

HIGH RESOLUTION TARGETED PROTEOMICS: BIOMARKER DISCOVERY IN A MOUSE TRANSGENIC MODEL OF MYOPATHY

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Identification of candidate biomarker of a mouse transgenic model of myopathy by high resolution LC-MS/MS and the use of Pinpoint software for targeted quantitation. Autoimmune muscle diseases are associated with abnormal over-expression of molecules of the MHC-I on muscle surface. Here a proteomic study was performed on mouse muscle tissue in order to evaluate the consequences of a continuous MHC-I expression. An experimental workflow was designed including protein digestion and label free qualitative and quantitative analysis by high resolution accurate mass LC-MS/MS. 3 muscle tissues from MHC-I inducible mouse were compared to 2 healthy controls using this targeted differential analysis approach.

Mice were sacrificed and muscle tissues were collected. Protein samples were digested with trypsin after reduction and alkylation. Peptides were analyzed by LC-MS/MS with a LTQ-Orbitrap Velos (Thermo Fisher Scientific). Database search of the 5 LC-MS/MS runs resulted in the identification of 1471 proteins and 5948 unique peptides with FDR<1%. 457 proteins were identified with more than 5 peptides and subsequently selected for targeted quantitation. Pinpoint was used to automatically create a list of peptides to serve as surrogates for the target proteins (proteotypic peptides). The peptide list was refined by only selecting peptides which were already identified by Sequest. In total, 414 proteins and 1632 peptides were selected for targeted quantitation. Quantitation was performed with Pinpoint. Peptides were quantified by extracting the first and second isotope of each charge state from Orbitrap full scan MS1 spectra.

A Total of 411 proteins and 1632 peptides were successfully quantified and relative quantitation was performed between the 2 sample groups. An S-plot of the ratio was generated showing a Gaussian distribution with a median of 1.1. 6 proteins were found to be down-regulated with ratios < 0.2. A concentration ratio of 33 proteins was above 5, showing up-regulation related to MHC-I induced mouse. A few proteins were found to be highly up-regulated like Protein-glutamine gamma-glutamyltransferase 2 which shows good consistency between all its peptides ratios.