Actin dynamics at focal adhesions: a common endpoint and putative therapeutic target for proteinuric kidney diseases

Sanja Sever[1] and Mario Schiffer[2]

[1]Department of Medicine, Division of Nephrology, Harvard Medical School, Boston, Massachusetts, USA; and [2]Department of Nephrology, Hannover Medical School, Hannover, Germany

Proteinuria encompasses diverse causes including both genetic diseases and acquired forms such as diabetic and hypertensive nephropathy. The basis of proteinuria is a disturbance in size selectivity of the glomerular filtration barrier, which largely depends on the podocyte: a terminally differentiated epithelial cell type covering the outer surface of the glomerulus. Compromised podocyte structure is one of the earliest signs of glomerular injury. The phenotype of diverse animal models and podocyte cell culture firmly established the essential role of the actin cytoskeleton in maintaining functional podocyte structure. Podocyte foot processes, actin-based membrane extensions, contain 2 molecularly distinct "hubs" that control actin dynamics: a slit diaphragm and focal adhesions. Although loss of foot processes encompasses disassembly of slit diaphragm multiprotein complexes, as long as cells are attached to the glomerular basement membrane, focal adhesions will be the sites in which stress due to filtration flow is counteracted by forces generated by the actin network in foot processes. Numerous studies within last 20 years have identified actin binding and regulatory proteins as well as integrins as essential components of signaling and actin dynamics at focal adhesions in podocytes, suggesting that some of them may become novel, druggable targets for proteinuric kidney diseases. Here we review evidence supporting the idea that current treatments for chronic kidney diseases beneficially and directly target the podocyte actin cytoskeleton associated with focal adhesions and suggest that therapeutic reagents that target the focal adhesion-regulated actin cytoskeleton in foot processes have potential to modernize treatments for chronic kidney diseases.

Kidney International (2018) **93,** 1298–1307; https://doi.org/10.1016/j.kint.2017.12.028

KEYWORDS: podocytes; actin; dynamin; Bis-T-23

Correspondence: *Sanja Sever, Department of Medicine, Harvard Medical School and Division of Nephrology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. E-mail: ssever@mgh.harvard.edu; or Mario Schiffer, Department of Nephrology, Hannover Medical School Hannover, Lower Saxony 30625 Germany. E-mail: Schiffer.Mario@mh-hannover.de*

Received 2 June 2017; revised 7 December 2017; accepted 13 December 2017; published online 17 April 2018

The presence of high-molecular-weight proteins like albumin in the urine (proteinuria) is the first sign that the selectivity of the kidney filtration barrier has been compromised. Presently, proteinuria is considered one of the strongest risk factors for future loss of kidney function. Most proteinuric kidney diseases and all nephrotic diseases (proteinuria $>$ 3.5 g/d) demonstrate a change in podocyte morphology on an ultrastructural level.[1,2] Podocyte structure is conventionally divided into 3 types of subcellular compartments: the cell body, the microtubule-driven membrane extensions termed primary processes, and the actin-driven membrane extensions termed foot processes (FPs). FPs of neighboring cells are connected by a podocyte-unique multiprotein complex termed "slit diaphragm." Podocyte injury often leads to loss of FPs, a process termed "FP effacement,"[3,4] and to molecular reorganization or loss of the slit diaphragm.[5] FP effacement is the result of dysregulation of the actin cytoskeleton, providing a direct link between proteinuria and actin cytoskeleton dynamics.

Given the connection between podocyte structure and function, concerted efforts have been made to decipher the 3-dimensional architecture of podocytes. Focused ion beam scanning electron microscopy has identified a tortuous ridge-like prominence which protrudes from either the basal surface of the primary processes or from the cell body.[6] The FPs, previously believed to be terminal projections that only protruded from the primary processes, also branched from this ridge-like prominence. This ridge-like prominence generated a more direct link between the cell body and the FPs than originally thought. The authors suggested that the ridge-like prominences might have served as an adhesion apparatus for the direct attachment of the cell body and the primary processes to the glomerular basement membrane (GBM) and as an apparatus to connect FPs to the cell body. Those novel insights suggest that, in addition to the structural role of podocytes in maintaining kidney filtration, the newly identified ridge-like prominence may play an important role in regulating global organization of the actin cytoskeleton in FPs, as well as cell signaling between neighboring podocytes, the GBM and endothelial cells.

Podocytes have remarkable transformational abilities in response to cellular stress. Although podocyte injury often leads to FP effacement, if the podocytes are still attached to the GBM, they have the ability to reform FPs.[7] Because FP

effacement and re-formation are membrane-modifying processes, they are both driven by distinct reorganization of the actin cytoskeleton. During FP effacement, the actin backbone is disassembled and the cellular protrusions lose their delicate appearance to a more squat form. At later stages, a dense layer of actin develops at the GBM-facing side of the cells.[8] This common stress response is seen only on ultrastructural analysis using electron microscopy and can be observed in most proteinuric diseases. During FP regeneration, the dense actin layer is dissolved, and novel FPs branch from the cells.[9] Re-formation of the FPs can be achieved only by induction of actin polymerization in the membrane vicinity. Both FP effacement and reformation require funneling distinct signals into a common hub(s) that controls assembly and disassembly of actin.[10,11] FPs contain 2 physically and molecularly distinct hubs that control actin dynamics, a slit diaphragm and focal adhesions.

Do current treatments affect the actin cytoskeleton in podocytes?

Despite the absence of inflammatory cells in biopsy samples, many diseases with direct podocyte involvement are considered mediated by immune cells because treatment with classic immunosuppressant drugs leads to remission in a significant number of patients. This paradigm has been deemed valid for more than 40 years.[12] A major conceptual shift from immune-cell-mediated to podocyte-mediated diseases occurred by realizing that many heritable forms of focal segmental glomerulosclerosis (FSGS) are caused by mutations in proteins that are important for podocyte function.[13] Those discoveries provided direct evidence of the essential role of podocytes in the glomerulus and suggested that direct targeting of the pathogenic pathways within podocytes may have a beneficial effect on glomerular diseases. This original idea was subsequently expanded to argue that current treatments for glomerular diseases, such as those involving angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, as well as diverse immunosuppressants, all exhibit their antiproteinuric effects in part by directly targeting actin cytoskeleton within the podocyte.[13]

One of the earliest attempts to link the effect of antiproteinuric drugs with actin cytoskeleton dynamics in podocytes was in 2008.[14] Cyclosporin A (CsA) is an inhibitor of serine/threonine phosphatase calcineurin, known for its role in regulating T-cell activation through regulation of the nuclear factor of activated T-cell (NFAT) signaling. It was suggested that CsA blocked calcineurin-mediated dephosphorylation of the podocyte-specific adaptor protein synaptopodin, which then protected synaptopodin from deleterious cathepsin L-mediated proteolysis in injured podocytes.[15] Because synaptopodin contributes to RhoA[16] and Cdc42[17]signaling, and because those GTPases are major regulators of the actin cytoskeleton in general (see below), these data suggested that CsA ultimately exhibited a protective effect on the actin cytoskeleton in podocytes by stabilizing the RhoA/Cdc42 signaling pathway. Moreover, CsA has a direct effect on cofilin-1 expression, and CsA also regulates phosphorylation of WAVE1, an actin nucleator and a key regulator of Arp2/3-mediated actin polymerization.[18,19]

Identification of B7-1 (CD80), a costimulatory receptor present on antigen-presenting cells required to induce T-cell activation also on podocyte membrane, provided the opportunity to test the idea that immunomodulators directly target podocytes in humans. Experiments using diverse animal models and podocyte tissue culture suggested that *de novo* expression of B7-1 in podocytes initiated their injury by deactivating essential $\alpha 3\beta 1$ integrin.[20,21] Abatacept (CTLA4-Ig), a fusion protein that blocks T-cell activation by binding to B7-1 and B7-2 (CD86) on antigen-presenting cells with high affinity, is a drug licensed for the treatment of rheumatoid arthritis. After an original promising study,[21] the responsiveness of FSGS patients to abatacept or to a newer version of the drug named belatacept has been discouraging.[22] In fact, although abatacept effectively reduced the level of activated $CD4^+$ T cells, its administration did not preserve kidney function in the streptozocin-induced model of diabetic nephropathy in mice,[23] further questioning the direct effect of abatacept on podocytes.

In contrast to the negative results with immunomodulators that act by blocking T-cell activation, the B-cell-targeting drug rituximab exhibited moderate success in some patients with nephrotic syndrome.[24,25] Rituximab is a chimeric monoclonal antibody against the protein CD20, which is found primarily on the surface of immune system B cells where it acts by destroying them. Fornoni *et al.*[26] suggested that, in CKD patients, rituximab operates in a B-cell-independent manner by directly targeting podocytes. Specifically, the authors hypothesized that rituximab binds sphingomyelin phosphodiesterase acid-like 3b (SMPDL3b), a lipid-modulating phosphodiesterase. Studies using podocytes in culture suggested that rituximab partially prevented SMPDL3b down-regulation, which was associated with recurrent FSGS. Because levels of SMPDL3b expression affected the actin cytoskeleton in podocytes, the study suggested that rituximab modulates podocyte actin cytoskeleton in an SMPDL3b-dependent manner.

The above-mentioned therapeutics were developed to specifically target the immune system. Presently, it is hard to conclude with certainty whether the antiproteinuric effects observed in a subset of patients and/or animal models are due to their effect on podocytes, to immunomodulation, or to both. In contrast to immunomodulators, renin angiotensin aldosterone system (RAAS) blockers are the only generally accepted and most widespread supportive antiproteinuric therapy agents that have a proven influence on kidney function.[27] Although their antiproteinuric effect is clearly due to alterations in the blood flow in the glomerulus due to change in blood pressure, it is not surprising that a number of studies also examined the ability of RAAS to alter the actin cytoskeleton in podocytes. Both angiotensin II