Peptide and PTM Biomarkers of Cardiovascular Disease in a Mouse Model

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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 analog. 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.

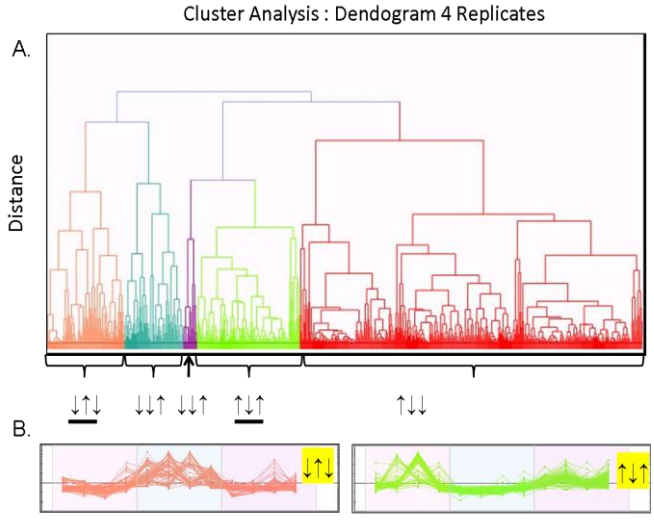
MS feature identification was enabled by analyzing the MS/MS data using Proteome Discoverer (Thermo-Fisher) and Mascot (Matrix Science) software, searching custom protein databases using both variable-modification and error-tolerant search modes. Label-free quantification was conducted using Progenesis LCMS (Nonlinear Dynamics). Collation and meta-analysis were conducted using the Trans Proteomic Pipeline (ISB), Scaffold (Proteome Software), STRAP (in-house) and Ingenuity Pathway Analysis (Ingenuity) software.

Results

Label-free analysis of serum peptides yielded 31401 total features with 4642 feature ANOVAs < 0.05; for 817 ANOVAs MS/MS yielded protein IDs. Hierarchical clustering yielded 5 distinct groups of data for respective groups (Fig. 1). A total of 961 features were observed with changes centered on this study and profiled as show in Fig. 1B: control, HFHS, HFHS+: 405 features = down/up/down, 556 = up/down/up.

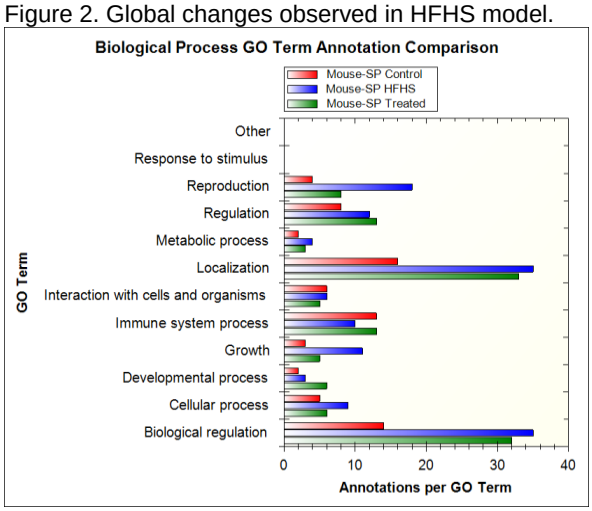
Other groups included increases or decreases with diet that did not change with treatment. A total of 221 features had peptide/protein ID via MS/MS.

Figure 1. HFHS model: mouse serum peptidomics



Further analysis by Gene Ontology (GO) indicated robustness of the data. Proteins with a biological function GO term which increased with the diet were observed to decrease with the treatment (Figure 2). These included proteins involved in biological regulation, cellular processes, immune response, and metabolic processes. This makes the further analysis of proteins that changed with treatment particularly interesting.

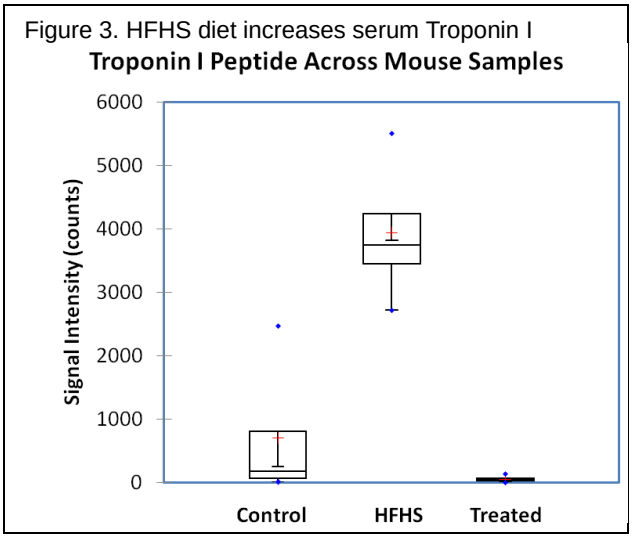
Figure 2. Global changes observed in HFHS model.



Ingenuity Pathway Analysis allowed thorough interrogation of the data for putative biomarkers and yielded >40 markers associated with the following: cardiac hypertrophy, chronic obstructive pulmonary disease, coronary artery disease, diastolic dysfunction, heart failure, hypertension, hypertrophy, myocardial infarction, systolic dysfunction, atherosclerosis, coronary disease, dilated cardiomyopathy, hypertrophic cardiomyopathy, hypoplasia, ischemic stroke, obesity and pulmonary hypertension.

Of particular note, troponin I (TNNI_MOUSE) was identified in the group of serum proteins that increased with HFHS diet and decreased with resveratrol treatment (Fig. 3). Troponin I levels increased not only in patients with 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 and supports the proof of principle that cardiac proteins can be detected in the blood of mice with metabolically diseased hearts. Further analysis of OPTMs on troponin peptides observed in the blood correlates with the fact that HFHS-fed mice have abundant OPTMs in the heart.

Figure 3. HFHS diet increases serum Troponin I



Conclusions

Development of a CVD-specific protein panel obtained from mouse models will afford the first step in biomarker panel development such that disease diagnosis and progression may be performed directly at the molecular level. Correlation of proteomics data with phenotype and genomics data will increase understanding of the etiology of CVD disease and host physiological response to CVD pathogenesis. The discovery of potential biomarkers will afford earlier detection, improved diagnosis and treatment of CVD and other oxidative stress-induced protein changes correlated to CVD and may be directly applied to many heart diseases.

Acknowledgements

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