METABOLIC AND EPIGENETIC REGULATORS AS PATHOLOGY AND THERAPY BIOMARKERS IN PRECLINICAL RESEARCH ON DUCHENNE MUSCULAR DYSTROPHY: NEW INSIGHTS INTO THE GENE AND PROTEIN EXPRESSION PROFILE IN MURINE MODELS

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Background and aims: In the past few years, the potential role of epigenetic modulators such as histone deacetylases (HDAC) in the pathogenesis of Duchenne Muscular Dystrophy (DMD) has been widely investigated. In detail, HDAC IIa, a class of proteins acting in macromolecular complexes as epigenetic silencers, modulates the expression of some myogenic regulatory factors with a primary role in skeletal muscle cells proliferation and differentiation. Furthermore, the HDAC IIa are important targets of adenosine monophosphate-activated protein kinase (AMPK), a master metabolic regulator that targets a plethora of downstream effectors involved in muscle metabolism. AMPK activation, occurring during exercise, induces the phosphorylation of the HDAC IIa and their retention in cytoplasm, thus inhibiting their function of transcriptional repressors. Our recent findings support the view that a key mechanism of damage in dystrophic myofibers is an insufficient mechanical-metabolic coupling. We hypothesized that an improper stimulation of AMPK may contribute to a failure of the HDAC IIa inactivation and downregulates the pathways involved in oxidative metabolism and myogenesis. Our current research is focused on the expression, at gene and protein level, of key metabolic and epigenetic players in various experimental conditions in murine models of DMD, in order to identify pathology and therapy biomarkers useful in translational research.

Methods: We performed gene expression (SYBR Green—Real Time PCR) in gastrocnemius (GC) muscles of C57BL/10 mdx sedentary mouse (6and 12months of age). In addition, we analyzed GC muscles of DBA/2J mdx mice (6months), an animal model characterized by a more severe phenotype due to a polymorphism in the gene of latent Transforming growth factor beta (TGF-b) binding protein. GC muscles of C57BL/10 wild type (WT) and DBA/2J WT age-matched mice were used as control groups. b-actin and b2-microglobulin were adopted as housekeeping genes to normalize the expression level of targets of interest. Western Blot analyses are underway to assess the correlate of mainly involved genes at protein level.

Results and discussion: The first step of our study consisted of analyzing the expression of genes involved in oxidative metabolism and myogenesis. MyoD, a myogenic factor whose expression is improved by HDAC inhibitors in DMD myofibers, was not modified in GC of C57/BL/10 mdx mice of 6months, while it was markedly upregulated at 12months. This suggest that MyoD expression may be strictly related to the cycles of degeneration/regeneration occurring in mdx myofibers, likely under the control of various epigenetic regulators in a time-dependent manner. However, MyoD was remarkably downregulated in D2/mdx mice vs WT. This result suggests a greater role of HDAC in the impaired muscle regenerative capacity of this specific dystrophic phenotype. Interestingly, another key myogenic factor silenced by HDAC IIa, Myocyte Enhancer Factor 2C (MEF2c), was considerably downregulated in both mdx phenotypes; the same result was evident for the expression of Myostatin (Mstn), a negative modulator of muscle mass. Finally, the expression of Liver kinase B1(LKB1), an enzyme involved in the phosphorylation and activation of AMPK, was unaffected at any age in C57BL/10 mdx mice ages whereas it was significantly lower in D2/mdx mice.

Conclusions: Our preliminary results pave the way for further experiments aimed at gaining insight into the role of AMPK and HDAC IIa signaling in the mechanical-metabolic uncoupling of dystrophic muscle. In particular, experiments have been planned to confirm specific impairment in more severe pathology states, i.e. due to chronic exercise, or to assess the outcome of treatment with metabolic enhancers such as AMPK activator metformin. Our understanding of the altered HDAC IIa - AMPK pathway in models of DMD might be useful for better addressing pharmacological interventions in translational research. (supported by PRIN-MIUR 2017FJSM9S_005)