Biomarkers of HIV Latency in Tissues from People with HIV

0517

Xinlian Zhang[1], Andrew Qazi [2], Roni Lobato Ventura [2], Antoine Chaillon[1], Davey Smith[1], Sara Gianella[1], **Nadejda Beliakova-Bethell**[1,2,3]
[1]University of California San Diego, La Jolla, CA, USA, [2]Veterans Medical Research Foundation, San Diego, CA, USA, [3]VA San Diego Healthcare System, San Diego, CA, USA

BACKGROUND

- Identifying biomarkers of the persistent HIV reservoir is essential for its elimination.
- In this study, we defined latency as transcriptionally active provirus in the absence of detectable viremia.
- Transcriptional profiles of latent HIV+ cells varied depending on the environment, though certain genes were shared across conditions (PMID 38156317).

HYPOTHESIS

In tissues from people with HIV (PWH), both shared and tissue-specific biomarkers exist.

METHODS

- We collected fresh lymph nodes, spleen, and liver via rapid research autopsy (<6h postmortem) from 4 PWH (3 men and 1 woman) enrolled in the Last Gift cohort, all on ART with undetectable HIV RNA at death.
- CD4+ T cells were isolated by negative selection from freshly dissociated tissues and subjected to single-cell RNA-seq (10X Genomics, Inc.)
- Reads were mapped to the combined human and autologous HIV genomic reference using CellRanger (v7.1.0), and data were analyzed using Seurat v5 and scType in Bioconductor R (**Figure 1**).
- Biomarkers were defined as genes expressed differentially between cells with detectable HIV RNA and cells with undetectable HIV RNA (Bonferroni p<0.05).

SIMILAR CD4+ T CELL PHENOTYPES ACROSS TISSUES AND THE DETECTION OF HIV+ CELLS

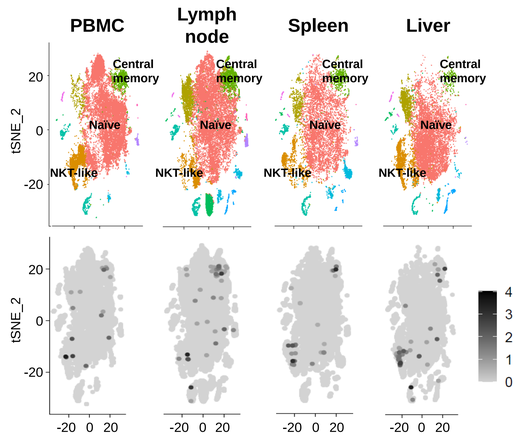


Figure 1. Cell clustering by tissue (top) and detection of cells expressing low levels of HIV RNA (bottom). *NKT-like*, natural killer T cell-like; HIV expression is $\log_2$ transformed.

Shared biomarkers were identified in latently infected cells from PBMC, lymph nodes, spleen, and liver; but tissue microenvironments affect biomarker expression.

BIOMARKERS SHARED ACROSS TISSUES

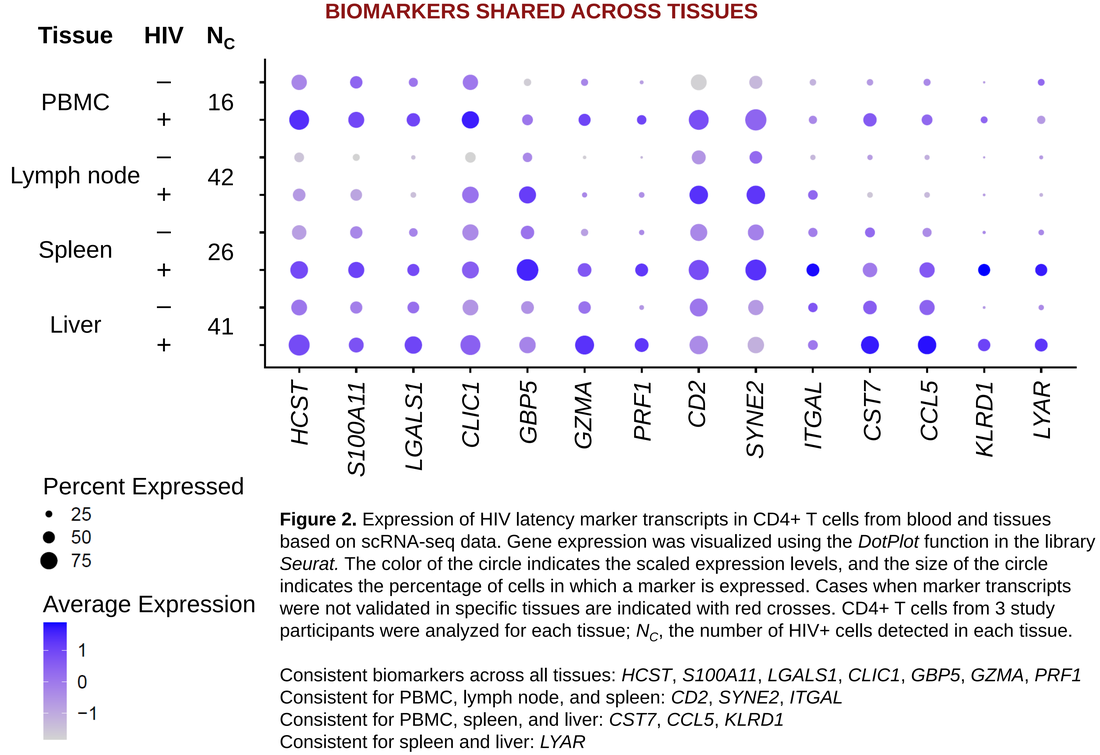


Figure 2. Expression of HIV latency marker transcripts in CD4+ T cells from blood and tissues based on scRNA-seq data. Gene expression was visualized using the *DotPlot* function in the library *Seurat.* The color of the circle indicates the scaled expression levels, and the size of the circle indicates the percentage of cells in which a marker is expressed. Cases when marker transcripts were not validated in specific tissues are indicated with red crosses. CD4+ T cells from 3 study participants were analyzed for each tissue; N_C, the number of HIV+ cells detected in each tissue.

Consistent biomarkers across all tissues: *HCST*, *S100A11*, *LGALS1*, *CLIC1*, *GBP5*, *GZMA*, *PRF1*
Consistent for PBMC, lymph node, and spleen: *CD2*, *SYNE2*, *ITGAL*
Consistent for PBMC, spleen, and liver: *CST7*, *CCL5*, *KLRD1*
Consistent for spleen and liver: *LYAR*

RESULTS

- A total of 59,152 cells were analyzed, of which 125 cells had detectable HIV RNA. **Table 1** shows cell numbers by tissue and by participant.

Table 1. Total cells sequenced and analyzed by tissue and study participant.

Tissue	Participant	Total cells sequenced	Total cells analyzed	# of cells with HIV RNA
PBMC	P1	2195	1670	10
	P2	9287	8884	3
	P3	2195	6756	3
Lymph node	P1	6568	5925	36
	P2	9275	8133	3
	P4	6326	5182	3
Spleen	P1	5006	4363	20
	P2	6079	5349	1
	P3	514	427	5
Liver	P1	6673	5250	37
	P2	4182	3374	1
	P3	5156	3839	3

- The numbers of differentially expressed genes (Bonferroni p<0.05) by tissue were:
  PBMC – 180; lymph node – 281; spleen – 141; liver – 128.
- Most of the significant genes were tissue-specific; however, shared marker transcripts were identified (**Figure 2**), including those found previously *in vitro* and new ones (*PRF1*, *GBP5*).
- Multiple genes functioning in cell-cell adhesion (*LGALS1*, *S100A11*, *CD2*, and *ITGAL*) and cytotoxicity (*HCST*, *GZMA*, *KLRD1*, and *PRF1*) were present among the shared marker transcripts.

CONCLUSIONS

- These studies validated biomarkers previously identified in HIV+ CD4+ T cells *in vitro* and found new biomarkers in CD4+ T cells from people with HIV.
- Shared biomarkers across tissues were identified.
- Tissue-dependent variations underscore the complexity of HIV latency.
- Tissue-specific biomarkers must be considered when designing strategies to target and eliminate the HIV reservoir.

ACKNOWLEDGEMENTS

We would like to thank he study participants who donated their bodies after death to research. The participants were from the Last Gift Cohort, which is supported by P01 AI169609 from NIAID. The biomarker identification studies were supported by the VA Merit Review Award I01 BX005285.