Slit or pore? A mutation of the ion channel TRPC6 causes FSGS

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Podocytes are delicate creatures that, together with the basement membrane and the fenestrated capillary endothelium of the renal glomerulum, ensure that waste products exit the circulation via the kidney, retaining essential plasma constituents such as albumin. The task is formidable; 180 liters of plasma need to be depleted of albumin and other plasma proteins every day. Failure to get this job done results in a loss of protein with the urine. Typically, a proteinuria of >3.5 g/day causes a nephrotic syndrome characterized by hypoalbuminaemia, oedema and hyperlipidaemia.

**The nephrin–podocin complex**

The podocyte attaches to the glomerular basement membrane via foot processes and, in almost all instances of proteinuria, these processes are lost, a disturbance termed foot process effacement by the renal pathologist. Although several agents are known to trigger foot process effacement (e.g. drugs, viral infections and malignancy), the molecular mechanism leading to this morphological alteration has remained elusive. The recent identification of gene products mutated in familial forms of proteinuria has not only highlighted the importance of the podocyte, but has focused attention on a small structure formed in between foot processes of neighbouring podocytes, the slit diaphragm. Nephrin, an integral membrane protein mutated in NPSH1 (Finnish type) [1], is a member of the immunoglobulin (Ig) superfamily that together with podocin (mutated in NPSH2, steroid-resistant nephrotic syndrome) [2] localizes to the slit diaphragm. The extracellular Ig domains of nephrin engage in homophilic interactions, and form heterodimers with the Ig domains of NEPH family members [3–7]. The Ig domains of slit diaphragm proteins appear to contribute to the porous scaffold that bridges the 40 nm wide gap between two foot processes [8]. Podocin, a stomatin family member with a predicted hairpin-like structure, recruits nephrin to lipid rafts in the plasma membrane [9–11]. Lipid rafts are cholesterol-enriched microdomains that may enable members of the Src-kinase family such as Fyn to phosphorylate tyrosine residues in the C-terminal domain of nephrin [12–14], thereby creating binding sites for additional signalling molecules such as phosphoinositide 3-kinase (PI3K) [15,16]. Since experimental proteinuria rapidly disrupts the nephrin–podocin complex [17], assembly of a multimeric nephrin signalling complex appears essential for normal podocyte homeostasis [18]. However, which are the crucial cellular programmes regulated by nephrin and other components of the slit diaphragm?

**Podocyte protection against apoptosis and detachment**

Mice lacking the nephrin-interacting adaptor protein CD2AP develop nephrotic syndrome and glomerulosclerosis [19,20]. Podocytes from these mice reveal an increased susceptibility to apoptotic stimuli, for example after exposure to transforming growth factor-β [21]. CD2AP facilitates recruitment of PI3K to nephrin and activation of AKT, a protein kinase that exerts its anti-apoptotic action through phosphorylation of BAD [15]. Nephrin-dependent inhibition of apoptosis can protect podocytes against injury inflicted by either filtered toxins or mechanical stress that threaten to detach the podocytes from the glomerular basement membrane. It is quite remarkable that podocytes, otherwise robust culture cells, are exquisitely sensitive to anoikis (detachment-induced apoptosis) [15]. Lesions which compromise the structural (rather then a functional) integrity of podocytes appear to result in a slowly progressive loss of podocytes, the hallmark...
of focal segmental glomerulosclerosis (FSGS) [22]. Consistent with this hypothesis, mutations of the ACTN4 gene, encoding one of four highly homologous actins (α-actinin-4), cause autosomal dominant FSGS [23]. Mutant α-actinin-4 forms aggregates, compromising the normal actin cytoskeleton of podocytes [24]. Interestingly, α-actinin-4 deficiency not only causes recessive glomerular disease, but also increases cellular motility [25].

TRPC6, a new player from the family of TRP cation channels

Most families with autosomal dominant FSGS do not map to ACTN4 (chromosome 19q13). In one kindred, the FSGS-causing mutation localizes to chromosome 11q [26]. Winn et al. have now reported the identification of the gene that is responsible for 11q-linked FSGS [27]. To everyone’s surprise, the gene responsible for this form of familial FSGS encodes TRPC6, a member of the transient receptor potential (TRP) family of non-selective cation channels. The mutation is located in the first of three ankyrin repeats, replacing a conserved proline at position 112 with a glutamine. Addressing the question of why this mutation causes autosomal dominant disease, Winn et al. found that TRPC6P112Q increased peak calcium concentrations after stimulation with diacylglycerol, a well-established activator of TRPC6 [28]; TRPC6P112Q also potentiated angiotensin II-mediated calcium signalling in HEK 293 cells. The P112Q mutation enhanced cell surface expression of TRPC6, suggesting that the dominant effect of this mutation is the result of an increased number of ion channels at the cell surface rather than altered gating of TRPC6. The mutation is located in close proximity to a tyrosine-based sorting motif (position 108–111) responsible for interaction with the μ subunit of AP adaptor proteins, and could potentially interfere with clathrin-mediated endocytosis. Proline substitution at position 112 abolishes a binding site for SH2-containing proteins (Tyr108), potentially abrogating the interaction with proteins involved in the trafficking of TRPC6. TRPC6 contains a putative AKT phosphorylation site (Ser94) that creates a 14-3-3-binding site. Since 14-3-3 can modulate ion channel trafficking [29], it will be interesting to test whether nephrin-mediated AKT activation affects the subcellular localization of TRPC6. Although the ankyrin repeats of other TRP channels have been implicated in self-assembly [30] and stretch-mediated changes in pore characteristics [31], the function of the ankyrin repeats present in the canonical TRP family members remains largely elusive. Both the overall domain architecture and the proline are conserved in TRPC3 (Pro53 in TRPC3); TRPC3 and TRPC6 form heterodimers in the apical membrane of tubular epithelial cells [32]. If TRPC3P53Q displays similar properties to TRPC6P112Q, it will be important to investigate whether TRPC3 mutations can cause familial FSGS.

Relation between TRPC6’s gain of function and FSGS

While the paper by Winn et al. nicely addressed the gain-of-function mechanism through which TRPC6P112Q exerts its dominant effect [27], it remains unclear how the exaggerated calcium response triggered by TRPC6P112Q translates into FSGS. The authors speculate that TRPC6P112Q disrupts glomerular homeostasis and/or causes apoptosis as described for LTRPC2 [33]. Cytoplasmic calcium concentrations are tightly regulated to prevent cellular damage. A baseline and/or activation-triggered increase in calcium uptake may alter the threshold for apoptosis, for example by altering the calcium content of the endoplasmic reticulum [34]. Thus, it will be important to study the rate of apoptosis in TRPC6 mutant podocytes.

Although TRPC6P112Q-mediated apoptosis as a pathogenetic mechanism for FSGS is conceptually intriguing, an alternative model is posed by the function of slit diaphragm proteins in Caenorhabditis elegans. Here, members of the immunoglobulin superfamily related to nephrin/NEPH proteins provide spatial cues, and act as guideposts for synapse formation [35,36]. Coincidentally, both TRPC3 and TRPC6 play an essential role in axon guidance in response to BDNF [37]. Little is known about the cellular programmes that govern podocyte foot process formation; however, it can be anticipated that remodelling of podocyte foot processes is highly dynamic, adjusting to the constantly changing demands of glomerular filtration and injury-inflicted podocyte damage. Rather than serving as mechanical scaffolds, slit diaphragm molecules aided by TRPC6 may guide the delicate web of podocyte feet to seal the filtration barrier. Winn et al.’s discovery has not only shed new light on the pathogenesis of FSGS, but has opened novel avenues of approach to this disease from a therapeutic view. It will be interesting to test whether taming TRPC6’s excessive activity ameliorates familial as well as acquired FSGS, a disease that has been notoriously difficult to treat.

Conflict of interest statement. None declared.

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