FEBS Letters 579 (2005) 4792–4796 FEBS 29857

Disruption of perlecan binding and matrix assembly by post-translational or genetic disruption of dystroglycan function

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Received 13 June 2005; revised 19 July 2005; accepted 25 July 2005

Available online 11 August 2005

Edited by Micheal R. Bubb

Abstract **Dystroglycan is a cell-surface matrix receptor that requires LARGE-dependent glycosylation for laminin binding. Although the interaction of dystroglycan with laminin has been well characterized, less is known about the role of dystroglycan glycosylation in the binding and assembly of perlecan. We report reduced perlecan-binding activity and mislocalization of perlecan in the LARGE-deficient Large^{myd} mouse. Cell-surface ligand clustering assays show that laminin polymerization promotes perlecan assembly. Solid-phase binding assays provide evidence for the first time of a trimolecular complex formation of dystroglycan, laminin and perlecan. These data suggest functional disruption of the trimolecular complex in glycosylation-deficient muscular dystrophy.**

Keywords: Dystroglycan; Laminin; Perlecan; Basement membrane; Congenital muscular dystrophy; Large^{myd} mouse

1. Introduction

Dystroglycan (DG) is a transmembrane protein that links the extracellular matrix (ECM) to the cellular cytoskeleton, and it has multiple roles in various tissues [1]. DG consists of the extracellular alpha subunit (α-DG), and the transmembrane beta subunit (β-DG), which are encoded by the same mRNA and cleaved in post-translational processing [2]. β-DG binds to intracellular dystrophin or utrophin, which then binds to actin filaments and extracellular α-DG. α-DG binds to several ECM proteins that contain laminin globular (LG) domains such as laminins, agrin, and perlecan [3]. Using recombinant LG domains, laminins and perlecan have shown to compete for binding to α-DG [4].

Recent studies demonstrate that the *O*-glycosylation essential for ligand-binding activity of α-DG takes place on the mucin-like domain [5]. Detailed analyses indicate that the N-terminal domain of α-DG is necessary for molecular recognition by a glycosyltransferase, LARGE, and that the DG-LARGE interaction is critical for the functional expression of DG [5]. Mutations in the *LARGE* gene have been found in human congenital muscular dystrophy type 1D, as well as in the Large^{myd} mouse [6,7]. Furthermore, recent studies suggest that the DG post-translational glycosylation pathway is a convergent target for several human muscular dystrophies, classified as ''dystroglycanopathies'' [3]. Hypoglycosylation of α-DG in dystroglycanopathies and Large^{myd} mice has been observed in conjunction with a reduction of laminin-binding activity [8].

Here, we investigate roles of DG in assembly of perlecan on the cell surface. Reduced perlecan-binding activity of DG and abnormal laminin/perlecan complexes were detected in Large^{myd} mice. By controlling ligand concentration and molecular interaction, we provide evidence for the first time of a trimolecular complex of DG, laminin, and perlecan. These data demonstrate the mechanism of the trimolecular complex formation and suggest its disruption in the pathogenesis of glycosylation-deficient muscular dystrophy.

2. Materials and methods

2.1. Animals, antibodies, and proteins

Wild type (C57BL/6) and Large^{myd} mice were bred at The University of Iowa from stock originally obtained from Jackson Laboratories (Bar Harbor, ME). All animal studies were authorized by the Animal Care Use and Review Committee at The University of Iowa.

Monoclonal antibody IIH6 against α-DG and rabbit polyclonal antibodies against perlecan (anti-PGI and anti-PGV) were described previously [8–10]. Anti-laminin and perlecan antibodies were obtained from Sigma and Chemicon, respectively.

Perlecan fragments domain I (PGI) and domain V (PGV) were prepared as previously described [9,10]. Laminin-1 and heparan sulfate proteoglycan (HSPG), derived from Engelbreth–Holm–Swarm (EHS) mouse sarcoma, were obtained from Biomedical Technologies Inc.

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0014-5793/$30.00
doi:10.1016/j.febslet.2005.07.059

and Sigma, respectively. Purification of α-DG and enrichment of DG with wheat germ agglutinin (WGA)-beads were also described previously [5,8]. *p*-Aminoethylbenzenesulfonyl fluoride (AEBSF)-treated laminin-1 was prepared using the method described by Colognato et al. [11].

2.2. Solid-phase binding assay

The solid-phase binding assay was described previously [8]. To measure IIH6-sensitive binding, IIH6 was used at 0.06 mg/ml. In cases where the DG-laminin complexes were tested, laminin was preincubated with immobilized DG in 3% BSA-LBB (10 mM triethanolamine, pH 7.6, 140 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$) for 16 h prior to the addition of tested ligand. Ligand binding was detected by incubating with primary antibodies followed by HRP-conjugated secondary antibodies. All data were triplicate means.

2.3. Miscellaneous

Immunofluorescence analysis [8] and laminin-clustering assay [12] were described previously.

3. Results

3.1. Reduction of perlecan-binding activity of DG in Large^{myd} mice

To examine perlecan-binding activity of DG in Large^{myd} mice, DG preparations were enriched with WGA-chromatography from skeletal muscle. Western blotting with antibodies to the α-DG core protein confirmed that nearly all of the DG in the muscle sample bound to WGA-beads (data not shown). Solid-phase PGV binding assays showed a reduction of more than 80% of PGV binding in Large^{myd} mice (Fig. 1). This indicates that perlecan-binding activity requires the specific carbohydrate modification of α-DG. Therefore, we hypothesized that reduced ligand-binding activity of DG may cause displaced localization of ligand proteins.

3.2. Mislocalization of perlecan/laminin in Large^{myd} mice

We have reported previously that the Large^{myd} mouse has a disruption of glia limitans, the surface basement membrane in the brain, which leads to abnormal neuronal migration [8]. Here, we show that perlecan does not localize in regions where the glia limitans is disrupted (Fig. 2). In addition, the Large^{myd} mouse presents with an abnormal appearance of punctuate

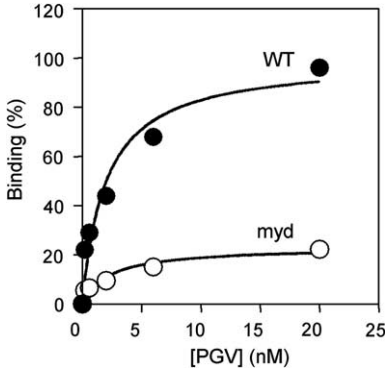

Fig. 1. *Reduction of perlecan-binding activity of DG in Large^{myd} mice.* WGA-bound materials from Large^{myd} (myd) or littermate control (WT) skeletal muscle extracts were immobilized and then incubated with various concentrations of PGV. Binding was detected with anti-PGV antibody. The maximal binding to the control preparations was set as 100%.

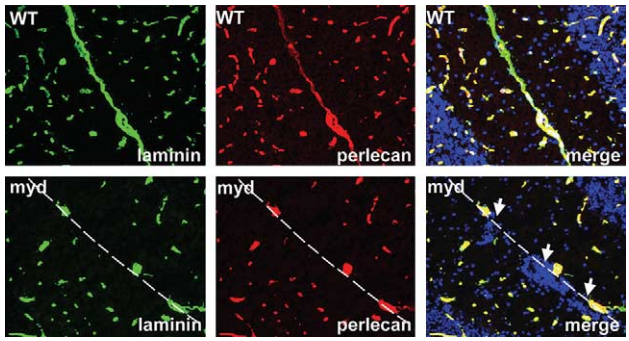

Fig. 2. *Immunolocalization of laminin and perlecan in Large^{myd} cerebellum.* Cryoselections from Large^{myd} (myd) and littermate control (WT) mice were stained with antibodies against laminin and perlecan. Staining in blue denotes DAPI. Dotted line indicates location of disrupted glia limitans between of cerebellar lobules. Arrows indicate clusters of abnormally migrated granule cells.

accumulations of laminin/perlecan throughout the cerebral cortex in locations where DG is not normally localized, whereas laminin/perlecan staining on microvessels appears normal (double arrows) (Fig. 3). We also observed similar punctuate accumulations of laminin and perlecan in brain-specific DG-null mice (data not shown). This suggests that perlecan interacts with laminin independently of DG, however DG may be required for proper location of perlecan.

3.3. Cell surface assembly of perlecan with DG-facilitated laminin-network

In order to understand the role of DG and laminin in membrane assembly of perlecan, cell-surface ligand clustering assays were performed. We observed defects of the cell surface laminin-clustering on Large^{myd} myoblast and fibroblast, however perlecan was not detected in these cell culture system (R.B, M.K, and K.P.C unpublished data). Since LARGE-deficiency results in a DG functional-null phenotype, we have used genetically engineered embryonic stem (ES) cells as a model system. We previously reported that that DG was required for the formation of laminin clusters on the surface of individual ES cells [12]. The clusters have been classified on the basis of three distinct morphologies: dots, lines, and plaques [13]. Fig. 4A shows colocalization of clusters of DG, laminin, and

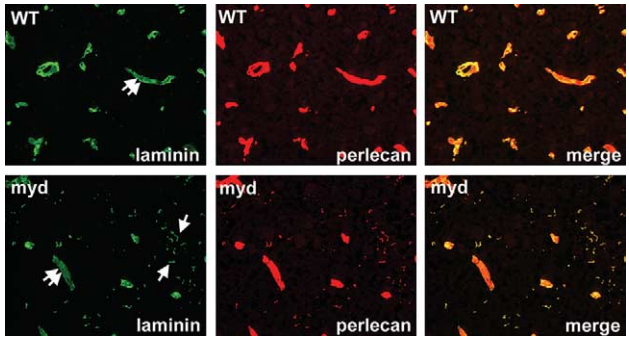

Fig. 3. *Immunolocalization of laminin and perlecan in Large^{myd} cerebral cortex.* Cryosections from Large^{myd} (myd) and littermate control (WT) mice were stained with antibodies against laminin and perlecan. The double arrow indicates normal staining of laminin and perlecan at microvessels within the cerebral cortex. The single arrow indicates areas of abnormal punctuate accumulations of laminin and perlecan in Large^{myd} cerebral cortex.