Institutionen för Medicinsk Biokemi och Biofysik

Identification and characterization of kidney glomerulus-associated genes and proteins

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SUMMARY

The kidney glomerulus is a micro-organ comprised of a molecular filtration barrier that prevents the loss of blood proteins into the primary filtrate. This function is dependent on the coordination of its three constituent layers: the endothelium, the glomerular basement membrane and the podocytes. While each of the three layers contributes to the permselectivity of the glomerular filtration barrier, the podocyte forms the final barrier to filtration.

Many glomerulus-enriched proteins have been implicated in pathogenesis of renal diseases. We have identified over 300 glomerulus-upregulated genes using expressed sequence tag profiling and microarray analysis, in order to discover new genes with important roles in glomerular filtration. Two of these proteins, Plekhh2 and Schip1, were characterized further in this study.

Plekhh2 is an intracellular protein with two PH, MyTH and FERM domains, highly enriched in the podocytes and testes, for which no function has previously been ascribed. We studied by immunoelectron microscopy Plekhh2 distribution in the human glomerular filter and found that its expression was reduced in focal segmental glomerulosclerosis. Heterologously expressed Plekhh2 localizes to the peripheral regions of lamellipodia in cultured podocytes and its PH1 domain contains a PIP3 consensus-binding site. The N-terminal half of Plekkh2 is not required for targeting to lamellipodia but it rather mediates Plekhh2 self-association. By yeast two-hybrid we identified two Plekhh2 interacting partners: Hic-5, a focal adhesion protein, and actin. Plekhh2 and Hic-5 coprecipitate and colocalize at the soles of podocyte foot processes in situ. Strikingly, endogenous Hic-5 partially relocalizes from focal adhesions to lamellipodia in Plekhh2-expressing podocytes. We found also that Plekhh2 stabilizes the cortical actin cytoskeleton by attenuating actin depolymerization. Plekhh2, Hic5 and actin show parallel expression changes in two mouse models of glomerular damage.

Schip1 is a coiled-coil protein previously discovered through association studies with schwannomin (Nf2, merlin) in the mouse brain, shown to be responsive to PDGFβ stimulation. We have identified Schip1 as a highly enriched kidney glomerulus transcript in the podocytes, and investigated its functions in this context. We show that Schip1 promotes migration of cells in response to PDGFβ stimulation and accumulation of cortical actin. In cultured podocytes, Schip1 localizes to lamellipodia periphery, closely overlapping the cortical actin. Actin disassembly by latrunculin A treatment, could not be prevented by Schip1 expression, but the protein colocalized to remaining actin fibers. Strikingly, by yeast two-hybrid, coprecipitations and FRET we discovered that Schip1 interacts with Nherf2/ezrin. This is a well characterized podocyte protein complex, forming a supporting net for docking actin filaments in the foot processes by binding to podocalyxin and/or PDGFrβ cytoplasmic tails. Furthermore, we show by comparative microarray studies, that the expression of Schip1 and its associated proteins is affected in a similar manner in several mouse models of human glomerular diseases.

Our experiments suggest that both Plekhh2 and Schip1 are involved in actin assembly dynamics at the leading edge of cellular extensions. We propose that these proteins are associated to the complicated podocyte foot process actin network. The discovery and characterization of novel glomerular genes and proteins presented in this thesis, has contributed to our understanding of glomerular biology and pathophysiology of renal diseases.

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