T-TRACE ENABLES SINGLE-CELL MULTI-OMICS ANALYSIS OF HIV RESERVOIR CELLS FROM TISSUES

421

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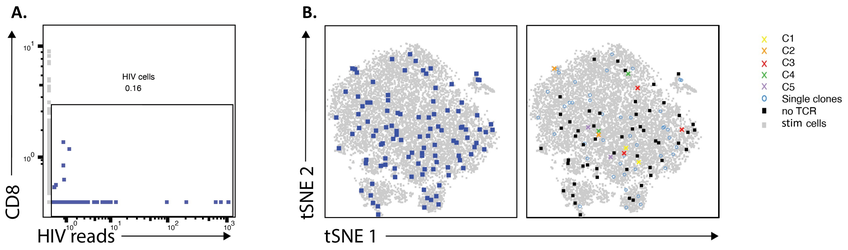
BACKGROUND

Single-cell RNA sequencing of HIV-infected cells from people with HIV (PWH) has for the most part been restricted to analyses of viremic individuals and of *ex vivo* stimulated reservoir cells from ART-suppressed individuals, and to the blood compartment. We describe application and optimization of T-TRACE as a multiomics approach to simultaneously characterize the transcriptome (scRNAseq), surface proteome (CITE-seq), and clonal expansion (VDJ-seq) of unstimulated HIV reservoir cells, from both blood and tissue cells of ART-suppressed PWH.

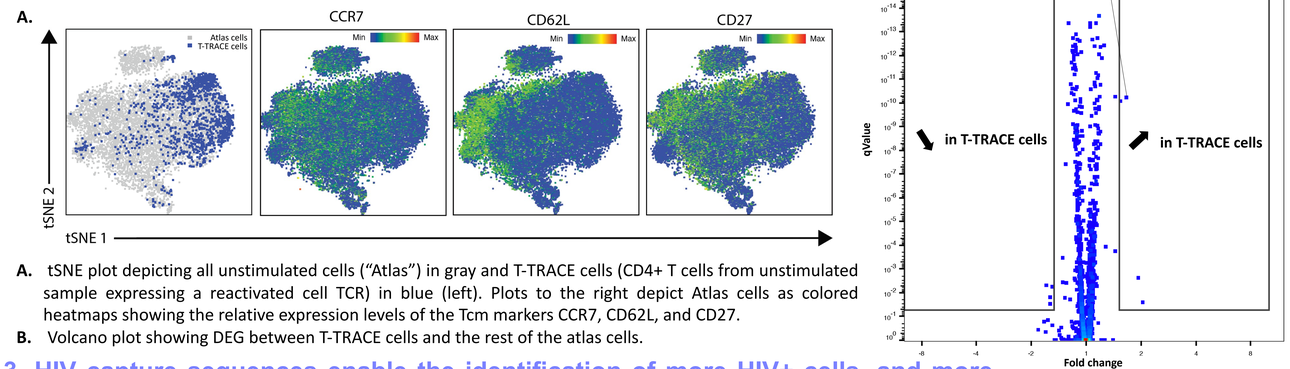
RESULTS

1. Stimulation of CD4+ T cells from ART-suppressed PWH identifies 30 different clonotypes of reservoir cells



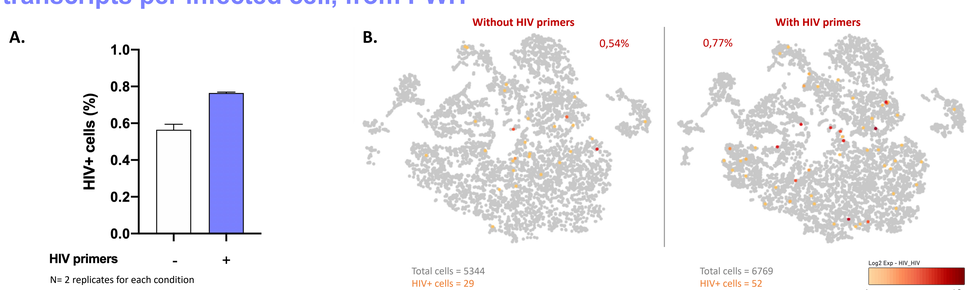
A. Gating on HIV+ cells identified from a stimulated sample from ART-suppressed donor.
B. tSNE plots depicting all stimulated cells in gray and HIV+ cells in blue (left) or colored by clonotype (right).

2. T-TRACE cells exhibit a Tem phenotype and a cytolytic signature



A. tSNE plot depicting all unstimulated cells ("Atlas") in gray and T-TRACE cells (CD4+ T cells from unstimulated sample expressing a reactivated cell TCR) in blue (left). Plots to the right depict Atlas cells as colored heatmaps showing the relative expression levels of the Tcm markers CCR7, CD62L, and CD27.
B. Volcano plot showing DEG between T-TRACE cells and the rest of the atlas cells.

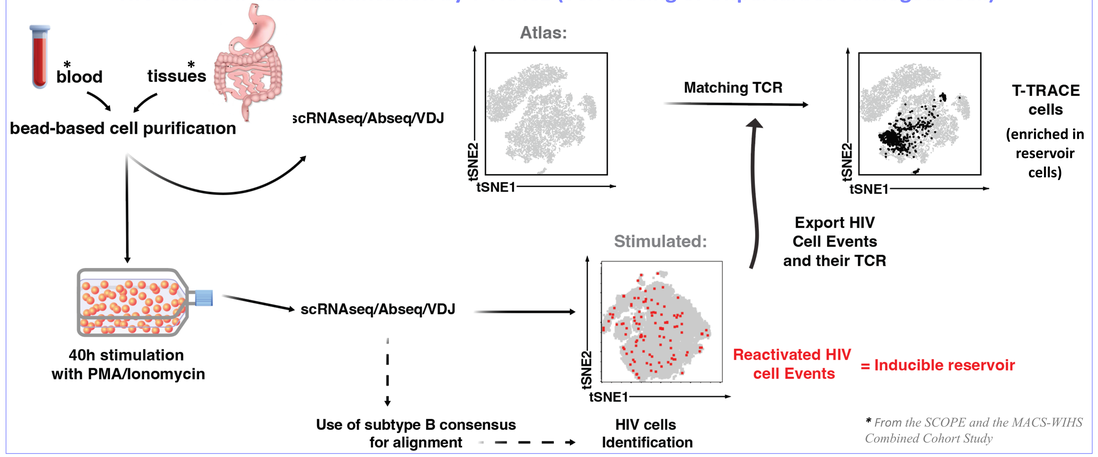
3. HIV capture sequences enable the identification of more HIV+ cells, and more transcripts per infected cell, from PWH



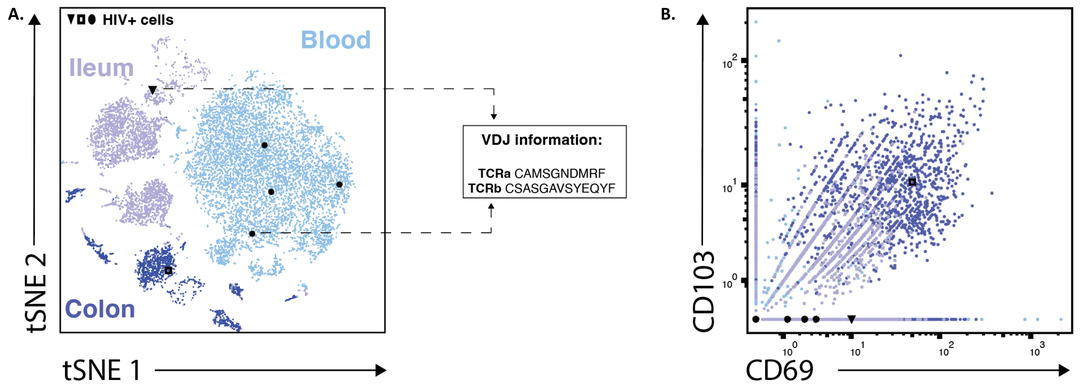
A. Bar graph showing the frequency of detectable HIV RNA+ cells from PWH, with or without the addition of HIV capture sequences.
B. tSNE of CD4+ T cells from viremic samples with or without the addition of HIV capture sequences. HIV+ cells are colored in heatmap fashion from orange to red depending the the number of HIV reads detected.

METHODS

HIV reservoir cells identification by T-TRACE (TCR Tracing of Unperturbed Analogous Cell)



4. Transcriptionally active reservoir cells between gut and blood share the same VDJ sequence but exhibit distinct phenotypes



A. tSNE showing CD3+/CD8- blood cells, colonic cells, and ileum cells from an ART-suppressed PWH. HIV RNA+ cells are shown in black. The sequence of a shared TCR detected on HIV RNA+ cells from ileum and blood is indicated.
B. 2D plot showing levels of expression of T resident memory markers CD69 and CD103.

CONCLUSIONS

Implementation of T-TRACE with HIV capture sequences allows efficient in-depth transcriptomic and surface phenotypic analysis of transcriptionally-active reservoir cells, along with examination of the clonal expansion histories of these cells. Application of the approach on specimens from ART-suppressed PWH revealed that clones of HIV reservoir cells were shared between gut and blood, and these clones exhibit distinct phenotypes.

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