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Single cell analysis of quiescent HIV infection reveals host transcriptional profiles that regulate latency

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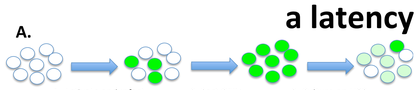
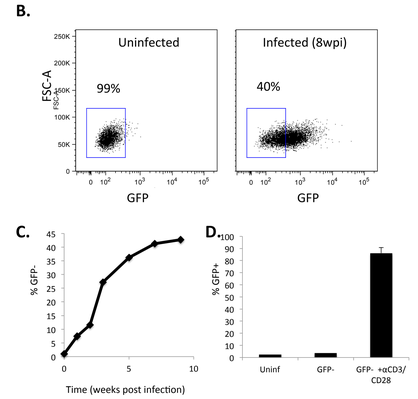
Abstract

The latent HIV reservoir is diverse, but most studies of HIV latency have used bulk cell assays. Here we characterized a primary cell model of HIV latency with single cell qPCR (sc-qPCR) for viral RNA (vRNA), and single cell RNAseq (scRNAseq). sc-qPCR revealed distinct populations of cells transcribing vRNA across a wide range of levels. Strikingly, scRNAseq of latently infected primary cells revealed a relationship between vRNA levels and the transcriptomic profiles within the population. Cells with the greatest level of HIV silencing expressed a specific set of host genes including markers of central memory T cells. By contrast, latently infected cells with higher levels of HIV transcription expressed markers of activated and effector T cells. These data reveal that heterogeneous behaviors of HIV proviruses within the latent reservoir are influenced by the host cell transcriptional program. Therapeutic modulation of these programs may reverse or enforce HIV latency.

Primary cell model of latency

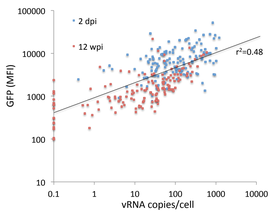
Heterogeneous silencing of HIV gene expression in a latency model





(A). Graphical depiction of primary cell model of latency. Cells were infected with pNL4-3-Δ6-dreGFP, an eGFP expressing HIV clone, and infected (GFP+) cells isolated by flow sorting. After 8-12 weeks of co-culturing the sorted GFP+ population with H80 cells, cells displayed heterogeneous levels of virally encoded GFP expression, with 40% now being GFP-. **(B)**. The percent GFP- cells over time was determined by flow cytometry. Data shown is a representative sample from one of three separate donors. **(C)**. Latently infected cells were isolated by flow sorting of GFP- cells from the infected cell culture. These cells were then stimulated with anti-CD3/CD28 beads for 3 days. GFP expression was measured by flow cytometry before (GFP-) and after stimulation (GFP- + αCD3/CD28), and compared to uninfected cells (uninf).

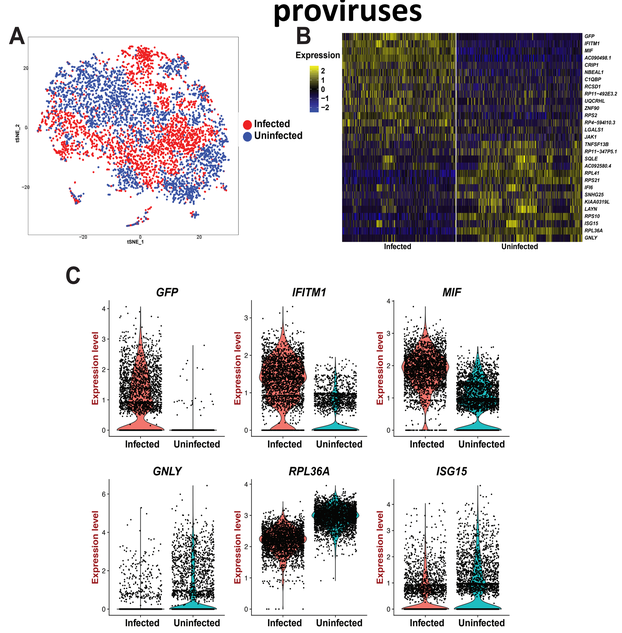
Single cell analysis shows heterogeneous viral RNA expression in a latency model



Single infected primary CD4 T cells at 2 dpi or 12 wpi were flow sorted and analyzed for vRNA expression by sc-qPCR. GFP fluorescent intensity (MFI) for each cell was plotted against vRNA copies per cell. Each dot represents data from an individual cell. Linear regression was used to generate a trendline and r2 value for correlation between vRNA and GFP fluorescence.

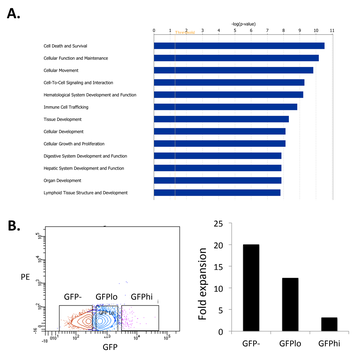
Single RNAseq analysis of latency

Limited reprogramming of infected cells by latent proviruses



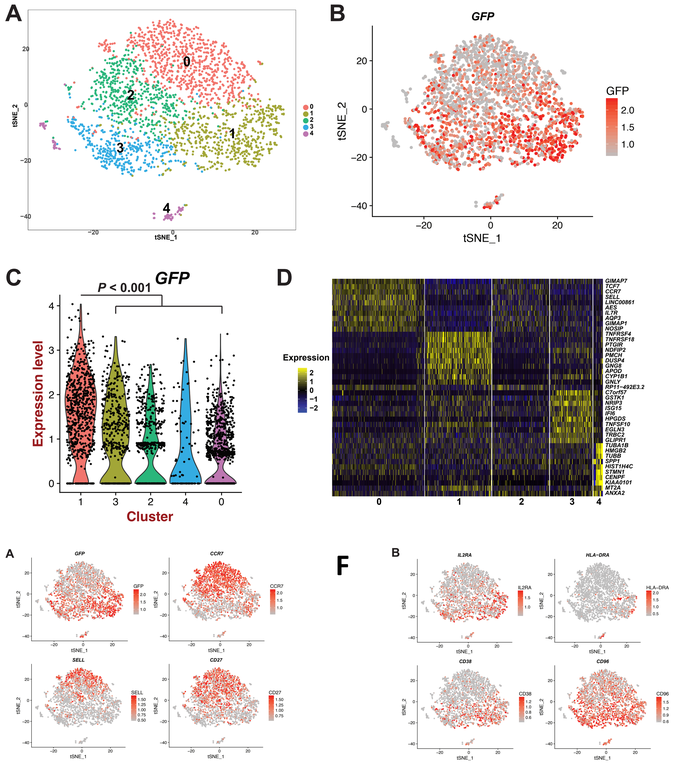
(A). Two-dimensional plot from unsupervised clustering by t-distributed stochastic neighbor embedding (tSNE) of the single-cell transcriptomes from 2,676 infected (red) and 3,097 uninfected (blue) primary CD4 T cells. **(B).** Heatmap of significantly changed transcripts between infected and uninfected cells (likelihood ratio test; $P \leq 0.05$). Each pixel column is the expression of an individual cell. Transcripts are on the rows with the normalized expression (Z-scores) colored by the legend (yellow, more upregulated; blue, more downregulated). **(C).** Violin plots detailing the expression levels of the top 3 upregulated and downregulated transcripts within infected cells.

HIV is preferentially silenced in cells with greater proliferative



(A). To identify cellular pathways/functions that were enriched in genes associated with HIV silencing, Ingenuity Pathway analysis (IPA) was used to analyze the set of genes that are differentially expressed between cells with active viral gene expression (GFP+) and those with silenced viral gene expression (GFP-), The top 13 most prominently affected cellular functions are shown. **(B.)** An infected culture of primary CD4 T cells at 12 weeks post infection was sorted into three populations based on the level of GFP expression. – GFP-, GFPlow and GFPhigh. Sorted cells were then stimulated with αCD3/αCD28 beads for three days and then expanded in 100 U/mL IL-2 for two weeks. At the end of this period the fold expansion for each culture was calculated. Data shown are representative of two independent experiments.

A host cell transcriptional signature correlates with latency



(A). Graph-based clustering of 2,676 infected cells represented on the tSNE plot. **(B).** Normalized transcript expression level of GFP on the tSNE plot representation. Legends indicate normalized transcript values. **(C).** Violin plots of GFP expression within the identified clusters. **(D)**. Heatmap of the top 10 transcripts that define each cluster. Panels represent individual clusters and each pixel column is the expression of individual cells. Transcripts are on the rows with normalized expression (Z-scores) colored by the legend (yellow, more upregulated; blue, more downregulated). Normalized expression pattern for selected genes whose expression negatively **(E)**, or positively **(F)**, correlated with viral gene expression (GFP) plotted on the tSNE plot representation. Each dot represents and individual cell. Legends indicate normalized transcript values.

Summary

- In a primary cell model of HIV latency, cells exhibit variegated levels of silencing after extended periods of time in culture.
- scRNAseq shows HIV silencing in latently infected primary cells is correlated with a host cell transcriptional signature.
- The latency associated signature is expressed in Tn and Tcm cells.
- HIV is preferentially silenced in cells with greater proliferative potential.
- These data indicate a connection between HIV silencing and the transcriptional program of the host cells.

Acknowledgements

Evan Trudeau (UNC Flow sorting core), UNC CFAR, Darrell Bigner (Duke), Robert Siliciano (JHU), David Margolis (UNC)
CARE Collaboratory 1UM1AI126619