The podocyte as a direct target for treatment of glomerular disease?

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Mallipattu SK, He JC. The podocyte as a direct target for treatment of glomerular disease? Am J Physiol Renal Physiol 311: F46–F51, 2016. First published April 20, 2016; doi:10.1152/ajprenal.00184.2016.—The Centers for Disease Control and Prevention estimates more than 10% of adults in the United States, over 20 million Americans, have chronic kidney disease (CKD). A failure to maintain the glomerular filtration barrier directly contributes to the onset of CKD. The visceral epithelial cells, podocytes, are integral to the maintenance of this renal filtration barrier. Direct podocyte injury contributes to the onset and progression of glomerular diseases such as minimal change disease (MCD), focal segmental glomerular sclerosis (FSGS), diabetic nephropathy, and HIV-associated nephropathy (HIVAN). Since podocytes are terminally differentiated with minimal capacity to self-replicate, they are extremely sensitive to cell stress. Podocyte injury is integral to the pathogenesis of primary glomerulopathies. Since podocytes are terminally differentiated epithelial cells with minimal capacity to regenerate, they are exquisitely sensitive to cell stress. Podocyte injury is integral to the pathogenesis of primary glomerulopathies, such as focal segmental glomerular sclerosis (FSGS), minimal change disease (MCD), and membranous nephropathy. Although the inciting injury to the podocyte may vary between these glomerular diseases, the inevitable consequence of podocyte injury is actin cytoskeleton derangement, apical redistribution or loss of slit diaphragm proteins, and loss of structural integrity, leading to eventual foot process effacement and podocyte detachment or apoptosis. Regardless of the type of insult, the podocyte ultimately loses its functional capacity to maintain the glomerular filtration barrier, leading to progressive kidney disease. Consequently, therapies that prevent podocyte injury by enhancing their structural integrity, preventing their loss, or promoting their regeneration will have a major clinical impact in our management of glomerular disease (Table 1). The focus of this Perspectives article is to provide an overview of key therapeutic agents in which the podocyte serves as their primary target of action in the treatment of primary glomerulopathies.

Inhibiting Renin-Angiotensin System

In addition to the antihypertensive effects of renin-angiotensin system (RAS) inhibitors, angiotensin-converting enzyme inhibitors, and ANG II receptor blockers play a critical role in mitigating podocyte injury and proteinuria in glomerular disease. Recent studies demonstrated that mechanical strain on podocytes results in upregulation of ANG II type 1 receptor (AT₁R), with eventual foot process effacement and proteinuria (9, 22). In addition, podocyte-specific overexpression of AT₁R resulted in marked podocyte effacement and proteinuria without significant changes in systolic blood pressure (SBP) (9, 22). Consequently, the utilization of RAS inhibitors may have direct effects on the podocyte by inhibiting the effects mediated by AT₁R. For instance, administration of aldosterone antagonists, such as spironolactone, increased AKT phosphorylation, leading to actin stabilization in the podocyte with reduction in proteinuria in the Ren2 rat model of hypertensive nephropathy (55). This improvement in podocyte injury and proteinuria was independent of changes in SBP (55). Furthermore, direct renin inhibitors, such as aliskiren, attenuated proteinuria and oxidative stress, while improving nephrin expression in this Ren2 rat model, independent of AT₁R blockade (24, 56). Interestingly, aliskiren-induced podocyte recovery was similar to that of irbesartan, despite not having as pronounced an effect in SBP reduction (24, 56). Since the (pro)renin receptor is present in human podocytes and activation of the (pro)renin receptor triggers the generation of angiotensin peptides, as well as MAPK/ERK signaling, blockade of the (pro)renin receptor directly attenuates proteinuria in murine models of diabetic and hypertensive nephropathy, independent of changes in blood pressure (24). Interestingly, aliskiren reduced intracellular ANG II levels without inhibiting (pro)renin-induced MAPK/ERK signaling in cultured human podocytes, suggesting an independent pathway that can be targeted in RAS signaling (44). Nonetheless, the salutary benefits to the podocyte derived indirectly from the antihypertensive effects of inhibiting the RAS signaling cannot be
neglected. For instance, the addition of AT1R antagonists
Glucocorticoids (16, 20, 38, 51, 52, 57, 58)
Rat model (Ren2, transgenic), murine and human
podocytes

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inhibitors
Calcineurin
inhibitors (12, 45, 53)
Mouse model (LPS, transgenic mice), murine and human
podocytes

RAS inhibitors (24, 44, 55, 56)
Rat model (Ren2, transgenic), murine and human
podocytes

Table 1. Therapeutic agents with direct effects on the podocyte

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| Calcineurin inhibi-
| Refrences | Models | Molecular Target in Podocytes |
| (12, 45, 53)       | Mouse model (LPS, transgenic mice), murine and human podocytes | • Inhibit MAPK/ERK signaling |
|                   | Human podocytes, FSGS patient cohorts | • Increase RhoA activity with stabilization of actin cytoskeleton |
| Rituximab          | (15)       | Human podocytes, FSGS patient cohorts | • Reduces podocyte apoptosis |
| Abatacept          | (41, 59)   | Mouse model (LPS), human podocytes, FSGS patient cohorts | • Restore Bcl-2 expression |
| ACTH               | (5, 28, 39)| Rat model (PHN), pediatric cohorts with nephrotic syndrome | • Reduce p21, p53, IL-6, Vegf expression |
| Retinoic acid      | (21, 27, 30, 32, 37, 40, 50, 61–63) | Human podocytes, murine models (HIVAN, ADR, LPS) | • Restores synthesis of glycosylated nephrin |
|                    |            |        | • Restore ZO-1 expression |

**Glucocorticoids**

Glucocorticoids (GCs) remain the initial and primary

endothelial nitric oxide synthase signaling, contributing to
glomerular injury (1, 6). Taken together, inhibition of RAS
signaling in the podocyte at multiple levels can attenuate
podocyte injury and eventual loss by a wide range of mech-

anisms, which include enhancing actin stabilization and reduc-
ing oxidative stress under models of podocytopathy.

Glucocorticoids (GCs) remain the initial and primary

immunosuppressive therapy in the treatment of primary

glomerulopathies (49). In many instances, alternate immuno-

suppressives are evident in proinflammatory states (4, 42), GCs exhibit a therapeutic benefit in primary

glomerulopathies, such as MCD and FSGS, where a proin-

flammatory milieu is not readily apparent. In fact, the initial
treatment option for MCD is high-dose GC therapy and, in

many instances, alternate immunosuppressive therapy is
typically not considered until GC therapy has failed to be effective in patients (49).

GCs regulate gene expression by initially binding to the

glucocorticoid receptors (GR) in the cytoplasm, which subse-

quently undergo dimerization and translocation to the nucleus

and bind to glucocorticoid response elements on target genes. Previous studies have described the potential for GCs to have a direct effect on the podocyte by rearrangement of the actin cytoskeleton, inhibiting apoptosis and regulating protein trafficking of critical slit diaphragm proteins in murine and human podocytes (20, 38, 46, 51, 52, 57). In addition, GR, as well as the major components of GR complex, are expressed in human podocytes (20, 57, 58). Furthermore, GCs have also been implicated in ameliorating podocyte injury (38, 57) and improving podocyte survival (51) in murine and human cell culture models. Although, there is some evidence to suggest that GCs may have an anti-apoptotic effect by restoring Bcl-2 expression and reducing p53 levels in cultured podocytes treated with puromycin (51, 52), the mechanism mediating this process remains largely unexplored. In addition, others have demonstrated that GCs prevent podocyte cell death by down-regulating cyclin kinase inhibitor p21 and IL-6 levels in cell culture models of podocyte injury (57).

Vascular endothelial growth factor (VEGF) is predomin-
nantly expressed in podocytes in normal glomeruli. However, in diabetic nephropathy, VEGF is markedly upregulated in podocytes, and crosstalk with endothelial cells contributes to vasculogenesis and angiogenesis with subsequent vascular leakage and vasodilatation (3, 47, 57). In cultured human podocytes, treatment with GCs demonstrates a reduction in VEGF expression (57). However, others have demonstrated that targeting specific isoforms of VEGF is critical since complete inhibition of VEGF conversely leads to podocyte loss.
and proteinuria (11). In addition, VEGF-A and VEGF-C expression are essential for podocyte survival (35).

Endoplasmic reticulum (ER) stress can exacerbate podocyte injury (16). Specifically, ER stress leads to a failure in the synthesis of glycosylated nephrin, which is retained in the ER and fails to translocate to the podocyte membrane. By using a cell culture model of energy depletion, the authors provide evidence that GCs reverse podocyte injury by restoring the synthesis of glycosylated nephrin by stimulating production of ATP (16).

Despite several groups demonstrating that the podocyte may serve as a direct target of GCs, a majority of the studies have been conducted in cell culture systems. A recent study by Zhang et al. (60) demonstrated that administration of prednisone attenuated glomerulosclerosis in a proteinuric murine model of FSGS. The authors observed that prednisone reduced apoptosis and increased podocyte progenitors by activating ERK signaling. However, additional studies are required to validate these findings and to identify whether key signaling molecules are required to mediate the effects of GCs in the podocyte. Combined, these studies demonstrate that the podocyte serves as a direct target of GCs, independent of its immunomodulatory effects.

Calcineurin Inhibitors

Although GCs remain the initial treatment in patients with primary FSGS, only 25% of patients respond to initial therapy (8). In the GC nonresponders, the next step in management is calcineurin inhibitors, where 40–50% of patients are expected to achieve partial or complete remission (17). Calcineurin is a serine/threonine phosphatase that dephosphorylates nuclear factor of activated T-cell (NFAT), which leads to nuclear translocation and activation of T-cell-induced immune response (2). Although calcineurin inhibitors, such as cyclosporine A and tacrolimus, exhibit an immunosuppressive effect by inhibiting NFAT signaling in T cells, recent studies have determined that their therapeutic benefit may extend beyond immune cells.

Calcineurin is expressed in podocytes, and calcineurin inhibitors have been demonstrated to ameliorate podocyte injury by restoring the expression of podocyte-specific proteins, such as zonula occludens-1 and synaptopodin (12, 25). Faul et al. (12) determined that the beneficial effects of cyclosporin A in proteinuric murine models is directly related to stabilization of actin cytoskeleton in the podocyte under cell stress and independent of NFAT signaling in the podocyte. Specifically, the authors showed that cyclosporin A prevents synaptopodin from cathepsin-L-mediated degradation, leading to preservation of phosphorylated synaptopodin and subsequent RhoA-mediated stabilization of actin stress fibers under cell stress (12).

Because the podocyte membrane mediates the calcium influx required for activation of calcineurin, calcineurin inhibitors may also ameliorate podocyte injury by reducing the intracellular influx of calcium (26, 34, 45, 48). In addition to the effect on actin cytoskeleton, subsequent studies demonstrated that inhibition of NFAT signaling in the podocyte may also serve as a mechanism by which calcineurin inhibitors inhibit podocyte injury and eventual glomerulosclerosis (53).

Calcineurin inhibitors are associated with nephrotoxicity due to its proapoptotic effects in tubular and interstitial cells, which may potentially negate the therapeutic benefits in the podocyte. In cultured murine podocytes, Faul et al. (12) demonstrated that cyclosporin A increased actin stress fibers without inducing apoptosis. In contrast, other laboratories showed a dose-dependent increase in apoptosis with cyclosporin A treatment in cultured murine podocytes (14). Consequently, further studies are required to resolve these conflicting observations. Nonetheless, calcineurin inhibitors have a clear role in a subset of patients and should be included in the armament of therapeutic strategies in primary glomerulopathies.

Rituximab

Several novel biological agents have emerged in recent years as potential therapies in patients with podocytopathies. Of these agents, rituximab has some promise in attenuating podocyte injury in patients with primary glomerulopathies who have failed GC or calcineurin inhibitor therapy. Rituximab is a chimeric monoclonal antibody directed at the CD20 glycoprotein on the surface of B cells. In addition to the immunosuppressive role of rituximab, recent studies demonstrate that the podocyte cytoskeleton is a direct target of Rituximab in patients with recurrent FSGS after renal transplantation (15). Specifically, Fornoni et al. determined that rituximab binds to the putative acid-sphingomyelinase, sphingomyelinase-like phosphodiesterase receptor (SMPDL-3b), on the podocyte membrane and prevents actin cytoskeleton derangement in recurrent FSGS (15). Despite these findings, rituximab has only been evaluated in small patient cohorts with primary FSGS. The response rate has only been 20–30%, suggesting that rituximab may only have a therapeutic benefit in a subset of patients with primary FSGS (13). Consequently, further studies are required to validate whether SMPDL-3b can identify the subset of patients that are likely to respond to rituximab therapy.

Abatacept

Costimulatory modulators, such as B7-1 and B7-2, are expressed on the surface of antigen-presenting cells and serve to regulate T-cell-mediated immunity (43). Consequently, they have been primarily utilized for their immunomodulatory effects in kidney transplantation and other systemic anti-inflammatory disorders (7, 33). Interestingly, B7-1 is also expressed in podocytes and is significantly increased in models of podocyte injury (41). In addition, it has been demonstrated that urinary B7-1 levels correlate with the progression of MCD. Furthermore, Reiser et al. (41) determined that knockdown of B7-1 prevented destabilization of the actin cytoskeleton in the podocyte and attenuated podocyte effacement and proteinuria in proteinuric murine models. Most recently, Yu et al. (59) demonstrated that abatacept, B7-1 inhibitor, restored B1 integrin levels, reversed podocyte migration, attenuated proteinuria, and reversed glomerular injury in a small cohort of patients with primary FSGS and recurrent FSGS after kidney transplantation (59). Although these findings need to be validated in larger cohorts, a subset of patients with B7-1-positive staining in primary FSGS may benefit from treatment with abatacept.

ACTH

Studies conducted more than a half century ago identified the potential therapeutic benefits of ACTH in pediatric cohorts.
with nephrotic syndrome (5, 39). ACTH is a pituitary neuro-immunoendocrine polypeptide that has been demonstrated to attenuate proteinuria in patients with nephrotic syndrome (19). Although its use in the treatment of podocytopathies has fallen off over the last century, it has recently emerged as a gel formulation in the United States. Although studies have identified the potential role of ACTH in patients with steroid-resistant FSGS, response rate remains less than 30%, and its use as primary therapy has yet to be proven (23). Furthermore, the mechanism(s) by which ACTH attenuates podocyte injury remains unclear. Although some have postulated its direct role on the podocyte via the melanocortin 1 receptor (MC1R) in rat models of membranous nephropathy (28, 29), these findings were not observed in the proteinuric murine model of FSGS (29). However, Elvin et al. (10) demonstrated that MC1R agonists only attenuated podocyte injury in cultured murine podocytes with overexpression of human MC1R. Future studies will need to focus on whether the conditional knockdown of the MC1R in podocytes attenuates the salutary effects of MC1R agonists. Detailed reviews have been published recently on the role of MC1R in podocyte injury and kidney disease (18, 19).

Retinoic Acid

Retinoic acid (RA), derivative of vitamin A, is critical for cell differentiation, regulation of apoptosis, and inhibition of inflammation and proliferation. In the setting of podocyte injury, RA attenuates proteinuria and kidney injury in several animal models of proteinuric kidney disease (21, 27, 30–32). RA restores podocyte differentiation markers and may restore lost podocytes by inducing differentiation of kidney progenitor cells in proteinuric murine models. In addition, treatment with RA attenuates inflammation and podocyte apoptosis in these proteinuric murine models.

Earlier studies demonstrate that treatment with RA reduced markers of proliferation and preserves podocyte-specific differentiation markers in models of HIV-associated nephropathy (HIVAN) (50). Since podocyte injury is due to a loss of podocyte differentiation markers in murine models of HIVAN, we determined that treatment with RA in cultured podocytes abrogates this process in a CAMP-dependent manner and via the induction of a kidney-enriched zinc-finger transcription factor, Krüppel-like factor 15 (21, 32). Similarly, the use of cAMP inhibitors diminished RA-induced podocyte differentiation (21, 27, 30). Furthermore, the use of phosphodiesterase-4 inhibitor increased CAMP levels and enhanced RA-induced differentiation markers in HIV-1-infected human podocytes (21). We also demonstrated that retinoic acid receptor alpha (RARα) is the key molecule mediating the renoprotective effects of RA, and treatment with Am580, a RARα agonist, restores podocyte differentiation markers in proteinuric models of podocyte injury (40). In addition, the combined administration of phosphodiesterase inhibitor, Roflumilast, with Am580 resulted in a synergistic attenuation of proteinuria and podocyte injury in murine models of HIVAN (62). Combined, these findings suggest that treatment with RA restores podocyte differentiation markers via the RARα-mediated CAMP-dependent pathway (21, 30).

RA has also been critical to the transition of parietal epithelial cells to podocytes in models of podocyte injury (37, 61). For instance, in a rat model of membranous nephropathy, RA restored podocyte number by increasing these epithelial transition cells in the glomerulus colocalized to paired box gene 2, parietal epithelial cell marker, and Wilms-Tumor 1 (WT1), podocyte-specific marker (61). Similarly, the authors confirmed this, using an experimental model of FSGS to illustrate that the beneficial effect of RA in podocyte regeneration (61).

Conclusions

Our understanding of the pathophysiology and management of podocytopathies has dramatically changed over the last decade. In recent years, drugs repurposed to treat primary glomerulopathies demonstrate that their salutary benefits are, in part, due to their direct action on the podocyte. On the basis of these data, future studies must focus on identifying druggable downstream targets of these systemic agents that are specific to the podocyte with minimal systemic toxicity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.K.M. and J.C.H. conception and design of research; S.K.M. performed experiments; S.K.M. analyzed data; S.K.M. and J.C.H. interpreted results of experiments; S.K.M. prepared figures; S.K.M. drafted manuscript; S.K.M. and J.C.H. edited and revised manuscript; S.K.M. and J.C.H. approved final version of manuscript.

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