Editorial Review

The nephrin-based slit diaphragm: new insight into the signalling platform identifies targets for therapy

Petri Aaltonen¹ and Harry Holthöfer¹,²

¹The Haartman Institute, University of Helsinki, Finland and ²Center for BioAnalytical Sciences, Dublin City University, Ireland

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Introduction

The global epidemic of chronic kidney disease (CKD) is progressing at an alarming rate. Ranging from mild to a most severe state, already up to 11% of the general population is affected in the US, Australia, Japan and Europe [1,2]. With a simultaneous steady increase of type II diabetes and its associated kidney complications, particularly in India, China and South-East Asia, kidney-related diseases globally are rapidly escaping any present treatment options and resources.

In most cases of CKD, the first clinical sign is proteinuria. Thus, a better understanding of mechanisms controlling the kidney permeability barrier in health and disease is essential to design better early diagnostics and therapies, to act while permanent damage may still be preventable. The key to success should be in bringing the latest genetic and molecular findings efficiently into translational platforms and finally into clinical verification. The meticulously collected large patient databases should be an essential part of this process.

Focus on the podocyte

While some key molecules of the filtration barrier have been identified and firmly documented and their number is rapidly increasing [3–5], crucial questions on their interactions and functionality in disease conditions remain unanswered.

The morphologically distinct glomerular visceral epithelial cell, podocyte, has proven to be an irreplaceable cell type maintaining permeability properties of the filtration barrier. Obviously, the other filter components, including the glomerular basement membrane and the endothelial cells of glomerular capillaries, are also major contributors to the permeability properties, Podocyte is a cell with distinct domains, including the interdigitating foot processes, their strict division to apical and basal aspects and the interconnecting filtration slits, together with dynamic connections into the cytoskeletal machinery. Interestingly, this complex cell has provided some recent important clues on how some of the thus far identified core molecules play together, at least in vitro.

The inducer of the ongoing rapid progress in revealing molecular mechanisms of proteinuria was, after years of convergent findings, the identification of NPHS1 gene-encoding nephrin [6], the key protein mutated in the congenital nephrotic syndrome of the Finnish type, the prototype disease of pure proteinuria. The full role of nephrin in various more common clinical entities is still evolving, but already, results suggest its functional involvement e.g. in diabetes [7,8] and hypertension [9]. What also makes nephrin so interesting is its apparent role as a molecular scaffold at the slit diaphragm, serving to link molecules like podocin, CD2AP and others [10,11]. Thus, an ever deeper understanding of detailed nephrin functions may also be the key to understanding the pathophysiological role of the whole molecular complex maintaining the patent filtration properties.

What, then, makes nephrin and this molecular complex in the slit diaphragm so special? First, nephrin structure appears to be preserved throughout evolution [12]. From studies in lower vertebrates and invertebrates, hints of its linkage to cell-cell interactions are emerging [13,14]. Also, nephrin is shown to have distinct outside-in and inside-out signalling functions [11,15]. However, the in vivo ligands and effects need to be characterized in detail. As is true for many key functional molecules, nephrin has also shown to present with unique patterns of regulation, including alternative usage of exons to yield tissue specificity [16].
Nephrin-based slit diaphragm

Nephlin mRNA also shows a typical splicing pattern apparently regulated in different disease entities [17], and a very unique regulation by natural antisense mRNA [18] and bidirectional regulation with a closely related molecule, filtrin [19]. It has also become evident that nephlin may leak out from podocytes in distinct ailing circumstances and, subsequently, be found in urine in typical fragmented patterns [7,20]. Whether these truly are shed or actively shed by-products of an active vesicular transport process from podocyte foot processes as suggested earlier [21], secreted from perturbing podocytes in specific exosomes, or are released during apoptotic process after podocyte detachment and leakage into urine [22], is currently uncertain.

What appears interesting, however, is the fact that in addition to the embryonic lethality of nephlin knockout mouse, reintroduction of functional nephlin specifically into podocytes of this knockout mouse line appears to rescue from lethality [23]. This result suggests that it is indeed the nephlin scaffold in the kidney which is crucial to functions, while the nephlin in its other sites of expression, like the pancreatic beta cells [24] may still have some as yet identified major functions.

Together, these findings suggest that there are many good reasons to focus major research activity on the nephlin protein complex and its key role in maintenance of the functional filtration barrier.

The nephlin-link, a target for therapy

Nephlin has been shown to mediate outside-in signalling of podocytes to maintain the connection to the intracellular actin-paved cytoskeleton responsible for the complex podocyte structure, and to participate in its rapid change in proteinuria. Enabling this signalling function, the intracellular part of nephlin has the tyrosine residues required for the action of the specific kinases of the Src family [25], including Fyn. The phosphorylation of Fyn modulates podocyte gene expression and interaction of nephlin with podocin [26].

The phosphorylation of nephlin is known to activate at least two pathways (Figure 1). First, the phosphorylation of nephlin by the Fyn kinase leads to the activation of the phosphoinositide 3-OH kinase (PI3K), as the regulatory subunit p85 of PI3K recognizes the phosphorylated nephlin-CD2AP complex and allows the catalytic subunit p110 to act on the phospholipids of the inner leaflet of the cell membrane [27]. This leads to the downstream phosphorylation and inactivation of the apoptotic factor Bad via the serine-threonine kinase AKT.

The second pathway involves a key mediator Nck, as recently demonstrated [28,29]. It is shown that the Nck adaptor protein is involved in the regulation of actin-cytoskeletal link to nephlin. Nck proteins can bind to the nephlin phosphotyrosines via their SH2 domain, while the SH3 domains can mediate actin polymerization. The specific binding sites of Nck1 and Nck2 isotypes on nephlin have been mapped, and mutations in these sites prevent the interaction with nephlin and, subsequently, the mediation of nephlin signalling to the structurally and functionally important podocyte cytoskeleton [29].

With this information now available, completely new approaches can be designed to modulate the nephlin-cytoskeleton interaction in situations of proteinuria.

Conclusion

Taken together, nephlin appears to be an important scaffold, into which other molecules of the slit diaphragm are associated into structural-functional complexes, which appears an important target structure for pharmacologic molecules but also for diagnostic platforms [5]. Clearly, all the previously generated transgenic and nephlin knockout mouse lines and particularly the podocyte-specific inducible nephlin knockout mouse line avoiding the embryonic lethality will be critically important in further studies. We are now beginning to understand how extracellular stimuli are transduced intracellularly in the podocyte, to maintain structural integrity of the filtration slit in response to blood pressure, proteinuria, and engagement by antibodies or other factors. Future steps to design molecules to modulate these interactions are yet to be taken.

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References


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