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HDAC6 Inhibition Improves Stress-Induced Cardiomyocyte Restructuring in Heart Failure with Preserved Ejection Fraction (HFpEF) in a Multimodal Manner Based on Single Cell RNA-seq (scRNA-seq) Analyses

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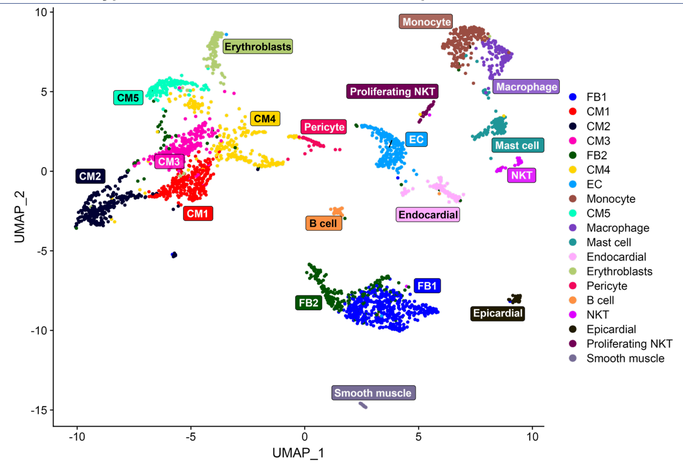
Introduction

In the high fat diet (HFD)/L-NAME mouse model of HFpEF, we have previously shown that pharmacological inhibition of HDAC6 improves functional and structural cardiac metrics, together with improved oxidative phosphorylation, improved contraction, and reduced fibrosis. Tissue level as well as single cell level transcriptional studies of control, HFD + L-NAME HFpEF, and HFD + L-NAME HFpEF treated with either HDAC6 inhibitor TYA-018 demonstrated extensive reversal of disease phenotypes in mice treated with TYA-018. This compound is a member of the same chemical series and as TN-301, currently in clinical development of the treatment of HFpEF. Based on scRNA-Seq analyses, we report characterization of cardiomyocyte (CM) subtypes in HFpEF that establish specific and substantial cell-cell interactions with their surrounding fibroblasts and endothelial cells. These interactions initiate CM-subtype-specific signaling cascades, leading to emergence of 'stressed' hypoxic CM and hypercontractile CM subtypes. We show that these cell types increase in the HFpEF disease state. The proportion of 'stressed' CMs at tissue level corresponds to the worsening cardiac functional measures, and their levels normalize after TYA-018 treatment. These findings suggest HDAC6 inhibition may ameliorate undesirable HFpEF phenotype characteristics such as dysregulated metabolism, fibrosis and hypertrophy, and also present detailed cell-level evidence of the favorable effects of HDAC6 inhibition on metabolic syndrome.

Mouse Heart Single Cell Transcriptional Diversity

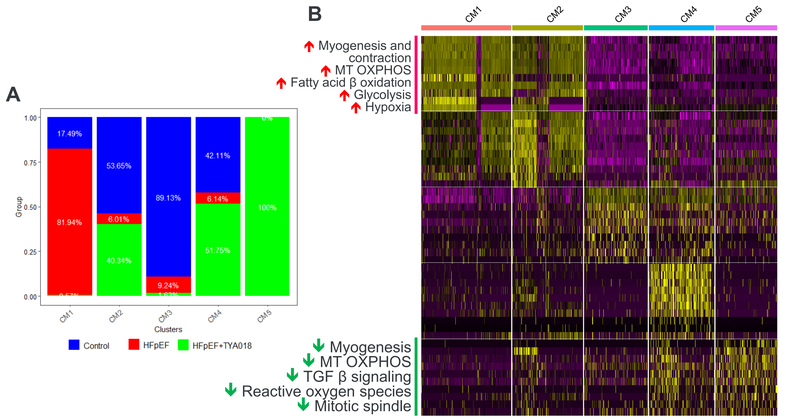
Methods: As part of the HFpEF program at Tenaya, we studied the single-cell transcriptional landscape in 5 C57BL/6 mice at Tenaya (wild type (WT) healthy animals (n=2), HFD + L-NAME HFpEF + vehicle (n=1), and HFD + L-NAME HFpEF +TYA-018 (n=1). Mice were sacrificed 9 weeks after start of the treatment. The analysis was performed using ICELL8 system for single-cell Next-Gen Sequencing (NGS) for single-cell dispensing, allowing for intact cardiomyocyte cell studies instead of single-nucleus studies using 10x Genomics platform. Library prep was performed using SMART-Seq Pro kit (Takara Bio) and was sequenced by Illumina NovaSeq using SP flow cell.

Identified cell types and their clusters in Control, HFpEF, and TYA018-treated mice hearts



HFpEF-enriched cardiomyocyte subtypes normalize after treatment with HDAC6 inhibitor

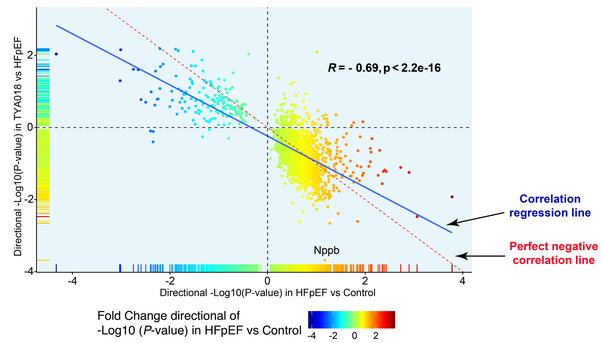
CM1 cardiomyocyte subtype shows significant enrichment in HFpEF CMs, and near complete reversal in CM5 with enrichment in TYA018-treated CMs.



Results 1. Clustering of cardiomyocytes from WT Control, HFpEF, and HFpEF + TYA018 groups. (A) CM1 cluster shows enriched proportion in HFpEF group while CM5 cluster is only present in TYA018-treated CMs. (B) Cardiomyocytes in CM1 demonstrate significant functional enrichment in contraction, oxidative phosphorylation and fatty acid metabolism, and exhibit hypoxic features. In contrast, CM5 cluster is characterized by reduced myogenesis, reduced oxidative phosphorylation, and loss of TGF β signaling.

HDAC6 inhbition reverses HFpEF-specific transcriptional changes in cardiomyocytes

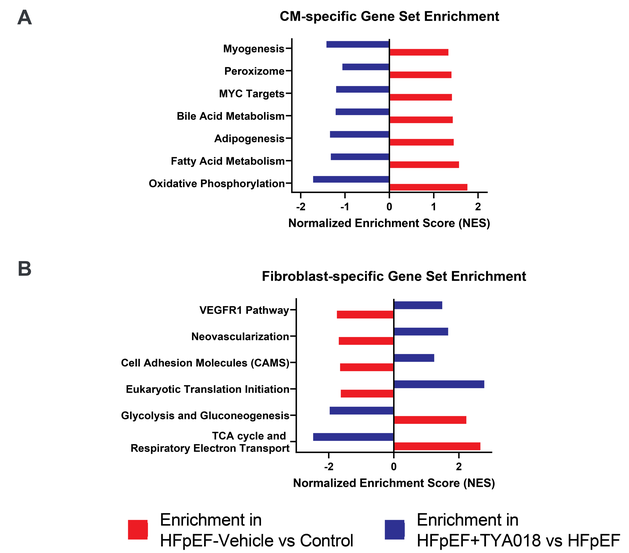
Differential gene expression analysis in cardiomyocytes demonstrates extensive reversal of HFpEF-specific alterations under TYA-018 HDAC6 inhibitor



Results 2. Correlation of direction and statistical significance of gene-wise changes in comparisons. A) HFpEF vs Control (on X axis) and B) HFpEF + TYA-018 vs HFpEF (on Y axis). Each point is a gene with differential gene expression statistical significance in at least on of the A or B comparisons (BH-adjusted FDR < 0.05). X and Y coordinates are calculated by multiplying change direction in –Log10(P-value) in the respective comparison. Correlation is calculated using pearson correlation and represented as correlation coefficient.

Transcriptional program enrichment in HFpEF pre- and post- TYA018 treatment

HFpEF CM- and FB- specific differential gene set enrichments demonstrate a strong reversal trend after treatment with TYA-018 HDAC6 inhibitor.



Results 3. Gene Set Enrichment Analysis of key gene sets in cardiomyocytes and fibroblasts. (A) CM-specific GSEA enrichment , and (B) FB-specific GSEA enrichment. Gene sets unbiasedly selected from Hallmark and C2CP collections in MsigDB database, prioritized by their enrichment in HFpEF group vs Control group. Enrichment shown as Normalized Enrichment Score (NES) and only shown if FDR Q-value < 0.25 in GSEA analysis.

Summary and Future Directions

These studies provide evidence on development of a stressed, hypermetabolic, and hypercontractile cardiomyocyte subtype in HFpEF using the HFD/L-NAME mouse model, directly contributing to the disease clinical outcome. Our single cell gene expression analyses suggest that HDAC6 inhibition by TYA-018 substantially reverses the HF-associated transcriptional profiles in CMs as well as fibroblasts. Further studies will be performed to expand our knowledge on how HFpEF specific cell-cell interactions and regulations before and after HDAC6 inhibition will alter disease pathophysiology.

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