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Strategy for cell therapy evaluation in GRMD dog (model of Duchenne Muscular Dystrophy): quantitative proteomic analysis of skeletal muscle

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Introduction

Duchenne Muscular Dystrophy (DMD), the most common of inherited neuromuscular disorder, is caused by mutations in the dystrophin gene leading to the absence of the protein. Membrane permeabilization and subsequent alterations in signaling pathways and energy metabolism play important roles in muscle fiber necrosis. Here, using the clinically relevant Golden Retriever Muscular Dystrophy (GRMD) dog model, we employed combined proteomic approaches to compare changes in protein expression profiles of GRMD versus healthy dog muscles. We found that the set of over-expressed proteins was composed of factors involved in apoptosis, calcium signaling and myoblast development/differentiation. On the other hand, the set of under-expressed proteins appeared primarily composed of metabolic ones, many of which have been shown to be regulated by the transcriptional coactivator peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α). Thus, for the evaluation of novel therapeutic approaches, it is essential to analyse the direct reversal of dystrophin lack and the associated consequences in treated muscles. In this context, we used isotope-coded protein labelling to study the global proteome after systemic delivery of MuStem cells, a progenitor cell population that showed its therapeutic efficacy after systemic delivery. LC-MS/MS analyses performed on an ESI-LTQ-Orbitrap mass spectrometer led to the relative quantification of 750 proteins out of 1750 identified proteins. Moreover, same samples were analyzed at transcriptional level through a dedicated expression microarray to identify differentially expressed genes after treatment. The set of signature molecule identify by combined "omics" approaches will allowed us to evaluate the MuStem cell-based therapy approach.

Physiopathology of GRMD dog muscle: Proteomic approaches

Proteomic analysis using antibody array, ICAT and ICPL approaches

Decreased protein level and altered transcriptional regulation of PGC-1α

MuStem cells, a potential candidate for DMD therapy

MuStem cells provide an attractive therapeutic avenue for DMD patients

Strategy for the biochemical evaluation of experimental cell therapy

Outline of the «omics» analysis

Discussion

Several strategies have been recently set up in order to rescue dystrophin synthesis in animal models of DMD. The GRMD dog model has increasingly been used to assess efficacy of a range of gene-and cell-based therapy approaches. One of the major problems in comparing the benefit of different therapeutic treatments is to find common outcome measurements. In order to identify reliable biomarkers of the disease and to characterize the global perturbations of the dog model we combined «OMICS» approaches. Our strategy using protein array, ICAT and ICPL technologies provides sensitive and quantitative approaches to establish the pathophysiological events that trigger the fibrosis degeneration. We identify differences in the representation of key proteins between healthy and GRMD muscles, arguing that secondary changes play an important role in the dystrophic process. Defective energy metabolism is a hallmark of the disease progression and PGC-1α may be at the origin of the general metabolic crisis that characterize this disease. The aim of our study is to show how transcriptomic and proteomic profiling could be used for the proper evaluation of novel therapeutic approaches. Our strategy for MuStem cell therapy evaluation in GRMD dog analysed the reversal abnormalities in treated muscles with non-dedicated approaches.

By jointly using genomics, proteomics and bioinformatics there is a great potential to make considerable contribution to biomarker identification and to open up new avenues for characterization of the pathophysiological events and evaluation of new therapies.

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