

Dystroglycan induced muscular dystrophies – a review

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Abstract. – Dystroglycanopathies are muscular dystrophies caused by mutations in genes involved in O-linked glycosylation of α -dystroglycan. Severe forms of these conditions result in abnormalities in brain and ocular development too, in addition to muscular dystrophy. The full spectrum of developmental pathology is caused mainly by loss of dystroglycan from Bergmann glia. Moreover, cognitive deficits are constant features of severe forms of dystroglycanopathies. However, the precise molecular mechanism leading to neuronal dysfunction in these diseases is not fully known yet. The present review article will discuss the importance of dystroglycan in cerebellar development and associated pathological states.

Key Words:

Dystroglycan, Cerebellar development, Dystroglycanopathies.

Introduction

Cobblestone lissencephaly belongs to a category of disorders characterized by structural malformation of the brain, caused by defects in neuronal migration during early development¹. The cobblestone phenotype is the result of neuroglial over migration into the subarachnoid space, leading to the formation of an additional cortical layer; the ramification is heterotopia that produces an unusually “bumpy” brain surface. Clinical representations of this disorder include cortical dysplasia, dysmyelination, and pontocerebellar dysplasia. The presence of cobblestone lissencephaly is pathognomonic of a group of congenital muscular dystrophies: Walker-Warburg syndrome, muscle-eye-brain disease, and Fukuyama congenital muscular dystrophy. These diseases are the severe manifestations of a heterogeneous group of autosomal recessive disorders termed “dystroglycanopathies” – muscular

dystrophies caused predominantly by mutations in genes involved in the O-linked glycosylation of α -dystroglycan².

In addition to muscle dystrophy, a constellation of brain anomalies, ranging from mild cognitive impairment to severe mental retardation, with or without structural malformations, is presented in these maladies; yet what role dystroglycan plays in the central nervous system remains largely undiscovered. Thus, understanding how the protein dystroglycan contributes to brain development in general, and cerebellar histogenesis in particular, is imperative to elucidate the mechanisms leading to dystroglycanopathy-associated cerebellar dysplasia. The present review shall be focused on the above theme and will explore all important associated areas.

Dystroglycanopathy and Congenital Muscular Dystrophy

The common biochemical hallmark for these diseases is hypoglycosylation and reduced ligand-binding affinity of α -DG³. These dystrophies present a broad clinical spectrum, ranging from congenital muscular dystrophy (CMD) with brain and eye involvement in severe cases to limb-girdle muscular dystrophy (LGMD) in milder instances⁴. CMD is a heterogeneous group of diseases with autosomal recessive inheritance, characterized by the onset of hypotonia, muscle weakness, contractures at birth or within the first few months of life, and by dystrophic changes visible in the muscle biopsy. The clinical diversity of CMD is shown by the different degrees of delay in motor developmental, physical disability, muscle pathology, and by presence or absence of mental retardation.

Within the brain, dystroglycan is expressed in astrocytic endfeet abutting the glia limitans and the intracerebral vasculature⁵. In addition to being localized at the inner limiting membrane and the basal laminae of blood vessels, DG is

expressed by photoreceptor cells in the outer plexiform layer of the retina⁶. In addition to this, DG has been confirmed to be expressed important brain parts including all major neurons and glia in the developing central nervous system⁷. On the other hand, a study in recent past has confirmed its presence in the developing cerebellum, in granule cell precursors, Purkinje cells, radial glia, and Bergmann glia⁸. Furthermore, in the BM receptors, dystroglycan is present as dystrophin-glycoprotein Complex comprised of an extracellular α -subunit and a transmembrane β -subunit⁹. The physiological studies about its functional evaluation confirmed the involvement of DG in diverse cellular functions like skeletal muscle membrane integrity maintenance, structural as well as functional regulation of the CNS and skeletal muscle regulation^{10,11}. Besides this, the precise function of the DGC remains to be described, but it is suggested to bestow sarcolemmal stability during muscle contraction. So, far it is now certain that genetic causes of various reported muscular dystrophies are associated with mutations in various components of DGC.

In addition to DG and dystrophin, the core proteins of the DGC in skeletal muscle are the sarcoglycans-sarcospan sub-complex, whose purported function is to stabilize the whole DGC within the sarcolemma. Indeed, mutations in either sarcoglycans or dystrophin reduce the expression or perturb the formation of the entire DGC, and are thought to be one of the underlying mechanisms responsible for their respective muscular dystrophies. Additional components of the DGC include dystrobrevins and syntrophins, two types of dystrophin-binding proteins that do not appear to have direct roles in the mechanical function of the DGC, but rather serve as docking sites for other intracellular signaling proteins¹². Mutation in the dystroglycan gene (DAG1) itself or mutations in genes encoding known and putative glycosyltransferases – enzymes involved in the post-translational modifications of α -DG – lead to a group of heterogeneous muscular dystrophies; these dystrophies have a spectrum of clinical manifestations ranging from limb-girdle phenotypes to severe brain and eye malformations and mental retardation¹³. Hypoglycosylation of α -DG and ensuing reduced ligand binding affinity is the underlying mechanism leading to disease manifestation. Meanwhile, mutations in the LAMA2 gene encoding the $\alpha 2$ subunit of laminin (merosin),

the protein component of the basal lamina which surrounds muscle fibers, cause merosin-deficient muscular dystrophy^{14,15}. This corroborates the perception that any disruption to the DGC-linked ECM and muscle fiber cytoskeleton, whether it be minimized expression of the protein component of the basement membrane, or diminished ligand binding capability of DG, or reduced expression of the DGC, is sufficient to cause muscular dystrophy.

Post-translational modification of α -Dystroglycan

Dystroglycan (DG) is a glycoprotein that undergoes glycosyltransferase-mediated N-glycosylation, mucin-type O-glycosylation, O-mannosylation, and an identified phosphorylated O-mannosyl glycan bearing xylose and glucuronic acid-containing polysaccharide. Loss of N-linked glycosylation on DG has no effect on its ability to bind its ligand; however, loss of O-linked glycosylation appears to disrupt DG-ligand binding¹⁶. As it happens, defects in O-mannosyl glycan synthesis, caused by mutations in genes required for α -DG glycosylation, give rise to congenital disorders termed dystroglycanopathies. Biochemical analysis on skeletal muscle biopsy from these patients revealed the hallmarks of dystroglycanopathy: hypoglycosylated α -DG, reduced expression of laminin $\alpha 2$, and reduced laminin-binding capability, suggesting a connection between CDG and dystroglycanopathy.

Extracellular Interactions of Dystroglycan

Dystroglycan (DG) is ubiquitously expressed in a number of tissues and has, thus, been associated with several proteins of multiple functions. Notwithstanding the omnipresent distribution and the numerous binding partners, DG has been studied most extensively in muscle, particularly in relation to its role in the dystrophin-glycoprotein complex and associated muscular dystrophies, while much less is known about its functions in the brain¹⁷. This section will describe the significance of DG-ligand interplays in the context of brain development and disease, focusing on extracellular interactions via the numerous glycans on α -DG. The mucin domain of α -DG is in fact decorated with O-linked glycans, amongst which the phosphorylated O-mannose polysaccharide was recently identified to be crucial for α -DG's ligand-binding affinity¹⁸. α -DG binds to laminin G (LG) domain-containing proteins in

the extracellular space, including components of the basement membrane (BM) such as agrin, laminin, and perlecan, synaptic adhesion molecules α -neurexins and β -neurexins, pikachurin at the photoreceptor ribbon synapse, and slit at the spinal cord ventral midline¹⁹⁻²¹. Collectively, studies of α -DG interactions with LG-domain-containing proteins in the brain support the notion that DG is crucial for the proper laminar organization and structural development of the CNS.

Laminin, perlecan, and agrin are protein components of the BM, matrix extension of the plasma membrane which serves to protect tissue from physical stress as well as provide structural support for cell differentiation, polarization, migration, and survival²². Laminin is a heterotrimer consisting of one each of five α , four β , and three γ subunits joined at the coiled-coil domain. The N-terminals of the three laminin chains (α , β , and γ) form ternary nodes that are essential for laminin polymerization, whereas only the C-terminal of laminin α chain participates in cell surface adhesion via interactions between the last two of its LG domains (LG4, LG5) and α -DG²³. Perlecan and agrin are both LG domain-containing heparan sulfate proteoglycans (HSPG) that bind to both laminin and α -DG, forming a collateral linkage between laminin network and the cell surface. These HSPGs are capable of binding to collagen, the only other polymerizing component of the BM other than laminin, bridging and compacting the two superstructures. Interactions between α -DG and these components of the BM are thus critical for proper BM assembly and maturation.

Role of Neurexins

Neurexins are presynaptic adhesion molecules that form trans-synaptic complexes with postsynaptic neuroligins through which synaptic transmissions between neurons are mediated²⁴. Neurexin-neuroligin interaction is required for proper synaptic function and cognition, as mutations in neurexins and neuroligins are implicated in autism spectrum disorders²⁵. There are two types of neurexins: larger α -neurexins containing six LG domains intercalated by EGF-like domains, and shorter β -neurexins containing a single LG domain²⁶. Both α - and β -neurexins bind to the mucin domain of α -DG, but the exact physiological functions of these interactions are not known. Pikachurin is an ECM-like retinal protein localized to the synaptic cleft in the photorecep-

tor ribbon synapse²⁷. In the visual system, photoreceptors detect and distinguish a wide range of light intensities, then transmit the received light information to bipolar and horizontal cells in the retina using a special type of synapse called ribbon synapse. The ribbon synapse enables the graded and rapid release of synaptic vesicles and faithfully transmits a broad range of light intensity information.

DG is expressed at the presynaptic terminals of photoreceptors and forms complexes with pikachurin at the synaptic cleft via interaction between α -DG and LG domains of pikachurin²⁸. Oligomerization of pikachurin induces DG clustering on the photoreceptor surface; conversely, DG expression is required for the accumulation of pikachurin. Such bidirectional interaction is required for proper ribbon synapse's structural formation and subsequent retinal electrophysiology. Slits are secreted proteins at the floor plate that act as chemorepellents for commissural axonal projections from spinal cord neurons²⁹. Roundabouts (Robo) are canonical transmembrane receptors for Slits; Slit-Robo binding is further stabilized by the HSPG syndecan³⁰. Commissural axons are initially attracted to the midline by the chemoattractant Netrin acting through its receptor, deleted in colorectal carcinoma (DCC), and are insensitive to Slits³¹. During midline crossing commissural axons respond to Slits' repulsion due to upregulation of their surface expression of Robo and, thus, are forced to exit from the midline to the contralateral side; at the same time, they are prevented from crossing back to the ipsilateral side. After crossing over, these axonal projections correspond to additional cues, including Slits, to turn and proceed to their synaptic targets.

Conclusions

The above researches make it clear that a lot of work has been recorded in the literature revealing importance of dystroglycan in cerebellar development and disease. However, further studies are still required to design efficient drugs for better management of the affected patients.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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