A data independent LC-MS based method for a multi-omic approach to investigate obesity treatment within a mouse model

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Introduction

Obesity is one of the risk-factors associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. It has previously been reported that glycosphingolipids play a crucial part in metabolic syndrome. The manipulation of the function of glycosphingolipids with small molecule drug compounds within mouse models has shown that symptoms can be negated. Knowledge relating to the proteome, metabolome and lipidome during development is still to be fully explored. The work presented here is to provide a multi-omic analysis of protein and lipid liver extracts from control and obese mouse models undergoing treatment to prevent or revert obesity.

Methods

Lipid and protein extracts were generated from liver tissue originating from 3 control and 3 obese mice models. Protein extracts were proteolysed with trypsin and the resulting peptides separated over a 90 minute linear reversed-phase nanoscale LC gradient, whilst the extracted lipids were separated over a 20 minute reversed-phase LC gradient. Data were acquired using a data independent acquisition approach, whereby the collision energy was switched between a low and elevated energy state during alternate scans. Proteomic acquisitions also utilized ion mobility in the acquisition scheme. The acquired data were processed and searched using Progenesis QI and dedicated protein sequence and lipid compound databases, providing normalized label-free quantitation results for both datasets.

Preliminary Data

Proteomic samples were based on 100 ng loadings and analyzed as triplicate technical replicates in a randomized order. Processing and searching the data using Progenesis QI resulted in over 1250 curated proteins being identified, across all technical replicates and biological conditions. Over 300 proteins exhibit a fold change greater than 2 with significant analysis of variance. Unique peptides were used for relative label-free quantitation with median abundance normalization performed across all samples. Lipid extracts were prepared using 500 µL IPA/water (50:50), of which 2 µL were injected on-column and analyzed in triplicate. Samples were also acquired in a random order with a QC comprised of all samples in equal amounts and injected every 5 injections. Lipid data analysis was conducted in a similar manner with Progenesis QI used for processing and searching. Interrogation of the data revealed over 500 potential identifications for combined positive and negative ion acquisitions with mass errors less than 2 ppm. Compound searches provided a range of lipid classes including free fatty acids, ceramides, triglycerides, sphingomyelins and glycosphingolipids. Identification scores are based on mass accuracy, isotoptic fit and fragmentation. Unsupervised multvariate analyses showed clear distinction between obese and control groups in both proteomic and lipidomic experiments. Pathway analysis tools were used to review the complimentary datasets and hence provide an understanding of the underlying biology of differentially expressed proteins and lipids.

Novel Aspect

A multi-omic, biochemical and network investigation for the study of obesity using a mouse model.