Transcriptomic HIV Reservoir Profiling Reveals a Role for Mitochondrial Functionality in HIV Latency

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Background and aim of the study

A proper understanding of the viral reservoir is crucial for HIV monitoring and evaluation of the efficacy of cure strategies. The heterogeneous composition and variable phenotype of the reservoir underline its complexity and emphasize the need for further characterization. Here, we aimed to:

- Develop a novel assay for identification and isolation of viral reservoir cells from PWH without *ex vivo* reactivation
- Gain insight into the unaltered transcriptome of the transcriptionally active and latent HIV reservoir and identify underlying mechanisms of latency

Methods

- Flow cytometry-fluorescent *in situ* hybridization (flow-FISH) was used to detect transcriptionally latent or active HIV+ cells.
- TAR/Gag flow-FISH was used to sort HIV+ (transcriptionally latent and active), and double probe-negative (DN) CD4 T cells from PBMCs of ART-naïve PWH (N=5). Isolated cells were processed for 3' RNA sequencing (Qiagen UPX 3' Transcriptome kit; NextSeq® 500 Illumina, 100x27bp, 5M reads). Transcriptomic analysis was performed using the mixOmics and EdgeR packages in R. Pathway overrepresentation was performed with Ingenuity Pathway Analysis (IPA).
- HIV transcriptional activity in PBMCs following overnight treatment with antioxidants was determined by RT-qPCR (*pol* gene).

1. Flow-FISH detection of unstimulated HIV+ CD4 T cells of ART-naïve PWH *ex vivo*

The design of flow-FISH probes was based on the presence of abortive TAR and/or elongated Gag transcripts found in the HIV reservoir. **TAR/Gag flow-FISH detected 7-303 HIV+ cells per 10^4 CD4+ T cells in PBMCs of ART-naïve PWH *ex vivo*, and was able to distinguish between transcriptionally latent (TAR+Gag-) and active (TAR+Gag+) HIV+ cells.**

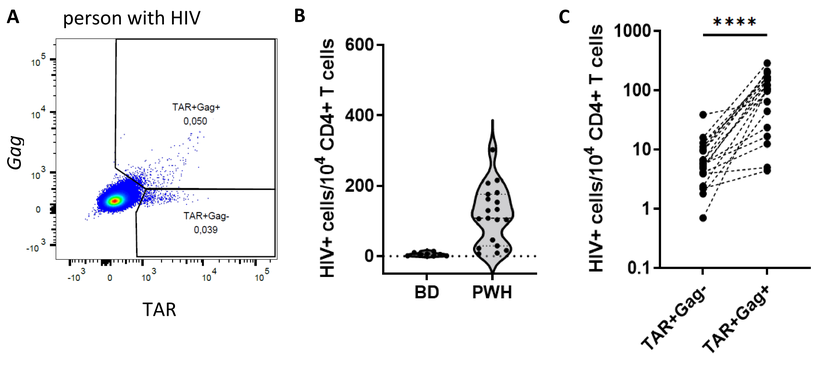


Fig 1. (A) Flow cytometry plot of representative person with HIV, with gating based on background signal in blood donors (BDs). (B) Total number of HIV+ cells per 10^4 CD4 T cells in PBMCs from ART-naïve PWH (N=19), with BDs (N=8) included as control. (C) Total number of detected TAR+Gag- and TAR+Gag+ HIV+ cells per 10^4 CD4 T cells. ****p <0.0001, Wilcoxon matched-pairs signed-rank test.

2. Transcriptional profiling of HIV+ CD4 T cells from ART-naïve PWH

The transcriptomic profiles of the isolated cell populations (i.e. latent, active, DN) were assessed by unsupervised PCA, which showed **major clustering of samples from the same individuals**. This suggests that supervised analysis might be more appropriate for the identification of differences between fractions. Using partial least squares discriminant analysis (PLS-DA), **we were able to circumvent the transcriptomic similarity of samples within individuals and thus separate the populations and obtain transcriptomic profiles.**

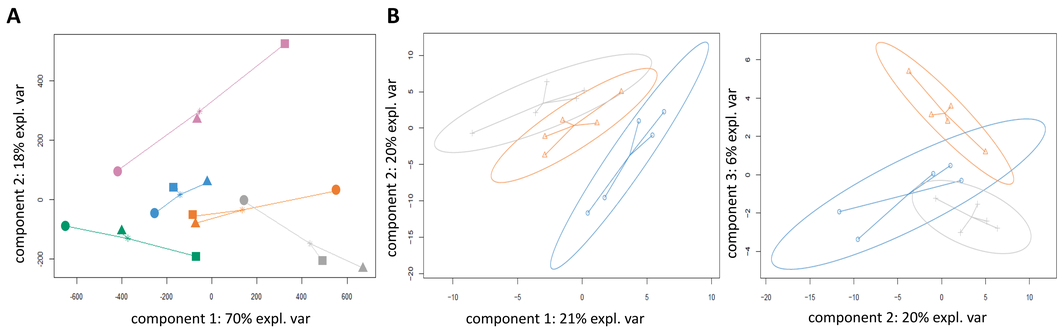


Fig 2. (A) PCA plot, with populations indicated by symbols, individuals by color, and samples from the same individuals connected by lines. (B) PLS-DA plots with separation of latent (blue), active (orange), and DN populations (grey).

3. Differentially expressed genes between transcriptionally latent and active HIV+ cells

Paired group comparisons were performed to identify differentially expressed genes (DEGs; logFC ≥0.5 and p <0.1) in HIV+ compared to HIV- cells, and in transcriptionally latent compared to transcriptionally active HIV+ cells. **The most significant DEGs were found to be mitochondrial genes. Top enriched canonical pathways were found to be related to mitochondrial functions and machinery as determined by IPA.**

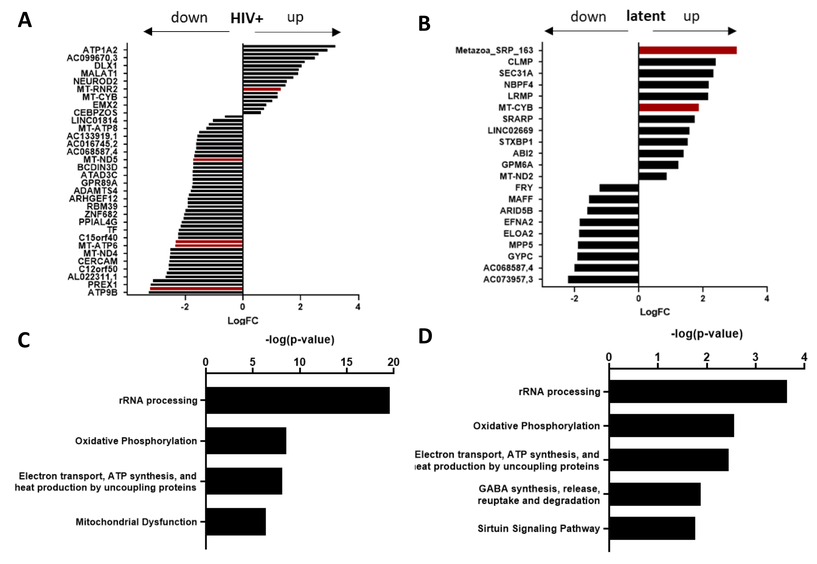


Fig 3. Barplots showing up- and downregulated DEGs for HIV+ versus HIV- (A), and latent versus active population (B), with red bars denoting DEGs with FDR <0.05. Horizonal barplots showing the top canonical pathways for DEGs found in HIV+ versus HIV- (C), and latent versus active population (D).

4. Mitochondrial activation of PBMCs from PWH increases HIV transcriptional activity

Based on the identified DEGs, we assessed whether induction of mitochondrial metabolism affects latency. After overnight mitochondrial stimulation of PBMCs from PWH (N=17), **we observed an induction of HIV transcription in 53% and 30% of the individuals in response to both or one stimulus, respectively.**

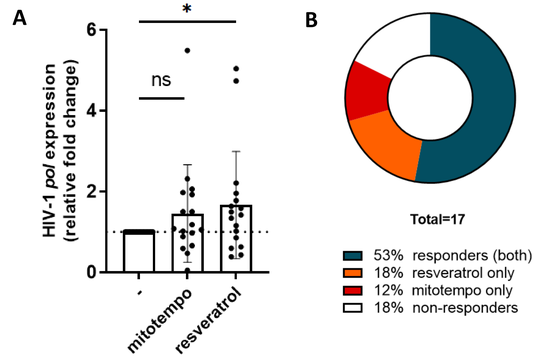


Fig 4. (A) Gene expression of HIV *pol* in PBMCs of PWH after stimulation with MitoTEMPO (10 µM) or trans-resveratrol (10 µM), relative to untreated. *p <0.05, Wilcoxon matched-pairs signed-rank test. (B) Pie chart depicting the fraction of individuals with increased HIV transcriptional activity upon treatment (single and double responders) or without induced activity (non-responders).

Conclusion

- **TAR/Gag flow-FISH detected and differentiated between cells with transcriptionally latent or active HIV in PBMCs from ART-naïve PWH without *ex vivo* stimulation.**
- **Transcriptomic profiling showed an association between mitochondrial functioning and the transcriptional activity of the viral reservoir.**
- **HIV transcriptional activity increased with antioxidant stimulation, confirming a role for mitochondrial processes in HIV+ CD4 T cells and in reactivating viral latency.**

Our findings underline the relevance of cellular metabolism in HIV infection, and warrant the therapeutic targeting or reversal of latency through metabolic modulation.

Acknowledgements

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