Epigenetic modifications of the PGC-1Î± promoter in Limb Girdle Muscular Dystrophy 2D account for impaired mitochondrial biogenesis: new perspective in HDAC inhibitors as therapeutic strategy of muscular dystrophy.

I. Di Renzo[1], C. Perrotta[1], M. Giovarelli[1], S. Pambianco[1], D. Cervia[1,2], C. De Palma[1,3], E. Clementi[1,3,4]

[1] Department of Biomedical and Clinical Sciences 'Luigi Sacco' (DIBIC), Università degli Studi di Milano, Milano, Italy
[2]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Università degli Studi della Tuscia, Viterbo, Italy
[3] Unit of Clinical Pharmacology, University Hospital 'Luigi Sacco'-ASST Fatebenefratelli Sacco; National Research Council-Institute of Neuroscience; Department of Biomedical and Clinical Sciences 'Luigi Sacco' (DIBIC), Università degli Studi di Milano, Italy
[4] Scientific Institute IRCCS Eugenio Medea, Bosisio Parini, Italy

Mitochondrial defects have been shown to accompany several forms of muscular dystrophies.
In Limb Girdle Muscular Dystrophy (LGMD) the loss of Î± sarcoglycan (Î±-SG) as the loss of dystrophin or other components of the dystrophin-glycoprotein complex, leads to a severe alteration in the physical properties of the sarcolemmal and to mitochondria swelling and dysfunction. However, the mechanism underlying this alteration has not been investigated yet. To address this issue in LGMD-2D we relied on the Î±-SG null mouse model of the disease. In these mice we identified a persistent impairment of mitochondrial biogenesis.
This defect was characterized by a reduction of mitochondrial number that was persistent during muscle development in mice from 1.5 to 5 months old. According to these data, the mRNA levels of the peroxisome proliferator-activated receptor-gamma coactivator 1 Î± (PGC-1Î±) the master regulator of of mitochondrial biogenesis, and of its downstream targest, *i.e.* the nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM), were significantly reduced in diaphragm and TA of 5 month-old Î±-SG null mice when compared to age-matched wild-type mice. Evaluating the response to cold exposure, a classical mitochondrial biogenetic stimulus, we observe no increase in mRNA levels of PGC-1Î± and NRF1 and mitochondrial content in muscles of Î±-SG null mice with respect to wild-type, thus demonstrating directly that mitochondrial biogenesis was altered in LGMD-2D.
To understand the mechanism underlying the persistent impairment of mitochondrial biogenesis in Î±-SG null mice we examined the distribution patterns of three regulatory histone modifications at PGC-1Î± locus, methylation of lysine 4 (Lys4) and lysine 27 (Lys27), H3K4me3 and H3K27me3 respectively, as well as a pan H3 acetylation (AcH3), by chromatin immunoprecipitation analysis (ChIP). Increased histone H3 acetylation was observed only in diaphragms from 5 months old wild-type control, but not in Î±-SG null mice. Histone acetylation represents a chromatin epigenetic tag reducing the interactions between histones and DNA, opening the DNA structure and allowing higher levels of gene transcription. These data indicate that the impairment of mitochondrial biogenesis in Î±-SG null mice is accompanied by a chromatin conformation of the PGC-1Î± promoter predictive of gene repression.
To determine whether this epigenetic modification caused the defect in mitochondrial biogenesis we reversed it with the class I and II deacetylase inhibitor Trichostatin A (TSA). Increased histone acetylation, which reflects the bioactivity of deacetylase inhibitors, was observed in the PGC-1Î± promoter by TSA treatment. Consistently, the expression of PGC-1Î±, TFAM, NRF1 and CYT B were significantly enhanced after TSA administration, indicating the reactivation of the gene expression program leading to mitochondrial biogenesis.
Taken together these data point out the importance of mitochondria pathways activation in muscular dystrophy like LGMD-2D. TSA, while a valid experimental tool, cannot be developed as a therapeutic strategy in muscle dystrophy because of safety concerns. In view of that, other HDAC inhibitors, currently under development, may be considered valuable options to be explored in the treatment of muscular dystrophies alone or in combination therapy.