BET ON AUTOPHAGY IN THE RACE AGAINST MUSCULAR DYSTROPHIES

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Duchenne muscular dystrophy (DMD) is a lethal muscle degenerative disease caused by mutations in the dystrophin gene. Early in life, DMD patients can temporarily compensate for the continuous degenerative process imposed on contractile activity of dystrophin-deficient myofibers, through a compensatory regeneration mediated by adult muscle stem (satellite) cells. However, the regenerative potential of satellite cells eventually declines at later stages of DMD and muscle fibers are replaced by fibrotic tissue, calcium deposits, and fat infiltration.[1] Treatment with corticosteroids results in short-term improvements at the cost of steroid-related side effects, but there is no cure for DMD. Several strategies have been employed to prolong ambulation and to delay the onset of secondary complications. Among these are gene and cellular therapies and pharmacologic strategies resulting in the replacement of a modified dystrophin protein or aimed at reducing the inflammatory cascade and enhancing muscle regeneration.[2] The *mdx* mouse and the golden retriever muscular dystrophy dog (GRMD) are the models most commonly used to study DMD, with the former displaying a milder phenotype compared with human patients.[3] Phenotype variability at the individual and muscle level has been described in the GRMD, making this model suitable to study the pathophysiology of muscular dystrophy beyond the primary effects of dystrophin loss. On the other hand, the same variability may distort assessment of outcome measures in preclinical trials.

The autophagic machinery has been recently identified among the secondary therapeutic targets. Indeed, autophagy has been shown to be involved in many cellular processes to protect cells in stress conditions, including: providing amino acids to sustain vitality in the face of nutrient stress; and removing non-functional organelles, damaged mitochondria, and pathogens after intense exercise, endoplasmic reticulum stress, hypoxia, oxidative stress, or infections.[4] There is clear evidence that autophagy is required to maintain muscle mass and myofiber integrity. Muscle-specific deletion of the crucial autophagy genes autophagy related 7 (ATG7) and autophagy related 5 (ATG5) was shown to result in muscle atrophy and age-dependent decreases in force production.[5] On the other hand, overexpressing forkhead box O3 (FOXO3)–BCL2 interacting protein 3 (BNIP3) as a key pathway regulating autophagy during muscle wasting leads to an atrophic phenotype.[6] Hence, it is possible to outline a dual role of autophagy in muscle homeostasis: Defective autophagy compromises the clearance of damaged proteins, toxic compounds, and organelles, whereas excessive autophagy leads to muscle loss and atrophy, pointing to autophagy as a sensitive process that needs to be fine-tuned to guarantee proper muscle function.[7]

In muscular dystrophies, autophagy has been reported to be impaired in muscles lacking collagen VI that accumulate dysfunctional organelles, thus triggering apoptosis and muscle wasting. Reactivation of autophagic flux by either nutritional or pharmacologic and genetic tools has been shown to ameliorate the dystrophic phenotype by removing dysfunctional mitochondria.[8] Recent findings highlighted a crucial role of autophagy in DMD.[9–11] For example, a low-protein diet has been shown to rescue the muscular defects in *mdx* mice.[9] In addition, treatment with the protein kinase AMP-activated catalytic subunit alpha 1 (AMPK) agonist 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR induces autophagy and improves muscle structure and strength without inducing muscle fiber atrophy.[10]

Additional data demonstrate that increased reactive oxygen species (ROS) production by CYBB/NOX2 leads to autophagic impairment in muscles from the *mdx* mouse. Treatment with simvastatin, an β-hydroxy β-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitor that inhibits Cytochrome b subunit beta (CYBB)/NADPH oxidase-2 (NOX2) and

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oxidative stress, restores the autophagic process and improves muscle function in *mdx* mice.[12]

Spermidine, a polyamine involved in cell metabolism that naturally induces autophagy, has been shown to play a pivotal role in protection against heart diseases and in longevity.[13] Systemic administration of spermidine reactivates autophagy, ameliorating the histologic and ultrastructural muscle defects in collagen VI null mice.[14]

Rapamycin is a treatment used to stimulate autophagy. When administered systemically to *mdx* mice, it reduces muscle fiber necrosis, providing a better balance between effector T cells and Treg cells and improved diaphragm muscle histopathology.[15] As an alternative approach to rescuing defective autophagy in *mdx* mice, rapamycin can be loaded into nanoparticles and administered orally or systemically, resulting in a reproducible increase of skeletal and cardiac muscular strength and performance.[16]

In our group, we monitored autophagy in muscles of *mdx* mice and human DMD patients at different stages of disease, showing that autophagy is activated during the early, compensatory regenerative stages and decreases during disease progression, in association with the functional exhaustion of satellite cell–mediated regeneration and the development of fibrosis.[17] Moreover, pharmacologic manipulation of autophagy can influence disease progression in *mdx* mice, supporting the notion that interventions that enhance activation of autophagy may be beneficial in the treatment of DMD[17] and making the autophagic process a "disease modifier" that can be targeted by interventions aimed to promote regeneration and to delay disease progression in DMD. In agreement with these concepts, numerous *in vitro* and *in vivo* therapeutic strategies have been described to address autophagy dysregulation in age-related degenerative processes and DMD.

In this issue of the Journal, Stoughton and colleagues[18] investigate the autophagic process in phenotypically distinct skeletal muscles from dystrophic (GRMD) and normal dogs at different ages. The aim of their work is to evaluate the correlation between autophagy and the variable GRMD phenotype. In line with the impairment of autophagy in muscles from *mdx* mice and DMD boys, the expression of autophagy markers (-microtubule associated protein 1 light chain 3 beta (MAPILC3b), autophagy related 12 (ATG12), beclin 1 (BECN1), and BNIP3) in 2 different skeletal muscles, cranial sartorius (CS) and vastus lateralis (VL), of 3- and 6-month-old GRMD dogs was lower in comparison with normal dogs. Further, increased accumulation of the protein sequestosome 1 (SQSTM1)/p62 in 6-month-old GRMD dogs is indicative of damaged protein aggregate accumulation due to impaired autophagic flux. On the other hand, the increased levels of microtubule associated protein 1 light chain 3 beta (LC3B-II) in 6-month-old GRMD CS are ascribed by the authors to an inefficiency in clearing cellular debris and damaged organelles or to an overloaded autophagic machinery, supporting the downregulation of autophagy genes at 6 months through a negative feedback mechanism. Next, the authors evaluate the functional correlation between impaired autophagy and phenotypic data, establishing an association between impaired autophagic activity and inappropriate muscle hypertrophy (different from the true hypertrophy seen in this muscle by Kornegay and colleagues[19]).

Stoughton *et al.* provide additional evidence by analyzing the LC3B accumulation to form puncta in muscle tissue sections isolated from CS and VL muscles of 6-month-old GRMD dogs, placing particular emphasis on the distinction between slow- and fast-twitch fibers. They demonstrate the reduced accumulation of LC3B-positive structures predominantly in slow/regenerative twitch myofibers. Accordingly, the fast myofibers exhibit LC3B-positive puncta, suggesting that the autophagic dysregulation is selective for a specific fiber type. This hypothesis matches with the observation that *mdx* muscles display altered oxidative metabolism[20] and are confirmed in a proteomic analysis made in dystrophic dog muscles that demonstrated the alteration of metabolic pathways,[21] suggesting a "metabolic crisis" as a general feature of the dystrophic phenotype.

In line with these observations, a fiber-type shift of fast to slow has been described in *mdx* mice overexpressing peroxisome activator–proliferated receptor gamma coactivator-1α (PGC-1α), leading to increased oxidative capacity[22] and further emphasizing the functional relationship among fiber type, oxidative status, metabolic conditions, and the rescue of dystrophic-associated defects. It is for this reason that the modulation of the autophagic process, for example, by a specific dietary regimen, may be used as part of a therapeutic strategy to ameliorate the dystrophic muscle phenotype.

Ethical Publication Statement: We (the authors) confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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