Defective glycosylation of α-dystroglycan contributes to podocyte flattening

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In addition to skeletal muscle and the nervous system, α-dystroglycan is found in the podocyte basal membrane, stabilizing these cells on the glomerular basement membrane. Fukutin, named after the gene responsible for Fukuyama-type congenital muscular dystrophy, is a putative glycosyltransferase required for the post-translational modification of α-dystroglycan. Chimeric mice targeted for both alleles of *fukutin* develop severe muscular dystrophy; however, these mice do not have proteinuria. Despite the lack of a functional renal defect, we evaluated glomerular structure and found minor abnormalities in the chimeric mice by light microscopy. Electron microscopy revealed flattening of podocyte foot processes, the number of which was significantly lower in the chimeric compared to wild-type mice. A monoclonal antibody against the laminin-binding carbohydrate residues of α-dystroglycan did not detect α-dystroglycan glycosylation in the glomeruli by immunoblotting or immunohistochemistry. In contrast, expression of the core α-dystroglycan protein was preserved. There was no statistical difference in dystroglycan mRNA expression or in the amount of nephrin and α3-integrin protein in the chimeric compared to the wild-type mice as judged by immunohistochemistry and real-time RT-PCR. Thus, our results indicate that appropriate glycosylation of α-dystroglycan has an important role in the maintenance of podocyte architecture.

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Podocytes are highly differentiated cells that possess specialized projections called foot processes. Adhesion molecules of podocytes are likely to have an important role in the maintenance of podocyte morphology by anchoring of these cells to the glomerular basement membrane (GBM).[1,2] To date, two major adhesion protein systems have been identified. As the first step, $\alpha_3\beta_1$-integrin was localized exclusively to the podocyte basal membrane domains in normal and flattened human podocytes and was speculated to contribute to the firm adhesion of the podocytes to the GBM matrix proteins.[3–5]

Recently, α- and β-dystroglycans were discovered in podocytes, and were found to be localized to the podocytes basal membrane domain.[6,7] Dystroglycan is composed of a transmembrane, heterodimeric complex of α- and β-subunits that link the extracellular matrix to the cell cytoskeleton. Gene knockout experiments have not been helpful for understanding the role of dystroglycans in podocyte function, because knockout results in early embryonic death, long before kidney glomeruli develop, and because Reichert's membrane, the first canonical basement membrane produced by the embryo, is disorganized, and it fails to withstand the blood pressure of maternal circulation.[8]

Fukuyama-type congenital muscular dystrophy (FCMD), discovered in Japan, is a severe muscular dystrophy with central nervous system involvement.[9] Fukutin, named after the gene responsible for FCMD, is a putative glycosyltransferase required for post-translational modification of α-dystroglycan.[10,11]

Chimeric mice generated using embryonic stem cells targeted for both *fukutin* alleles develop severe muscular dystrophy associated with the defective glycosylation of α-dystroglycan.[12] In this study, we investigated fukutin-deficient chimeric mice to clarify the role of α-dystroglycan glycosylation for maintaining podocyte architecture.

RESULTS

Chimeric mice showed muscle weakness, were unable to walk in a straight line, and dragged their feet as previously described.[12]

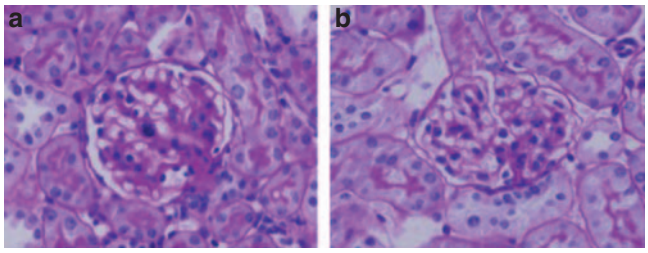

Figure 1 | Photomicrographs of periodic acid Schiff stain. Representative photomicrographs of wild-type (**a**) and the fukutin-deficient chimeric mice (**b**). Glomeruli from the fukutin-deficient chimeric mice have no abnormalities by light microscopy. Original magnification × 200.

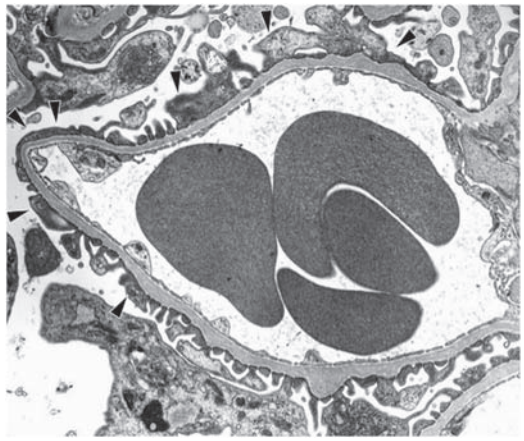

Figure 2 | Transmitted electron photomicrograph of the fukutin-deficient chimeric mice. The flattening of foot processes was observed in segmental lesion of capillary lumens (arrowheads). No detachment of foot processes from the GBM was observed. Original magnification × 4000. GBM, glomerular basement membrane.

Light microscopy of kidney specimens showed minor abnormalities (Figure 1) together without massive albuminuria. Electron microscopy revealed morphological changes in podocytes such as vacuolization, microvillous transformation, and segmental foot process effacement. However, the detachment of foot processes from the GBM was not observed (Figure 2). The number of foot processes along the GBM decreased significantly in chimeric mice (1.39 ± 0.18/ μm GBM; Figure 3b) compared with wild-type mice (2.09 ± 0.01/μm GBM; Figure 3a) ($P = 0.039$; Figure 3c). In addition, the GBM was thickened and the three-layered structure of the GBM was lost under the lesion of flattened foot processes in chimeric mice (Figure 3b). The thickness of the GBM was increased significantly in chimeric mice (0.30 ± 0.03 μm) compared with wild-type mice (0.15 ± 0.01 μm) ($P = 0.043$; Figure 3d).

Immunohistochemistry using the IIH6 monoclonal antibody against the laminin-binding carbohydrate residues of α-dystroglycan (Figure 4a) and rabbit polyclonal antibody AP1530 against the 34 amino acids in the C-terminal domain of human α-dystroglycan indicated that the α-dystroglycan was localized along the glomerular capillary walls in a linear manner in wild-type mice kidneys (Figure 4c). In contrast, the expression of α-dystroglycan laminin-binding carbohydrate residues decreased in chimeric mice (Figure 4b). Immunohistochemistry revealed that expression of the core α-dystroglycan protein was relatively preserved in chimeric mice (Figure 4d).

In wild-type mice, immunoblotting detected a broad band around 150 kDa representing α-dystroglycan (Figure 4e). Expression of laminin-binding carbohydrate residues was reduced in chimeric mice (Figure 4e), whereas that of the core α-dystroglycan protein was relatively preserved in chimeric mice. An additional band around 75 kDa was detected in chimeric mice, suggesting that considerable parts of α-dystroglycan were hypoglycosylated (Figure 4f). There was no statistical difference in the mRNA expression of dystroglycan between the wild-type and chimeric mice (Figure 4g).

In wild-type mice, a laminin overlay assay detected a broad band around 150 kDa corresponding to α-dystroglycan, whereas the overlay assay revealed a deficiency in the laminin-binding activity of α-dystroglycan in chimeric mice (Figure 4h).

The expression of α_3-integrin (another adhesion molecule localized to the podocytes basal membrane domain) and nephrin (which is thought to be the main component of the slit diaphragm)[13] was also investigated. The expression levels of α_3-integrin and nephrin were unaltered in chimeric mice using immunohistochemistry and real-time RT-PCR (Figure 5a–d, g, and h).

Tetramethylrhodamine (TRITC)-conjugated wheat germ agglutinin (WGA) staining was performed to detect sialic acid and *N*-acetyl-glucosamine (GlcNAc) oligomer. WGA staining was observed along the glomerular capillary walls in a linear manner in wild-type mice. Similar staining was observed in chimeric mice, suggesting that fukutin deficiency did not affect GlcNAc oligomers and sialic acid (Figure 5e and f).

DISCUSSION

Dystroglycan is expressed in many cell types such as skeletal muscles, various epithelia, and in the nervous system.[14,15] α-Dystroglycan is heavily glycosylated by *O*-mannosyl glycosylation. It is speculated that α-dystroglycan carbohydrate residues are bound to the cationic LG domain common to several matrix proteins such as laminin and agrin.[16] In the glomerulus, α-dystroglycan is localized to basal cell membrane domains of the podocyte, stabilizes podocytes on the GBM, and presumably is involved in the pathogenesis of foot process flattening.[17]

Using the IIH6 monoclonal antibody, we found that the expression of α-dystroglycan laminin-binding carbohydrate residues decreased in fukutin-deficient chimeric mice, whereas expression of the core α-dystroglycan protein was preserved. These results confirmed that fukutin is involved in the modification of α-dystroglycan laminin-binding carbohydrate residues. We performed the laminin blot overlay assay to evaluate the binding activity of α-dystroglycan to laminin and revealed a severe reduction in the binding