LGMD2D myotubes

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LGMD2D (LGMD-R3 according to the new nomenclature) is a rare autosomal recessive disease affecting striated muscle. It belongs to the group of limb girdle muscular dystrophies because of the involvement of the proximal musculature of the shoulders and pelvic girdle. LGMD2D is caused by mutations in the SGCA gene coding for α-sarcoglycan (SG).

Keywords: rare disease ; muscular dystrophy ; folding defective protein ; pathogenic mechanism ; endoplasmic reticulum associated degradation ; small molecules ; pharmacological chaperons ; proteostasis regulators ; therapy ; myogenic cells

1. Introduction

The Limb girdle muscular dystrophy type 2D (LGMD2D) is a rare autosomal recessive disease affecting mainly the upper and lower hind limb musculature, with no cure presently available. The disease, also known as α-sarcoglycanopathy is due to mutations of the *SGCA* gene coding for the membrane protein α-sarcoglycan (SG). Together with β-, γ- and δ-SG, α-SG forms the SG-complex that concurs with other dystrophin-associated proteins (DAPs) in the stabilization of the sarcolemma during the contraction of the striated muscle. The absence or strong reduction of the SG-complex is the main feature of LGMD2D that results in increased sarcolemma fragility and consequent progressive muscle degeneration. Most of the *SGCA* defects are missense mutations originating a folding defective, although potentially functional protein recognized by the endoplasmic reticulum (ER) quality control system and eliminated through the ER associated degradation (ERAD). To recover the mutants and escape SG-complex disruption, an approach was based on the use of protein folding correctors belonging to the CFTR (cystic fibrosis transmembrane regulator) modulators family, first developed to treat cystic fibrosis. Several of such compounds rescued different α-SG mutants expressed in cell models, and importantly their administration in primary myotubes of a LGMD2D patient improved the mutant folding, and the assembly and traffic of the SG-complex. The rescued complex was stable and functional and improved the sarcolemma behavior, even though containing a mutated subunit. Understanding the mechanism of action of CFTR correctors in sarcoglycanopathy will be mandatory; however, these findings suggest that these small molecules have the potential to progress as therapeutics for LGMD2D caused by missense mutations.

2. Description

LGMD2D (LGMD-R3 according to the new nomenclature [1]) is a rare autosomal recessive disease affecting striated muscle. It belongs to the group of limb girdle muscular dystrophies because of the involvement of the proximal musculature of the shoulders and pelvic girdle [2]. LGMD2D is caused by mutations in the *SGCA* gene coding for α-sarcoglycan (SG) [3][4][5]. This protein, together with β-, γ- and δ-SG, forms the SG complex, a key component of the dystrophin associated protein complex, significantly helping to preserve sarcolemma from contraction-induced stress. Moreover, a number of direct or indirect regulative roles have been attributed to the SG-complex [6][7]. LGMD2D, although heterogeneous, is often characterized by early onset and rapid progression, with people affected becoming wheelchair-bound during the adolescence [8]. Presently, no effective therapy is available for LGMD2D as well as for the other three forms of sarcoglycanopathy (LGMD2E, 2C and 2F, due to mutations in SGCB, SGCG and SGCD genes, respectively [9]). Most of the gene defects responsible for the onset of sarcoglycanopathy are missense mutations [10][11][12][13]. In the last few years, the pathogenic mechanism of the forms of sarcoglycanopathy due to this type of genetic defect has been disclosed. It has been observed that many sarcoglycans with an amino acid substitution are unable to properly fold, are recognized by the quality control system of the cells and delivered to a premature degradation [14][15][16][17]. Consequently, the correct assembly, traffic and localization of the SG-complex is impaired, leading to a global reduction in the structural stability of the sarcolemma. An interesting point is the possibility to rescue the defective sarcoglycan as well as the entire SG-complex, by preventing the degradation of the mutant, acting either at the initial [15][16], intermediate [17] or final step [14] of the pathway. On these premises, a novel strategy of therapeutic intervention was elaborated [18] also taking

advantage of the tremendous work done on another genetic disease, cystic fibrosis that shares with sarcoglycanopathy a similar pathogenic mechanism [19]. This approach is based on the use of small molecules known as cystic fibrosis transmembrane regulator (CFTR) correctors, which were originally selected and developed to improve folding and traffic of defective CFTR protein (type II mutants) [20][21][22]. In cystic fibrosis, some of these compounds act as pharmacological chaperones, directly binding to the mutated CFTR [23], while others play an indirect action, as modulators of the cell proteostasis [24]. Regardless of the mechanism, CFTR correctors have been proven effective not only on CFTR mutants but also on structurally correlated [25] as well as structurally uncorrelated defective proteins [26][27] such as α-SG [18,28]. Indeed, the outcome of the administration of CFTR correctors to LGMD2D myotubes is the enhanced assembly of the SG-complex that re-gains the ability to traffic toward the sarcolemma. Once at the final location the SG-complex containing the corrected subunit seems stable and functional. The combined administration of two CFTR correctors may result in additive/synergistic effects [18][28].These data support the view that several CFTR corrector could be effective in conditions different from cystic fibrosis, such as LGMD2D, suggesting new therapeutic opportunities for orphan diseases currently incurable.

References

1. Straub V, Murphy A, Udd B, group Lws: 229th ENMC international workshop: Limb girdle muscular dystrophies - Nomenclature and reformed classification Naarden, the Netherlands, 17-19 March 2017. Neuromuscul Disord 2018, 28(8):702-710.
2. Bushby K, Norwood F, Straub V: The limb-girdle muscular dystrophies - Diagnostic strategies. Bba-Mol Basis Dis 2007, 1772(2):238-242.
3. Romero NB, Tome FMS, Leturcq F, Elkerch F, Azibi K, Bachner L, Anderson RD, Roberds SL, Campbell KP, Fardeau M et al: Genetic-Heterogeneity of Severe Childhood Autosomal Recessive Muscular-Dystrophy with Adhalin (50 Kda Dystrophy-Associated Glycoprotein) Deficiency. Cr Acad Sci Iii-Vie 1994, 317(1):70-76.
4. Allamand V, Leturcq F, Piccolo F, Jeanpierre M, Azibi K, Roberds SL, Lim LE, Campbell KP, Beckmann JS, Kaplan JC: Adhalin Gene Polymorphism. Human Molecular Genetics 1994, 3(12):2269-2269.
5. Nigro V, Savarese M: Genetic basis of limb-girdle muscular dystrophies: the 2014 update. Acta Myol 2014, 33(1):1-12.
6. Sandona D, Betto R: Sarcoglycanopathies: molecular pathogenesis and therapeutic prospects. Expert Rev Mol Med 2009, 11:e28.
7. Tarakci H, Berger J: The sarcoglycan complex in skeletal muscle. Front Biosci (Landmark Ed) 2016, 21:744-756.
8. Kirschner J, Lochmuller H: Sarcoglycanopathies. Handb Clin Neurol 2011, 101:41-46.
9. Carotti M, Fecchio C, Sandona D: Emerging therapeutic strategies for sarcoglycanopathy. Expert Opin Orphan D 2017, 5(5):381-396.
10. Ginjaar HB, van der Kooi AJ, Ceelie H, Kneppers AL, van Meegen M, Barth PG, Busch HF, Wokke JH, Anderson LV, Bonnemann CG et al: Sarcoglycanopathies in Dutch patients with autosomal recessive limb girdle muscular dystrophy. J Neurol 2000, 247(7):524-529.
11. Vainzof M, Passos-Bueno MR, Pavanello RC, Marie SK, Oliveira AS, Zatz M: Sarcoglycanopathies are responsible for 68% of severe autosomal recessive limb-girdle muscular dystrophy in the Brazilian population. J Neurol Sci 1999, 164(1):44-49.
12. Duggan DJ, Gorospe JR, Fanin M, Hoffman EP, Angelini C, Pegoraro E, Noguchi S, Ozawa E, Pendlebury W, Waclawik AJ et al: Mutations in the sarcoglycan genes in patients with myopathy. New Engl J Med 1997, 336(9):618-624.
13. Xie Z, Hou Y, Yu M, Liu Y, Fan Y, Zhang W, Wang Z, Xiong H, Yuan Y: Clinical and genetic spectrum of sarcoglycanopathies in a large cohort of Chinese patients. Orphanet J Rare Dis 2019, 14(1):43.
14. Gastaldello S, D'Angelo S, Franzoso S, Fanin M, Angelini C, Betto R, Sandona D: Inhibition of proteasome activity promotes the correct localization of disease-causing alpha-sarcoglycan mutants in HEK-293 cells constitutively expressing beta-, gamma-, and delta-sarcoglycan. Am J Pathol 2008, 173(1):170-181.
15. Bartoli M, Gicquel E, Barrault L, Soheili T, Malissen M, Malissen B, Vincent-Lacaze N, Perez N, Udd B, Danos O et al: Mannosidase I inhibition rescues the human alpha-sarcoglycan R77C recurrent mutation. Hum Mol Genet 2008, 17(9):1214-1221.
16. Soheili T, Gicquel E, Poupiot J, N'Guyen L, Le Roy F, Bartoli M, Richard I: Rescue of sarcoglycan mutations by inhibition of endoplasmic reticulum quality control is associated with minimal structural modifications. Hum Mutat 2012,

33(2):429-439.

17. Bianchini E, Fanin M, Mamchaoui K, Betto R, Sandona D: Unveiling the degradative route of the V247M alpha-sarcoglycan mutant responsible for LGMD-2D. Hum Mol Genet 2014, 23(14):3746-3758.
18. Carotti M, Marsolier J, Soardi M, Bianchini E, Gomiero C, Fecchio C, Henriques SF, Betto R, Sacchetto R, Richard I et al: Repairing folding-defective alpha-sarcoglycan mutants by CFTR correctors, a potential therapy for limb-girdle muscular dystrophy 2D. Human Molecular Genetics 2018, 27(6):969-984.
19. Gelman MS, Kannegaard ES, Kopito RR: A principal role for the proteasome in endoplasmic reticulum-associated degradation of misfolded intracellular cystic fibrosis transmembrane conductance regulator. Journal of Biological Chemistry 2002, 277(14):11709-11714.
20. Rogan MP, Stoltz DA, Hornick DB: Cystic Fibrosis Transmembrane Conductance Regulator Intracellular Processing, Trafficking, and Opportunities for Mutation-Specific Treatment. Chest 2011, 139(6):1480-1490.
21. Birault V, Solari R, Hanrahan J, Thomas DY: Correctors of the basic trafficking defect of the mutant F508del-CFTR that causes cystic fibrosis. Curr Opin Chem Biol 2013, 17(3):353-360.
22. Miquéias Lopes-Pacheco; CFTR Modulators: Shedding Light on Precision Medicine for Cystic Fibrosis. *Frontiers in Pharmacology* **2016**, *7*, 275, 10.3389/fphar.2016.00275.
23. Okiyoneda T, Veit G, Dekkers JF, Bagdany M, Soya N, Xu HJ, Roldan A, Verkman AS, Kurth M, Simon A et al: Mechanism-based corrector combination restores Delta F508-CFTR folding and function. Nat Chem Biol 2013, 9(7):444-U469.
24. Lopes-Pacheco M, Sabirzhanova I, Rapino D, Morales MM, Guggino WB, Cebotaru L: Correctors Rescue CFTR Mutations in Nucleotide-Binding Domain 1 (NBD1) by Modulating Proteostasis. Chembiochem 2016, 17(6):493-505.
25. Sabirzhanova I, Pacheco ML, Rapino D, Grover R, Handa JT, Guggino WB, Cebotaru L: Rescuing Trafficking Mutants of the ATP-binding Cassette Protein, ABCA4, with Small Molecule Correctors as a Treatment for Stargardt Eye Disease. Journal of Biological Chemistry 2015, 290(32):19743-19755.
26. Sampson HM, Lam H, Chen PC, Zhang DL, Mottillo C, Mirza M, Qasim K, Shrier A, Shyng SL, Hanrahan JW et al: Compounds that correct F508del-CFTR trafficking can also correct other protein trafficking diseases: an in vitro study using cell lines. Orphanet J Rare Dis 2013, 8.
27. van der Woerd WL, Wichers CGK, Vestergaard AL, Andersen JP, Paulusma CC, Houwen RHJ, van de Graaf SFJ: Rescue of defective ATP8B1 trafficking by CFTR correctors as a therapeutic strategy for familial intrahepatic cholestasis. J Hepatol 2016, 64(6):1339-1347.
28. Carotti M, Scano M, Fancello I, Richard I, Risato G, Bensalah M, Soardi M and Sandonà D. Combined Use of CFTR Correctors in LGMD2D Myotubes Improves Sarcoglycan Complex Recovery Int. J. Mol. Sci. 2020, 21, 1813