Histone PTM Profiling Of Human Heart Failure Biopsies Reveals H3K27ac-mediated Metabolic Dysfunction

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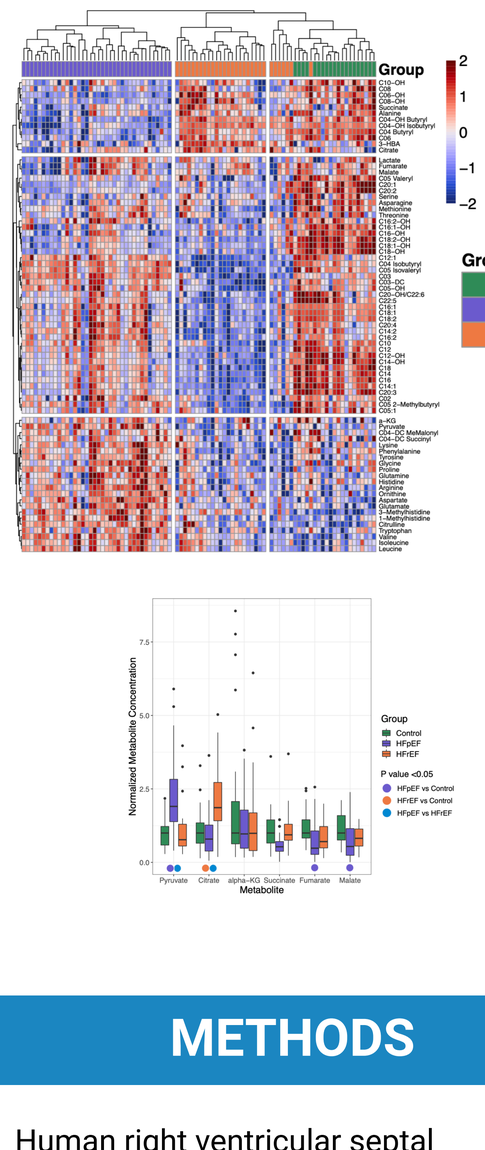
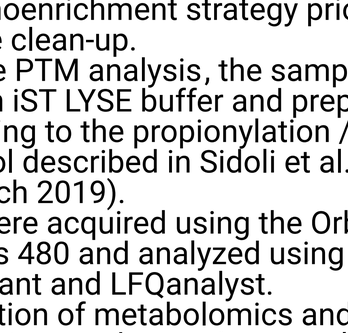
INTRODUCTION

Mass spectrometry-based global profiling of histone post-translational modifications (PTMs) has opened up new investigations of metabolic-epigenetic cross-talk. These mechanisms likely play pivotal roles in the Heart Failure with Preserved Ejection Fraction (HFpEF) syndrome, a disease that represents the greatest unmet therapeutic need in cardiology given its intimate relationship to obesity and metabolic syndrome. Preliminary data show depressed TCA cycle metabolites in human HFpEF endomyocardial biopsies. Our hypothesis is that obesity alters histone PTMs such as H3K27ac and H3K36me3, driving aberrant gene expression programs that regulate myocardial metabolism and worsen HFpEF. To address this complex question, we optimized mass-spectrometry-based histone PTM profiling in HFpEF patient biopsies from minute amounts of material.

Background Data. HFpEF displays a distinct metabolomic signature with depressed TCA cycle intermediates.

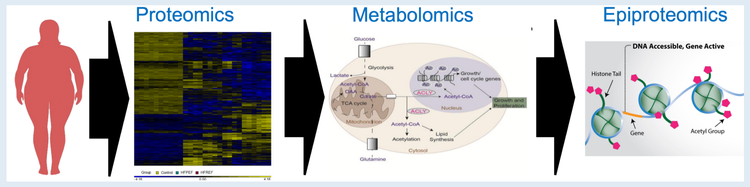




METHODS

- Human right ventricular septal endomyocardial biopsies from patients meeting consensus criteria for HFpEF (n=32) and donor controls (n=12) were obtained in collaboration with the Center For Heart Failure with preserved ejection fraction at the Johns Hopkins Hospital.
- Epiproteomics experiments consisted of based metabolomics, proteomics, and histone PTM analysis.
- For the proteomics experiment, samples were prepared with iST-NHS labeled with TMT-16plex followed by TiO_2 phosphoenrichment strategy prior to peptide clean-up.
- Histone PTM analysis, the samples were lysed in iST LYSE buffer and prepared according to the propionylation /trypsin protocol described in Sidoli et al. (Genome Research 2019).
- Data were acquired using the Orbitrap Exploris 480 and analyzed using MaxQuant and LFQanalyst.
- Integration of metabolomics and histone PTM data was done using corrplot in R.

RESULTS



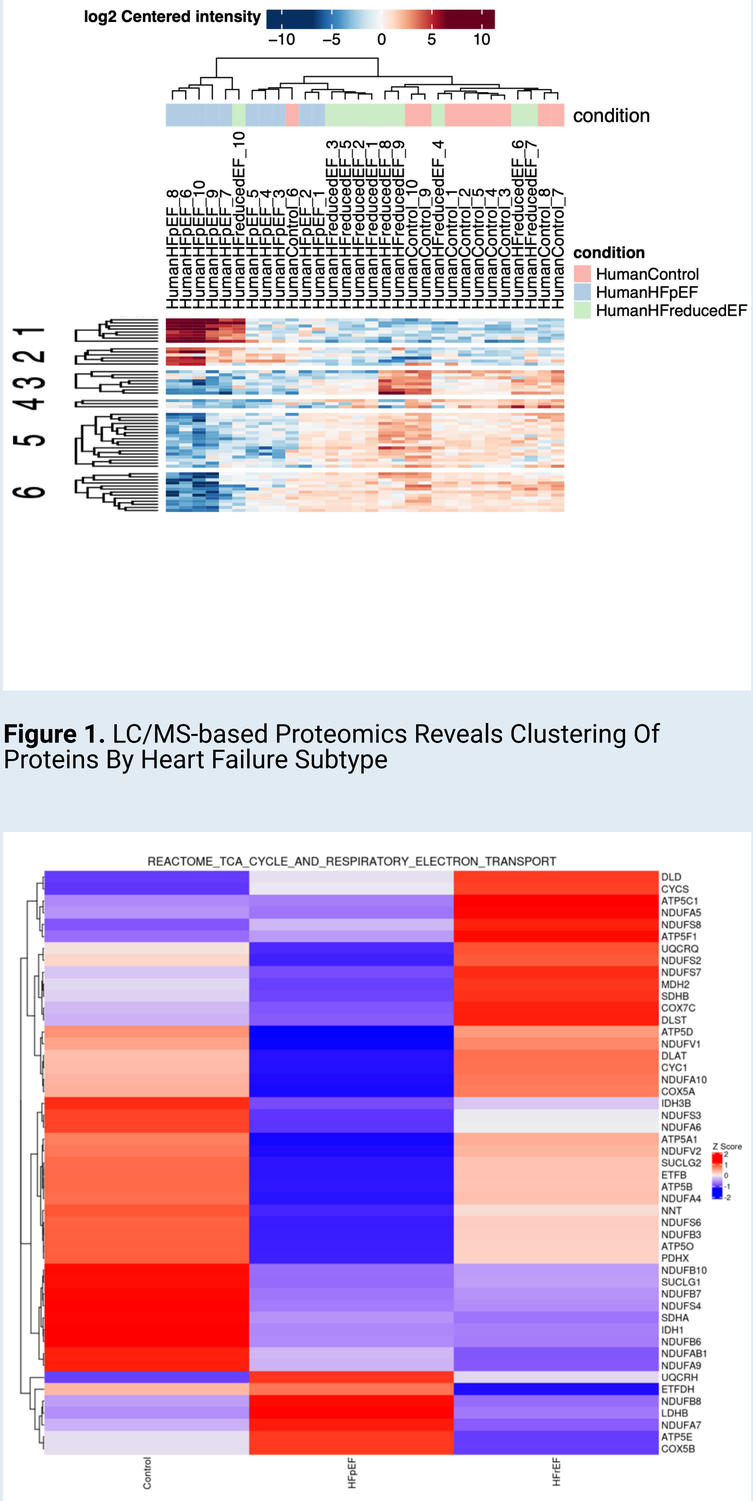


Figure 1. LC/MS-based Proteomics Reveals Clustering Of Proteins By Heart Failure Subtype

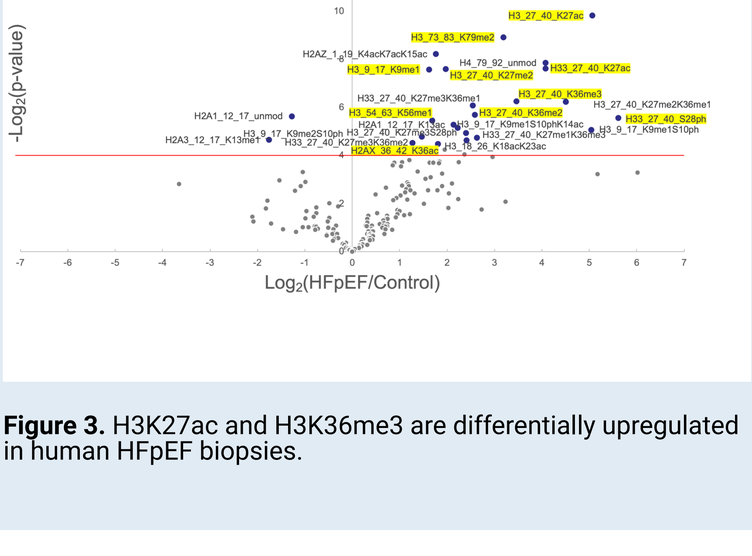


Figure 2. TCA Cycle and Oxidative Phosphorylation Proteins Are Depressed In HFpEF

Figure 3. H3K27ac and H3K36me3 are differentially upregulated in human HFpEF biopsies.

RESULTS

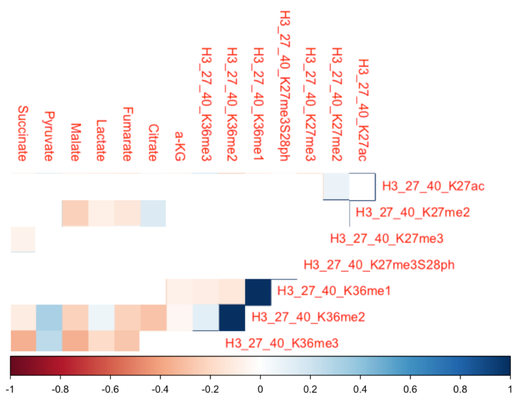


Figure 4. Integrated metabolomics and histone PTM analysis reveals significant correlations between TCA intermediates and histone H3 PTMs.

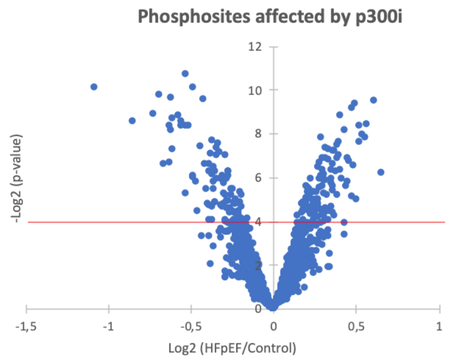


Figure 5. Histone acetyltransferase p300i affected the phosphoproteome of human HFpEF. In total ~1 300 phosphosites were identified and ~190 of them are highly regulated.

CONCLUSIONS

- A multi-omics approach reveals that histone PTMs are deposited as consequence of metabolic changes and regulates gene expression human HFpEF.
- Histone PTM profiling revealed a 16-fold upregulation of H3K27ac ($p<0.001$), and significant upregulation of other active transcriptional markers like H3K36me3 and H3S28ph.
- TCA Cycle and Oxidative Phosphorylation is depressed in HFpEF and correlates with elevated H3K27ac and H3K36me3 marks.
- Analysis of HFpEF-obesity animal models disclosed that the L-NAME/HFD mouse had striking histone PTM similarity to human HFpEF (-77 integrated distance units, $p < 0.005$).
- Small-molecule inhibitor phosphoproteomics screens identified histone modulators that could potentially be therapeutic. Inhibition of p300i identified over 1 300 phosphosites from them 193 are up-/down-regulated.

ACKNOWLEDGEMENT

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