TRPC6 channel as an emerging determinant of the podocyte injury susceptibility in kidney diseases

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Ilatovskaya DV, Staruschenko A. TRPC6 channel as an emerging determinant of the podocyte injury susceptibility in kidney diseases. Am J Physiol Renal Physiol 309: F393–F397, 2015. First published June 17, 2015; doi:10.1152/ajprenal.00186.2015.—Podocytes (terminally differentiated epithelial cells of the glomeruli) play a key role in the maintenance of glomerular structure and permeability and in the incipience of various renal abnormalities. Injury to podocytes is considered a major contributor to the development of kidney disease as their loss causes proteinuria and progressive glomerulosclerosis. The physiological function of podocytes is critically dependent on proper intracellular calcium handling; excessive calcium influx in these cells may result in the effacement of foot processes, apoptosis, and subsequent glomeruli damage. One of the key proteins responsible for calcium flux in the podocytes is transient receptor potential cation channel, subfamily C, member 6 (TRPC6); a gain-of-function mutation in TRPC6 has been associated with the onset of the familial forms of focal segmental glomerulosclerosis (FSGS). Recent data also revealed a critical role of this channel in the onset of diabetic nephropathy. Therefore, major efforts of the research community have been recently dedicated to unraveling the TRPC6-dependent effects in the initiation of podocyte injury. This mini-review focuses on the TRPC6 channel in podocytes and colligates recent data in an attempt to shed some light on the mechanisms underlying the pathogenesis of TRPC6-mediated glomeruli damage and its potential role as a therapeutic target for the treatment of chronic kidney diseases.

TRPC6; intracellular calcium; glomerulosclerosis; podocyte; glomerulus; diabetic nephropathy; proteinuria

Role of Podocytes in the Development of Kidney Diseases

Current discussions of the mechanisms of glomerular barrier injury and subsequent proteinuria (a core component of chronic kidney disease) tend to focus on the role of glomerular epithelial cells: podocytes. Indeed, podocytes are key components of the renal filtration barrier; their impairment has been reported to underlie glomerulopathies in such diseases as hypertension, preeclampsia, and diabetes mellitus (51, 61). Podocyte loss is one of the primary characteristics of focal segmental glomerulosclerosis (FSGS), a histological pattern of renal injury that can arise from a diverse range of causes and mechanisms and is a common cause of nephrotic syndrome (19, 32, 50). Basically, podocytes and their foot processes function as a final frontier which prevents the leak of plasma proteins into primary urine. Podocytes express numerous receptors and respond to various factors and metabolic products; however, these cells have limited proliferative capacity, and when glomerular growth and hemodynamic stresses exceed the ability of podocytes to undergo hypertrophy, they become irreversibly injured and detach. Podocytopenia is contemporaneous with proteinuria, which, if left untreated, can rapidly progress to end-stage renal disease that warrants dialysis and renal transplantation (6). Normal renal filtration is critically dependent on podocyte function, and it is of crucial importance to better understand the mechanisms of podocyte injury, apoptosis, and detachment to develop new therapeutic intervention strategies.

Transient Receptor Potential Canonical Channel 6 as a Determinant of Podocyte Injury

Transient receptor potential canonical channel (TRPC) proteins, which belong to the larger TRP superfamily of channels, form Ca2+-permeable channels that are important players in the pathogenesis of renal and cardiovascular diseases (3, 13, 64). An association between altered TRPC channels function and/or expression with the development of various renal complications occurring due to podocytopenia has garnered the attention of many investigators (2, 15, 21–24, 33, 40–42, 47–49, 58). The TRPC family is composed of seven structurally related channels (TRPC1–7) (11, 64). It was reported that TRPC1, TRPC3, TRPC4, TRPC5, and TRPC6 are expressed in podocytes (20, 28, 58). However, the role of the various members of the TRPC family in podocytes in normal and disease conditions is still somewhat controversial. To date, at least TRPC3 (35, 61), TRPC5 (23, 49, 58), and TRPC6 (28, 29, 31) channels have been functionally and pharmacologically
shown to be involved in calcium entry in the podocytes. In other types of cells (for instance, smooth muscle cells of the aorta) (12), an interdependent functional profile of TRPC channels was revealed (smooth muscle cells of TRPC6-deficient mice have been shown to have higher basal cation entry, which was abolished by TRPC3-specific siRNA). This observation has not been confirmed in the TRPC6−/− podocytes (29); however, the compensatory pathways existing within the TRPC family of channels should be always kept in mind while knockout models are being studied.

While compelling data indicate the role of TRPC5 in maintaining of calcium flux in podocytes and development of proteinuric kidney disease (49, 58), a large body of data that have emerged recently allowed pointing at TRPC6 as a promising member of the TRPC family, which could play a role in podocyte depletion in disease conditions. First, several independent laboratories have reported the identification of gain-of-function mutations of TRPC6 associated with autosomal dominant FSGS (25, 46, 62). In addition, changes in channel expression may also contribute to the disease (62); furthermore, it was proposed that TRPC6 is involved in the pathogenesis of the nongenetic forms of proteinuric disease: increased TRPC6 expression is found in glomeruli from patients with such renal pathologies as membranous glomerulonephritis and minimal-change disease (41). Furthermore, overexpression of wild-type or mutant (overactive) TRPC6 in podocytes is sufficient to cause a kidney disease consistent with FSGS (36). Nevertheless, both increased TRPC6 channel activity and expression lead to a pathologically high calcium influx in podocytes, which eventually causes their loss either through apoptosis, detachment, or lack of proliferation (24, 37, 53). Therefore, direct inhibition of TRPC6 channels may be of therapeutic benefit in various glomerulopathies (63).

TRPC6 is located on the podocyte membrane, where it is integrated into a signaling complex that interacts with nephrin, podocin, α-actinin-4, and some other proteins critical for podocyte function (11, 27, 40, 41, 46). As one of many examples, it was discovered that a certain mutant of podocin (P118L) fails to activate TRPC6 channels, and this may compromise the function of the slit diaphragm protein complex and aggravate proteinuria, progressive podocyte loss, and glomerulosclerosis (10). It was also reported that podocin acts as a switch which determines the preferred mode of TRPC6 activation; knockdown of podocin markedly increased stretch-evoked activation of TRPC6, but nearly abolished TRPC6 activation initiated by a diacylglycerol analog (4). It should be noted that TRPC6 channels are usually silent in the absence of stimuli; therefore, TRPC6 activation is important under physiological conditions, and normal functionality of the channel contributes to the integrity of the kidney filtration barrier. On the other hand, it should be emphasized that various stimuli in pathological conditions (or genetic liability) can lead to hyperactivity of the channel, which significantly contributes to podocyte depletion. While known gain-of-function mutations in the TRPC6 gene result only in a small fraction of known cases of FSGS, mutations in other genes such as NPHS2, ACTN4, INF2, and APOL1 might also result in calcium overload in podocytes via activation of TRPC6, producing the same pathological effect as gain-of-function mutations in the TRPC6 gene. Importantly, excessive calcium flux in podocytes mediated by TRPC6 channels is deleterious not only in FSGS but also in many other kidney diseases such as diabetic nephropathy (1, 33, 34, 43, 54, 57, 60, 66).

Potential Stimuli Causing Excessive Calcium Influx Through TRPC6 in Disease Conditions

Recent studies suggested several major activators of the TRPC6 channels which are reported to be increased in disease conditions and could mediate enhanced calcium influx in the podocytes. One of the likely triggers of a calcium-dependent pathway of programmed podocyte death is angiotensin II (ANG II); TRPC channels have been associated with ANG II-induced calcium influx in many renal cell types (14, 16–18, 22, 55). ANG II released into the renal interstitium is one of the key mediators of renal inflammation and fibrosis in progressive chronic nephropathies. Studies in models of chronic hypertension and protein-induced renal damages revealed that inhibition of ANG II receptors (ATRs) is effective against proteinuria (8, 9, 59). Angiotensin-converting enzyme (ACE) inhibitors and ATR blockers can attenuate progressive glomerulosclerosis in disease models and slow disease progression in humans (48). It was also shown that ANG II enhances albuminuria by activating TRPC6 channels in podocytes (15). Furthermore, alteration of TRPC6 expression and Ca2+ influx are involved in ANG II-induced apoptosis (65). Also, it was demonstrated that the deleterious effects of ANG II on podocytes and its pathogenic role in glomerular diseases involve enhanced TRPC6 expression (42). Therefore, the association between ANG II and TRPC6 channel is well established (1, 5, 15, 29, 42, 58). Recent data revealed that G protein-coupled receptors (GPCRs) linked to Gq signaling, which causes activation of receptors for ANG II, endothelins, thromboxanes, and some other GPCR agonists, induce glomerular injury by activating TRPC6 (60). Our data also provided evidence that ANG II increases native TRPC channel activity in the podocytes of freshly isolated glomeruli (this effect was lacking in the TRPC6−/− knockout mice) (29) (see Fig. 1A).

Furthermore, ANG II causes an acute release of H2O2 in the kidney (44). This observation is largely in line with the report which showed that ANG II-dependent activation of TRPC6 channels in rat podocytes requires generation of reactive oxygen species (ROS) (47). Furthermore, Kim et al. (34) have provided compelling evidence of the fact that in the podocytes ROS-producing NADPH oxidases are part of a complex with TRPC6 channels (when podocin is present) and contribute to the channel’s activation. Interestingly, TRPC6 expression and/or calcium influx in the podocytes has been shown to be induced by glucose (38), insulin (33), and ATP (30, 47), and these processes have also been associated with ROS production. Additionally, the role of TRPC6 channels in oxidative stress-induced podocyte ischemic injury has been recently demonstrated (67). ROS are ubiquitous cellular signals, which are closely associated with the development and progression of glomerular sclerosis, and elimination of ROS can be protective against kidney injury (7, 39, 45, 52, 56). These findings are indeed very intriguing and allow us to further speculate that ROS production (caused by various stimuli) is a common mechanism of TRPC6 channel activation in the podocytes (see Fig. 1B). However, by no means should we say that the mechanisms described above are the only signaling pathways critical for TRPC6 (as well as other TRPC channels in podo-
cytes) regulation. For instance, there is some uncertainty whether TRPC6 channels are intrinsically mechanosensitive (4, 61), or the GPCRs and/or phospholipases respond to mechanical stimuli and then activate TRPC6; however, there is no controversy about the fundamental observation that TRPC6 is certainly a component of mechanotransduction cascades, which become overly active during hyperfiltration, undoubtedly an issue in diabetes and chronic kidney disease (26).

**Conclusion**

Recent joint efforts of many research teams have led to critical advances in our understanding of the podocyte biology and its role in the maintenance of the kidney filtration barriers. To date, it is widely accepted that increased calcium influx through the TRPC6 channels is one of the major determining factors of podocyte injury in various renal pathologies, including FSGS, diabetic nephropathy, and nephrotic syndrome. Various animal models useful for the studies of TRPC6 have been created to date; for instance, global TRPC6 knockout mice (in which TRPC6 is knocked out in all cell types of the whole body, including podocytes) are viable and show no gross phenotype, besides the slightly higher mean arterial blood pressure (12). Furthermore, mice with podocyte-specific overexpression of TRPC6 [B6.Cg-Tg(NPHS2-Trpc6)F419Walz/J]
are commercially available. These transgenic mice exhibit albuminuria, podocyte structural injuries, glomerular lesions, tubulointerstitial damage, and other pathological features of FSGS (36) and represent an excellent model for the study of TRPC6 under FSGS conditions. In the light of the recent observations showing the interchangeable functionality of TRPC family members in other cell types, it would be rather intriguing to study calcium entry in the podocytes on the basis of a multiple TRPC channel knockout model.

Major research efforts are currently focused on exploring cellular pathways which transduce the activating signal to the TRPC6 channel. Selective manipulation of these pathways may be an effective means of modulating kidney injury; however, specific mechanisms of these processes and many questions, like whether TRPC6 channels are susceptibility or initiation factors of renal disease progression, remain uncertain. Nevertheless, it is clear that the ability of podocytes to precisely regulate the intracellular Ca$^{2+}$ level plays a crucial role in glomerular diseases; manipulating Ca$^{2+}$ levels by inhibiting TRPC channels or targeting their upstream effectors holds strong promise for treating patients with chronic kidney disease and preventing podocyte depletion at early stages of renal diseases, for instance, in diabetes. Efforts to understand the role of ANG II, ROS, ATP, and other stimuli in the regulation of TRPC channels in healthy and pathophysiological states have a strong potential for scientific and medical implications in furthering our understanding of TRPC-mediated diseases.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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REFERENCES
