ABNORMAL IMMUNOMETABOLISM AND GENE ACCESSIBILITY IN ALVEOLAR MACROPHAGES IN HIV

Sara C. Auld[1,2], Jolyn Fernandes[1], Mariam Ahmed[1], Neel R. Gandhi[1,2], Samantha M. Yeligar[1,3], Bashar S. Staitieh[1]
[1]Department of Medicine, Emory University School of Medicine, Atlanta, GA, [2]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, [3]Atlanta VA Medical Center, Atlanta, GA

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Introduction

- Widespread implementation of antiretroviral therapy (ART) has reduced, but not eliminated, the risk of death from pulmonary infections such as pneumonia and tuberculosis in people with HIV
- People with HIV also have a greater risk of non-infectious pulmonary diseases like emphysema, pulmonary hypertension, & pulmonary fibrosis
- Decreased lung function is associated with greater mortality in HIV
- Mechanisms underlying these risks remain poorly understood
- Alveolar macrophages, a pulmonary reservoir for HIV, exhibit derangements in antioxidant balance and innate immune function in HIV
- We studied the effects of HIV on alveolar macrophage immunometabolism
- In parallel, we characterized chromatin accessibility in alveolar macrophages from people with and without HIV to identify biological processes altered in HIV

Methods

- **Study Design and Sample Collection**: Five participants with HIV (on ART ≥ 18 months, CD4 ≥ 350 cells/µl, undetectable viral load), and five without HIV (matched by age, sex, race and smoking status) consented to a research bronchoscopy study in Atlanta, GA (Emory IRB #00088993)
- Bronchoalveolar lavage was performed and alveolar macrophages were isolated and washed before plating for analysis of mitochondrial bioenergetics and chromatin accessibility in RPMI-1640 (ATCC) with 10% FBS and gentamycin/amphotericin
- **Mitochondrial Bioenergetics**: Oxygen consumption rate (OCR) was measured using the Seahorse XFp Extracellular Flux Analyzer Cell Mito Stress Test
- Rate of change in OCR was normalized to protein concentrations in the same samples, analyzed using a student's t-test and data presented as mean ± SEM
- **ATAC-seq**: Cells were prepared with a transposition reaction prior to PCR amplification and sequencing using the Illumina NextSeq platform
- Peaks were called using edgeR, annotated using HOMER, pathway enrichment analysis using GSEA, heatmap using R, and functional annotations using Gene Ontology Tools

Results

Table 1: Characteristics of study participants

Characteristic	HIV-positive (n = 5)	HIV-negative (n = 5)
Age (median (range))	48 (32-62)	41.6 (7.4)
Female (n (%))	4 (80)	4 (80)
Black race	4 (80)	4 (80)
BMI	35 (22-52)	30 (25-38)
Smoker	4 (80)	4 (80)
Alcohol use	2 (40)	2 (40)
CD4 count**	966 (728-1067)	n/a
Viral load undetectable**	2 (40)	n/a

Results

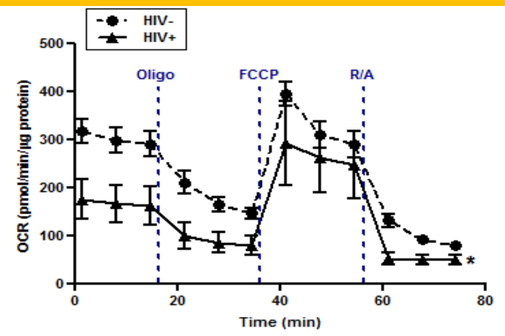

Figure 1: Alveolar macrophages from people living with HIV demonstrate defects in mitochondrial bioenergetics. Although maximal respiration was relatively unchanged in subjects with HIV, basal and ATP-linked respiration were significantly impaired and spare respiratory capacity was significantly increased. *p<0.05 vs HIV negative subjects.

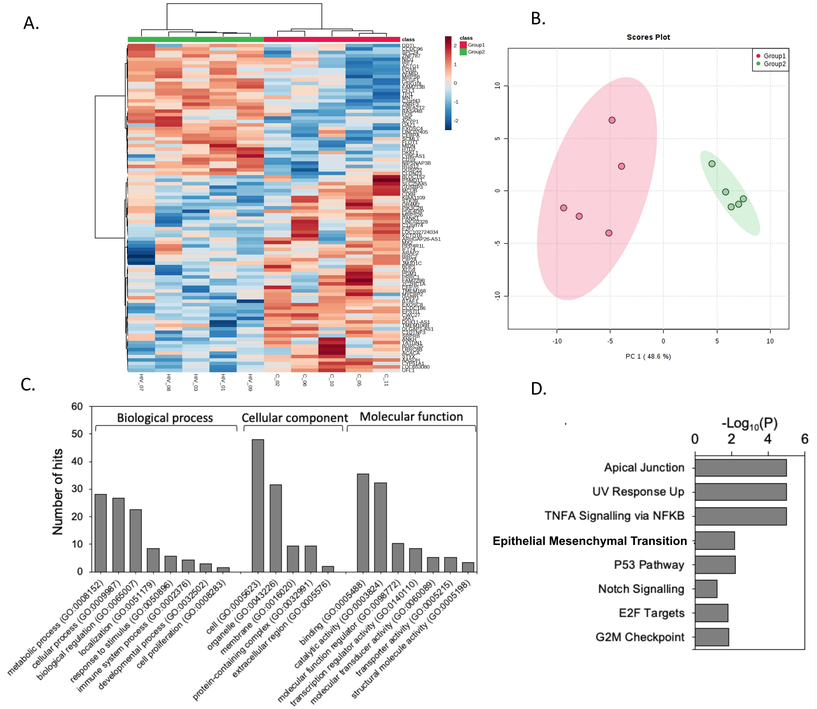

Figure 2: Alveolar macrophages from people living with HIV demonstrate significant differences in chromatin accessibility by ATAC-seq. (A) Overall, altered chromatin accessibility was found in 98 genes by ATAC-seq. A heat map demonstrates clear separation between subjects with HIV and control subjects. (B) A PCA plot demonstrates separation in chromatin accessibility with largest separation along principal component 1 (48.6%) and shows distinct clustering of samples from subjects with and without HIV. (C) Functional annotation revealed changes in HIV chromatin accessibility in genes controlling 20 key cellular processes. (D) Pathway analysis also reveals changes in specific cellular pathways, including NF-κB signaling (FDR < 0.25, p < 0.05).

Results

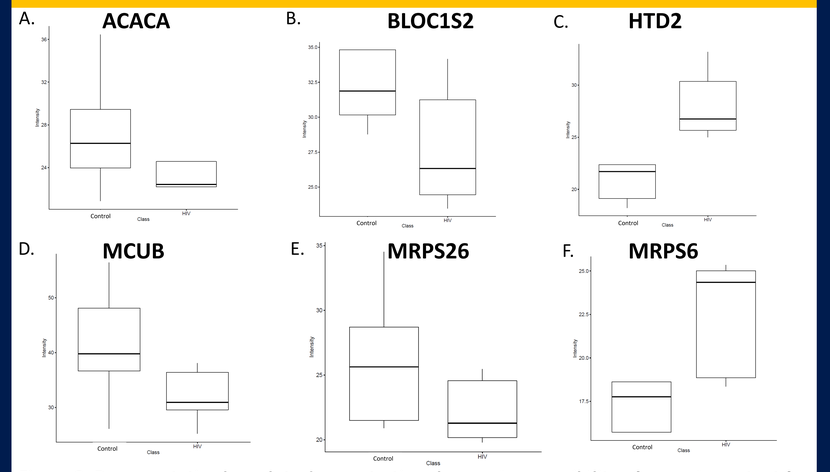

Figure 3: Representative box plots demonstrating changes in accessibility of genes important for mitochondrial homeostasis, including (A) Acetyl Co-A carboxylase alpha (ACACA), (B) Biogenesis of lysosome-related organelles complex 1 subunit 2 (BLOC1S2), (C) hydroxyacyl-thioester dehydratase type 2 (HTD2), (D) mitochondrial calcium uniporter subunit B (MCUB), (E) mitochondrial ribosomal protein S26 (MRPS26), (F) mitochondrial ribosomal protein S6 (MRPS6).

Summary and Conclusions

- Alveolar macrophages from people with well-controlled HIV demonstrated significant impairments in mitochondrial bioenergetics
- ATAC-seq analysis identified alterations in chromatin accessibility for multiple genes associated with mitochondrial function
- Mitochondrial derangements in alveolar macrophage function, which would reduce the ability of these cells to respond to infectious and non-infectious insults, may contribute to the elevated risk of pulmonary disease among people with HIV
- ATAC-seq identified 20 biological processes, cellular components, and molecular functions with differentially accessible genes in macrophages from people with and without HIV
- Further work is necessary to determine which mitochondrial genes and pathways are most responsible for the observed functional abnormalities and whether these impairments can be restored

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