Knocking Out Podocyte Rho GTPases: And the Winner Is... 

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Here are three rudimentary podocyte-related facts to consider. First, the podocyte plays an essential role in determining the functional properties of the glomerular filtration barrier. Second, the cytoskeleton of the podocyte is critical to this cell's ability to form foot processes and slit diaphragm junctions that ultimately determine glomerular permselectivity. Third, members of the Rho guanosine triphosphatase (GTPase) family of intracellular signaling molecules are master regulators of actin cytoskeletal dynamics. Therefore, given these three basic tenets, where would you place your bets to the following question: which of the ubiquitously expressed Rho GTPase subfamily members (RhoA, Rac1, cdc42) is the most likely candidate to elicit congenital nephrotic syndrome when conditionally deleted in the podocyte lineage? Not an easy wager, but rest easy, Scott et al. provide us with the answer in this issue of JASN.

Much of ESRD originates in kidney glomeruli, which are microvascular units inherently charged with the primary task of filtering the blood and elaborating a primary urine ultrafiltrate that subsequently undergoes modification by the tubular system. The glomerulus is able to achieve its remarkable functionality through the integrated efforts of the tripartite glomerular filtration barrier (GFB), which is comprised of endothelial and podocyte cell types that medially lay down the constituents of the glomerular basement membrane. Although each layer of the GFB contributes to its overall structural and functional integrity, the podocyte is recognized as the principal culprit in the majority of cases of congenital nephrotic syndrome, as well as a number of other glomerular diseases.

The podocyte’s unique ability to size-selectively filter blood solutes is a paragon of the intricate relationship between form and function. The morphology of the in vivo podocyte with its cell body and major processes that furcate into tertiary foot processes that interlace with those of neighboring podocytes is what gives us the beautiful scanning electron microscopy images that permit us a structural understanding of its functional design. Between juxtaposed foot processes lies the slit diaphragm (SD), a highly specialized cellular junction comprised of a host of targeted protein species, among them, nephrin. Notably, the constellation of proteins that comprise the SD form a signaling node that communicates information from the external milieu to the underlying actin cytoskeleton, and it is the cytoskeleton that is absolutely required for the formation and extension of foot processes. Understanding the cellular mechanisms that integrate the SD with the podocyte cytoskeleton is one of the primary challenges to understanding the etiology of glomerular disease.

The Rho GTPases are a family of intracellular signaling proteins that can be defined as much by their amino acid sequence homology as by their unifying roles in regulating the actin cytoskeleton. The most evolutionarily conserved Rho GTPases, including RhoA, Rac1, and Cdc42, are all key determinants of actin polymerization that regulate a variety of cellular processes such as adhesion, migration, division, and polarity. These Rho GTPases cycle between GTP- and GDP-loaded activity states, as determined by the concerted activities of guanine nucleotide exchange factor (GEF), GTPase-activating protein (GAP), and guanine nucleotide dissociation inhibitor (GDI) regulatory molecules, and ultimately exert their cellular roles through GTPase effector molecules that directly bind to the GTPase and mediate downstream signaling events. Cell biology textbooks provide us the dogma that active RhoA, Rac1, and Cdc42 lead to stress fiber, lamellipodia, and filopodia formation, respectively, but this view is highly simplified. The basic fundamental requirement of the prototypical Rho GTPases can be appreciated by the embryonic lethality that is observed in full Rac1- and Cdc42-null mice (there is no published account of a RhoA knockout) and has brought about the study of their in vivo function using Cre/loxP mouse conditional knockout methodologies.

To understand their individual roles in podocyte biology, Scott et al. conditionally deleted each of the prototypical Rho GTPases in the podocyte lineage and found that only deletion of Cdc42 resulted in any overt changes to podocyte morphology and overall glomerular function. The resulting phenotypes are noteworthy in their two extremes. Whereas Cdc42 deletion results in massive neonatal proteinuria, kidney failure, and associated death within 2 weeks of birth, podocyte-specific RhoA and Rac1 deletion mutants are conspicuously healthy. At the cellular level, podocytes of Cdc42-deleted mice are unable to properly form the thin foot process extensions normally seen in the healthy glomerulus and instead display dramatically broadened epithelial sheets with irregular, apically located cellular...
junctions that likely represent mislocalized pseudo-SDs. Furthermore, it appears in vitro that Cdc42, but neither RhoA nor Rac1, is required for actin polymerization as induced by nephrin clustering in mouse embryonic fibroblasts deleted of each Rho GTPase.

The results of this study provide us with a significant contribution to our knowledge regarding the importance, or lack thereof, of the individual Rho GTPases in podocyte development in vivo, but are nevertheless largely descriptive in nature. This, however, gives us the opportunity to speculate on the mechanistic details that will likely unfold in future studies. As notably pointed out, the glomerular phenotype observed in mice with Cdc42-deleted podocytes is remarkably similar to that previously observed in mice harboring atypical protein kinase CA/t conditionally deleted podocytes and is consistent with the notion that Cdc42 is a key regulatory component of the podocyte cell polarity complex that is keenly necessary for the formation of mature foot processes, as well as the correct targeting of SD protein components. Just how Cdc42-dependent cytoskeleton regulation is integrated with podocyte polarity determination is open to speculation. The authors go on to cite evidence from the literature supporting the dispensability of RhoA and Rac1 in podocytes, and there is mounting evidence indicating that, in contrast to Cdc42, it is the inappropriate activation of these Rho GTPases and not their inactivation that is most often associated with glomerular dysfunction.

However, the situation is not likely so straightforward, and, given compelling evidence indicating that many of the Rho GTPases crosstalk and cooperate with polarity proteins in defining cell polarization, a wider spectrum of possible explanations and considerations for the dichotomous renal phenotypes resulting from podocyte-specific Rho GTPase deletion is warranted. The first among these deals with the topic of molecular redundancy by functionally similar proteins. Here, it is noteworthy that, whereas Rac1 shows >90% sequence similarity to Rac2 and Rac3 and RhoA shows 84% and 92% sequence similarity to RhoB and RhoC, respectively, Cdc42 shares only approximately 60% homology with its two most closely related subfamily members. It is therefore not unlikely that the lack of phenotype in Rac1 and RhoA podocyte-deletion mutants is a result of functional compensation by closely related members and, conversely, that the podocyte dysfunction seen in Cdc42-deleted podocytes is obviated as a result of inadequate functional coverage by its less homologous subfamily species.

Another interesting topic of consideration has to do with the RhoGDIs, a family of proteins endowed with the capacity to regulate the expression, membrane localization, and activation state of the Rho GTPases. In a very relevant in vitro study, it was shown that there exists a competitive, stoichiometric balance between individual Rho GTPases for sequestration and protection from proteasomal degradation by RhoGDI, which accordingly acts as a key determinant of Rho GTPase activity and homeostasis. In an analogous manner, one can predict that deleting the expression of any single Rho GTPase in the podocyte will result in the generation of vacant RhoGDI molecules that can subsequently accommodate interactions with the other remaining Rho GTPases. In other words, following Cdc42 deletion, one must consider the possibility that altered activities of Rac1 and RhoA (perhaps their activation as a result of protection from proteasomal degradation by increased RhoGDI-dependent sequestration) may contribute to podocyte dysfunction and the overall observed phenotype.

Finally, although podocyte-specific RhoA- and Rac1-deleted mice have no observable phenotype, the possibility exists that their deletion perturbs Rho GTPase homeostasis and that these mice may be more or less susceptible to secondary renal challenges. In support of this possibility, the Kretzler laboratory has shown that podocyte-specific Rac1-deleted mice are protected from protamine sulfate–induced foot process effacement, whereas ongoing studies from our laboratory reveal that podocyte-specific RhoA-deleted mice are synergistically albuminuric when bred onto a Rhophilin-1–deleted mouse strain. (M.A. Lal et al. unpublished data, 2012). With these caveats in mind, as well as the fact that there exist >100 GEF/GAPs to regulate RhoGTPase signaling, it is a pretty safe bet to say that we are only beginning to define the repertoire of cytoskeletal and polarity components that are required to establish and maintain the podocyte’s unique morphology, which is essential for determining its permselectivity properties, and that new players and mechanisms await discovery.

DISCLOSURES
None.

REFERENCES