

Mitochondrial Dysfunction in Muscular Dystrophies

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INTRODUCTION

Mitochondria are being regarded as key players in the pathogenesis of Muscular Dystrophies (MDs). Mitochondria, as seen by the electron microscope, are stiff elongated cylindrical organelles located in the cytoplasm of eukaryotic cells that play a vital role in energy metabolism [1]. Mitochondrial function is an important determinant of skeletal muscle homeostasis and its abnormalities have been associated with a series of MDs, such as dysregulation in mitochondrial Oxidative Phosphorylation (OXPHOS) as well as mitochondrial complex activity is associated with various skeletal muscle pathologies [2]. In addition, MDs harbor mitochondrial structure abnormalities [3], genomic mutations in mtDNA [4], altered energy metabolism, defect in mitochondrial fusion and fission [5] and calcium ion signaling [6]. Presented below are some reports implicating mitochondrial dysfunction in MDs for some of the experimentally studied MDs.

DUCHENNE MUSCULAR DYSTROPHY

Dystrophin, the gene of which its mutation is implicated in the most common X-linked muscle myopathies (**DMD/BMD**) is a subsarcolemmal protein that, through linkage of the actin cytoskeleton with the extracellular matrix by dystroglycans, is critical for the integrity of muscle fibers. Although an alternative version of the shortest dystrophin isoform, DP71 has been identified in the mitochondria from rat brain over a decade ago and from mitochondria from Hek293 cells (E.T.E.Niba, A. Nishida and M. Matsuo, unpublished data), the precise role of mitochondrial dysfunction in DMD has not been very obvious. However, certain mechanisms have been proposed and some experimental evidence presented.

Exercise induced damage through muscle contractions induces stress on dystrophic muscle membranes and provokes micro-lesions that could eventually disrupt calcium (Ca^{2+}) homeostasis, and cell death. The increased permeability of Ca^{2+} into the muscle affects the Ca^{2+} buffering system of the mitochondria and hence Ca^{2+} overload in the mitochondria. The result is mitochondrial swelling and functional abnormalities [7] leading to increased muscle cell apoptosis and necrosis [8]. This triggers the inappropriate opening of the mitochondrial permeability transition pore (mPTP), an inner membrane channel that plays a role in several forms of cell death [8,9]. In addition, Onopiuk and team observed reduced amounts of specific subunits of the mitochondrial respiratory complexes I, III and IV and ATP-synthase as well as disorganized mitochondrial network in dystrophic mouse [10]. Furthermore, melanocytes from DMD patients displayed latent mitochondrial dysfunction similar to observations of mitochondria from patients with Ullrich congenital muscular dystrophy [11]. A F_1F_0 ATP synthase inhibitor oligomycin, led to depolarization of mitochondria from melanocytes and myoblast from DMD patients, results that were contrary to those from healthy donors, suggesting a role of ATP synthase dysfunction in dystrophic tissue [12] that maybe caused by complex 1 insufficiency.

In mdx mice, a model of DMD, mitochondrial-dependent necrosis was eliminated by treating with the non-immunosuppressive derivative of Cyclosporin A (**CsA**), Debio-025 [13], that could selectively inhibit matrix cyclophilin D without inhibition of calcineurin [11] leading to reduced mitochondrial swelling and necrotic disease manifestations [8,13], an indication of mitochondrial dysfunction in the pathogenesis of DMD.

COLLAGEN VI MUSCULAR DYSTROPHIES

Mitochondrial defects have been well studied in the pathogenesis of Collagen VI muscular dystrophies that are caused by mutations in the genes encoding the extracellular matrix protein collagen VI (**ColVIa1**), ranging from the severe Ullrich Congenital Muscular Dystrophy (**UCMD**) to the less severe Bethlem Myopathy (**BM**), and Congenital Myosclerosis [14].

The pathogenesis of these diseases are unknown however many studies have implicated mitochondrial dysfunction as a key factor. Increased apoptosis and ultrastructural defects of

the mitochondria and sarcoplasmic reticulum were evident in skeletal muscles from mice with UCMD [8,15,16]. Moreover, the skeletal muscle from mice lacking ColVIa1 displayed a latent mitochondrial defect caused by inappropriate opening of the mPTP. Furthermore, cell-based as well as muscle biopsy-based ultrastructural studies of BM and UCMD patients, presenting swollen mitochondria with hypodense matrix, disorganized cristae and paracrystalline inclusions associated with dilated sarcoplasmic reticulum and apoptotic changes providing strong evidence for the implication of mitochondrial dysfunction in ColVI myopathies [16]. The connection between ColVI and mitochondria is not known although a role for intracellular Ca²⁺ signaling is plausible [15].

The identification of the pathogenic mechanism of ColVI myopathies has paved the way to several treatments to rescue the mitochondrial defect, using certain compounds. In *in vitro* and *ex vivo* conditions, CsA, an inhibition of Cyclophilin D (**Cyp-D**), a matrix protein that sensitizes the pore opening could greatly alleviate symptoms of mouse lacking the ColVI a gene [8,15,17]. CsA displayed similar results in a pilot study in BM and UCMD patients [17]. Furthermore, Debio025, alleviated mPTP function as well in ColVIa1^{-/-} myopathic mice [18]. In addition, treatment employing genetic manipulations such as by inactivating the Ppif gene encoding Cyp-D in the ColVIa1^{-/-} mouse model abolishes the disease phenotype of ColVI deficiency [19].

SPINAL MUSCULAR ATROPHY

A down regulation of mitochondrial biogenesis has been shown to contribute to the pathogenesis of disease in the muscle of patients with Spinal Muscular Atrophy (**SMA**), a disease characterized by muscle wasting from childhood [20-22]. In general, reduced activities of the respiratory chain in SMA patients was connected with the reduction of the content of mtDNA as compared to the nuclear DNA, in a study of 30 SMA patients and 20 control subjects [23]. The implication of mitochondria dysfunction was also specifically displayed in the SMA types I, II, and III, where muscle mtDNA content and cytrate synthase activity were reduced in all 3 types of SMA. Moreover, muscle samples from patients with SMA-I and SMA-II exhibited a deficiency in Cytochrome-c Oxidase (**COX**) [22].

OCULOPHARYNGEAL MUSCULAR DYSTROPHY

Oculopharyngeal Muscular Dystrophy (**OPMD**) is a genetic disease that leads to ptosis or eyelid drooping and swallowing difficulties due to dysphagia. Mutations in Poly(A) Binding Protein Nuclear 1 (**PABN1**), a nuclear protein involved in polyadenylation of messenger RNAs (**mRNAs**) and poly(A) site selection [24-26] is responsible for the disease. Although a short expansion of GCN triplet repeats in pabn1 gene is evident in OPMD [25,27]), the molecular mechanisms are still not well understood. Studies in monozygotic twins with identical GCN expansions of the Poly(A) Binding Protein 2, PABP2 gene, the PABPN1 homologue in *Drosophila*, indicated for the first time, the presence of mtDNA deletions by Southern blotting in individuals with OPMD [28]. Also findings from experiments with *drosophila* and mouse indicated that mitochondrial protein-

encoded nuclear genes were down-regulated in a *pabn1* mutant in both animal models however the mitochondrial mass as well as citrate synthase activity was not different [29].

Further evidence was presented with human samples using a proteomic technique on muscle biopsies. From the results, 58% of down-regulated proteins in OPMD patients were connected with mitochondrial dysfunction. The molecular defects observed in PABPN1-17ala-expressing muscles, reduction of mRNA poly(A) tail length and decreased efficiency of cleavage at poly(A) sites are similar to those observed in *Pabp2* loss-of-function mutants [30]. Since poly(A) tails play a vital role in mRNA stability, these defects enhance the decay of these mRNAs. The affected mRNAs encode for mitochondrial proteins, and hence mitochondrial activity is impaired in diseased muscles.

MYOTONIC MUSCULAR DYSTROPHY

The relation between mitochondrial dysfunction and the pathogenesis in myotonic dystrophy is only beginning to unfold. DM-patients' muscles show a reduced expression of DMPK, the product of the gene that is altered by (CTG)_n repeat expansion and mitochondrial accumulation in degenerated myofibrils. DMPK-A, the largest isoform of DMPK, supplies antioxidants and antiapoptotic signals needed for correct muscle fiber function and differentiation [31]. Mouse and human DMPK-A both localize to the mitochondria. However, binding and accumulation of DMPK-A in the mitochondrial outer membrane can lead to structural changes in mitochondria and the formation of perinuclear clusters of morphologically altered mitochondria, eventually inducing the activation of autophagy [32].

On the other hand, experiments with blood samples have confirmed an inverse correlation between Coenzyme Q10 (**CoQ10**) levels, a component of the electron transport chain that participates in aerobic cellular respiration and CTG expansion length in DM patients. Consistently, muscle from DM patients show reduced CoQ10 levels.

Through proteomic analyses of myotubes from DM2 patients, EFTu, HSP60, GRP75 and Dienoyl-CoA-Isomerase which are proteins involved in mitochondrial fatty acid degradation were reduced [33] as well.

DYSFERLINOPATHY

Dysferlinopathy is another muscle disease for which mitochondrial abnormalities have been rarely reported. COX deficiency, mtDNA copy number reduction, reduced expression of complexes I, III and IV were observed in the study of a cohort of dysferlinopathy patients [34].

CALPAINOPATHY

The cysteine protease, Calpain 3 (**CAPN3**) is the gene of which its mutation is the causative agent of the autosomal recessive Calpainopathy or Limb Girdle Muscular Dystrophy 2A (**LGMD2A**). The pathological mechanisms, mitochondrial activities inclusive, have not been well elucidated in this

disease. The implications of mitochondria in the pathogenesis of LGMD2A became clear through proteomic studies by the Spencer group. They identified a number of mitochondrial enzymes, particularly enzymes involved in β -oxidation of fatty acids, as potential substrates for CAPN3 proteolytic activity [35]. CAPN3 has been shown to possess both a catalytic and a proteolytic function, regulating Ca signaling [36] and interacting with dysferlin to regulate the dysferlin complex [37]. In another study, mitochondrial dysfunction was identified as a feature of LGMD2A [38]. In CKO3 mouse, a model for LGMD2A, presence of morphologically abnormal mitochondria, decreased ATP production and increased oxidative stress indicated association of mitochondria defects to the disease.

CONCLUSION

Mitochondrial defects have been associated with a series of MDs [39] and a substantial body of experimental work, derived from animal models, attests to a major role of mitochondria in the early process of muscle degeneration. Common mechanisms of mitochondria-related cell injury central to most MDs include reduction of mitochondrial mass and number, dysregulation of the mitochondrial permeability transition pore opening and defective respiratory chain activities (Figure 1). However, it is arguable if the mitochondrial defects play a primary or secondary role in the onset and or pathogenesis of MDs although the mechanism of mitochondrial fragility is similar in most if not all of the MDs.

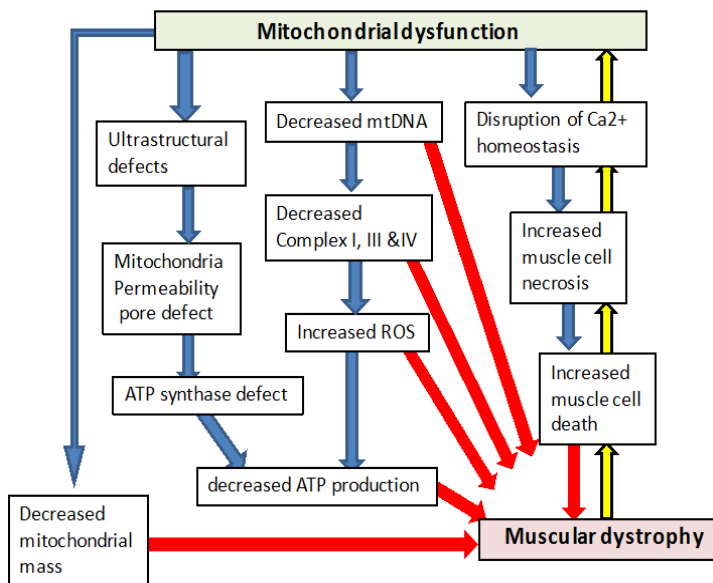


Figure 1: A schematic representation of a summary of the causative factors of muscular dystrophy as related to mitochondrial dysfunction. The various aspects of mitochondrial functioning that when defective lead a series of events that presents an adverse effect on the pathogenesis of muscular dystrophies are boxed. The blue arrows indicate the sequence of occurrence of each event in the case when mitochondrial dysfunction is primary while the yellow arrow represents a secondary involvement of mitochondria.

In the case of DMD, the concept that Ca²⁺-dependent mitochondrial dysfunction is a causative event in the onset of DMD has been put forward however, evidence displayed by Onopiuk and coworkers suggest that the increased membrane potential and the reduced respiratory complexes are primary factors in DMD pathology that promotes the Ca²⁺ overload. From this we can deduce that mitochondrial defect is primary to DMD. In addition, DMPK-A localizes in mitochondria and binds to the mitochondrial outer membrane, but evidence for the significance of DP71 being expressed in mitochondria is still missing although such evidence could strengthen the role for mitochondria in DMD as seen with DMPK-A in DM. Therefore, it will be worth elucidating the role for DP71 in mitochondria such as whether it interacts with any of the mitochondrial outer membrane proteins to sustain the mitochondria membrane.

In UCMD, it was observed that faulty mitochondria were a normal phenomenon in both homozygous and heterozygous mutation-carrying patients, an indication of the implication of defective mitochondria in the pathogenesis of all cases of UCMDs [11]. On the other hand, it is nonetheless ambiguous if there's a difference in the severity of the mitochondrial defect between DMD and BMD in myoblast and or melanocytes from such patients. Deciphering the implication of mitochondrial defect in either DMD or BMD in myoblast, melanocytes or most interestingly blood samples could provide novel ideas about biomarker development as well as another mode of distinction between the diseases for treatment establishment. MtDNA in most cases of SMA, does not play a primary role but it is involved subsequently. However, it may contribute to the neurodegenerative process [21] of the disease.

Taken together, mitochondrial defects might not be primary but improvement of mitochondrial function through use of CsA and Debio 025 have presented positive results in animal models of some of the MDs. Adding to this, activation of ATP Citrate Lyase (**ACL**) by IGF1 in skeletal muscle increases cardiolipin content and mitochondrial complex activity and improves mitochondrial function [2], because activation of IGF1/ACL/cardiolipin pathway induces mechanisms that increase ATP production through anaerobic signaling. Therefore, the therapeutic use of mitochondrial permeability transition pore modifiers, mitochondria protective drugs as well as other agents that improve mitochondrial function presents a great promise as therapies in various MDs [39]. Hence, it will be important to develop a better understanding of the role of mitochondrial dysfunction in MDs.

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