Peptide and PTM Biomarkers of Cardiovascular Disease in a Mouse Model

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Novel Aspect

label-free proteomics analysis of serum peptides in a mouse model identifies biomarkers and PTMs associated with metabolic disorder and CVD

Introduction

Unfavorable metabolic conditions (metabolic disorders) are associated with obesity, diabetes, and hyperlipidemia and are major causes for cardiovascular disease. One major environmental cause of this may be attributed to poor diet, aka the American diet model. Early detection and monitoring of these adverse effects on the heart and vasculature, although well studied, remain elusive. Our hypothesis is that nonspecific changes which occur in plasma proteins, indicators of inflammation and oxidants, may act as evidence of systemic metabolic disease. Here we explore the application of label-free proteomics using an American diet mouse model, with and without a resveratrol based treatment, to elucidate potential biomarkers of CVD including changes in circulating peptides and proteins and changes in observable post-translational modifications (PTMs).

Methods

Blood was from control mice, mice fed a high fat high sucrose diet (HFHS) and the same (HFHS) group treated with a resveratrol analogue. Serum peptides were obtained via membrane filter centrifugation. Select serum proteins were purified via antibody enrichment. Proteins were obtained via precipitation and digested with trypsin. LC-MS/MS analysis was carried out on an LTQ-Orbitrap coupled with a Waters NanoAcquity HPLC. MS feature identification was enabled by analyzing the MS/MS data using Proteome Discoverer (Thermo-Fisher) and Mascot (Matrix Science) software, searching custom protein databases using both variable-modification and error-tolerant search modes. Label-free quantification was conducted using Progenesis LCMS (Nonlinear Dynamics). Collation and meta-analysis were conducted using the Trans Proteomic Pipeline (ISB), Scaffold (Proteome Software), and STRAP (in-house) software.

Preliminary Data

Label-free analysis of serum peptides yielded 31401 total features with 4642 feature ANOVAs < 0.05 and 817 ANOVAs with matching protein ID via MS/MS. Hierarchical clustering yielded 5 distinct groups of data for respective groups. A total of 961 features were observed with changes centered on this study and profiled as follows (control, HFHS, HFHS+): down/up/down=405 features and up/down/up=556 features. Other groups included increases or decreases with diet that did not change with treatment. A total of 221 features had positive peptide/protein ID via MS/MS. Further analysis by Gene Ontology (GO) indicated robustness of the data. Proteins with a molecular function GO term which increased with the diet decreased with the treatment. This makes the further analysis of proteins that changed with treatment particularly interesting. Of particular note, troponin I (TNNI_MOUSE) was identified in the group of serum proteins that increased with diet and decreased with resveratrol treatment. Troponin I levels are increased in patients with not only myocardial infarction, but also heart failure. Our finding that troponin I is increased by HFHS diet and is associated with diastolic dysfunction validates the use of this mouse model as well as supports the proof of principal that cardiac proteins can be detected in the blood of mice with metabolically diseased heart. Analysis of OPTMs on troponin peptides in the blood correlates with the fact that HFHS fed mice have abundant OPTM in the heart. This suggests that studies to characterize OPTM on the circulating protein will be fruitful. Development of a CVD-specific protein panel obtained from these mouse models will afford the first step in biomarker panel development.

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