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

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ABSTRACT
Podocytes (terminally differentiated epithelial cells of the glomeruli) play a key role in the maintenance of glomerular structure and permeability and in the incipiency of various renal abnormalities. Injury to podocytes is considered a major contributor to the development of the 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 foot processes effacement, apoptosis and subsequent glomeruli damage. One of the key proteins responsible for calcium flux in the podocytes is TRCP6 (transient receptor potential cation channel, subfamily C, member 6) channel; 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 the podocyte injury. This mini-review focuses on the TRPC6 channel in podocytes and colligates recent data in attempt to shed some light on the mechanisms underlying the pathogenesis of TRPC6-mediated glomeruli diseases and its potential role as therapeutic target for the treatment of chronic kidney diseases.

Keywords: 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 the Focal Segmental Glomerulosclerosis (FSGS), a histologic 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, podocyte and its foot processes function as a final frontier which prevents the leak of plasma proteins into primary urine. Podocytes expresses 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 in order to develop new therapeutic intervention strategies.

TRPC6 calcium channel as a determinant of podocyte injury – Transient receptor potential canonical (TRPC) proteins, which belong to the larger TRP superfamily of channels, form Ca$^{2+}$-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 comprised 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 diseased 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 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 studying knockout models.

While compelling data indicate role of TRPC5 in maintaining of calcium flux in podocytes and development of proteinuric kidney disease (49, 58), a large body of data that has 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 or 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). However, discovery and ability to manipulate signaling upstream of TRPC6 activation could be of no less clinical significance.

TRPC6 is located on the podocyte membrane, where it is integrated into a signaling complex that interacts with nephrin, podocin, alpha-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 evoked 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 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 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 pathologic 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 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 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 angiotensin II receptors (ATRs) is effective against proteinuria (8, 9,
59). Angiotensin converting enzyme (ACE) inhibitors and ATRs blockers can attenuate
progressive glomerulosclerosis in disease models and slow disease progression in
humans (48). It was further shown that Ang II enhances albuminuria by activating
TRPC6 channels in podocytes (15). Furthermore, alteration of TRPC6 expression and
Ca^{2+} 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 GPCRs linked to G_{q} 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 channels activity in the podocytes of the freshly isolated glomeruli (this effect was lacking in the TRPC6^{-/-} knock out mice) (29) (see Fig. 1A).

Furthermore, Ang II causes an acute release of H_{2}O_{2} 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). Further, Kim et al 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 (34). Interestingly, TRPC6 expression and/or calcium influx in the podocytes have been shown to be induced by glucose (38), insulin (33) and ATP (30, 47), and these processes have been also 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 podocytes) 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 joined 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 determinative 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, tubule-interstitial damage, and other pathological features of FSGS (36) and represent excellent model to study TRPC6 under FSGS conditions. In the light of the recent observations showing the
interchangeable functionality of the 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 channels 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 act as susceptibility of initiation factors of renal disease progression, remain uncertain. Nevertheless, it is clear that the ability of podocytes to precisely regulate intracellular Ca$^{2+}$ level plays a crucial role in glomerular diseases; manipulating Ca$^{2+}$ levels by inhibiting TRPC channels or targeting their upstream effectors hold a strong promise for treating patients with CKD 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

The authors declare no competing interests.
FIGURE LEGENDS

Fig. 1. Effects of Ang II on TRPC6 channel activity and calcium influx in the podocytes.

A. Left panel: Representative current traces demonstrating the effects of Ang II on TRPC6 channels activity recorded in cell-attached patches made on podocytes of the freshly isolated glomeruli obtained from the wild type and TRPC6^{-/-} mice. Continuous current traces and addition of Ang II (1 µM) to the external bath solution are shown. All patches were held at a -60 mV test potential during the course of experiment. The c and o denote closed and open current levels, respectively (Figure adapted from Ilatovskaya et al (29)). Right panel (upper row): Representative images of the wild type mouse glomeruli stained with Fluo4 (green pseudocolor) and FuraRed (red) before and after application of 1 µM Ang II; podocytes are marked with arrows. Note an increase of green signal and a decrease of the red signal intensities upon addition of Ang II, which reflect an elevation in the intracellular calcium concentration. Graphs in the lower row demonstrate representative calcium transients caused by the application of 1 µM of Ang II in the podocytes of the wild type and TRPC6^{-/-} mouse glomeruli. Scale bar shown is 30 µm.

B: Scheme illustrating podocyte retraction as a result of the excessive calcium influx through the TRPC6 channels under a pathological stimulus (elevated Ang II levels) originated in a disease condition.
REFERENCES


**A**

Ang II

WT mouse

TRPC6/- mouse

Fluo4/FuraRed

Ang II 1 μM before Ang II

WT mouse

TRPC6/- mouse

**B**

Cell hypertrophy

Foot process effacement

Podocyte death

→ Podocyte depletion

→ Proteinuria

ATP

Ca^{2+}

TRPC6

ROS

capillary