Sensitizing the Slit Diaphragm with TRPC6 Ion Channels

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Members of the transient receptor potential (TRP) superfamily of ion channels are leading candidates for mediating compartmentalized Ca²⁺ release.¹ TRP channels are best known for their diverse physiologic role as cellular sensors contributing to thermal, tactile, taste, and osmolar and fluid flow sensing and mechanosensation. The discovery of the TRP family member TRPC6 in podocyte foot processes as part of the slit diaphragm protein complex² suggests that podocyte foot processes are functional Ca²⁺ compartments. This opens the question about the role of Ca²⁺ signaling in foot processes for normal podocyte structure and function and thereby for the maintenance of a proper glomerular filtration barrier.

The integral role for TRPC6 in kidney podocytes is underscored by the observation that mutations in several podocyte genes as well as specific molecular pathways have been identified as the cause for progressive kidney failure with urinary protein loss. Podocyte injury is a hallmark of glomerular disease, which is generally displayed by the rearrangement of the podocyte slit diaphragm and the actin cytoskeleton. Recent studies demonstrate a unique role for the Ca²⁺-permeable ion channel protein TRPC6 as a regulator of glomerular ultrafiltration. In both genetic and acquired forms of proteinuric kidney disease, dysregulation of podocyte TRPC6 plays a pathogenic role. This article illustrates how recent findings add to emerging concepts in podocyte biology, particularly mechanosensation and signaling at the slit diaphragm.


ABSTRACT

Physiologic permeability of the glomerular capillary depends on the normal structure of podocyte foot processes forming a functioning slit diaphragm in between. Mutations in several podocyte genes as well as specific molecular pathways have been identified as the cause for progressive kidney failure with urinary protein loss. Podocyte injury is a hallmark of glomerular disease, which is generally displayed by the rearrangement of the podocyte slit diaphragm and the actin cytoskeleton. Recent studies demonstrate a unique role for the Ca²⁺-permeable ion channel protein TRPC6 as a regulator of glomerular ultrafiltration. In both genetic and acquired forms of proteinuric kidney disease, dysregulation of podocyte TRPC6 plays a pathogenic role. This article illustrates how recent findings add to emerging concepts in podocyte biology, particularly mechanosensation and signaling at the slit diaphragm.


IMPLICATIONS OF THE INTERACTION OF TRPC6 WITH THE SLIT DIAPHRAGM

TRPC6 interacts with two proteins that are key effectors between podocytes: Podocin and nephrin.² The interaction of TRPC6 with the stomatin family member podocin, whose homologue mec-2 is a contributor to mechanosensation in the worm Caenorhabditis elegans, places TRPC6 at the site of a putative mechanosensor complex at the slit diaphragm (Figure 1). Being subject to mechanical force in a vertical direction from fluid flow across the membrane and in a lateral direction from contact between neighboring podocytes, such a complex

Published online ahead of print. Publication date available at www.jasn.org.

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could participate in monitoring glomerular pressure, or filtration rate, and help podocytes adapt to changes in the micro-environment of the glomerular filtration barrier. A report by Huber et al. substantiated this notion by demonstrating that podocin in heterologous expression systems regulates the activity of TRPC6 in a cholesterol-dependent manner.

In fact, several TRP channels have the capability to sense mechanical stretch directly; that is, conformational changes induced by stretch of the plasma membrane are sufficient to render the channel pore permeable. Spassova et al. showed that TRPC6 responds to pressure stimuli even in the presence of phospholipase C blockers, arguing against second-messenger activation and in favor of direct activation through membrane stretch. The authors of that study suggested a common molecular basis for lipid-mediated membrane stretch activation and the widely known activation of TRPC6 through diacylglycerol. In both cases, the mechanism underlying channel activation is a conformational change rendering TRPC6 channels permeable.

The concept of a mechanosensor at the slit diaphragm would be well in line with findings derived from human genetics; as is the case for TRPC6, it is widely known that mutations in the NPHS2 gene encoding podocin cause hereditary nephrotic syndrome in humans. Although TRPC6-associated disease displays a later onset of kidney failure and milder symptoms than the podocin disease, the similarity of the defects supports the concept that podocin modulates TRPC6 function. Also is the notion that TRPC6 function is partly compensated by other TRPC proteins, but there is no known podocin analogue that could explain the more severe phenotype for NPHS2 mutations as compared with TRPC6 mutations.

It is further suggested that the slit diaphragm serves not only as a molecular sieve but also—similar to other cell–cell junctions—as a platform mediating signals required for sustained podocyte structure and function. The slit diaphragm protein, nephrin, contributes to actin reorganization in podocytes through a phosphoinositide 3-kinase pathway. In addition, tyrosine phosphorylation, which is a key event in signal transduction, occurs at the slit diaphragm site: The Src family protein tyrosine kinase Fyn binds and phosphorylates nephrin. Upon phosphorylation by Fyn, nephrin can bind Nck adapter proteins, an event that in turn reorganizes the actin cytoskeleton. Interestingly, TRPC6 is also a target of phosphorylation by Fyn, enhancing TRPC6 channel activity. Together, these findings suggest TRPC6 is assembled in a complex together with nephrin and Fyn at the slit diaphragm, and this complex is partly regulated by tyrosine phosphorylation of nephrin and/or TRPC6.

TRPC6 as a component of a podocin-TRPC6-mechanosensor complex and/or nephrin-Fyn-TRPC6 signaling complex could be involved in monitoring the integrity of the slit diaphragm, which relates Ca\(^{2+}\)-signaling cascades into the foot process that are required to induce adaptive changes in the dynamic, contractile podocyte actin cytoskeleton. Under disease conditions, alteration of TRPC6 function directly affects the cytoskeletal response of podocytes and therefore play a major role during podocyte injury and foot process effacement (Figure 2). Given that Ca\(^{2+}\) responses can be subdivided into short-term, rapid responses that do not involve transcriptional changes and long-term modifications that require changes in gene
Models. TRPC6-deficient mice have drawn from the study of genetic animal diseases, important insights can be for studying the pathogenic mechanism. Along these lines, Kuwahara et al.12 found that TRPC6 is a key component of a Ca^{2+}-dependent regulatory loop that drives pathologic cardiac remodeling through the calcineurin-NFAT pathway. Given that one of the most widely used drugs in the treatment of idiopathic glomerular kidney disease are inhibitors of calcineurin, it will be very interesting to determine whether a similar pathway has physiologic relevance in podocytes and, if this is the case, to identify target genes of TRPC6-mediated transcriptional regulation.

**Figure 2.** Working model for the pathophysiology of podocyte TRPC6. Induction of wild-type TRPC6 protein or the presence of mutated TRPC6 can lead to glomerular disease. Whereas mutated TRPC6 leads to adult-onset FSGS, the induction of wild-type TRPC6 protein or the presence of mutated TRPC6 can cause rapid-onset glomerular disease. Modifying factors as detailed can affect the course and severity of proteinuric disease.

**ARE TRPC6^{−/−} MICE A SUITABLE MODEL FOR TRPC6-RELATED HUMAN DISEASE?**

For studying the pathogenic mechanisms behind many inherited human diseases, important insights can be drawn from the study of genetic animal models. TRPC6-deficient mice have been characterized.13 Young TRPC6^{−/−} mice lack an overt renal phenotype; however, thus far, no study has explicitly addressed the renal phenotype in TRPC6-deficient mice that are stressed. Moreover, given the late onset of the TRPC6-related human disease, aged deficient mice would have to be studied. Finally, the role of TRPC6 in the kidney may be different in human and mouse, and the findings in the animal model may simply not be directly applicable to the human disease phenotype. The latter notion is supported by our own studies in zebrafish, in which TRPC6 was absent in developing and adult podocytes.14

An explanation for the lack of an overt phenotype of TRPC6^{−/−} mice is provided by the second-hit hypothesis. In the context of mutations in the ACTN4 gene encoding the actin bundling protein (similar to mutations in TRPC6), α-actinin-4 results in late-onset FSGS in various degrees of severity. It has been suggested that, besides the sole presence of mutations, second physiologic hits such as variations in glomerular capillary pressure (e.g., caused by hypertension) or metabolic disturbance (e.g., caused by diabetes) may be required for the clinical manifestation of disease. Another possible disease modifier upstream of TRPC6 activity is the absence or presence of serum factors; for example, a yet-to-be-identified FSGS factor modulating TRPC6 stimulation. A second-hit hypothesis could explain the absence of a disease phenotype in TRPC6^{−/−} mice. The presence of second hits in different degrees could also explain why some individuals present with clinical symptoms much earlier than others with the same mutation. Furthermore, recent findings in bigenic mouse models of glomerular disease illustrate that mutations in genes other than TRPC6 may have to be taken into account when exploring the pathophysiology of TRPC6-related disease.15

For advancing our understanding of the role of TRPC6, the development of tissue-specific transgenic mice will be of great help. Podocyte-specific gene targeting became possible with cloning of podocyte-specific promoters.16 It will be very interesting to see whether podocyte-specific TRPC6^{−/−} mice, or mice overexpressing TRPC6 in a podocyte-specific manner, have a renal phenotype or respond differently to experimental challenge. Importantly, these animal models will also help us to distinguish between the pathophysiologic roles of TRPC6 in podocytes and in other cell types in the nephron, including glomerular endothelial and tubular epithelial cells.

**CONCLUSIONS**

The discovery of TRPC6 as part of the slit diaphragm in podocytes opens a new field for glomerular research, which is potentially amenable to therapy. The idea of a mechanos- or flow sensor at the level of the slit diaphragm is suggested by published data even though convincing *in vivo* studies are not yet available. TRPC6 mutations provide important clues toward the understanding of hereditary forms of proteinuric glomerular disease. Genotyping of patients with FSGS will likely result in the discovery of more TRPC6 mutations beyond the ones.
previously described. Expanded screening may also answer the question of whether polymorphisms of TRPC6 act as susceptibility or initiation factors for renal disease and may help to determine which patients would benefit from early, aggressive therapy. From the perspective of new biologic modifiers, studies indicating a role of TRPC6 in acquired glomerular disease enhance enthusiasm for developing TRPC6 antagonists.

ACKNOWLEDGMENTS

J.R. is supported by a research project grants from the National Institutes of Health (DK73495). The authors thank Jim Stanis for help with the illustration (Figure 1).

DISCLOSURES

None.

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