the GBM, interacts with glomerular endothelial cells and mesangial cells to ensure their normal function. The clearest example is the production of VEGF (vascular endothelial growth factor) by the podocyte, which traverses the GBM counter to the flow of glomerular filtration, to act on VEGF receptors on the endothelial cell, thus maintaining a healthy fenestrated endothelium. Another is during development when podocytes produce insufficient PDGF (platelet-derived growth factor), resulting in the absence of mesangial cells.
5. Immunological Function: podocytes may be a component of the innate immune system, possibly playing a surveillance role for pathogens or abnormal proteins in Bowman space. They constitutively express Toll-like receptor 4 (TLR4) which upon signaling stimulates chemokine production.⁶ Furthermore, lipopolysaccharide (LPS), a component of cell wall of gram-negative bacteria, triggers synthesis of B7.1, which (in addition to T cell co-stimulation) leads the actin cytoskeleton and slit diaphragm proteins to reorganize, resulting in the development of proteinuria.⁷

Podocyte Injury Leads to Proteinuria

The classic clinical feature of podocyte injury is proteinuria. Injury to mesangial or endothelial cells, which are in direct contact with the blood (containing leukocytes, complement, inflammatory proteins), typically leads to inflammatory kidney disease (nephritis). By contrast, as podocytes sit on the outside of the glomerular capillary, and are partitioned from the circulation by the GBM, injury usually does not lead to leukocyte recruitment and inflammation, but rather disrupts the glomerular filtration barrier, leading to proteinuria. (Table 1) This may be due to impaired slit diaphragm integrity or possibly alterations in podocyte charge or shape (effacement). Foot process effacement is an active process due to actin cytoskeleton rearrangement. Recent evidence links activation of the podocyte integrin, $\alpha V\beta 3$, by molecules such as the urokinase receptor to the development of foot process effacement and potentially the promotion of cell motility.⁸ The podocyte response to injury and resulting mechanisms of proteinuria are well described in recent reviews.^{5, 9}

Podocyte Loss Leads to Progressive Glomerulosclerosis

In the adult human kidney, there are roughly 500-600 podocytes per glomerular tuft, and their rate of turnover is very slow. When podocytes are lost through apoptosis or detachment into the urinary space, there is a very limited ability of remaining cells to proliferate and recover “bare” areas of GBM. This lack of coverage and support can lead to ballooning of the capillary loop, synechial attachment to Bowman capsule, and the development of FSGS.¹⁰ How then can injured or detached podocytes be replaced?

Podocyte Replacement—Recent interest has centered on the parietal epithelial cell’s role on the Bowman basement membrane. Along the vascular pole of the glomerular tuft, parietal epithelial cells on the Bowman basement membrane are in continuity with podocytes on the GBM. Indeed, within this region, podocytes can be found on the Bowman

basement membrane that bridge to the GBM and have been termed parietal podocytes. Some parietal epithelial cells express stem cell markers (CD24, CD133) and may act as renal progenitor cells. As they approach the vascular pole, they gradually lose these stem cell markers, acquire podocyte specific markers, and may migrate onto the GBM.¹¹ The ability of the podocyte to migrate within the glomerular tuft has been elegantly demonstrated using time lapse fluorescence microscopy.¹² Lineage tracing studies using parietal epithelial cells genetically tagged with β -galactosidase as a colorimetric marker have confirmed the recruitment of podocytes from parietal epithelial cells during the renal growth of juvenile mice.¹³ However, not all replacement podocytes are derived from resident renal cells. Transplant studies in which male recipients received female kidney allografts, have demonstrated the presence of podocytes derived from the recipient male in approximately 50% of cells, in keeping with a bone marrow derived stem cell source.¹⁴ Ohse *et al* showed that parietal epithelial cells express podocyte markers in several experimental models of podocyte disease, suggesting that the switch from parietal epithelial cell to podocyte occurs in disease states.¹⁵ We should note that this parietal epithelial cell to podocyte switch in phenotype may also have some adverse consequences for the glomerulus, possibly contributing to the development of adhesions connecting the glomerular tuft and the Bowman basement membrane, leading to segmental scars.

Podocyte Number Predicts Progression—The critical role of podocyte number and the development of glomerulosclerosis was demonstrated in an elegant study by Wharram *et al*.¹⁶ In their rat model with a site-specific transgene expressing the diphtheria toxin receptor only in podocytes, the administration of diphtheria toxin resulted in selective loss of podocytes with minimal inflammation. Higher doses of diphtheria toxin resulted in the loss of greater numbers of podocytes. This group was able to show that with loss of 10-20% of podocytes, the glomerulus could recover; however 20-40% loss resulted in FSGS, and greater than 50% resulted in irreversible glomerular injury. The role of podocyte number has been supported by clinical studies. In both type I and type II diabetic kidney disease, a decrease in podocyte number (podocytopenia) correlates with disease progression.^{17, 18}

Urine Podocyte Biomarkers—Proteinuria is the classic urinary biomarker predicting the progression of kidney disease. Recent refinements including the use of residual proteinuria after treatment and the character of the proteinuria (e.g. urinary excretion of IgG or β_2 -microglobulin) have been shown to better predict outcome in certain groups, but have not yet been embraced in clinical practice. As proteinuria is predominantly a podocyte disease, could there be podocyte-derived urine biomarkers for diagnosis or disease prognosis?

When podocytes detach into the urinary space, they may traverse the tubule and be excreted in the urine (podocyturia). Although not recognizable by direct urine microscopy, staining for specific podocyte markers allows the identification and quantification of these cells, a number of which are viable and can be grown in culture. Podocyturia has been described in diabetic kidney disease, FSGS, IgA nephropathy, and active lupus nephritis, but typically not in minimal change disease (MCD) or membranous nephropathy.¹⁹ Fragments of podocyte membranes in the urine have also been described and these urine microvesicles can be recovered by ultracentrifugation; this may prove a source for useful biomarkers.²⁰ Another clinical example is the recent identification of increased urinary CD80 (B7.1) excretion as a biomarker to differentiate MCD from primary FSGS.²¹

RECENT ADVANCES

Clinical Podocyte Disorders

Podocyte biology has advanced at breathtaking speed over the last decade. In this section we will review advances in the last two years that likely will translate from the bench to clinically applicable bedside tools.

Membranous Nephropathy—Membranous nephropathy is characterized by the presence of sub-epithelial (sub-podocyte) immune deposits with thickening of the glomerular capillary wall. The rat model of passive Heymann nephritis has many parallels with the human disease, but the antigenic target in this model, megalin, is absent from the human podocyte. Despite numerous efforts, the human podocyte antigen responsible for the characteristic glomerular IgG deposition and subsequent C5b-9 activation remained unidentified until very recently. Thus, possibly the most dramatic and clinically relevant advance in podocyte biology has been the identification by Beck *et al* of the M-type phospholipase A₂ receptor (PLA₂R) as the target antigen in the majority of cases of human membranous nephropathy.²² This group also described an association between the circulating anti-PLA₂R antibody level and disease activity, raising the prospect of targeting immunosuppression to those with active immunological disease.²² The finding of anti-PLA₂R in patients with nephrotic syndrome may also change the criteria for kidney biopsy. Clearly, these dramatic findings may revolutionize the treatment, diagnosis and possibly prognosis of membranous nephropathy in the very near future.

FSGS—FSGS has perhaps seen the biggest advances of all kidney disease in the past few years. Genetic studies in families with FSGS have now identified multiple disease causing genes that have greatly enhanced our understanding of podocyte biology (reviewed in²³). The most recent gene to be identified as a cause of autosomal dominant FSGS in young adults is *INF2*, which encodes a member of the formin protein family.²⁴ Mutations in genes encoding slit diaphragm's structural elements (nephrin, podocin, CD2AP [CD2-associated protein]), actin cytoskeleton (α -actinin 4), GBM (laminin β 2), the transcription factor WT1, the calcium channel TRPC6 and phospholipase C ϵ 1 have also been identified as causes of inherited FSGS. TRPC6 is of particular note, as upregulation of the wild type calcium channel has been noted in acquired podocyte disorders, and small molecule inhibition of these channels may be feasible.²⁵ These findings have rapidly translated to the bedside, as we now recognize that the majority of these hereditary podocytopathies are resistant to immunosuppression. Mutation analysis for most of these genes are now clinically available in the United States, and may help to identify patients in whom immunosuppression should be discontinued.

Although these hereditary podocytopathies are relatively rare, hypertensive kidney disease and FSGS are common, with an increased prevalence in patients of African ancestry. Recent seminal studies have identified genetic variations in the *APOL1* (apolipoprotein L1) gene in African American patients that account for a large part of this increased risk.²⁶ Notably, the ApoL1 variants associated with kidney disease lyse *Trypanosoma brucei rhodesiense* and the authors theorize that there is a survival benefit of this polymorphism in African people (similar to sickle cell trait and malaria) that may be responsible in part for the high rate of kidney disease in African Americans. It is yet to be determined if identifying sequence variations in this gene will allow us to tailor our therapy to individual patients.

HIV-Associated Nephropathy / Collapsing Glomerulopathy—The classic pattern of collapsing glomerulopathy seen in human immunodeficiency virus-associated nephropathy (HIVAN) is typified by glomerular collapse with extracapillary epithelial cell

proliferation. These cells have long been considered to be dedifferentiated podocytes, although recent data suggests a contribution from parietal epithelial cells / renal progenitor cells on the Bowman basement membrane. A similar histological pattern has been described in a mouse model in which VEGF is specifically overexpressed in podocytes.²⁷ Upregulation of VEGF and VEGF receptor (VEGFR2) expression on podocytes has now been described in patients with HIVAN.²⁸ Exogenous VEGF stimulates de-differentiation and proliferation of podocytes *in vitro*, which is blocked by neutralizing VEGFR2 antibodies.²⁸ It may become possible to develop therapies to block podocyte VEGFR2 signaling for patients with HIVAN. Care needs to be taken with this approach however, as current strategies to block VEGF activity in patients with cancer have resulted in proteinuria and hypertension due to renal thrombotic microangiopathy.²⁹

Obesity Related Glomerulopathy—With the growing epidemic of obesity, studies are now focusing on its effects on the kidney. Obesity related glomerulopathy presents with a pattern of glomerular enlargement that progresses to FSGS as a result of podocyte injury from chronic glomerular hyperfiltration. A new mechanism related to inadequate adiponectin signaling in podocytes has been proposed.³⁰ Fat cells act as endocrine organs and produce various adipokines (leptin, resistin and adiponectin). Low plasma adiponectin levels are associated with greater risk of type 2 diabetes and cardiovascular disease, and more recently, with albuminuria in a cohort of obese non-diabetic African American individuals.³⁰ The same group showed that adiponectin knockout mice have baseline albuminuria and foot process effacement, with an apparently normal glomerular endothelium. Notably, treatment with exogenous adiponectin has been shown to normalize the proteinuria and restored the podocyte foot processes.³⁰ Podocytes express the AdipoR1 receptor, and lack of ligand binding promotes oxidative stress via upregulation of Nox4 (NADPH oxidase 4). These data suggest that adiponectin may have protective effects on the podocyte, and treatment with adiponectin receptor agonists may become feasible.

MCD—MCD has classically been considered a podocyte disease, possibly mediated by a T cell lymphokine, and recent interest has focused on the role of IL-13 (interleukin 13). Serum IL-13 levels are elevated in patients with MCD, and rats overexpressing IL-13 develop a minimal change type lesion.³¹ Podocytes express IL-13 receptors, and ligand binding increases CD80 (B7.1) expression, which promotes alterations in slit diaphragm proteins and disorganization of the actin cytoskeleton.^{7, 31} Another molecule of great interest in MCD is ANGPTL4 (angiopoietin-like 4). Recently, podocyte over-expression of this molecule in rats has been shown to lead to a steroid sensitive nephrotic syndrome.³² A notable clinical advance has been the surprising efficacy of anti-B cell therapy with rituximab in patients with resistant or steroid dependent disease.³³

Diabetic Kidney Disease—Although mesangial and endothelial cell injury play a prominent role in diabetic kidney disease, podocyte injury (including foot process effacement, podocytopenia, and podocyte hypertrophy) is important in the pathophysiology of this condition.⁹ Hyperglycemia directly injures the podocyte secondary to increased oxidative stress, increased advanced glycation end products (AGE), activation of protein kinase C, activation of renin angiotensin system, and stimulation of growth factors such as transforming growth factor β (TGF β) and VEGF (reviewed in³⁴). But what about the role of insulin? The podocyte expresses the insulin receptor and is very insulin sensitive, expressing the glucose transporters (GLUT1, GLUT4) upon insulin signaling. So called podIRKO mice (which have a targeted deletion in the insulin receptor, specifically in podocytes) develop proteinuria by 5 weeks, and with aging develop features compatible with diabetic glomerulopathy including foot process effacement, GBM thickening and glomerulosclerosis.³⁵ This finding mirrors the cellular insulin resistance seen in type II

diabetes which may contribute to podocyte injury. Insulin sensitizing agents such as PPAR γ (peroxisome proliferator-activated receptor γ) agonists (e.g. pioglitazone) have been shown to enhance podocyte insulin sensitivity, and may have beneficial effects on decreasing proteinuria and slowing progression in type II diabetic kidney disease, independent of glucose control.³⁶

Crescentic Glomerulonephritis—Glomerular crescents typify a rapidly progressive glomerulonephritis secondary to a range of renal and systemic disorders. For many years the cellular composition of crescents has been considered to be a combination of macrophages, epithelial cells, and myofibroblasts, but the origin of the epithelial cell (parietal epithelial cell versus podocyte) has been debated. These data have been based mostly on immunohistochemical studies for cell specific markers; however, these cells may alter their cell specific markers in disease situations, either reverting to more primitive forms or undergoing epithelial mesenchymal transition and acquiring markers more characteristic of macrophages.

The podocyte had been considered to be virtually absent from crescents, due to the inability of these cells to proliferate and the absence of cells expressing any podocyte specific markers. Recent studies in human crescentic disease, using nestin, a more stable podocyte marker, have suggested that up to 50% of cells making up crescents may be podocyte derived.³⁷ However, mice cell lineage studies that tracked genetically tagged podocytes suggest that these extracapillary cells are predominantly derived from parietal epithelial cells, with podocytes contributing only 14% of these cells.³⁸ It seems likely that the majority of extracapillary cells in crescents are derived from renal progenitor cells on Bowman basement membrane which may partially transition to a podocyte phenotype.

Thrombotic Microangiopathy—This group of disorders is primarily associated with endothelial cell injury, so why include this in a review of podocyte biology? The critical role of VEGF, a pro-angiogenic factor, in podocyte and glomerular endothelial cell biology has become apparent over the last several years. VEGF is produced predominantly by podocytes, and although it may function as an autocrine-like signal within the podocyte, VEGF protein mainly seems to traverse the GBM and stimulate glomerular endothelial cells, promoting their survival and triggering fenestrae to enhance glomerular endothelial permeability. Heightened VEGF activity is associated with collapsing glomerulopathy, but recent studies have clarified the role of VEGF inhibition in some forms of thrombotic microangiopathy. Cancer studies in which the angiogenic effects of VEGF are inhibited with either anti-VEGF antibody (bevacizumab) or VEGF receptor antagonists have been associated with the adverse effects of proteinuria and hypertension. Biopsy studies have confirmed thrombotic microangiopathy as the primary kidney pathology in these patients.²⁹ Recombinational engineering of DNA with a conditional Cre-*lox* P system has been shown that inhibition of podocyte-derived (but not circulating) VEGF leads to the development of thrombotic microangiopathy and hypertension in mice, a finding that parallels the human pathology.²⁹

The Podocyte as a Target for Therapy

If we consider the patient in the case vignette, our thoughts as clinicians rapidly turn to therapy. What advances in our understanding of the therapy of podocyte diseases have occurred, and what promise does podocyte biology hold for future therapy? Are there new agents available to specifically treat the underlying cause of the disease, or agents available that augment the repair of podocytes?

Blockade of the Renin-Angiotensin-Aldosterone System—Renin-angiotensin-aldosterone system (RAAS) blockade has been shown to slow progression in both diabetic and non-diabetic kidney disease. Classic teaching from Brenner and others suggests that this renoprotective effect is primarily mediated by blockade of circulating angiotensin II, leading to a reduction in glomerular capillary pressure (and hence proteinuria), and to a decrease in pro-fibrogenic pathways.³⁹ Podocytes, however, also have a local intracellular RAAS that may be activated by glomerular hypertension or podocyte injury, and RAAS blockade may also act at this tissue level.

Exogenous angiotensin II binding to the angiotensin II type 1 (AT₁) receptor on the podocyte surface has been shown *in vitro* (i.e. independent of glomerular hemodynamics) to result in downstream events characteristic of podocyte injury including reorganization of the actin cytoskeleton, increased α 3(IV) collagen production, and decreased nephrin expression. Similarly, transgenic rats in which the AT₁ receptor is overexpressed selectively in podocytes go on to develop spontaneous glomerulosclerosis. However, the lesser contribution of local AT₁ receptor blockade to podocyte injury has been confirmed using a podocyte specific AT₁ receptor knockout mouse.⁴⁰ In this model, the knockout mice were not protected from angiotensin II infusion induced albuminuria or immune injury (anti-podocyte antibody) and, notably, angiotensin receptor blockers remained renoprotective in these animals.

What about other components of the RAAS? Evidence suggests a local role for aldosterone in glomerular injury and proteinuria (reviewed in⁴¹). In the subtotal nephrectomy model and the salt-loaded, spontaneously hypertensive rat model, the anti-proteinuric effects of ACE inhibitor and ARB treatment were negated by exogenous aldosterone administration. This deleterious role of aldosterone may be due to glomerular hemodynamics, but the mineralocorticoid receptor is expressed in podocytes, and exposure to aldosterone increases oxidative stress and upregulates podocyte SGK1 (serum/glucocorticoid regulated kinase 1). Lastly, the (pro)renin receptor is also expressed on podocytes and may promote glomerulosclerosis independently of angiotensin II and the AT₁ receptor.⁴² Mice deficient in angiotensinogen, ACE, or AT₁ receptor all have high renin levels and develop glomerulosclerosis. Aliskiren, a renin inhibitor, has been shown to have specific podocyte effects, reducing intracellular levels of angiotensin II, but it does not inhibit (pro)renin receptor MAPK (mitogen-activated protein kinase) signaling.⁴³

Taken together, multiple components of RAAS are activated in podocytes, and RAAS inhibitors clearly have local podocyte effects, in addition to their systemic actions.

Statins—HMG-CoA (β -hydroxy- β -methylglutaryl-CoA) reductase inhibitors (statins) reduce cardiovascular disease in patients with CKD, although notably not in patients on hemodialysis. Although rosuvastatin at higher doses has been associated with proteinuria (possibly by inhibiting albumin reuptake in the proximal tubule), large studies in CKD have suggested that statins may actually reduce the degree of proteinuria, although this does not seem to ameliorate the decline in kidney function.⁴⁴ The renoprotective effect seems to be independent of lipid lowering, implicating pleiotropic effects of statins, possibly on specific kidney cells.

Studies in animal models have attempted to elucidate some of these protective mechanisms. Statins have been shown to ameliorate several models of podocyte injury including 5/6 nephrectomy and puromycin aminonucleoside models. Specific effects on the podocyte include a pro-survival effect, decreased podocyturia, maintenance of slit diaphragm integrity, and prevention of foot process effacement.

Vitamin D—There is a great deal of interest in the protective role of vitamin D in cardiovascular disease and CKD. Calcitriol is a potent inhibitor of the renin-angiotensin system. Inhibition of calcitriol synthesis in mice increases renin expression, whilst injection of calcitriol suppresses renin expression,⁴⁵ both independent of calcium metabolism.

In the Third National Health and Nutrition Examination Survey (NHANES III), levels of 25 hydroxyvitamin D were shown to correlate with albuminuria, and preliminary interventional studies have suggested that in patients with CKD, paricalcitol⁴⁶ or calcitriol⁴⁷ supplementation reduce proteinuria. The mechanism of these observed effects may relate to RAAS suppression, but other mechanisms are described (reviewed in⁴⁸). The podocyte expresses the vitamin D receptor, and paricalcitol has been shown to reduce podocyte effacement, podocyte loss, and upregulate translation of slit diaphragm proteins in models of diabetic kidney disease.⁴⁹ Notably, these beneficial effects of vitamin D receptor activators occurred in the presence of an AT₁ receptor antagonist, but it remains unclear if the beneficial effects are related to more complete blockade of the RAAS (especially renin) or to a more direct podocyte effect.

Podocyte Specific Effects of Immunosuppressive Agents

Immunosuppressive therapy is fundamental to the treatment of MCD, idiopathic FSGS, and membranous nephropathy, and has been presumed to be directed at the underlying immune disorder. Recent evidence suggests that some of the anti-proteinuric effects of these agents may be related to direct podocyte effects.

Corticosteroids—Steroids, widely used in glomerular diseases, exhibit a broad range of immunosuppressive and anti-inflammatory effects. Noteworthy is that podocytes express glucocorticoid receptors, which translocate to the nucleus upon steroid binding. This might explain how steroids also are beneficial in models of podocyte injury (e.g. puromycin aminonucleoside model) in which immunological or inflammatory mechanisms are not prominent. In cultured podocytes injured with puromycin aminonucleoside, dexamethasone is both cytoprotective and enhances recovery after injury.⁵⁰ The protective mechanisms include a reduction in podocyte apoptosis, stabilization of actin filaments, increased RhoA GTPase activity, and stabilization of the slit diaphragm complex. These data support both a local and systemic benefit for steroids in podocyte diseases.

Calcineurin Inhibitors—The argument that MCD and FSGS are predominantly T cell mediated diseases, possibly due to soluble factors released by activated T cells, has been supported by the efficacy of calcineurin inhibitors in patients with steroid resistant disease. However, the fact that cyclosporin reduces proteinuria in non-immunological disorders, such as Alport syndrome, suggests that there may be specific glomerular effects. The vasoconstrictive effects on the afferent arteriole reducing glomerular capillary pressure are well appreciated, but are there specific effects on the podocyte?

An elegant study by Faul *et al*, demonstrated that the beneficial effects of cyclosporin are not dependent on the inhibition of NFAT (nuclear factor of activated T cells) signaling in T cells, but can result from local inhibition of calcineurin-mediated dephosphorylation of synaptopodin, the actin organizing protein.⁵¹ The complex architecture of the podocyte is maintained by the prominent actin cytoskeleton which is highly regulated by proteins including the GTPase dynamin and synaptopodin. Enzymatic degradation of dynamin or synaptopodin by cathepsin L leads to actin cytoskeleton reorganization promoting foot process effacement and proteinuria. Calcineurin inhibitors protect synaptopodin from degradation by cathepsin-L, and stabilize the actin cytoskeleton.⁵¹ We should also note that recent work has shown that enhanced NFAT signaling within podocytes (not T cells) may

also lead to glomerulosclerosis, and this local pathway would also be inhibited by cyclosporin.⁵²

mTOR Inhibitors—A deleterious role of mTOR (mammalian target of rapamycin) inhibitors on the podocyte was suggested by studies in transplant patients that showed the development of proteinuria or FSGS in patients treated with sirolimus. Following this, a randomized trial of sirolimus therapy in FSGS was stopped early secondary to nephrotoxicity.⁵³ These negative effects may be partly mediated by decreased VEGF expression, or decreased expression of a variety of slit diaphragm and cytoskeletal proteins.

Rituximab—Podocytes share some immunological features with B lymphocytes, but they do not express CD20, and there are no specific podocyte effects reported with rituximab. Despite this, a number of small studies are now demonstrating clinical efficacy with the use of rituximab in a range of podocyte disorders including MCD, FSGS, and membranous nephropathy.

Future Directions

The rapid advances in podocyte biology are paving the way for the development of novel diagnostic tools and therapeutic agents to manage proteinuria and glomerular disease. Therapies based on newly identified podocyte proteins / pathways are being closely examined. For example, activation of Notch signaling pathways in podocytes may promote apoptosis, and upregulation of the TGF β and VEGF pathways leading to proteinuria and glomerulosclerosis, and this can be ameliorated using available Notch signaling pathway inhibitors.⁵⁴ Other potential candidates include urokinase plasminogen activator receptor inhibitors, Cathepsin L inhibitors, and TRPC6 inhibitors, amongst others.

Another exciting idea is the direct targeting of therapeutic agents to the podocyte, thus limiting systemic adverse effects. The podocyte is a good target for directed therapy due to the combination of high renal blood flow, with the added component of glomerular filtration, which exposes the podocyte to large volumes of capillary filtrate containing small molecules. The targeting of larger molecules is more difficult as these must cross the first two layers of the glomerular filtration barrier (fenestrated endothelium and GBM); however, there is well known evidence of antibody delivery to the podocyte in the passive Heymann nephritis model. Macromolecules often require specific cell entry mechanisms to reach intracellular targets and it is notable that podocytes also have a prominent receptor dependent mechanism for uptake of macromolecules including albumin and immunoglobulins. Hauser *et al* demonstrated a novel delivery technique in which a sheep anti-mouse IgG that recognizes an unknown podocyte antigen is used to deliver small interfering RNA (siRNA) to the cell.⁵⁵ The IgG-siRNA complex was endocytosed into the podocyte and suppression of gene expression was confirmed.

SUMMARY

The podocyte, although located on the periphery of the glomerular capillary, is now central to the understanding of proteinuria and progressive kidney disease. The future for novel therapies, potentially targeted directly at podocyte biology, is bright!

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Abbreviations

GBM	glomerular basement membrane
VEGF	vascular endothelial growth factor
PDGF	platelet-derived growth factor
MMPs	matrix metalloproteinases

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Box 1

Major functions of the Podocyte

- Structural support of glomerular capillary loop
 - ◇ Prominent actin cytoskeleton
 - ◇ Mechano-sensing by slit diaphragm complex
- Synthesis and repair of GBM
 - ◇ Synthesis of $\alpha3\alpha4\alpha5$ network of type IV collagen
 - ◇ Remodeling of GBM by MMPs
- Major component of glomerular filtration barrier
 - ◇ Podocyte slit diaphragm outermost component of glomerular filtration barrier
- Cross talk with other glomerular cells
 - ◇ VEGF maintains glomerular endothelium
 - ◇ PDGF directs mesangial cells into developing glomerulus
- Immunological Functions
 - ◇ Expression of Toll-like receptor 4

Figure 1.

Glomerular capillary wall. This acts as the glomerular filtration barrier preventing proteins and large molecules from passing from the capillary lumen into the urinary space. The podocyte cell body lies with the urinary space, and the cell is attached to the GBM via the foot process. Adjacent foot processes are separated by the filtration slit, bridged by the slit diaphragm. Disruption of the glomerular filtration barrier leads to proteinuria.

Figure 2.

Podocyte and glomerular capillary. Electron micrograph showing glomerular capillary loops, one containing a red blood cell (RBC), with the glomerular basement membrane (GBM) lined by the glomerular endothelial cell (GEN) and the podocyte cell body (Podo) lying in the urinary space extending foot processes (FP) to the outer surface of two adjacent capillary loops.

Figure 3. Foot processes and slit diaphragm. The podocyte has a prominent actin cytoskeleton, stabilized by synaptopodin and α -actinin-4, and linked to the slit diaphragm via CD2AP. The foot processes are bound to the glomerular basement membrane via integrins (α 3 β 1, α V β 3) and α - and β -dystroglycans (DG). The major component of the slit diaphragm is nephrin, which bridges the filtration slit and is closely associated with several other proteins including podocin and CD2AP.

Table 1

Clinical Disorders of the Podocyte.

Clinical Presentation	Pathophysiology	Etiology
Proteinuria & Nephrotic Syndrome	Disruption of glomerular filtration barrier	Primary Kidney Disease: MCD, FSGS, membranous nephropathy, MPGN Systemic Disorders: diabetic kidney disease, amyloidosis, lupus (membranous), Fabry disease
Chronic Glomerulonephritis	Failure to synthesize structurally normal GBM	Type IV collagenopathies (Alport syndrome, thin basement membrane nephropathy) Nail patella syndrome Other hereditary nephropathies
Thrombotic microangiopathy	Blockade of podocyte derived VEGF	Pre-eclampsia, VEGF inhibitors (eg, bevacizumab)

Abbreviations: FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; MCD, minimal change disease; MPGN, membranoproliferative glomerulonephritis; VEGF, vascular endothelial growth factor.