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CHAPTER 22

Peripheral nerve dystroglycan: its function and potential role in the molecular pathogenesis of neuromuscular diseases

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Introduction

Dystrophin is a large cytoskeletal protein encoded by the Duchenne muscular dystrophy (DMD) gene [1,2]. In skeletal muscle, dystrophin exists in a large oligomeric complex tightly associated with several novel sarcolemmal proteins, including an extracellular glycoprotein of 156 kDa (α-dystroglycan) and a transmembrane glycoprotein of 50 kDa (adhalin) [3–8]. The 156 kDa α-dystroglycan binds laminin, a major component of the basement membrane [7,9,10]. It is heavily glycosylated, two-thirds of its molecular mass being accounted for by sugar residues [7,9,10]. The glycoprotein-binding site exists in the cysteine-rich/C-terminal domains of dystrophin [11,12]. Dystrophin also interacts with F-actin through the actin binding site(s) in the N-terminal domain [10,13–15]. These findings indicate that the dystrophin-glycoprotein complex spans the sarcolemma to link the subsarcolemmal actin-cytoskeleton with laminin meshwork in the basement membrane in skeletal muscle [6,7,10,16].

Utrophin, an autosomal homologue of dystrophin, is localized exclusively to the neuromuscular junction in adult skeletal muscle [17–19]. Utrophin, which has the cysteine-rich/C-terminal domains highly homologous to those of dystrophin [17], is associated with sarcolemmal proteins identical, or immunologically homologous to the dystrophin-associated proteins in the neuromuscular junction [19]. The recent demonstration that α-dystroglycan binds the basement membrane component agrin, which mediates the clustering of acetylcholine receptor in the neuromuscular junction, suggests that the utrophin-glycoprotein complex may play a role in peripheral synaptogenesis [20–23].

Distal transcripts of the DMD gene have been identified and their protein products have been shown to be expressed differently from the full-size 400 kDa dystrophin [24,28]. A protein of 71 kDa called Dp71 (also called apo-dystrophin-1) is expressed in non-muscle tissues, such as brain and liver [24–27]. A protein of 116 kDa called Dp116 (also called apo-dystrophin-2) is expressed in Schwann cells of peripheral nerve [28]. While physiological functions of these proteins remain unknown, the fact that both Dp71 and Dp116 share the cysteine-rich/C-terminal domains of dystrophin, which are involved in the interaction

with the glycoprotein complex, suggests that Dp71 and Dp116 may exist in a similar complex as the dystrophin-glycoprotein complex of skeletal muscle.

Results

Here we report on (1) the status of expression of α-dystroglycan, adhalin, dystrophin and utrophin, and (2) the biochemical characterization of α-dystroglycan, in peripheral nerve [29, 30]. Immunohistochemical analysis demonstrated intense staining of peripheral nerve by antibodies against the C-terminal domains of dystrophin or utrophin [29]. Staining was positive surrounding myelin sheath of nerve fibers [29]. A similar staining pattern was obtained with antibody against α-dystroglycan [29]. In sharp contrast, antibody against the N-terminal domain of dystrophin and antibody against adhalin did not stain peripheral nerve [29].

On immunoblot analysis of SDS-extracts of peripheral nerve, antibodies against the C-terminal domains of dystrophin stained a band with a molecular mass of 110–120 kDa, but full-size 400 kDa dystrophin was not detected [29]. This 110–120 kDa band was not stained by antibody against the N-terminal domain of dystrophin [29]. These findings, together with the immunohistochemical data described above, indicate that this 110–120 kDa band is Dp116. Antibody against the C-terminus of utrophin, on the other hand, stained full-size

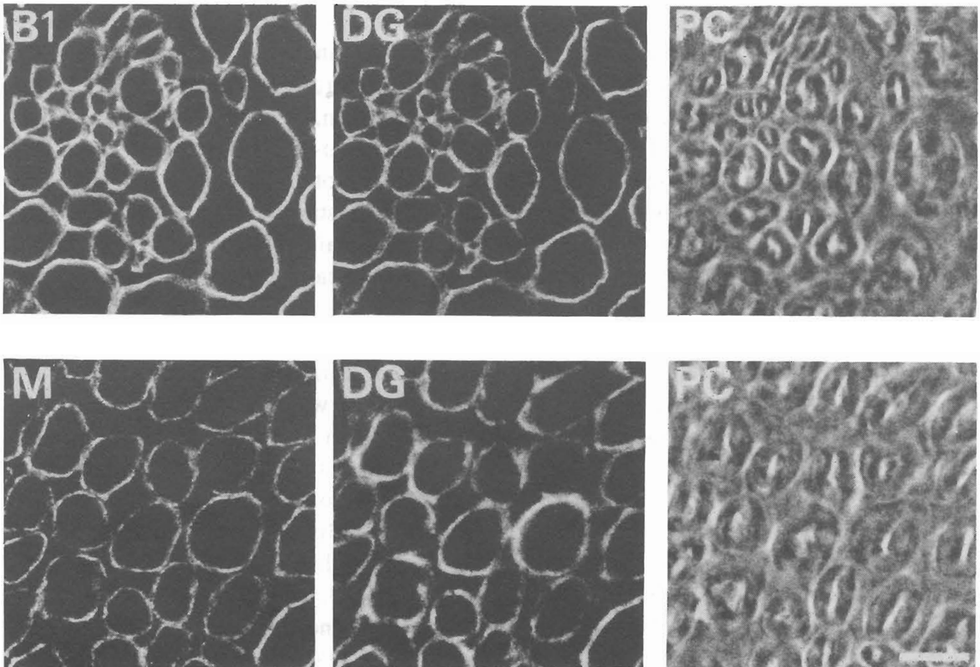

Fig. 1. Co-localization of dystroglycan and merosin in peripheral nerves. Shown are the double-immunostaining analyses of dystroglycan/merosin B1 chain and dystroglycan/merosin M chain. Confocal laser scanning microscopy fluorescent and phase-contrast (PC) images are shown. DG, B1 and M indicate dystroglycan, the M and B1 chains, respectively. Dystroglycan and merosin were co-localized surrounding myelin sheath of peripheral nerve fibers. Bar, 10 μm.

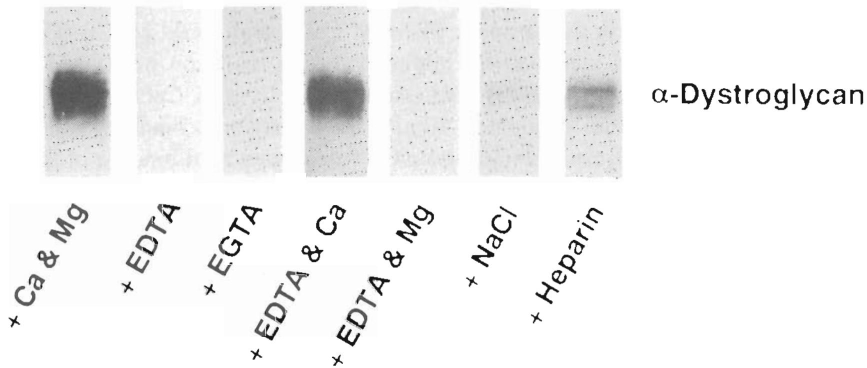

Fig. 2. Laminin-binding properties of peripheral nerve dystroglycan.. Nitrocellulose transfers of the digitonin extracts of peripheral nerve membranes were incubated, in the presence of 1 mM $CaCl_2$ and 1 mM $MgCl_2$, with mouse EHS laminin (+Ca and Mg). The binding of laminin to the 120 kDa peripheral nerve α-dystroglycan was inhibited by the inclusion of 10 mM EDTA (+EDTA) or EGTA (+EGTA) in the overlay medium. Addition of 20 mM $CaCl_2$ (+EDTA and Ca), but not 20 mM $MgCl_2$ (+EDTA and Mg), in the overlay medium containing 10 mM EDTA restored laminin binding. The binding of laminin was significantly reduced by the presence of 0.5 M NaCl (+NaCl) or 1 000-fold excess (wt/wt) of heparin (+Heparin) in the overlay medium.

400 kDa utrophin in peripheral nerve [29]. Antibody against α-dystroglycan stained a band of 120 kDa in peripheral nerve, while it stained a band of 156 kDa in skeletal muscle [29]. Antibody against adhalin did not stain a band in peripheral nerve while it stained a band of 50 kDa in skeletal muscle [29].

Taken together, these findings demonstrate the expression of Dp116, utrophin and α-dystroglycan surrounding myelin sheath of peripheral nerve fibers [29]. However, full-size dystrophin and adhalin are undetectable in peripheral nerve, suggesting that the putative complex which could involve Dp116, utrophin and α-dystroglycan may lack adhalin in peripheral nerve [29].

In the next step towards the elucidation of the cellular functions of this putative complex, we investigated the biochemical activities of the 120 kDa peripheral nerve α-dystroglycan. Immunocytochemical analysis demonstrated the co-localization of α-dystroglycan and merosin, a laminin heterotrimer comprised of the M, B1 and B2 chains [31–36], surrounding myelin sheath of peripheral nerve fibers (Fig. 1) [30]. Immunocytochemical analysis of teased peripheral nerve fibers demonstrated homogeneous staining for α-dystroglycan on the surface of myelin sheath but not in the node of Ranvier, suggesting the localization of α-dystroglycan in the outer membrane of myelin sheath [30].

Immunoblot analysis demonstrated that the 120 kDa α-dystroglycan was enriched in peripheral nerve membranes and was extracted by non-ionic detergent such as digitonin [30]. The 120 kDa α-dystroglycan was quantitatively absorbed by WGA-Sepharose, indicating that it is a membrane-associated glycoprotein with *N*-acetylneuraminic acid/*N*-acetylglucosamine residues [30]. In the presence of 1 mM $CaCl_2$ and 1 mM $MgCl_2$, laminin- and merosin-Sepharose removed the 120 kDa α-dystroglycan from the digitonin extracts of peripheral nerve membranes [30]. α-Dystroglycan was eluted by 10 mM EDTA [30]. When overlaid with laminin or merosin on the nitrocellulose transfers, the eluted α-dystroglycan bound both these proteins [30]. In order to confirm laminin and merosin binding of peripheral nerve α-dystroglycan, the nitrocellulose transfers of the digitonin extracts of peripheral

nerve membranes were overlaid with laminin or merosin. The 120 kDa α-dystroglycan bound laminin and merosin in the presence of 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (Fig. 2) [30]. The binding of laminin and merosin was inhibited by the inclusion of 10 mM EDTA or EGTA in the overlay medium (Fig. 2) [30]. Addition of 20 mM $CaCl_2$, but not 20 mM $MgCl_2$, in the overlay medium containing 10 mM EDTA restored binding of laminin and merosin (Fig. 2) [30]. These results demonstrate Ca^{2+}-dependency of binding of laminin and merosin. The binding of laminin and merosin was inhibited by the presence of 0.5 M NaCl or 1000-fold excess (wt/wt) of heparin in the overlay medium (Fig. 2) [30].

Discussion

Together, our results indicate that α-dystroglycan is a novel merosin-binding protein in peripheral nerve, most likely in the myelin sheath membrane. The size of α-dystroglycan varies among different tissues [7,9,10]. The molecular mass of peripheral nerve α-dystroglycan is comparable to that of brain form (120 kDa), and is smaller, by about 40 kDa, than that of striated muscle or lung form (156 kDa) [7,9,10,37]. This is presumed to be due to differences in the posttranslational modification, such as glycosylation, of α-dystroglycan [7,9,10,29,30]. However, the binding activities of laminin and merosin to the 120 kDa α-dystroglycan are similar to the laminin-binding activities of the 156 kDa α-dystroglycan [10,30].

Recently, the specific reduction of merosin M chain was demonstrated in skeletal muscle of patents with Fukuyama-type congenital muscular dystophy (FCMD), a disease characterized by brain anomaly and muscular dystophy [38]. The abnormality in the expression of α-dystroglycan in the sarcolemma of FCMD patients implicates the disturbance of the interaction between α-dystroglycan and merosin in the molecular pathogenesis of FCMD [39]. The M chain is also deficient in skeletal muscle and peripheral nerve of *dy* mice [40–42], which have muscular dystophy and peripheral nerve dysmyelination [40–42]. Intriguingly, laminin is known to promote Schwann cell myelination [43,44]. Furthermore, the expression of merosin is upregulated during development of peripheral nerve [31], and merosin is expressed in differentiated Schwann cell neoplasms, with the exception of 6 detectable in undifferentiated malignant Schwannomas [45]. These findings indicate that the expression of laminin/merosin in the basement membrane is associated with Schwann cell differentiation and myelination. Taken together, our results raise intriguing possibilities that α-dystroglycan may be involved in the regulation of Schwann cell myelination and that the disturbance of the interaction between α-dystroglycan and merosin may play a role in the pathogenesis of peripheral neuropathy in *dy* mice. At present it is unknown if α-dystroglycan exists in a complex associated with Dp116, utrophin or other dystrophin-associated proteins, or if this putative complex is involved in signal transduction. Further research is currently underway in our laboratory to address these important questions.

Finally, adhalin is deficient in the sarcolemma of patients with severe childhood autosomal recessive muscular dystophy with DMD-like phenotype (SCARMD) [46–51], and all the dystrophin-associated proteins, including adhalin, are greatly reduced in the sarcolemma of DMD patients [12,52]. These findings suggest that the deficiency of adhalin may be the common denominator leading to muscle cell necrosis in these diseases [16,46]. Interestingly, SCARMD patients never present with nervous system dysfunction which is found in a substantial percentage of DMD patients. Taking our results into consideration, one hy-

pothesis to explain this phenotypic difference could be that adhalin is not expressed in the nervous system [8,29,53,54], and thus, its specific deficiency causes only muscular dysfunction.

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