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Dystroglycan and muscolar dystrophies related to the dystrophin-glycoprotein complex

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Summary. - Dystroglycan (DG) is an adhesion molecule composed of two subunits, α and β, that are produced by the post-translational cleavage of a single precursor molecule. DG is a pivotal component of the dystrophin-glycoprotein complex (DGC), which connects the extracellular matrix to the cytoskeleton in skeletal muscle and many other tissues. Some muscular dystrophies are caused by mutations of DGC components, such as dystrophin, sarcoglycan or laminin-2, or also of DGC-associated molecules, such as caveolin-3. DG-null mice died during early embriogenesis and no neuromuscular diseases directly associated to genetic abnormalities of DG were identified so far. However, DG plays a crucial role for muscle integrity since its targeting at the sarcolemma is often perturbed in DGC-related neuromuscular disorders.

Key words: dystroglycan, muscular dystrophy, dystrophin-glycoprotein complex.

Riassunto *(Il distroglicano e le distrofie muscolari connesse al complesso di glicoproteine associate alla distrofina)*. - Il distroglicano (DG) è una molecola di adesione composta da due subunità, α e β, prodotte da un taglio post-traduzionale di un singolo precursore. Il DG è un componente centrale del complesso di glicoproteine associate alla distrofina (DGC) che ha il ruolo di connettere la matrice extracellulare al citoscheletro. Alcune distrofie muscolari sono causate da mutazioni nei componenti del DGC come la distrofina, i sarcoglicani e la laminina-2 o anche in proteine associate al DGC come la caveolina-3. Non si conoscono malattie neuromuscolari associate a mutazioni del DG e la distruzione *(knock-out)* del gene provoca l'arresto dello sviluppo embrionale del topo. Il DG ha comunque un ruolo cruciale per la stabilità della fibra muscolare poiché in diverse forme di distrofie muscolari connesse al DGC la sua localizzazione al sarcolemma è profondamente perturbata.

Parole chiave: distroglicano, distrofie muscolari, complesso di glicoproteine associate alla distrofina.

Introduction

Muscular dystrophies (MD) are a group of heterogeneous inherited disorders characterized by progressive muscle degeneration and frequently accompanied by cardiomyopathy or mental retardation. In many cases MD arise from perturbations of the connection between the cytoskeletal elements of striated muscle fibers and the surrounding basement membrane, which leads to muscle fragility and contraction-induced damage [1]. A primary contribution to this interaction is offered by the dystrophin-glycoprotein complex (DGC), a group of tightly associated transmembrane and cytoskeletal proteins that forms a molecular bridge between dystrophin and the extracellular matrix [2] (Fig. 1 and Table 1). DGC is formed by dystrophin, dystroglycan complex (α- and β-DG), sarcoglycans (α, β, γ and δ), sarcospan, syntrophins (α-1, β-1 and β-2) and dystrobrevins. Dystrophin binds to cytoskeletal F-actin and to the cytodomain of transmembraneous β-DG; the extracellular domain of β-DG binds to the peripheral membrane protein α-DG that interacts with several components of the basement membrane of the skeletal muscle, laminin, perlecan and agrin [3].

In 1987, dystrophin was identified as the protein product of the Duchenne MD locus [4]. Dystrophin is absent from muscle of patients affected by Duchenne muscular dystrophy (DMD) whereas the milder Becker muscular dystrophy (BMD) is caused by several mutations that result in a reduced expression of dystrophin or of truncated and only partially functional

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Table 1. - Components of dystrophin-glycoprotein complex (DGC), or DGC-associated, involved in muscular dystrophies

Subcellular localization	Protein	Disease
Cytoskeleton	Dystrophin	Duchenne muscular dystrophy
Sarcolemma	Sarcoglycans	Limb-girdle muscular dystrophy 2C-F
	Caveolin-3	Limb-girdle muscular dystrophy 1C
Extracellular matrix	Laminin-2	Congenital muscular dystrophy

Table 2. - Expression of dystroglycan (DG) subunits in various muscular dystrophies

Muscular dystrophy	α-DG	β-DG
DMD	Absent	Absent
LGMD	Absent	Normal
MCMD	Absent or reduced	Normal
MEB	Hypoglycosylated	Normal
FCMD	Hypoglycosylated	Normal
WWB	Hypoglycosylated	Normal

DMD: Duchenne muscular dystrophy; LGMD: limb-girdle muscular dystrophies; MCMD: merosin-deficient congenital muscular dystrophies; MEB: muscle-eye-brain disease; FCMD: Fukuyama congenital muscular dystrophy; WWS: Walker-Warburg syndrome.

isoforms of the protein [5]. Mutations in genes encoding sarcoglycans have been also shown to cause some of the recessive limb-girdle muscular dystrophies (LGMD) whereas no pathogenic mutations have been identified so far in genes encoding syntrophins or dystrobrevins [5, 6]. Furthermore, mutations in the gene encoding for the α-2 chain of the extracellular matrix protein laminin give rise to congenital muscular dystrophy (CMD) [7]. All the aforementioned pathologies can be defined as DGC-related neuromuscular disorders.

No naturally occurring mutations of the DG gene have been described so far. Only a mild form of muscular dystrophy associated with secondary β-DG deficiency has been described in a four-year-old Saudi boy [8]. On the other hand, especially in DGC-related MD, DG targeting at the sarcolemma is often perturbed, and recently it was discovered that alteration of its post-translational modification pattern represent important secondary effects leading to severe muscular diseases [9, 10] (Table 2). In DMD, both α- and β-DG are not properly targeted at the sarcolemma, but in LGMDs and in CMDs β-DG is still retained at the sarcolemma while α-DG is absent [5].

Dystroglycan

DG is encoded by a single gene as a precursor protein that is rapidly cleaved to generate two subunits, α- and β-DG [11, 12] (Fig. 2). DG is expressed in a wide variety of tissues: skeletal and cardiac muscles, epithelia, central and peripheral nervous systems [13].

Alpha-DG is a heavily glycosylated protein. Although its predicted molecular weight is ≈ 72 kDa, it appears as a broad smeared band on western blots with an apparent molecular weight of 156 kDa in skeletal muscle, 140 kDa in cardiac muscle and 120 kDa in brain. The nature of α-DG glycosylation is still largely unknown and few studies suggest that most glycans are O-linked mannose-type [14]. Alpha-DG binds a number of extracellular molecules such as laminin, agrin, perlecan, neurexin and biglycan [12]. Primary sequence analysis and electron microscopy showed that α-DG has a dumbbell-like shape in which two globular domains, N- and C-terminal domains, are connected by a central, elongated and highly glycosylated mucin-like region rich in prolines, serines and threonines [15]. Alpha-DG interacts non-covalently with the membrane-spanning β-DG. The binding epitope was mapped within the C-terminus of α-DG in a region highly conserved of 36 aminoacids [16, 17].

The cytodomain of β-DG contains many proline residues, which are likely to represent preferential sites for protein-protein interactions. In fact, β-DG interacts with different cytoplasmic proteins: dystrophin and dystrophin related proteins, rapsyn, caveolin-3 and Grb2 [18-20]. In addition to intracellular proteins, β-DG binds also to transmembrane proteins such as the sarcoglycans [21]. Several evidences suggest an involvement of DG in signal transduction, as indicated by the presence of potential SH2 and SH3 binding motifs within the cytodomain of β-DG. In fact, it was shown that phosporylation of Tyr^{892} is required for recruiting SH2 domain containing proteins [22, 23].

DG forms a linkage between the cytoskeleton and the extracellular matrix and it is crucial for the structural stability of the plasma membrane [24].

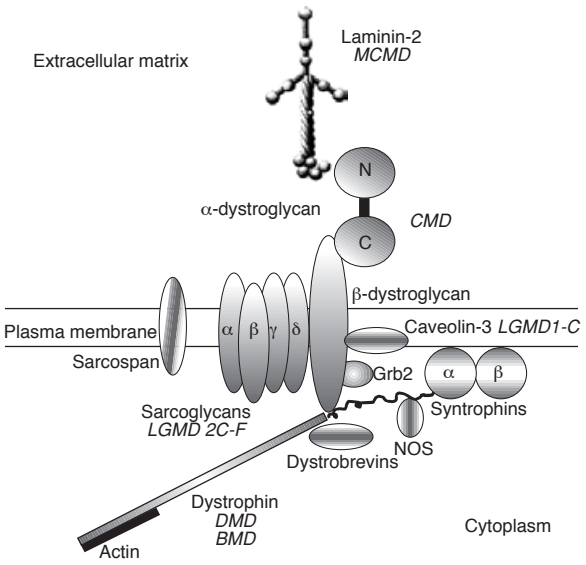

Fig. 1. - Dystrophin-glycoprotein complex (DGC). It is a group of peripheral and integral membrane proteins which forms a mechanical linkage between the F-actin cytoskeleton and the extracellular matrix. The DGC in skeletal muscle is formed by a) the cytosolic dystrophin, syntrophins and dystrobrevins; b) the heavily glycosylated dystroglycan-complex, formed by two subunits, α- and β-DG, and c) the four sarcoglycans, α, β, γ and δ, plus the non-glycosylated 25 kDa sarcospan, which belongs to the T4 transmembrane protein family. Many muscular dystrophies arise from mutations in DGC components and in related DGC proteins as illustrated. DMD, Duchenne muscular dystrophy, due to the absence of dystrophin; BMD, Becker muscular Dystrophies, due to the expression of truncated forms of dystrophin; LGMD, limb-girdle muscular dystrophies, due to mutations in one of the four sarcoglycans or in caveolin-3; CMD, due to the absence of laminin-2 (also named mMCMD, merosin congenital muscular dystrophy) or to defects in glycosyltransferases leading to hypoglycosylation of α-DG.

Moreover, DG plays an essential role also during the assembly of the first extra-embryonic basement membrane during mammalian morphogenesis: DG-null mouse embryos failed to further develop at day 6.5 when the Reichert's basement membrane is deposited [25] and embryoid bodies derived from DG knockout mice have basement membranes severely disrupted [26]. It has been proposed that the laminin binding to α-DG induces the self-assembly of laminin and a reorganization of the plasma-membrane receptors and the cytoskeletal elements in a polygonal network [27]. Chimaeric mice develop muscular dystrophy whereas their muscular basement membrane is correctly deposited and myogenesis is normal [28]. Recently, *via* a conditional knock-out approach, it has been confirmed that disruption of the DG gene results in the loss of the DGC in differentiated muscle but only in a mild muscular dystrophy phenotype [29]. Interestingly, it was found that satellite cells (the staminal cells of muscle), still expressing dystroglycan, are able to support a progressive regeneration of skeletal muscle fibers. Thus, the regenerative capacity of satellite cells expressing dystroglycan is likely to be responsible for the mild disease observed in mice [29]. DG is also involved in the maturation and stabilization of nicotinic acethylcoline receptors (nAChR) at the neuromuscular junction (NMJ) [30, 31].

Recently, new evidences of an involvement of DG in other pathological conditions emerged. DG acts as a receptor for *Mycobacterium leprae,* the causative agent of leprosy, and some arenaviruses, which cause haemorrhagic fevers [32, 33]. Recently it was observed that DG expression could be greatly altered in several carcinomas. A possible mechanism is that the extracellular region of β-DG is cleaved by a metalloprotease and α-DG would lose its normal membrane-associated location [34, 35].

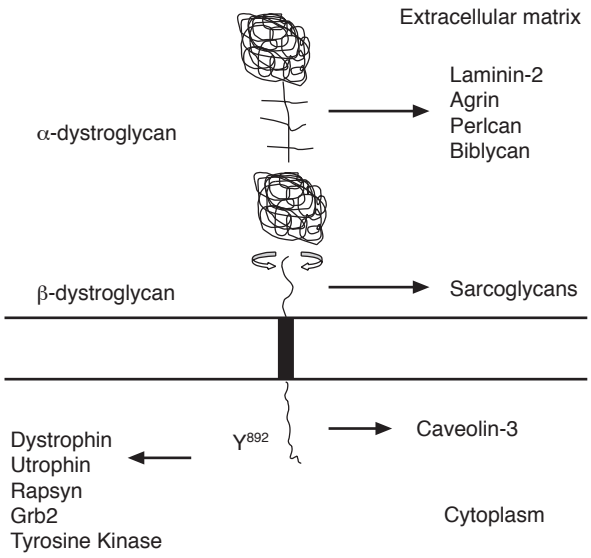

Fig. 2. - Dystroglycan complex. Dystroglycan (DG) represents a link between the extracellular matrix and the cytoskeleton and it is crucial for the structural stability of the plasma membrane. DG is composed of two subunits, α- and β-DG. Alpha-DG binds to a number of extracellular matrix molecules, laminin, agrin and perlecan and it interacts tightly with β-DG. The cytoplasmic tail of β-DG binds dystrophin, caveolin-3 and other cytoplasmic proteins involved in signal trasduction. The tyrosine 892 of beta-DG can be phosphorylated by a kinase belonging to the *src* family (see text).

Duchenne muscular dystrophy and Becker muscular dystrophy

Duchenne muscular dystrophy (DMD) is one of the most common and severe muscle disorders. Genetically, it is inherited as a X-linked recessive disease and it affects 1 out 3500 males. DMD is due to mutation in the gene encoding dystrophin, the large cytoskeletal protein that is located at the cytoplasmic face of sarcolemma and that links actin to the membrane [36].

In DMD muscle fibers, dystrophin is completely absent [37]. The milder allelic disorder Becker muscular dystrophy (BMD), is caused by mutations that result in reduced expression of dystrophin or expression of truncated partially functional forms of the protein [38]. Patients with DMD have a childhood onset phenotype and die before their twenties because of respiratory and cardiac problems whereas patients with BMD have moderate muscle weakness and may have a normal life.

The absence of dystrophin in skeletal muscle leads to a dramatic perturbation of the DGC: both α- and β-DG, together with the other DGC proteins, leave their normal sarcolemmal location [39]. The link between the cytoskeleton and the extracellular matrix is largely perturbed and muscle fibers become weak and degenerated. In tissue-section immunostaining, muscle appear necrotic with increased connective tissue within its fibers [40]. Moreover, the lack of dystrophin not only leads to a failure in forming a proper DGC but also to a reduction of the binding affinity of other proteins for the actin cytoskeleton [41].

It was shown that in *mdx* mice, the most common animal model for DMD, the overexpression of Dp71, a short isoform of dystrophin that contains the binding site for DG but not for actin, restored the DGC components [42]. However, Dp71 overexpression did not rescue the dystrophic phenotype probably for the inability of Dp71 to target actin at the inner face of sarcolemma [43]. This experiment pointed out the importance of dystrophin and DG for the proper localization of DGC and for the fully integrity of muscle fibers. On the other hand, a successful reduction of the dystrophic phenotype was obtained when utrophin, the autosomal homologue of dystrophin harbouring both DG and actin binding sites, was expressed in *mdx* mice [44].

During the last years, new approaches for rescuing the dystrophic phenotype that are based on the regenerative capacities of the muscle fibers are being experimented. Rosenthal and colleagues have demonstrated that the muscle specific overexpression of the insulin-like growth factor I (IGF-I) ameliorates muscular dystrophy in *mdx* mice [45]. IGF-I plays a critical role in muscle regeneration promoting the proliferation and differentiation of satellite cells [46]. Overexpression of IGF-I induced muscle hypertrophy and it defended muscle against the secondary symptoms associated with the disease such as muscle weakness. Recently, the same functional improvement was obtained by the pharmacological inhibition of myostatin [47]. Myostatin is a new member of the tumour growth factor-β family (TGF-β) which is expressed in skeletal musle where suppresses muscle growth [48]. Inhibition of myostatin in dystrophic muscle increases muscle mass and improves the muscle strength [47]. This experiment provides a novel pharmacological approach for the treatment of muscle diseases and circumvents the problems associated with conventional gene therapy.

Limb-girdle muscular dystrophies

Limb-girdle muscular dystrophies (LGMD) represent a large group of muscular dystrophies that shows different clinical severity. The mutated genes causing LGMD encode proteins with different locations within the skeletal muscle fibers, such as sarcolemma, sarcomeres and even nuclear proteins [1]. Alterations of DG targeting have been found only in two types of LMGD, sarcoglycanopathies and caveolin-3-deficient LMGD, which are both DGC-related.

Sarcoglycanopathies

Sarcoglycanopathies are a group of four autosomal recessive LGMD caused by mutations of the α, β, γ and δ sarcoglycan genes, respectively. The clinical phenotype of LGMD closely resembles that of DMD [49].

The sarcoglycans (SG) are a group of transmembrane glycoproteins which is tightly associated with the 25 kDa sarcospan, related to tetraspannins [12, 50]. Beta, γ and δ sarcoglycans interact with β-DG [51]. It was observed that in the muscles of patients with LGMD, besides the absence of one defective subunit, also the other SG and sarcospan are not found at the sarcolemma [52]. Therefore, mutation of one of the four SG gene results in the loss of the whole SG complex. Analysis of the SG knock-outs mice confirmed this hypothesis [53-55] (Table 3). Interestingly, sarcospan-deficient mice did not develop muscular dystrophy and maintain normal muscular functions [56].

The sarcolemmal location of sarcoglycans is a prerequisite for the stabilization of DG and accordingly in LGMD muscle DG is greatly reduced at the sarcolemma. For example, in the cardiomyopathic BIO 14.6 hamster, the animal model for LGMD-2F, the primary absence of δ-SG results in the lack of DG

Table 3. - Mouse models for muscular dystrophies related to the dystrophin-glycoprotein complex (DGC)

Genotype	Gene product	Phenotype
mdx	Dystrophin	Mild
UTRN$^{-/-}$	Utrophin	None
mdx/UTRN$^{-/-}$	Dystrophin/ utrophin	Severe
dy	Laminin-α2	Severe
DAG1$^{-/-}$	Dystroglycan	Embryonic lethal
DAG1$^{-/-}$ chimera	Dystroglycan	Severe
Scga$^{-/-}$	α-Sarcoglycan	Mild
Scgb$^{-/-}$	β-Sarcoglycan	Mild
Scgg$^{-/-}$	γ-Sarcoglycan	Severe
Scgad$^{-/-}$	δ-Sarcoglycan	Severe
Cav-3$^{-/-}$	Caveolin-3	Severe

[57]. Adenovirus-mediated gene transfer of δ-SG normalized the levels of α- and β-DG and the sarcolemma integrity [57]. In LGMD-2D the absence of α-SG causes the disruption of the entire SG complex and the consequent disruption of the interaction between α-DG and the muscle basement membrane [54].

Caveolin-3-deficient limbe-girdle muscolar dystrophies

Caveolins are widely expressed integral membrane proteins and are the main component of *caveolae*, vesicular invaginations of the plasma membrane which are involved in trafficking and signal trasduction events [58, 59]. The mammalian caveolin gene family consists of three members: caveolins-1 and 2, which are coexpressed in many tissues, and the muscle-specific caveolin-3. Despite caveolin-3 is not an integral component of the DGC, it was found a direct association between caveolin-3 and β-DG: this interaction involves the WW-like domain of caveolin-3 and the WW-binding consensus sequence within the cytoplasmic tail of β-DG (a WW domain harbors four conserved aromatic amino acids including two trypthophan residues) [60].

Loss or reduced expression of caveolin-3 leads to the autosomal dominant LGMD-1C [61]. The disassembly of caveolin-3 network dramatically affects the expression of α-DG. Although caveolin-3 and α-DG do not interact, α-DG is almost completely lost from the muscle fibers surface whereas β-DG is normally localized at the sarcolemma [62]. It was recently shown that double caveolin-1 and -3 knock out mice, *Cav-1-3*$^{-/-}$, develop a severe cardiomyophaty [63].

Interestingly, transgenic overexpression of caveolin-3 in muscle fibers induces a Duchenne-like muscular dystrophy phenotype, that is characterized also by an increased number of *caveolae* [64]. Up-regulation of caveolin-3 leads to the loss of dystrophin and to a dramatic reduction of the β-DG levels. The overexpression of caveolin-3 would alter the normal processing or stoichiometry of DGC leading to its degradation.

Congenital muscular dystrophies

The acronym CMD (congenital muscular dystrophy) is used to indicate another heterogeneous group of dystrophies that frequently leads to death in early childhood. Mutations in various genes may lead to the development of CMD [36]. Two major groups of CMD could be identified: the merosin-deficient congenital muscular dystrophies (MCMD) and three forms due to secondary defects in posttranslational modifications of α-DG due to abnormalities of some glycosyltransferase, muscle-eye-brain disease (MEB), Fukuyama congenital muscular dystrophy (FCMD) and Walker-Warburg syndrome (WWS).

Merosin-deficient congenital muscular dystrophy

Laminins are a family of large multidomain glycoproteins (≈ 800 kDa) representing one of the major components of basement membranes. Three polypeptide chains associate to generate at least twelve heterotrimeric (α, β, γ) isoforms, from laminin-1 to laminin-12 [65]. Laminins bind α-DG through their so-called G (globular) or LNS domains for their sequence (and structural) similarity with domains found also in neurexins and within the sex hormone-binding globulin (SHBG). LNS domains occur in tandem array in several extracellular molecules: laminin isoforms, agrin, perlecan and neurexins [66, 67]. In skeletal muscle and peripheral nerve, α-DG binds to laminin-2 (merosin), formed by α-2, β-1 and γ-1 chains. Alpha-DG interacts with α-2(LNS1-3) and α-2(LNS4-5) with high-affinity [66]. The dissociation constant, measured by solid-phase radioligand binding assays, between native α-DG, purified from skeletal muscle, and laminin-1 is 8 nM [69]. The interaction with LNS is calcium-dependent and it is likely to involve a positively charged surface of the LNS subdomains and the negatively charged carbohydrate groups of α-DG [68], as supported by the observation that full deglycosylation of native α-DG abolishes the interaction with laminin [70].

Mutations in the laminin-α2 chain gene cause a severe form of CMD, commonly referred to as merosin-deficient congenital muscular dystrophy (MCMD). About 50% of patients with MCMD show a primary deficiency of laminin-2 that brings to dramatic perturbations of the basement membrane molecular

architecture [37] (Fig. 3). Recently, the loss of α-DG from the membrane was observed [71], whereas older studies had previously emphasized that DG and DGC were unaffected in the MCMD phenotype [72, 73].

The molecular scenario underlining MCMD is certainly complex. It has been found that in MCMD muscle α-DG is significantly reduced from the sarcolemma whereas the transmembrane β-DG is still present and its expression level seems to be 3-fold higher than in normal muscle [71] (Fig. 4). It is likely that α-DG is greatly stabilized by the interaction with its extracellular matrix binding partners since it was shown that in dy^W transgenic mice (the animal model for MCMD (Table 3) the overexpression of the muscle isoform of agrin in a miniaturized form, harboring both laminin and α-DG binding sites, is able to significantly alleviate the dystrophic symptoms [71]. This experiment shows that the interaction between DG and its extracellular binding partners could be exploited as a new and elegant tool for rescuing muscular dystrophies.

Another molecular aspect of the dystrophic phenotype observed in MCDM and dy^W mice, depends on the different biochemical behavior of laminin isoforms. In fact, it was shown that the binding between α-DG and laminin-2 is not inhibited by heparin, which is instead able to significantly inhibit the interaction with other laminin isoforms [74]. In dy^W, the absence of laminin-2 leads to an overexpression of other laminin types (mainly laminin-4) [75], whose interaction with α-DG is strongly inhibited by heparin [74]. Heparin mimics the biological activities of the abundantly expressed heparan sulfate proteoglycans, therefore it was proposed that heparan sulfate proteoglycans may dramatically perturb α-DG binding to the laminin variants overexpressed in MCDM muscles. This differential heparin sensitivity may help to identify a mechanism for specifically modulating the interaction of α-DG to different extracellular ligands [75].

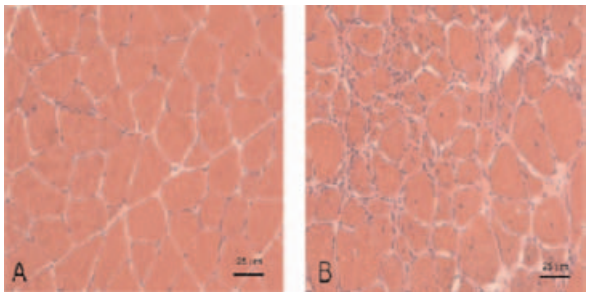

Fig. 3. - Hematoxylin-eosin staining of triceps muscle from wild-type and laminin-α2 deficient mice. (A) In a physiological condition, all the muscle fibres have a similar size and peripherally localized nuclei. **(B)** Laminin-α2 deficient muscle appears necrotic with central nuclei and increased connective tissue within the fibers.

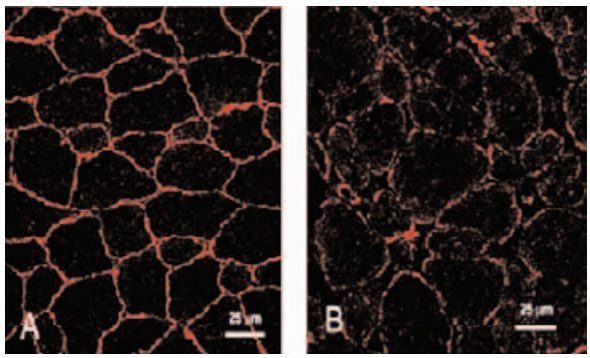

Fig. 4. - Alpha-DG immunostaing of triceps muscle from wild type and laminin-α2 deficient mice. (A) In wild type muscle α-DG is homogeneously localized at the sarcolemma of the muscle fibers. **(B)** In laminin-α2 deficient muscle the amount of alpha-DG is greatly reduced and its localization is not homogeneous.

Post-translational modifications of dystroglycan

For extracellular matrix molecules, glycosylation often represents the most important post-translational event since carbohydrates play an essential role for the *core protein* stability and may also modulate the interaction with binding partners. The proper post-translational maturation of α-DG was recently identified as a crucial step in order to achieve the final assembly and efficient function of muscle tissues [10]. Defects in a number of glycosyltransferases were identified as the causes of three recessive CMD, muscle-eye-brain disease (MEB), Fukuyama congenital muscular dystrophy (FCMD) and Walker-Warburg syndrome (WWS), all characterized by severe muscle weekness and mental retardation [10]. MEB and WWS are associated with mutations in the gene encoding two glycosyltransferase that participates in the synthesis O-mannosyl glycans, POMGnT1 and POMT1 respectively [76, 77].

FCMD, a disease only found in Japan, is due to mutations of the fukutin gene, an enzyme that is likely to be involved in the modification of cell-surface glycoproteins [78]. A fukutin-related protein (FKRP) gene was cloned by computational homology analysis and FKRP mutations were shown to be associated with CMD and with LGMD-2I [79]. An animal model supports the link between defective glycosylation and muscular dystrophy: the mice affected by myophaty (myd) harbors a mutation in the LARGE gene encoding for a glycosyltransferase [53].

In the aforementioned dystrophies and in the *myd* mice, α-DG is expressed in a hypoglycosylated form and this is thought to be a major secondary defect that leads to the dystrophic phenotype [80]. As mentioned

above, carbohydrate moieties of α-DG are believed to play a major role in the interaction between α-DG and laminin. Using overlay binding assays it was observed that, in MEB and FCMD patients and in *myd* mice, this interaction is dramatically reduced [80] The deglycosylation of α-DG weakens the interaction with laminin-2 and it is likely to represent a common molecular phenomenon in a large number of CMD. Recently, an amelioration of the dystrophic phenotype in *mdx* mice was obtained via the overexpression of GlaNac transferase which would restore the proper glycosylation of α-DG [81].

Concluding remarks

During the last years, it became evident that α- and β-DG can be differently perturbed in a number of muscular dystrophies. While the β-subunit is only absent in DMD, α-DG is greatly reduced or abnormally processed in all the muscular dystrophies which we have reviewed. The central role of α-DG in the pathogenesis of muscular dystrophies emphasizes the role of DG as a linker between the extracellular matrix and cytoskeleton. It was suggested that an increase of protease activity, typical of muscular dystrophy, leads to the unspecific degradation of proteins within the extracellular matrix [59]. This might explain the reason why different forms of muscular dystrophy display the secondary loss of α-DG. The failure in forming a strong interaction with laminin (as in congenital muscular dystrophies) could make α-DG prone to the action of proteolytic enzimes. In addition, also the hypoglycosylation observed in some neuromuscular disorders could both affect the interaction with basement membrane molecules and favour the action of proteases. Understanding at the molecular level the role of DG in muscle should be considered as one of the major factor in order to develop efficient therapeutical approaches for the treatment of a large number of muscular dystrophies.

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REFERENCES

1. Spence HJ, Chen YJ, Winder SJ. Muscular dystrophies, the cytoskeleton and cell adhesion. *BioEssays* 2002;24(6):542-52.

2. Ervasti JM, Campbell KP. Membrane organization of the dystrophin-glycoprotein complex. *Cell* 1991;66(6):1121-31.

3. Henry MD, Campbell KP. Dystroglycan inside and out. *Curr Opin Cell Biol* 1999;11(5):602-7.

4. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987;51(6):919-28.

5. Campbell KP. Three muscular dystrophies: loss of the cytoskeleton-extracellular matrix linkage. *Cell* 1995;80(5):675-9.

6. Hack AA, Groh ME, McNally EM. Sarcoglycans in muscular dystrophy. *Microsc Res Tech* 2000;48(3-4):167-80.

7. Miyagoe-Suzuki Y, Nakagawa M, Takeda S. Merosin and congenital muscular dystrophy. *Res Tech* 2000;48(3-4):181-91.

8. Salih MA, Sunada Y, Al-Nasser M, Ozo CO, Al-Turaiki MH, Akbar M, Campbell KP. Muscular dystrophy associated with beta-dystroglycan deficiency. *Ann Neurol* 1996; 40(6):925-8.

9. Durbeej M, Henry MD, Campbell KP. Dystroglycan in development and disease. *Curr Opin Cell Biol* 1998;10(5):594-601.

10. Muntoni F, Brockington M, Blake DJ, Torelli S, Brown SC. Defective glycosylation in muscular dystrophy. *Lancet* 2002; 360(9343):1419-21.

11. Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature* 1992;355(6362):696-702.

12. Winder S. The complexities of dystroglycan. *Trends Biochem Sci* 2001;26(2):118-24.

13. Durbeej M, Henry MD, Ferletta M, Campbell KP, Ekblom P. Distribution of dystroglycan in normal adult mouse tissues. *J Histochem Cytochem* 1998;46(4):449-57.

14. Chiba A, Matsumura K, Yamada H, Inazu T, Shimizu T, Kusunoki S, Kanazawa I, Kobata A, Endo T. Structures of sialylated O-linked oligosaccharides of bovine peripheral nerve alpha-dystroglycan. The role of a novel O-mannosyl-type oligosaccharide the binding of alpha-dystroglycan with laminin. *J Biol Chem* 1997;272(4):2156-62.

15. Brancaccio A, Schulthess T, Gesemann M, Engel J. Electron microscopic evidence for a mucin-like region in chick muscle alpha-dystroglycan. *FEBS Lett* 1995;368(1):139-42.

16. Sciandra F, Schneider M, Giardina B, Baumgartner S, Petrucci TC, Brancaccio A. Identification of the beta-dystroglycan binding epitope within the C-terminal region of alpha-dystroglycan. *Eur J Biochem* 2001;268(16):4590-7.

17. Bozzi M, Veglia G, Paci M, Sciandra F, Giardina B, Brancaccio A. A synthetic peptide corresponding to the 550-585 region of alpha-dystroglycan binds beta-dystroglycan as revealed by NMR spectroscopy. *FEBS Lett* 2001;499(3):210-4.

18. Cartaud A, Coutant S, Petrucci TC, Cartaud J. Evidence for *in situ* and *in vitro* association between beta-dystroglycan and the subsynaptic 43K rapsyn protein. Consequence for acetylcholine receator clustering at the synapse. *J Biol Chem* 1998; 273(18):11321-6.

19. Jung D, Yang B, Meyer J, Chamberlain JS, Campbell KP. Identification and characterization of the dystrophin anchoring site on beta-dystroglycan. *J Biol Chem* 1995;270(45):27305-10.

20. Yang B, Jung D, Motto D, Meyer J, Koretzky G, Campbell KP. SH3 domain-mediated interaction of dystroglycan and Grb2. *J Biol Chem* 1995;270(20):11711-4.

21. Yoshida T, Pan Y, Hanada H, Iwata Y, Shigekawa M. Bidirectional signaling between sarcoglycans and the integrin adhesion system in cultured L6 myocytes. *J Biol Chem* 1998;273(3):1583-90.

22. James M, Nuttall A, Ilsley JL, Ottersbach K, Tinsley JM, Sudol M, Winder SJ. Adhesion-dependent tyrosine phosphorylation of (beta)-dystroglycan regulates its interaction with utrophin. *J Cell Sci* 2000;113(Pt 10):1717-26.

23. Sotgia F, Lee H, Bedford MT, Petrucci T, Sudol M, Lisanti MP. Tyrosine phosphorylation of beta-dystroglycan at its WW domain binding motif, PPxY, recruits SH2 domain containing proteins. *Biochemistry* 2001;40(48):14585-92.

24. Henry MD, Campbell KP. Dystroglycan: an extracellular-matrix receptor-linked to the cytoskeleton. *Curr. Opin. Cell Biol* 1996; 8(5):625-31.

25. Williamson RA, Henry MD, Daniels KJ, Hrstka RF, Lee JC, Sunada Y, Ibraghimov-Beskrovnaya O, Campbell KP. Dystroglycan is essential for early embryonic development: disruption of Reichert's membrane in Dag1-null mice. *Hum Mol Genet* 1997;6(6):831-41.

26. Henry MD, Campbell KP. A role for dystroglycan in basement membrane assembly. *Cell* 1998;95(6):859-70.

27. Colognato H, Winkelmann DA, Yurchenco PD. Laminin polymerization induces a receptor-cytoskeleton network. *J. Cell Biol* 1999;145(3):619-31.

28. Cote PD, Moukhles H, Lindenbaum M, Carbonetto S. Chimaeric mice deficient in dystroglycans develop muscular dystrophy and have disrupted myoneural synapses. *Nat Genet* 1999;23(3):338-42.

29. Cohn RD, Henry MD, Michele DE, Barresi R, Saito F, Moore SA, Flanagan JD, Skwarchuk MW, Robbins ME, Mendell JR, Williamson RA, Campbell KP. Disruption of DAG1 in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. *Cell* 2002;110(5):639-48.

30. Gesemann M, Cavalli V, Denzer AJ, Brancaccio A, Schumacher B, Ruegg MA. Alternative splicing of agrin alters its binding to heparin, dystroglycan, and the putative agrin receptor. *Neuron* 1996;16(4):755-67.

31. Hopf C, Hoch W. Agrin binding to alpha-dystroglycan. Domains of agrin necessary to induce acetylcholine receptor clustering are overlapping but not identical to the alpha-dystroglycan-binding region. *J Biol Chem* 1996;271(9):5231-6.

32. Cao W, Henry MD, Borrow P, Yamada H, Elder JH, Ravkov EV, Nichol ST, Compans RW, Campbell KP, Oldstone MB. dentification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus. *Science* 1998;282(5396):2079-81.

33. Rambukkana A, Yamada H, Zanazzi G, Mathus T, Salzer JL, Yurchenco PD, Campbell KP, Fischetti VA. Role of alpha-dystroglycan as a Schwann cell receptor for *Mycobacterium leprae*. *Science* 1998;282(5396):2076-9.

34. Henry MD, Cohen MB, Campbell KP. Reduced expression of dystroglycan in breast and prostate cancer. *Hum Pathol* 2001;32(8):791-5.

35. Losasso C, Di Tommaso F, Sgambato A, Ardito R, Cittadini A, Giardina B, Petrucci TC, Brancaccio A. Anomalous dystroglycan in carcinoma cell lines. *FEBS Lett* 2000;484(3):194-8.

36. Koenig M, Monaco AP, Kunkel LM. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell* 1998;53(2):219-26.

37. Cohn RD, Campbell KP. The molecular basis of muscular dystrophies. *Muscle Nerve* 2000;23(10):1456-71.

38. Piccolo F, Moore SA, Mathews KD, Campbell KP. Limb-girdle muscular dystrophies. *Adv Neurol* 2002;88:273-91.

39. Ohlendieck K, Campbell KP. Dystrophin-associated proteins are greatly reduced in skeletal muscle from mdx mice. *J Cell Biol* 1991;115(6):1685-94.

40. Narita S, Yorifuji H. Centrally nucleated fibers (CNFs) compensate the fragility of myofibers in mdx mouse. *Neuroreport* 1999;10(15):3233-5.

41. Rybakova IN, Patel JR, Ervasti JM. The dystrophin complex forms a mechanically strong link between the sarcolemma and the costameric actin. *J Cell Biol* 2000; 150(5):1209-14.

42. Cox GA, Sunada Y, Campbell KP, Chamberlain JS. Dp71 can restore the dystrophin-glycoprotein complex in muscle but fails to prevent dystrophy. *Nat Genet* 1994;8(4):333-9.

43. Greenberg DS, Sunada Y, Campbell KP, Yaffe D, Nudel U. Exogenous Dp71 restores the levels of dystrophin-associated proteins but does not alleviate muscle damage in mdx mice. *Nat Genet* 1994;8(4):340-4.

44. Tinsley JM, Potter AC, Phelps SR, Fisher R, Trickett JI, Davies KE. Amelioration of dystrophic phenotype of mdx mice using a truncated utrophin transgene. *Nature* 1996;384(6607):349-53.

45. Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL, Rosenthal N. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 2001;27(2): 195-200.

46. Florini JR, Ewton DZ, Coolican SA. Growth hormone and the insuline-like growth factor system in myogenesis. *Endocr Rev* 1996;17(5):481-517.

47. Bogdanovich S, Krag TO, Barton ER, Morris LD, Whittemore LA, Ahima RS, Khurana TS. Functional improvement of dystrophic muscle by myostatin blockade. *Nature* 2002;420(6914):418-21.

48. Sharma M, Langley B, Bass J, Kambadur R. Myostatin in muscle growth and repair. *Exerc Sport Sci Rev* 2001,29(4):155-8.

49. Ozawa E, Noguchi S, Mizuno Y, Hagiwara Y, Yoshida M. From dystrophinopathy to sarcoglycanopathy: evolution of a concept of muscular dystrophy. *Muscle Nerve* 1998;21(4):421-38.

50. Crosbie RH, Heighway J, Venzke DP, Lee JC, Campbell KP. Sarcospan, the 25-kDa transmembrane component of the dystrophin-glycoprotein complex. *J Biol Chem* 1997;272(50):31221-4.

51. Noguchi S, Wakabayashi E, Imamura M, Yoshida M, Ozawa E. Formation of sarcoglycan complex with differentiation in cultured myocytes. *Eur J Biochem* 2000; 267(3):640-8.

52. Barresi R, Confalonieri V, Lanfossi M, Di Blasi C, Torchiana E, Mantegazza R, Jarre L, Nardocci N, Boffi P, Tezzon F, Pini A, Cornelio F, Mora M, Morandi L. Concomitant deficiency of beta- and gamma-sarcoglycans in 20 alpha-sarcoglycan (adhalin)-deficient patients: immunohistochemical analysis and clinical aspects. *Acta Neuropathol* 1997;94(1):28-35.

53. Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: an overview of current mouse model. *Curr Opin Genet Develop* 2002,12(3):349-61.

54. Duclos F, Straub V, Moore SA, Venzke DP, Hrstka RF, Crosbie RH, Durbeej M, Lebakken CS, Ettinger AJ, van der Meulen J, Holt KH, Lim LE, Sanes JR, Davidson BL, Faulkner JA, Williamson R, Campbell KP. Progressive muscular dystrophy in alpha-sarcoglycan-deficient mice. *J Cell Biol* 1998;142(6):1461-71.

55. Durbeej M, Cohn RD, Hrstka RF, Moore SA, Allamand V, Davidson BL, Williamson RA, Campbell KP. Disruption of the beta-sarcoglycan gene reveals pathogenetic complexity of limb-girdle muscular dystrophy type 2E. *Mol Cell* 2000;5(1):141-51.

56. Lebakken CS, Venzke DP, Hrstka RF, Consolino CM, Faulkner JA, Williamson RA, Campbell KP. Sarcospan-deficient mice maintain normal muscle function. *Mol Cell Biol* 2000;20(5):1669-77.

57. Holt KH, Lim LE, Straub V, Venzke DP, Duclos F, Anderson RD, Davidson BL, Campbell KP. Functional rescue of the sarcoglycan complex in the BIO 14.6 hamster using delta-sarcoglycan gene transfer. *Mol Cell* 1998;1(6):841-8.

58. Razani B, Lisanti MP. Caveolins and caveolae: molecular and functional relationships. *Exp Cell Res* 2001;271(1):36-44.

59. Galbiati F, Razani B, Lisanti MP. Cavolae and caveolin-3 in muscular dystrophy. *Trends Mol Med* 2001;7(10):435-41.

60. Sotgia F, Lee JK, Das K, Bedford M, Petrucci TC, Macioce P, Sargiacomo M, Bricarelli FD, Minetti C, Sudol M, Lisanti MP. Caveolin-3 directly interacts with the C-terminal tail of beta -dystroglycan. Identification of a central WW-like domain within caveolin family members. *J Biol Chem* 2000;275(48):38048-58.

61. Minetti C, Sotgia F, Bruno C, Scartezzini P, Broda P, Bado M, Masetti E, Mazzocco M, Egeo A, Donati MA, Volonte D, Galbiati F, Cordone G, Bricarelli FD, Lisanti MP, Zara F. Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. *Nat Genet* 1998;18(4):365-8.

62. Herrmann R, Straub V, Blank M, Kutzick C, Franke N, Jacob EN, Lenard HG, Kroger S, Voit T. Dissociation of the dystroglycan complex in caveolin-3-deficient limb girdle muscular dystrophy. *Hum Mol Genet* 2000;9(15):2335-40.

63. Park DS, Woodman SE, Schubert W, Cohen AW, Frank PG, Chandra M, Shirani J, Razani B, Tang B, Jelicks LA, Factor SM, Weiss LM, Tanowitz HB, Lisanti MP. Caveolin-1/3 double-knockout mice are viable, but lack both muscle and non-muscle caveolae, and develop a severe cardiomyopathic phenotype. *Am J Pathol* 2002;160(6):2207-17.

64. Galbiati F, Volonte D, Chu JB, Li M, Fine SW, Fu M, Bermudez J, Pedemonte M, Weidenheim KM, Pestell RG, Minetti C, Lisanti MP. Transgenic overexpression of caveolin-3 in skeletal muscle fibers induces a Duchenne-like muscular dystrophy phenotype. *Proc Natl Acad Sci USA* 2000;97(17):9689-94.

65. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. *Dev Dyn* 2000;218(2):213-34.

66. Talts JF, Andac Z, Gohring W, Brancaccio A, Timpl R. Binding of the G domains of laminin alpha1 and alpha2 chains and perlecan to heparin, sulfatides, alpha-dystroglycan and several extracellular matrix proteins. *EMBO J* 1999;18(4):863-70.

67. Sugita S, Saito F, Tang J, Satz J, Campbell KP, Sudhof TC. A stoichiometric complex of neurexins and dystroglycan in brain. *J Cell Biol* 2001;154(2):435-45.

68. Brancaccio A, Schulthess T, Gesemann M, Engel J. The N-terminal region of alpha-dystroglycan is an autonomous globular domain. *Eur J Biochem* 1997;246(1):166-72.

69. Tisi D, Talts JF, Timpl R, Hohenester E. Structure of the C-terminal laminin G-like domain pair of the laminin alpha2 chain harbouring binding sites for alpha-dystroglycan and heparin. *EMBO J* 2000;19(7):1432-40.

70. Ervasti JM, Campbell KP. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *J Cell Biol* 1993;122(4):809-23.

71. Moll J, Barzaghi P, Lin S, Bezakova G, Lochmuller H, Engvall E, Muller U, Ruegg MA. An agrin minigene rescues dystrophic symptoms in a mouse model for congenital muscular dystrophy. *Nature* 2001;413(6853):302-7.

72. Sunada Y, Bernier SM, Kozak CA, Yamada Y and Campbell KP. Deficiency of merosin in dystrophic dy mice and genetic linkage of laminin M chain gene to dy locus. *J Biol Chem* 1994;269(19):13729-32.

73. Vachon PH, Xu H, Liu L, Loechel F, Hayashi Y, Arahata K, Reed JC, Wewer UM Engvall E. Integrins (alpha7beta1) in muscle function and survival. Disrupted expression in merosin-deficient congenital muscular dystrophy. *J Clin Invest* 1997; 100(7):1870-81.

74. Andac Z, Sasaki T, Mann K, Brancaccio A, Deutzmann R, Timpl R. Analysis of heparin, alpha-dystroglycan and sulfatide binding to the G domain of the laminin alpha1 chain by site-directed mutagenesis. *J Mol Biol* 1999;287(2):253-64.

75. McDearmon EL, Burwell AL, Combs AC, Renley BA, Sdano MT, Ervasti JM. Differential heparin sensitivity of alpha-dystroglycan binding to laminins expressed in normal and dy/dy mouse skeletal muscle. *J Biol Chem* 1998;273(37):24139-44.

76. Yoshida A, Kobayashi K, Manya H, Taniguchi K, Kano H, Mizuno M, Inazu T, Mitsuhashi H, Takahashi S, Takeuchi M, Herrmann R, Straub V, Talim B, Voit T, Topaloglu H, Toda T, Endo T. Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev Cell* 2001;1(5):717-24.

77. Beltran-Valero De Bernabe D, Currier S, Steinbrecher A, Celli J, Van Beusekom E, Van Der Zwaag B, Kayserili H, Merlini L, Chitayat D, Dobyns WB, Cormand B, Lehesjoki AE, Cruces J, Voit T, Walsh CA, Van Bokhoven H, Brunner HG. Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg Syndrome. *Am J Hum Genet* 2002; 71(5):1033-43.

78. Kobayashi K, Nakahori Y, Miyake M, Matsumura K, Kondo-Iida E, Nomura Y, Segawa M, Yoshioka M, Saito K, Osawa M, Hamano K, Sakakihara Y, Nonaka I, Nakagome Y, Kanazawa I, Nakamura Y, Tokunaga K, Toda T. An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* 1998;394(6691):388-92.

79. Brockington M, Yuva Y, Prandini P, Brown SC, Torelli S, Benson MA, Herrmann R, Anderson LV, Bashir R, Burgunder JM, Fallet S, Romero N, Fardeau M, Straub V, Storey G, Pollitt C, Richard I, Sewry CA, Bushby K, Voit T, Blake DJ, Muntoni F. Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. *Hum Mol Genet* 2001;10(25):2851-9.

80. Michele DE, Barresi R, Kanagawa M, Saito F, Cohn RD, Satz JS, Dollar J, Nishino I, Kelley RI, Somer H, Straub V, Mathews KD, Moore SA, Campbell KP. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature* 2002;418(6896):417-22.

81. Nguyen HH, Jayasinha V, Xia B, Hoyte K, Martin PT. Overexpression of the cytotoxic T cell GalNAc transferase in skeletal muscle inhibits muscular dystrophy in mdx mice. *Proc Natl Acad Sci U S A* 2002;99(8):5616-21.