Catalase Corrected Metabolic Syndrome Induced Protein/PTM Changes in a Mouse Model of CVD

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Introduction

Metabolic syndrome is associated with unfavorable metabolic conditions which may lead to obesity, diabetes, and hyperlipidemia, all of which are major causes for cardiovascular disease (CVD). A major environmental cause of metabolic syndrome may be attributed to a poor diet consisting of high fat and high sucrose (HFHS) intake: aka the American diet. Previously we have shown changes in protein and PTM expression in HFHS mouse models of metabolic induced CVD. Select changes are observed due to an increase in reactive oxygen species (ROS) and hence an increase in oxidative stress. In this study we expand our model of CVD to include mice with cardiac-specific overexpression of catalase (CatTG) which through enzymatic catalysis will enhance the decomposition of ROS, protect against cardiac hypertrophy and dysfunction and reduce the incidence of CVD. Determination of specific protein and PTM changes will allow us to expand our understanding of the underlying mechanisms of CVD and determine putative markers of metabolic syndrome-related CVD.

Methods

Heart, left ventricles (n=5), were obtained from control mice, mice fed a high fat high sucrose diet (HFHS), CatTG transgenice mice, and CatTG mice fed the same HFHS diet. Tissue samples were prepared using standard procedures and peptides were subjected to proteomics analyses. LC-MS/MS analysis was carried out on a Q Exactive mass spectrometer 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 both Scaffold (Proteome Software) and Progenesis LCMS (Nonlinear Dynamics). Collation and meta-analysis were conducted using the Trans Proteomic Pipeline (ISB), Scaffold and STRAP PTM (in-house) software.

Preliminary Results/Abstract

Label-free analysis of peptides yielded >65,000 total aligned features with >4,600 features with $p<0.05$ with matching peptide ID via MS/MS. Hierarchical clustering yielded 4 distinct groups of data. More than 3,100 features were observed with changes centered on this study: control, control+HFHS, CatTG, CatTG+HFHS. Other groups included increases/decreases with diet that did not change with treatment. From more than 700 protein changes, a subset of 277 proteins changed with $p<0.01$. Specific to this study, Troponins T, I and C changed with diet and were corrected with catalase overexpression. Protein changes were mapped to 189 canonical pathways (Ingenuity Pathway Analysis) including mitochondrial dysfunction; metabolic pathways: TCA Cycle II, Fatty Acid Beta-Oxidation, Glycogenesis-I; and signaling pathways: Calcium signaling, RhoGDI signaling and Epithelial Adhesion Junction Signaling. Coverage was observed for all 5 complexes of the ETC. Network analysis yielded representation of 433 proteins across 13 interconnected networks including Metabolic Disease, Cardiovascular Disease, Energy Production and Lipid Metabolism. An abundance of PTMs were observed to change on select proteins which correlated with our model. These included specific PTM changes on specific locations on Troponins T, I and C, and indicate that, in addition to protein expression changes, we also observe specific changes in PTMs across the different states. In summary, we observed protein/peptide and PTM changes which were reflective of CVD, were specific to our model and confirmed the cardio-protective benefit of over expression of catalase in the CatTG mice. Peptide/ PTM changes correlated well with our other models of CVD and increased our understanding of the mechanisms involved with progression of CVD. Specific peptides and PTMs have been highlighted as putative biomarkers and will be used to develop a CVD-specific panel, the first step in biomarker panel development.

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Novel Aspect
label-free proteomics analysis of a mouse model identifies biomarkers and PTMs associated with metabolic disorder and CVD