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Expression Profiling of HIV Latently-Infected Cells via NanoString and Mass Cytometry

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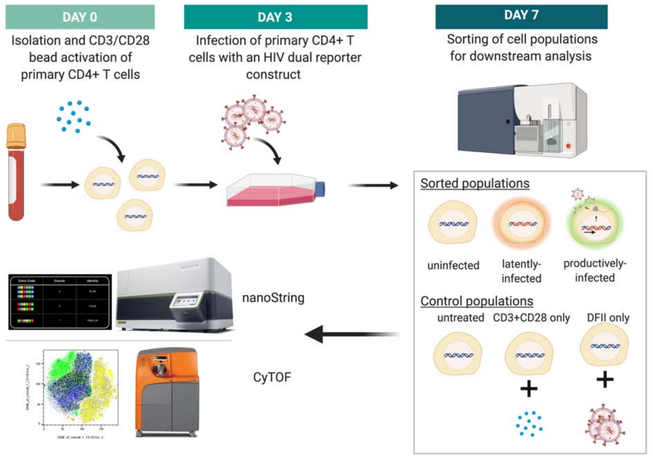
BACKGROUND

The main barrier to an HIV cure is the latent HIV reservoir. Long-lived HIV latently-infected cells remain invisible to the host immune system and persist during antiretroviral therapy. In this study, we characterized latently-infected cells by implementing combined transcriptomic and proteomic profiling to identify unique expression signatures and reliable biomarkers that can be exploited to target and eliminate the latent reservoir.

METHODS



Primary CD4+ T cells were purified from six healthy donors, activated using CD3/CD28 beads for 72h, and were infected afterwards with a dual-reporter HIV construct for 96h that enables the isolation of HIV latently-infected and productively-infected cells by flow cytometry. The populations were characterized using NanoString hybridization and fluorescence-based digital counting technology allowing for simultaneous detection of 770 mRNA and 30 surface protein targets, and mass cytometry (CyTOF), measuring 40 proteins. Target expression levels were compared between populations using false discovery rate (FDR<0.1), cellular pathways were analyzed using global significance scores, and upstream regulatory networks and causal relationships were deciphered using Ingenuity Pathway Analysis (IPA).

RESULTS

TCR stimulation and donor effects dominate expression signatures

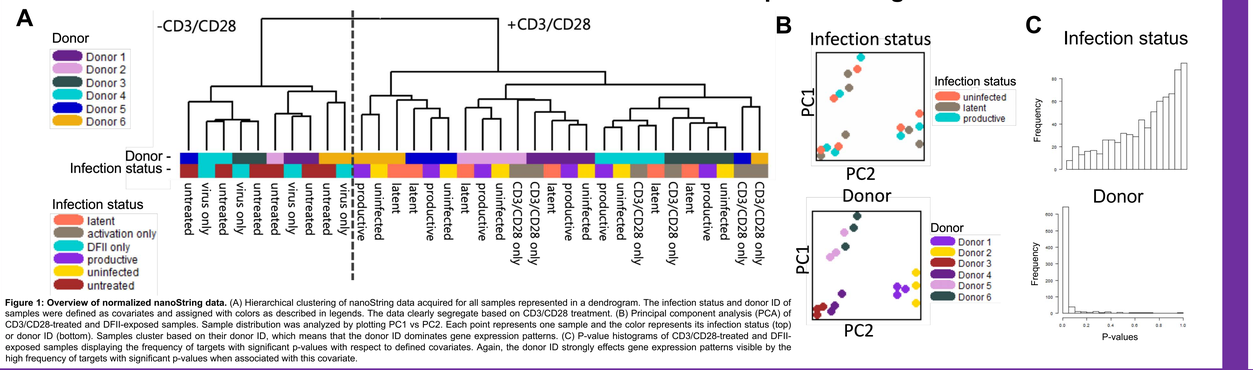
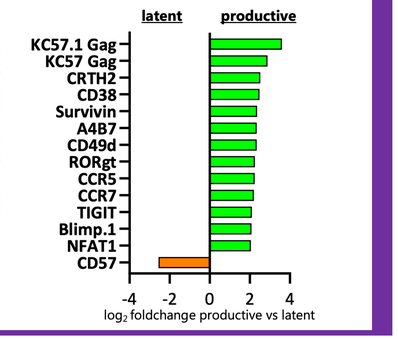


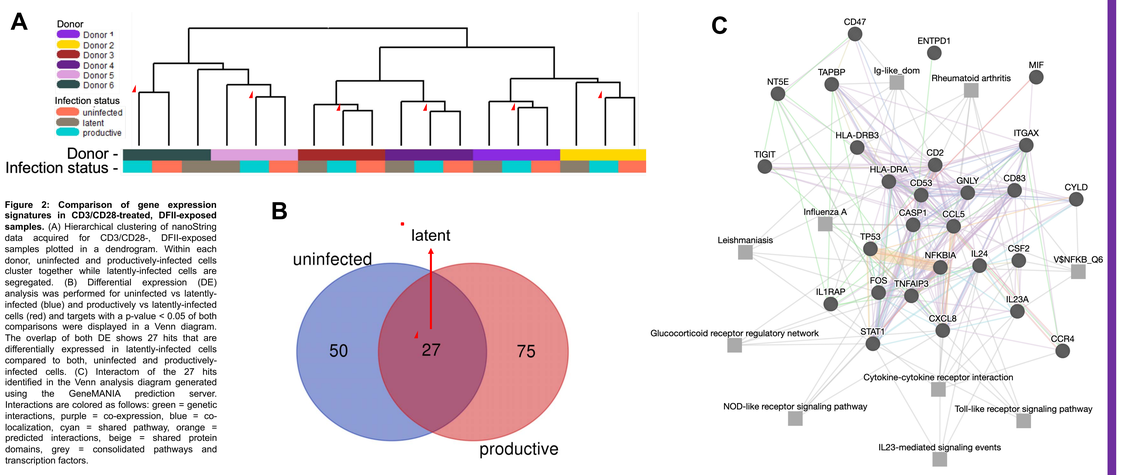
Figure 1: Overview of normalized nanoString data. (A) Hierarchical clustering of nanoString data acquired for all samples represented in a dendrogram. The infection status and donor ID of samples were defined as covariates and assigned with colors as described in legends. The data clearly segregate based on CD3/CD28 treatment. (B) Principal component analysis (PCA) of CD3/CD28-treated and DFII-exposed samples. Sample distribution was analyzed by plotting PC1 vs PC2. Each point represents one sample and the color represents its infection status (top) or donor ID (bottom). Samples cluster based on their donor ID, which means that the donor ID dominates gene expression patterns. (C) P-value histograms of CD3/CD28-treated and DFII-exposed samples displaying the frequency of targets with significant p-values with respect to defined covariates. Again, the donor ID strongly effects gene expression patterns visible by the high frequency of targets with significant p-values when associated with this covariate.

CyTOF reveals factors that are differentially expressed in HIV latently-infected cells

Figure 4: Differential expression of proteins in latently-infected vs productively-infected cells. The expression of 40 proteins was measured in latently-infected and productively-infected cells using a T cell focused CyTOF panel. Expression levels were then compared between productively- and latently-infected cells using a $\log_2$ fold change cutoff at 2. As expected, the two HIV-Gag proteins were the strongest hits in productively-infected cells. Interestingly, CD57, the Beta-1,3-Glucuronyltransferase 1, was strongly overexpressed in latently-infected cells when compared to productively-infected cells.



HIV latently-infected cells are highly distinct from uninfected and productively-infected cells



IL8, CD39, and CD73 are differentially expressed in HIV latently-infected cells

Figure 2: Comparison of gene expression signatures in CD3/CD28-treated, DFII-exposed samples. (A) Hierarchical clustering of nanoString data acquired for CD3/CD28-, DFII-exposed samples plotted in a dendrogram. Within each donor, uninfected and productively-infected cells cluster together while latently-infected cells are segregated. (B) Differential expression (DE) analysis was performed for uninfected vs latently-infected (blue) and productively vs latently-infected cells (red) and targets with a p-value < 0.05 of both comparisons were displayed in a Venn diagram. The overlap of both DE shows 27 hits that are differentially expressed in latently-infected cells compared to both, uninfected and productively-infected cells. (C) Interactom of the 27 hits identified in the Venn analysis diagram generated using the GeneMANIA prediction server. Interactions are colored as follows: green = genetic interactions, purple = co-expression, blue = co-localization, cyan = shared pathway, orange = predicted interactions, beige = shared protein domains, grey = consolidated pathways and transcription factors.

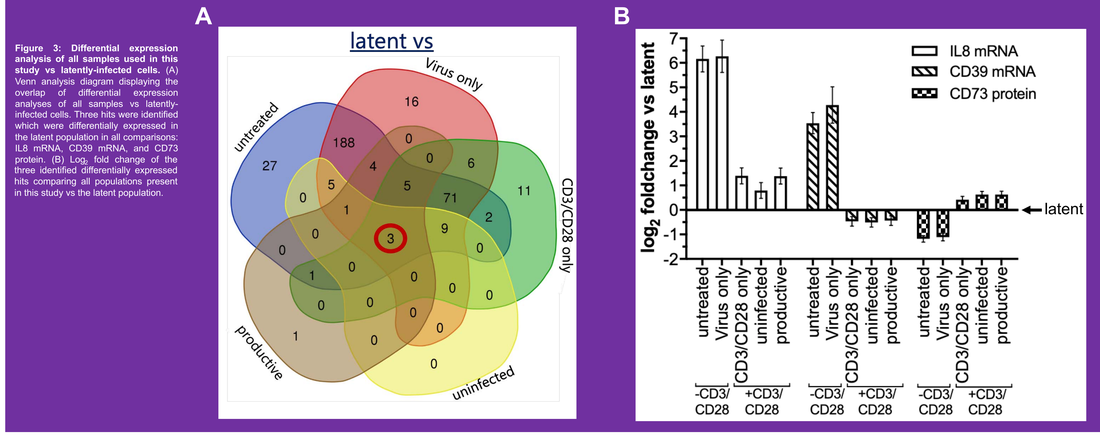


Figure 3: Differential expression analysis of all samples used in this study vs latently-infected cells. (A) Venn analysis diagram displaying the overlap of differential expression analyses of all samples vs latently-infected cells. Three hits were identified which were differentially expressed in the latent population in all comparisons: IL8 mRNA, CD39 mRNA, and CD73 protein. (B) Log_2 fold change of the three identified differentially expressed hits comparing all populations present in this study vs the latent population.

CONCLUSIONS

- Inter-donor differences dominate expression patterns, which complicates the discovery of signatures of HIV latently-infected cells shared across donors.
- Expression levels of IL8 mRNA, CD39 mRNA, and CD73 protein are signatures of latent infection.
- Hypoxia and adenosine signaling may be involved in HIV persistence.
- NanoString combined with CyTOF is a powerful approach to decipher HIV latency.

NEXT STEPS

- Validate signatures of HIV latency using samples from ART-suppressed HIV-infected individuals
- Perform CRISPR/Cas9-mediated knockouts of signature factors to determine effects on HIV latency
- Investigate the relationship between hypoxia, adenosine signaling, and HIV persistence

ADDITIONAL INFORMATION

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