

Podocyte directed therapy of nephrotic syndrome—can we bring the inside out?

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Abstract Several of the drugs currently used for the treatment of glomerular diseases are prescribed for their immunotherapeutic or anti-inflammatory properties, based on the current understanding that glomerular diseases are mediated by immune responses. In recent years our understanding of podocytic signalling pathways and the crucial role of genetic predispositions in the pathology of glomerular diseases has broadened. Delineation of those signalling pathways supports the hypothesis that several of the medications and immunosuppressive agents used to treat glomerular diseases directly target glomerular podocytes. Several central downstream signalling pathways merge into regulatory pathways of the podocytic actin cytoskeleton and its connection to the slit diaphragm. The slit diaphragm and the cytoskeleton of the foot process represent a functional unit. A breakdown of the cytoskeletal backbone of the foot processes leads to internalization of slit diaphragm molecules, and internalization of slit diaphragm components in turn negatively affects cytoskeletal signalling pathways. Podocytes display a remarkable ability to recover from complete effacement and to re-form interdigitating foot processes and intact slit diaphragms after pharmacological intervention. This ability indicates an active inside-out signalling machinery which stabilizes integrin complex formations and triggers the recycling of slit diaphragm molecules from intracellular compartments to the cell surface. In this review we summarize current evidence from patient studies and model organisms on the direct impact of immunosuppressive and supportive drugs on podocyte signalling

pathways. We highlight new therapeutic targets that may open novel opportunities to enhance and stabilize inside-out pathways in podocytes.

Keywords Podocyte · Actin cytoskeleton · Treatment · Nephrotic syndrome · Foot process effacement

Introduction

Proper podocyte function and structure plays an important role in determining the integrity of the glomerular filtration barrier. Podocytes are highly differentiated epithelial cells with interdigitating foot processes that form a network on the outer surface of the filter slit diaphragms connecting neighbouring foot processes. Podocytes are terminally differentiated cells with no regenerative capacity. Therefore, a reduction in podocyte number beyond a critical threshold leads to glomerular disease progression in experimental rodent models [1, 2]. However, as long as the podocytes are just effaced and not lost, they display a remarkable capacity to recover foot processes within a short period of time, as occurs in minimal change disease, the classical example of the potential of podocytes to recover. However, effacement caused by other diseases also has the capability to recover, as described in a recent case of nephrotic range proteinuria following a Hantavirus infection which completely resolved within a few weeks [3].

Ultrastructural findings in this case report [3] document the cytoskeletal phenotype of foot process effacement as well as a redistribution of slit diaphragm proteins to the intracellular compartment of podocytes. This process clearly indicates that slit diaphragm components are not degraded immediately after internalization but may be transferred to intracellular storage compartments from where they can be recycled from the inside back out to the cell surface. Transient proteinuria

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models in rodents, such as the protamine sulphate model in rats and the lipopolysaccharide (LPS) model in mice display rapid but reversible foot process effacement and proteinuria. Even more striking, PS infusions in rodents can result in the fusion of podocyte foot processes, and this phenotype is reversible within minutes after infusion of heparin [4, 5]. Therefore, experimental evidence also suggests that a molecular machinery in podocytes exists to internalize slit diaphragm components and recycle them rapidly back to the cell surface. Most likely, as we recently demonstrated, those mechanisms are orchestrated by posttranslational modifications [6]. The targeting or supporting of these “inside-out” pathways could be a novel anti-proteinuric and podocyte-specific treatment concept. Other mechanisms of recovery involve glomerular cell replacement from stem cells. A glomerular stem cell niche was recently described which implies that lost podocytes could be replaced by cells from the parietal layer [7–9]. Whether this concept is only part of a physiological replenishment or holds true in disease states is a controversial discussion since parietal epithelial cells are clearly involved in the disease process itself [10–13].

In this review we summarize current evidence supporting the hypothesis that the established treatment regimens for nephrotic syndromes might be interfering directly with cellular pathways that stabilize and support the recovery of the actin cytoskeleton and the slit diaphragm of podocytes. If this hypothesis were to be valid, drugs commonly used in the clinic to treat our patients would have to be put into a new context, and there would be a need for novel and cell-specific therapeutic approaches since it most likely would be unnecessary to target the general immune system and accept the systemic side effects of immunosuppressive drugs.

The podocyte cytoskeleton plays a key role in proper glomerular function

A central aspect of studies in podocyte cell biology, which involves convergence of several signalling pathways of the deregulated immune system, is the actin cytoskeleton.

Podocyte foot processes consist of cortical actin filaments and actin-associated proteins, such as myosin, α -actinin and synaptopodin, which ensure the dynamic maintenance and reorganization of the cytoskeleton. The important role of the podocyte actin cytoskeleton and podocyte–glomerular basement membrane (GBM) interactions for the development of foot process effacement is supported by several genetic loss- or gain-of-function models affecting the cytoskeleton and recapitulating genetic human diseases [14–19]. It would therefore seem reasonable to search for supportive therapies that would directly interfere with pathways regulating slit diaphragm protein expression and cytoskeletal pathways that activate or support the recovery potential of stressed podocytes.

In the following section we discuss widely subscribed drugs which have direct positive or negative (side-) effects on podocyte structures. Table 1 summarizes current knowledge.

Commonly prescribed drugs directly targeting the podocyte

Renin–angiotensin–aldosterone system blockers

The renin–angiotensin–aldosterone (RAAS) system plays a crucial role in kidney disease. The major effector molecule of the RAAS is angiotensin II that mediates its function through angiotensin II type-1 and type-2 receptor (AT1-R and AT2-R). Immunofluorescence studies showed that both AT1-R and AT2-R are expressed in podocytes and that their expression is elevated in the proteinuric state [20]. Angiotensin II depolarizes podocytes directly through the opening of chloride ion channels and the resulting increased chloride conductance. The activation of chloride ion conductance is mediated by an AT1-R [21]. Angiotensin II infusion has been shown to induce proteinuria independent of any pressure effects [22] and to induce reorganization of the actin cytoskeleton and increase intracellular cAMP in cultured glomerular epithelial cells [23]. Durvasula et al. reported that exposure to cyclic stretch increased both the production of endogenous angiotensin II and the expression of AT1-R in cultured rat podocytes, and in vivo after 5/6 nephrectomy in rats [24]. In another study, the tissue RAAS of the kidneys was activated in diabetic nephropathy and immortalized murine podocytes, with higher concentrations of angiotensinogen, angiotensin II and AT1-R expressed under high glucose conditions [25].

RAAS blockers have beneficial effects on proteinuria and progression to renal failure. Lewis et al. showed a renoprotective effect of angiotensin-converting enzyme (ACE) inhibitors in type 1 diabetes [26]. RAAS blockers also have beneficial renal effects in type II diabetes [27]. The ACE inhibition in the Progressive Renal Insufficiency (AIPRI) study demonstrated that ACE inhibitors are able to delay the loss of renal function in non-diabetic patients.

The anti-proteinuric effect of RAAS blockers is primarily thought to be mediated by a reduction in glomerular perfusion pressure. However, growing evidence suggests that RAAS inhibition might also act on the podocyte cytoskeleton and on components of the glomerular slit diaphragm directly. In podocytes RAAS inhibition results in decreased cell tonus, rearrangement of the actin cytoskeleton and decreased rate of apoptosis and protein leakage [24]. A direct effect of RAAS inhibition on the slit diaphragm is that it prevents redistribution of zona occludens 1 (ZO-1) in rats [28] and restores the expression of nephrin in experimental models of diabetic nephropathy [29]. Both of these proteins are important components for the proper filtration functioning of the glomerulus

Table 1 (Side-)effects and podocytic target structures of commonly used drugs

Currently used drugs	Effects on podocytes	Target structures/pathways in podocytes
Cyclosporine A (calcineurin inhibitor)	Positive	Synaptopodin–integrin interaction, actin cytoskeleton [41]
Doxorubicin (anthracycline antibiotic)	Negative	Actin cytoskeleton [72]
Everolimus, sirolimus (mTOR inhibitors)	Positive (diabetes) [55]/ negative (other glomerular disease) [56, 57]	Actin cytoskeleton, VEGF [53, 61], synaptopodin, podocin, nephrin, apoptosis [60]
Glitazones (PPAR γ agonists)	Positive	SUMOs [75], mitochondrial function [73]
Glucocorticoids	Positive	Glucocorticoid receptor, gene expression, actin rearrangement [34–36]
RAAS blockade (ACE inhibitor, AT1-R blocker)	Positive	AT1-R, actin cytoskeleton [23, 24], ZO-1 [28]
Rituximab (CD20 antibody)	Positive	SMPDL-3b, actin cytoskeleton [50, 51]
Statins (HMG-CoA reductase inhibitors)	Positive/ uncertain	Mitochondrial function [65], β -integrin [66, 67]
VEGF-R blocker	Positive (diabetes) [86, 87], negative (other glomerular disease) [80, 81, 88, 89]	Apoptosis, podocyte effacement, nephrin [79]

PPAR γ , peroxisome proliferator-activated receptor gamma; RAAS, renin–angiotensin–aldosterone system; ACE, angiotensin-converting enzyme; AT1-R, angiotensin II type 1 receptor; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA lyase reductase; VEGF-R, vascular endothelial growth factor receptors; SMPDL-3b, sphingomyelin phosphodiesterase, acid-like 3B

(Fig. 1). The selective aldosterone blocker eplerenone also ameliorates podocyte injury, proteinuria and salt-evoked nephropathy [30, 31].

Glucocorticoids

The current paradigm is that glucocorticoids act in glomerular disease by reducing the release of pathogenic factors of circulating T lymphocytes that are thought to be one of the major causes of some forms of idiopathic nephrotic syndromes. Glucocorticoids bind to the cytoplasmic glucocorticoid receptor that is translocated to the nucleus after dimerization. In the nucleus glucocorticoids bind to glucocorticoid response elements on the DNA or interact with other transcription factors [32]. However, glucocorticoids might have important direct effects on the cells as well (Fig. 1). Human podocytes also express the glucocorticoid receptors [33] and in vitro, dexamethasone treatment protects podocytes from puromycin aminonucleoside (PAN) injury by inhibiting actin filament disruption and PAN-induced apoptosis [34, 35]. Similar to the PAN model, dexamethasone can also rescue podocytes from adriamycin-induced actin rearrangement by stabilizing the expression of α -actinin-4 [36].

In a differential proteomic analysis of dexamethasone-treated cultured murine podocytes ciliary neurotrophic factor, α B-crystallin and heat shock protein 27 were upregulated by dexamethasone. These three proteins play a well-known role in protecting cells from injury, and current data suggest a direct effect of steroids on podocytes in nephrotic diseases [34]. Most of the in vivo studies of experimental nephrotic syndrome induced in rats by PAN-injection performed to date have demonstrated that glucocorticoid treatment reduces proteinuria and attenuates podocyte foot process effacement [37, 38]. In humans, glucocorticoid therapy remains the primary treatment option for nephrotic syndrome, and in many cases of minimal change disease, steroid treatment induces remission and restoration of the slit diaphragm architecture, leading to the term “steroid-sensitive nephrotic syndrome” [39]. Steroid-resistant cases usually receive combination treatments with addition of calcineurin inhibitors (CNIs).

Calcineurin inhibitors

Calcineurin inhibitors (CNIs), such as cyclosporine A or Fk506, lower the activity of T cells. The immunosuppressive actions of cyclosporine were originally attributed to the dephosphorylation of the nuclear factor of activated T cell (NFAT) family members (Fig. 2) as CNIs lead to nuclear translocation and activation of early genes of the T cell-driven immune response [40]. However, Faul et al. showed that the beneficial effect of cyclosporine A on proteinuria is not dependent on NFAT inhibition in T cells. In podocytes, calcineurin dephosphorylates synaptopodin and makes it more susceptible

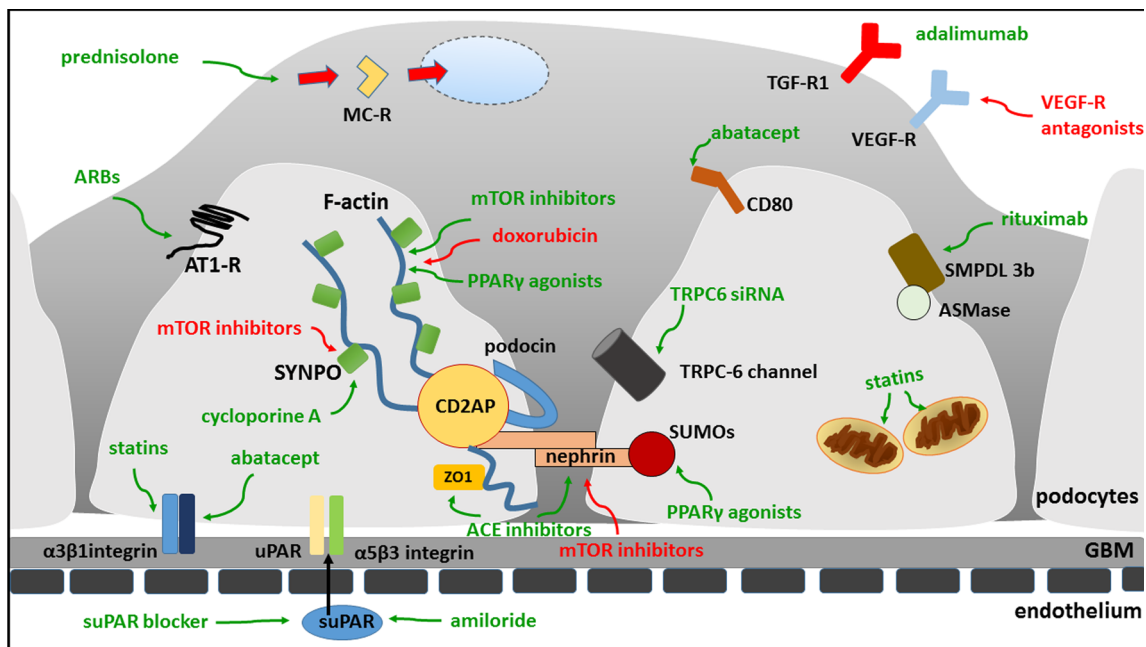


Fig. 1 Illustration of different drug targets in the podocyte. Positive effects of drugs on podocyte function are shown in green and negative effects of drugs on podocyte function are shown in red. Please note that mammalian target of rapamycin (*mTOR*) inhibitors are mentioned in both colours as they have pro-survival effects in diabetic nephropathy but can cause negative side effects on podocytes in other clinical settings. Here *mTOR* inhibitors can lead to proteinuria and podocyte apoptosis by decreasing the expression of synaptopodin, podocin and nephrin. *ARBs* Angiotensin receptor blocker, *ASMase* acid sphingomyelinase, *AT1-R*

angiotensin 1 receptor, *CD2AP* CD2-associated protein, *CTLA-4* cytotoxic T lymphocyte-associated protein 4, *MC-R* mineralocorticoid receptor, *PPARγ* peroxisome proliferator-activated receptors γ , *SMPDL 3b* sphingomyelin phosphodiesterase acid-like 3b protein, *SUMOs* small ubiquitin-like modifiers, *SYNPO* synaptopodin, *suPAR* soluble urokinase plasminogen activator receptor, *TGF-R* transforming growth factor receptor, *TRPC-6* transient receptor potential cation channel 6, *VEGF-R* vascular endothelial growth factor receptor

to cathepsin L-mediated degradation. By inhibiting calcineurin, cyclosporine A stabilizes the actin cytoskeleton in podocytes

by preserving the phosphorylation-dependent synaptopodin-14-3-3 β integrin interaction [41] (Fig. 1). In addition to the

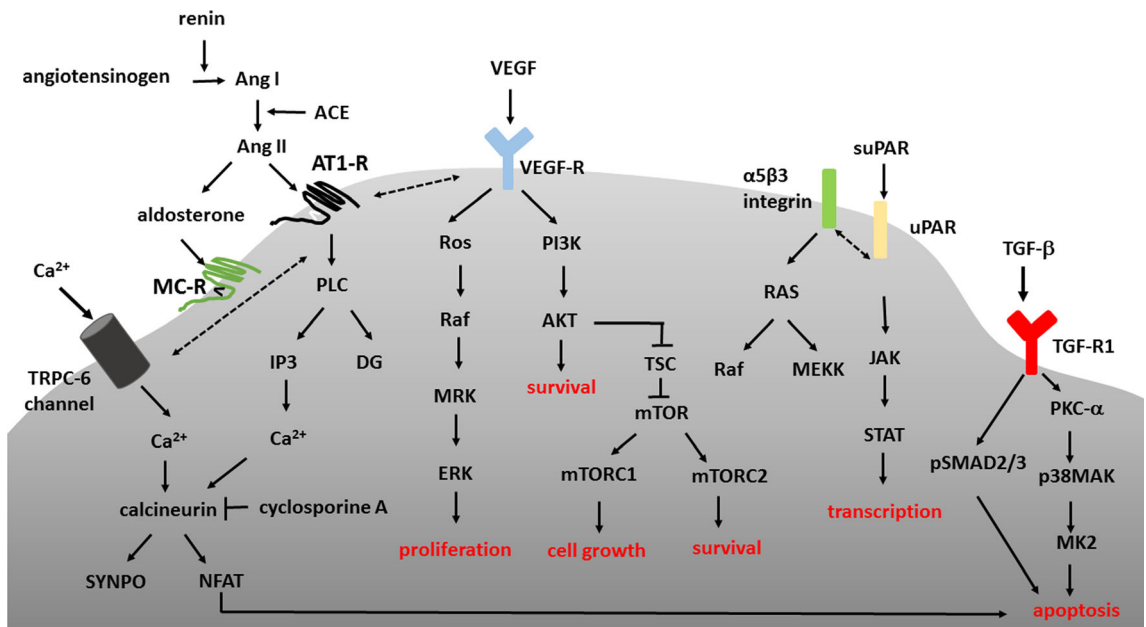


Fig. 2 Signalling pathways activated by different drugs in podocytes. *AKT* Protein kinase B, *Ang I/II* angiotensin I/II, *DG* diacylglycerol, *ERK* extracellular signal-regulated kinases, *IP3* inositol triphosphate 3, *JAK* janus kinase, *MAK* mitogen-activated protein kinases, *NFAT* nuclear

factor of activated T-cells, *PI3K* phosphatidylinositol-4,5-bisphosphate 3-kinase, *PKC-α* protein kinase C alpha, *PLC* phospholipase C, *STAT* signal transducer and activator of transcription, *TSC* tuberous sclerosis complex. For other abbreviations, see caption to Fig. 1

effectiveness in glomerular diseases that are thought to be immunologically mediated, CNIs are also used to reduce proteinuria in Alport syndrome, which is a clear non-immunological disease caused by mutations of the type IV collagen of the GBM [42–44], further supporting the notion of additional CNI-mediated effects independent of T cells. Additional evidence derives from studies in children with genetic podocytopathies. Here cyclosporine A was demonstrated to have an anti-proteinuric effect in cases with mutations in the WT-1, podocin and phospholipase C epsilon genes [45–47].

Rituximab

Rituximab is a monoclonal antibody directed against the CD20 receptor expressed on B lymphocytes but has also been demonstrated to be effective in glomerular diseases that are not related to B cells, such as recurrent focal segmental glomerulosclerosis (FSGS) after transplantation [48]. As recently reported by Ruggenti et al., rituximab can also reduce the number of relapses in patients with minimal change disease (MCD) and FSGS [49]. Interestingly, rituximab binds not only to CD20 but also to other molecules, including sphingomyelin phosphodiesterase acid-like 3b protein (SMPDL-3b), in immune cells [50]. Interestingly, the authors of a recent study suggested that rituximab controls actin cytoskeleton remodelling in podocytes through the stabilization of sphingolipid-related enzymes [51] (Fig. 1). In this study, SMPDL-3b downregulation after exposure to FSGS patient sera rendered podocytes more susceptible to actin remodelling, and rituximab partially preserved the disruption of stress fibers through the stabilization of SMPDL-3b [51].

Mammalian target of rapamycin inhibitors

Mammalian target of rapamycin (mTOR) is a serine–threonine kinase, which controls cell growth and metabolism. mTOR also has a central role in cell survival. It mediates its functions through mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) as well as through regulatory-associated protein of mTOR (Raptor) and rapamycin-insensitive companion of mTOR (Rictor) [52]. Moreover, mTOR regulates vascular endothelial growth factor (VEGF) that is essential for podocyte survival through autocrine and paracrine pathways [53]. Current data on the function of mTOR inhibitors in renal disease are conflicting.

It has been shown that hyperactivation of the mTOR pathway in diabetic nephropathy plays a pivotal role in the hypertrophy of glomerular cells and is associated with podocyte injury and the progressive decline of glomerular filtration rates [54] and that inhibition of the mTORC1 pathway with rapamycin has reno-protective effects on the progression of diabetic nephropathy [55]. However, the induction of proteinuria after treatment with mTOR inhibitors, such as sirolimus

and everolimus, is a typical side effect [56, 57]. In a knock-out model Raptor deletion induced proteinuria and glomerular lesions in mice, and concomitant Rictor deletion exacerbated this phenotype [52, 58, 59]. Sirolimus has also been shown to reduce the expression of synaptopodin, podocin and nephrin and in addition to increase podocyte apoptosis [60]. VEGF expression was reduced in the glomeruli of patients with sirolimus-induced thrombotic microangiopathy [61]. Thus, mTOR inhibitors seem to have direct effects on podocyte integrity. However, on the one hand mTOR inhibitors have the side effect of proteinuria; on the other hand they have been shown to have beneficial effects in diabetic nephropathy. The conflicting data of mTOR regarding glomerular function might indicate that a delicate balance of mTOR and/or subtype-specific receptor activity is necessary for proper podocyte function. In this regard mTOR function seems to be similar to VEGF function (see below) that also requires an orchestrated activity in the glomerulus. To date it is not clear whether there is a specific mTOR pathway that should be targeted depending on the disease that should be treated. More studies on mTOR subtype-specific functions are needed.

3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors—also known as statins—are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase that plays a central role in the production of cholesterol in the liver. HMG-CoA reductase inhibitors have been shown to decrease the rate of decline of kidney function and to reduce proteinuria in patients with chronic kidney disease stages I–III [62, 63]. These effects are independent of serum lipid levels, implicating pleiotropic effects of statins in the kidney [64]. In addition to their general effect on lipid metabolism, statins seem to have beneficial effects on mitochondrial function in podocytes. Rosuvastatin attenuated angiotensin II-dependent increases in NADPH oxidase activity and reactive oxygen species (ROS) generation in cultured podocytes [65]. In line with these findings are reports that fluvastatin is able to attenuate injury induced by PAN and to increase the production of β 1-integrin in human podocytes in vitro, which is thought to be an ROS activity-inhibiting mechanism [66, 67]. Rosuvastatin has also been found to display pro-survival activities in injured podocytes through a p21-dependent anti-apoptotic pathway [68].

Peroxisome proliferator-activated receptors γ agonists

Thiazolidinediones (TZDs)—also known as glitazones—are anti-diabetic agents which activate the peroxisome proliferator-activated receptors γ (PPAR γ), leading to

modified gene transcription that improves insulin resistance, among others. Growing evidence suggests that TZDs also may affect PPAR γ -independent pathways in glomerular disease. TZDs have been shown to reduce podocyte injury and proteinuria in diabetic nephropathy [69, 70]. In two studies, the development of PAN-induced glomerulosclerosis could be ameliorated by pioglitazone which also reduced apoptosis and necrosis in cultured podocytes [70, 71]. Similar, rosiglitazone was observed to attenuate the development of proteinuria and glomerulosclerosis in doxorubicin-induced FSGS in rats [72]. Rosiglitazone is able to protect podocytes against damage caused by mitochondrial dysfunction [73]. Moreover, TZDs can prevent actin filament redistribution induced by PAN [74] and directly influence expression of small ubiquitin-like modifiers (SUMOs) [75], which we recently demonstrated are essential for the cellular surface expression of nephrin in podocytes (Fig. 1) [6].

Thus, PPAR γ agonists do not only lower blood glucose levels in diabetes but are also beneficial in diabetic nephropathy by improving podocyte mitochondrial function, slit diaphragm integrity and stability of the actin cytoskeleton.

VEGF inhibitors

Podocytes are the major source of VEGF production in the glomerulus. We and others have shown that podocytes express different VEGF isoforms and also VEGF receptors (VEGF-R) [76, 77]. Furthermore, VEGF-A and VEGF-C have an important autocrine role in the podocyte, and VEGF inhibition leads to the activation of pro-apoptotic pathways in cultured human podocytes [77]. Podocyte-specific heterozygous and homozygous deletions of VEGF results in proteinuria and endotheliosis in mice [78]. VEGF also has direct beneficial effects on podocyte survival independent of nephrin expression [79]. An endogenous condition when VEGF-A levels are reduced is preeclampsia. Here a soluble VEGF-R1 (sFLT-1) produced by the placenta blocks VEGF in the circulation and causes proteinuria, hypertension and endotheliosis [80, 81]. Serum from women with preeclampsia and with high sFLT-1 concentrations can directly alter podocyte structure and function whereas supplementing these sera with exogenous VEGF can directly reverse its effects [82]. These studies clearly indicate that podocyte function depends on a certain level of autocrine VEGF-R activation. The delivery of VEGF₁₂₁ via the tail vein normalizes VEGF levels and prevents the late-gestational spike in blood pressure and proteinuria in a murine model for preeclampsia [83]. In a rat model for preeclampsia the treatment with VEGF₁₂₁ reverses hypertension, proteinuria and glomerular endotheliosis [84]. To our knowledge, VEGF-A treatment has not yet been used in humans to treat proteinuria. However, because of the negative effects seen in diabetic nephropathy (see below) more work has to be done to

elucidate more clearly the precise tuning of VEGF amount and different isoforms before VEGF infusion can be tried in patients. Based on our own work treatment with VEGF-C could be a short-term alternative [77].

The levels of VEGF, while downregulated in preeclampsia VEGF levels, are upregulated in diabetic nephropathy. Moreover, podocyte VEGF₁₆₄ overexpression in mice is sufficient to induce structural and functional abnormalities in the glomerular filtration barrier similar to those in diabetic nephropathy [85]. Anti-VEGF antibodies reduce the severity of diabetic nephropathy in rodents [86, 87], whereas they can induce hypertension, proteinuria and glomerular disease in human patients [88]. Anti-VEGF strategies that either block the extracellular binding of VEGF to its receptor (anti-VEGF antibodies) or inhibit intracellular signalling pathways of VEGF-R (receptor tyrosine kinase inhibitors) are widely used in the clinical setting to inhibit angiogenesis in different types of cancer. In patients treatment with VEGF inhibitors can lead to podocyte effacement. Our group was the first to describe podocyturia (loss of podocytes in the urine) in patients receiving VEGF ablation therapy with bevacizumab or sunitinib [89].

Proteinuria recovery following the discontinuation of VEGF ablation therapy indicates the direct beneficial effect of this treatment and underlines the remarkable recovery potential of podocytes.

The conflicting data on the VEGF system in different species and different glomerular diseases suggest that a dynamic and context-related balance of VEGF levels and isoforms is necessary to maintain proper glomerular function. Consequently, this molecule is a difficult potential drug target.

New strategies to directly target the podocyte

Notch inactivation

Therapies based on newly identified podocyte proteins and signalling pathways might be the future for the treatment of glomerular disease. For example, inactivation of Notch signalling pathways in podocytes, which promotes apoptosis, could be a promising target pathway to ameliorate the damage caused by glomerular disease [90]. A major problem with this therapeutic strategy is that many of the pathways which are upregulated or reactivated in glomerular disease might be essential for maintaining homeostasis in other tissues. Therefore, a more podocyte-directed therapy is highly desirable. In the following sections we present new therapeutic strategies by directly targeting the podocyte. Table 2 summarizes the effects of new anti-proteinuric strategies that potentially target podocyte structures and pathways.

Table 2 Effects and target structures of new anti-proteinuric strategies on podocytes

New anti-proteinuric strategies	Target structures/pathways on podocytes
Abatacept (CTLA-4-Ig)	B7-1, integrin signalling [4, 93, 96]
Adalimumab (Anti-TGF- β)	TGF- β /SMAD, apoptosis [111]
Amiloride (suPAR blocker)	β 3-integrin signalling [99, 100]
Oral ManNAc	Angptl 4 [116]
Ruboxistaurin (selective PKC- β inhibitor)	(Among others) extracellular matrix synthesis/turnover [120, 121]
Saquinavir (protease inhibitor)	NF- κ B/I κ B α [122, 123]
TRPC6 siRNA	Ca ²⁺ levels, actin cytoskeleton rearrangement [107–110]

Ig, Immunoglobulin; TGF- β , transforming growth factor beta, MANNAc, *N*-acetyl-D-mannosamine; protein kinase C beta; TRPC6, transient receptor potential cation channel, subfamily C, member 6; siRNA, small interfering RNA; Angptl 4, angiopoietin-like 4; NF- κ B/I κ B α , nuclear factor kappa-light-chain-enhancer of activated B cells/inhibitor of kappa B

Abatacept

Abatacept (CTLA-4-Ig) consists of a fusion protein of the extracellular domain of CTLA-4 and human immunoglobulin (Ig) G1. CTLA-4 transmits an inhibitory signal to T cells and through its binding to CD80 (also called B7-1) on antigen-presenting cells, it prevents the delivery of the co-stimulatory signal to the T cell [91, 92]. Abatacept is used in glomerular disease to suppress the T cell activity that is thought to be a major cause in some of these diseases. For example, MCD is commonly thought to be mediated by a circulating factor released by T cells. However, a couple of years ago an unanticipated novel role for CD80 in podocytes as an inducible modifier of glomerular permselectivity was described [4]. While CD80 expression is absent in normal podocytes, CD80 was found to be induced in podocytes in various animal models of proteinuria and was detected in podocytes from patients with glomerular diseases.

Increased podocytic CD80 expression has been found in genetic, drug-induced, immune-mediated and bacterial toxin-induced experimental nephrotic syndrome [4]. The exposure of podocytes to LPS rapidly unregulate CD80 in mice and leads to actin reorganization and nephrotic-range proteinuria, whereas mice lacking CD80 are protected from this damage [4]. Sera from MCD patients in relapse, but not in remission, were found to stimulate CD80 expression in cultured podocytes [93]. In addition, urinary concentrations of soluble CD80 in patients with relapsed MCD were significantly higher than those in patients with MCD in remission and with other glomerular diseases including FSGS and in healthy controls [94]. Serum and urinary CTLA-4 levels tend to be low in MCD patients in relapse, and the urinary CTLA-4 level returns to higher levels during remission [95]. Yu et al. described the use of abatacept in inducing remission in five patients with FSGS based on positive staining for CD80 in glomerular podocytes, reporting a clinically significant reduction in proteinuria in all patients [96]. However, the validity of these findings were questioned by other researchers [97, 98]. An ongoing clinical phase III trial that is evaluating abatacept for the treatment of lupus class III

and IV is also analysing the outcome of proteinuria (<https://clinicaltrials.gov/ct2/show/NCT01714817>).

Soluble form of the urokinase plasminogen activator receptor blockers

Another potential therapeutic option might be the soluble form of the urokinase plasminogen activator receptor (suPAR) blocker. SuPAR secretion by T lymphocytes has been suggested to be a “permeability factor” in the pathogenesis of FSGS. It binds to α 5 β 3 integrin in the podocyte membrane, thereby leading to podocyte contraction and loss of podocytes from the GBM [99, 100]. Amiloride inhibits the synthesis of uPAR and suPAR secretion by T lymphocytes and thereby decreases α 5 β 3 integrin activation [101, 102].

However, the role of suPAR in glomerular disease has been questioned in recent years. Even though experimental data suggest that suPAR therapy may also cause proteinuria and FSGS by alternated β 3-integrin signalling, these findings cannot be easily translated to routine clinical care [103]. Higher serum or plasma suPAR levels have also been demonstrated in patients with other diseases, such as cancer, sepsis and atherosclerosis [104, 105]. Future studies are ongoing to specifically reduce suPAR levels in patients with primary or recurrent FSGS.

Transient receptor potential cation channel 6 small interfering RNA

Another exciting concept is the direct and specific targeting of therapeutic agents to the podocyte, thereby limiting systemic adverse effects. One such example has been described for the transient receptor potential cation channel 6 (TRPC6). TRPCs are non-selective cationic channels which play a major role in chemo- and mechanosensation. In podocytes, TRPC6 is a slit diaphragm-interacting protein [106] which may regulate changes in calcium ion (Ca²⁺) levels and in actin cytoskeleton rearrangement [107]. Overexpression of TRPC6 in mice

induces glomerular disease [108]. In humans gain-of-function mutations in TRPC6 have been associated with familial forms of FSGS [109]. Novel therapeutic options might involve small interfering RNAs (siRNAs) coupled with a podocyte-specific delivery system. A TRPC6 siRNA coupled with a sheep anti-mouse podocyte antibody has already been shown to reduce TRPC6 protein expression in podocytes: the IgG–siRNA complex was endocytosed into the podocyte and interfered with protein expression [110]. To date, there has been no clinical trial with TRPC6 siRNA.

Anti-transforming growth factor beta therapy

Transforming growth factor beta-1 (TGF- β 1) and Smad7 induce apoptosis in podocytes through different downstream pathways leading to podocyte depletion and progressive glomerulosclerosis [111]. In recent years case reports have shown a reduction of proteinuria in patients receiving anti-TGF- β therapy. TNF- α blockade was successfully used to treat nephrotic syndrome in a patient with AA amyloid due to TNF receptor-associated periodic syndrome [112], and complete reversal of nephrotic syndrome secondary to amyloidosis was observed in a patient with inflammatory bowel disease and ankylosing spondylitis after treatment with infliximab (anti-TNF- α antibody) [113]. In a small study with 15 patients with renal amyloidosis, anti-TNF therapy had positive effects on the reduction of proteinuria in some patients [114]. Alternatively, Stokes et al. reported five patients with long-term rheumatoid arthritis who developed new-onset glomerular disease while receiving TNF- α antagonist. The authors suggested an induction of rheumatoid arthritis-related nephropathy or de novo autoimmune disorders due to the anti-TNF therapy [115].

The aim of a phase II trial carried out between 2008 and 2014 was to test whether adalimumab and/or galactose can safely reduce proteinuria and protect kidney function better than standard treatment for patients with FSGS (<https://clinicaltrials.gov/ct2/show/NCT00814255>). The study has been completed, but the results are not yet available.

Oral *N*-acetyl-D-mannosamine

Sialic acids are essential for a variety of cellular functions, including cell adhesion and signal recognition as well as the formation and progression of tumors. Disruption of sialic acids can result in severe proteinuria. *N*-acetyl-D-mannosamine (ManNAc) is the precursor of all physiological sialic acids. The discovery of a central, mechanistic role played by two different forms of angiotensin-like 4 (Angptl 4) in human and experimental glomerular disease has opened new treatment avenues. Localized upregulation of a hyposialylated form of glycoprotein Angptl 4 secreted by podocytes induces the cardinal features of human MCD, suggesting that glycoprotein Angptl 4 upregulation is a

significant contributor toward proteinuria in experimental diabetic nephropathy. Oral treatment with ManNAc improves sialylation of Angptl 4 in vivo and reduces proteinuria by over 40 % [116]. Oral ManNAc is currently being tested in two phase I clinical trial for the treatment of the rare disorder distal myopathy with rimmed vacuoles–hereditary inclusion body myopathy (<http://clinicaltrials.gov/NCT01236898> and NCT01359319) [117]. To our knowledge, there is no ongoing study involving ManNAc in patients with glomerular disease.

Isoform-specific protein kinase C inhibitors

Among various podocyte kinases, proper protein kinase C (PKC) signalling plays a critical role in podocyte function. Where some PKC isoforms are indispensable for proper glomerular development, others might be harmful to the glomerulus when activated in diabetes [118]. Moreover, a combined deletion of atypical PKC- λ/ι and atypical PKC- ζ isoforms in podocytes is associated with incorrectly positioned centrosomes and Golgi apparatus and mis-localized molecules of the slit diaphragm, leading to proteinuria [14]. Menne et al. reported that diabetic PKC- α isoform-null mice are resistant to albuminuria and glomerular upregulation of the VEGF system [119].

The lack of PKC- β can provide protection against diabetes-induced renal hypertrophy and glomerular hyperfiltration. [120]. The ability of isoform-specific PKC inhibitors to antagonize diabetes-induced glomerulonephropathy might be a new avenue for future therapeutic options. A selective PKC- β inhibitor (ruboxistaurin) has been used in clinical trials to determine its efficacy in diabetic macular edema, retinopathy, neuropathy, nephropathy and endothelial dysfunction in patients with type I or type II diabetes. The results of a phase II clinical trial suggest that ruboxistaurin can decrease the loss of glomerular filtration rate and proteinuria in diabetic patients who are already receiving as therapy the optimal RAAS blockade [121].

Protease inhibitors

It has been suggested that alterations in the NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells)/I κ B α (inhibitor of kappa B) regulatory feedback loop contribute to immunologic abnormalities observed in MCD [122]. NF- κ B is centrally regulated by the proteasome. Saquinavir is a protease inhibitor usually used to treat human immunodeficiency virus. In a small case series of patients with steroid-dependent and steroid-resistant nephrotic syndrome, the addition of saquinavir to therapy with other immunosuppressive drugs reduced proteinuria and had a steroid-sparing effect [123]. These results give hope to a new management strategy for nephrotic patients.

Conclusions

For years nephrologists have successfully used immunosuppressive therapy for the treatment of glomerular diseases, based on the rationale that many glomerular diseases are autoimmune-mediated. However, recent research has revealed that many of these drugs have direct effects on the podocyte - mostly through alterations of the actin cytoskeleton. Figures 1 and 2 summarize our current understanding of how drugs directly act on podocytes and inform the affected downstream signalling pathways. This information is of special importance because in some cases podocyte injury can be reversible, and the actin cytoskeleton has the ability to reorganize and restore interdigitating foot processes and recycle slit diaphragm proteins back to the cell surface.

The roles of the mTOR and the VEGF signalling pathways in this context are controversial. It now appears that both pathways require a fine-tuned, balanced baseline activation to maintain glomerular physiology. Thus, the question of whether parts of these signalling cascades can be safely targeted, and if so which parts, remains to be answered.

A close crosstalk between researchers and clinicians is of particular importance for the development of novel podocyte-specific drugs and for the identification of podocyte-specific drug targets in addition to those already widely used to treat glomerular disease, with the aim to minimize the general side effects of therapy in our patients.

Key points

1. A crucial structure for podocyte maintenance is the actin cytoskeleton.
2. It is apparent that many of the drugs currently used to treat glomerular diseases have direct effects on the podocytes and their cytoskeleton, but are still not podocyte-specific.
3. Some drugs have direct side effects on podocytes, causing proteinuria and loss of podocytes from the GBM.
4. The discovery of novel genes and signalling pathways involved in glomerular diseases will facilitate the future development of podocyte-specific drugs.

Multiple choice questions (answers are provided following the reference list)

1. Which of the following answers is correct
 - a) The mTOR pathway is inactivated in diabetes.
 - b) mTOR inhibitors can induce proteinuria.
 - c) mTOR inhibitors have only immune modulatory functions.
 - d) mTOR inhibitors bind to special receptors on the podocyte surface.
2. Which of the following proteins is neither a unit of the podocyte cytoskeleton nor has a direct interaction with it?
 - a) Actin
 - b) Synaptopodin
 - c) ZO-1
 - d) Nephlin
3. Which of the following is a podocyte-specific drug?
 - a) Cyclosporine
 - b) Prednisolone
 - c) Rituximab
 - d) mTOR inhibitors
 - e) None of the above
4. Which of the following statements is correct
 - a) Human podocytes express CD20.
 - b) Human podocytes do not express the angiotensin II type 1 receptor
 - c) Human podocytes express the glucocorticoid receptor
 - d) Human podocytes express the abatacept-binding partner under physiological conditions.
5. Which statement is correct?
 - a) VEGF inhibitors only have renal side effects on the glomerular endothelium
 - b) Bevacizumab has a protective effect on podocytes
 - c) VEGF inhibitors can lead to foot process effacement
 - d) VEGF receptors are only expressed on glomerular endothelial cells

Conflict of interest The authors declare no Conflict of interest.

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Answers

1. b
2. d
3. e
4. c
5. c