Is podocyte shape controlled by the dystroglycan complex?

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Abstract

α- and β-dystroglycan were detected at the base of foot processes of human glomeruli by immunoelectron microscopy. Perfusion of isolated rat kidneys with the polycationic compound protamine sulfate was found to induce rapid (i.e. within 15 min) flattening of foot processes in an energy- and actin-dependent fashion. Here we provide evidence that (i) glomeruli possess large amounts of a specifically composed complex; (ii) this complex may undergo changes in human glomerular disease; and (iii) flattening of foot processes is directly associated with dissociation of laminin–dystroglycan complexes.

Keywords: dystroglycan; podocyte

Extensive flattening of podocyte foot processes and increased permeability of the glomerular capillary filter are the major pathological features of minimal change nephrosis (MCN) and focal segmental glomerulosclerosis (FSGS) [1–3]. Adhesion proteins anchor and stabilize podocytes on the glomerular basement membrane (GBM), and presumably are involved in the pathogenesis of foot process flattening. So far, αβ1-integrin has been localized to basal cell membrane domains [4,5]. In this report, α- and β-dystroglycan were detected at precisely the same location by immunoelectron microscopy, and the presence of α- and β-dystroglycan chains was confirmed by immunoblotting on isolated human glomeruli. As the major dystroglycan-binding partners in the GBM (laminin, agrin and perlecan) and the intracellular dystrophin analogue utrophin are also present in glomeruli, it appears that podocytes adhere to the GBM via dystroglycan complexes, similar to muscle fibres in which actin is linked via dystrophin and dystroglycan to the extracellular matrix. In analogy to muscle cells, it is therefore plausible that podocytes also use precisely actin-guided dystroglycan complexes at their ‘soles’ actively to govern the topography of GBM matrix proteins. Expression of the α/β-dystroglycan complex was reported to be reduced in muscular dystrophies, and therefore we searched for similar pathological alterations in archival kidney biopsies from patients with MCN (n = 16) and FSGS (n = 8) by quantitative immunoelectron microscopy. The density of α-dystroglycan on the podocyte’s soles was significantly reduced to 25% in MCN, while it was not different in normal controls and FSGS. The expression of α-dystroglycan was reduced to > 50% in MCN, and slightly increased in FSGS. Levels of dystroglycan expression returned to normal in MCN after steroid treatment (n = 4). Expression of β1-integrin remained at normal levels in all conditions. These findings pinpoint different potentially pathogenic mechanisms of foot process flattening in MCN and FSGS [6].

β-Dystroglycan is associated laterally with members of the sarcoglycan family of proteins that mediate interconnections to several other proteins [7,8]. We have localized by immunocytochemistry δ-sarcoglycan only, but no other members of the sarcoglycan family. It is of interest that δ-sarcoglycan specifically links β1-integrins to the dystroglycan complex, and it is possible that it serves a similar function in basal glomerular cell membranes.

A major goal of the study of the glomerular dystroglycan complex is to understand better the molecular mechanisms of foot process flattening that occurs invariably in association with proteinuria. Mice lacking dystroglycan die in early fetal life, due to general lack of basement membrane organization [9]. In glomeruli, utrophin replaces dystrophin as the linker between dystroglycan and actin [10]. Therefore, we have investigated the shape of foot processes in utrophin ‘knockout’ mice that, however, fail to develop a renal phenotype. We conclude that the components of the glomerular dystroglycan complex are different from those in muscle, and we speculate that their function may be guaranteed by redundancies with other proteins not discovered so far.

Perfusion of isolated rat kidneys with the polycationic compound protamine sulfate was found to induce rapid (i.e. within 15 min) flattening of foot
processes in an energy- and actin-dependent fashion [11,12]. We therefore have followed the redistribution of the components of the dystroglycan complex by light and electron microscopic immunohistochemistry. While dystroglycan was distributed in the basement membrane in an almost perfectly linear pattern in controls, it was observed within intracellular vacuoles in podocytes after protamine perfusion. This pattern corresponded by immunoelectron microscopy to numerous vesicles in podocytes that sometimes were joined to pre-lysosomal multivesicular bodies, and were emptied with them into the urinary space. Dystroglycan on the base of other foot processes was redistributed to patches and clumps. This raised the question of whether dystroglycan could be detached from the basal membrane matrix. We have addressed this question in an in vitro solid phase assay in which purified laminin was immobilized; $\alpha$-dystroglycan was bound and detected by monoclonal anti-$\alpha$-dystroglycan antibody. Rising concentrations of protamine sulfate were found to compete for laminin–$\alpha$-dystroglycan binding in an approximate dose–response relationship. Moreover, pre-formed complexes on laminin and $\alpha$-dystroglycan were dissociated by protamine sulfate at concentrations similar to those that caused flattening of foot processes in vivo.

Taken together, the presently available data suggest that (i) glomeruli possess large amounts of a specifically composed dystroglycan complex; (ii) this complex may undergo changes in human glomerular disease; and (iii) flattening of foot processes is directly associated with dissociation of laminin–dystroglycan complexes.

References

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