A Cohort-Based Study of Host Gene Expression: Tumor Suppressor and Innate Immune/Inflammatory Pathways associated with the HIV Reservoir Size

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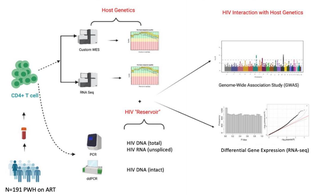
BACKGROUND

- Although lifelong HIV antiretroviral therapy (ART) suppresses the virus, the major barrier to an HIV cure is the persistence of infected cells, "the HIV reservoir." [1-2]
- Several host factors have been shown to influence the size of the HIV reservoir, such as the timing of ART initiation since HIV infection, pre-ART viral load, ethnicity, and sex.[3-4]
- There have been few cohort-based studies evaluating host genomic or transcriptomic predictors of the HIV reservoir.[5-8]
- Here, we performed a large cross-sectional study of 191 people with HIV (PWH) on ART to determine host gene expression associated with measures of the peripheral CD4+ T cell reservoir.

METHODS

- PWH suppressed on ART for at least 24 months were sampled from the University of California San Francisco SCOPE and Options HIV+ cohorts.
- Peripheral CD4$^+$ T cells were enriched from cryopreserved peripheral blood mononuclear cells (PBMCs) via immunomagnetic selection (StemCell)
- DNA and RNA were extracted from CD4$^+$ T cells using the AllPrep Universal kit (Qiagen).

Figure 1. Study design



- Three measures of the HIV reservoir size were quantified:
  - **HIV unspliced (us)RNA** and **HIV total (t)DNA** were quantified using quantitative (q)PCR using primer probe sequences near the Gag-LTR junction, a highly conserved region among all group M HIV-1 sequences.[9]
  - **HIV intact DNA** was quantified from the remaining extracted DNA, using a multiplexed digital droplet PCR (ddPCR) assay targeting five regions of the HIV-1 genome.[10]
- Host bulk RNA sequencing was performed from CD4+ T cells using the NEBNext Ultra II RNA Library Prep Kit (New England Biolabs) and sequenced on a NovaSeq6000 instrument.
- Plasma protein validation was performed from a subset of N=175 participants for whom cryopreserved plasma samples were available, using the electrochemiluminescence-based multi-plex Meso Scale Discovery (MSD) platform.
- Intracellular protein validation was performed from a subset of N=40 participants in whom additional PBMCs were available, using specific ELISA kits (LSBio and others).

- To our knowledge, this is the largest cohort-based transcriptomic study of **host genetic predictors of the HIV reservoir size**.
- Individuals with higher expression of 2 tumor suppressor genes had smaller total HIV reservoir size (**HIV total DNA**).
- Individuals with more "transcriptionally active" reservoirs **(HIV unspliced RNA)** had decreased expression of several inflammatory genes.

RESULTS

Table 1. Descriptive statistics of study population

Characteristic[a]	Total (N=191)	Early-Treated[b] (N=54)	Later-Treated[b] (N=137)
Male (%)	183 (96%)	54 (100%)	129 (94%)
Age (years)	47 (13)	44 (12)	47 (13)
Nadir CD4+ T cell count (cells/mm^3)	352 (251)	522 (346)	304 (190)
Maximum pre-ART HIV RNA (log$_{10}$copies/mL)	5.1 (0.9)	5.6 (0.7)	5.0 (0.8)
Duration of ART suppression (years)	5.1 (4.2)	6.0 (4.3)	4.7 (4.2)
Timing of ART initiation (years)	2.0 (4.6)	0.20 (0.19)	3.5 (4.3)

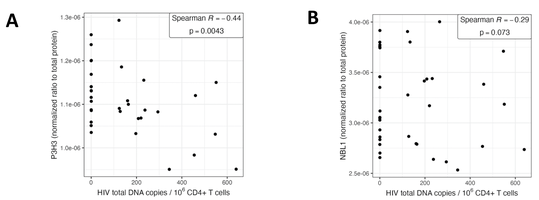
[a] Medians (with standard deviation or %). [b] Early-treated = Individuals who initiated ART within 6 months of HIV infection; later-treated = Individuals who initiated ART after 6 months of HIV infection.

Table 2. ↑Host RNA expression of tumor suppressor genes (*NBL1, P3H3)* was associated with ↓ HIV total DNA

Gene	Gene Name	p[a]	q[b]	FC[c]	%Change[d]	TPM[e]
NBL1	Neuroblastoma suppressor of tumorigenicity 1	6.14E-07	0.012	0.982	-1.8	3.37
P3H3	Prolyl 3-Hydoxylase 3	1.25E-06	0.012	0.984	-1.6	2.10

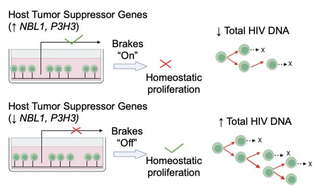
[a] p = two-sided p-value. [b] q = two-sided false discovery rate (FDR) Benjamini-Hochberg q-value. [c] FC = fold-change in host gene expression per two-fold change in copies of HIV from multivariate model adjusted for age, sex, nadir CD4+ T cell count, timing of ART initiation, ancestry (PCs), and residual variability (probabilistic estimation of expression residuals, PEERs). [d] % Change = percent change in host gene expression per two-fold change in copies of HIV. [e] Transcripts per million.

Figure 2. Protein validation from peripheral CD4$^+$ T cells showed ↑ intracellular P3H3 and NBL1 associated with ↓ HIV total DNA



Intracellular protein expression from peripheral CD4+ T cells among a subset of 40 participants in the study.

Figure 3. Proposed model of the inverse association of P3H3 and NBL1 and HIV total DNA



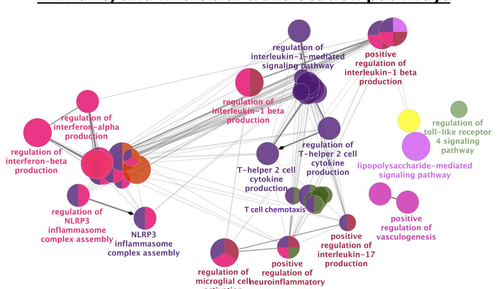
Proposed model for the observed inverse associations between HIV total DNA and tumor suppressor gene (*NBL1, P3H3*) expression (q<0.05). Given the known function of these genes, we hypothesize that these tumor suppressor genes might act as "checks" on cell proliferation, thereby restricting the size of the total HIV DNA reservoir.

Table 3. ↑ HIV unspliced RNA was associated with ↓ inflammatory genes and ↓ 2 membrane channel genes

Gene	Gene Name	p[a]	q[b]	FC[c]	%Change[d]	TPM[e]
KCNJ2	Potassium Inwardly Rectifying Channel Subfamily J Member 2, kir2.1	1.49E-07	0.003	0.903	-9.7	2.06
IL1A	Interleukin-1 alpha	1.55E-06	0.012	0.904	-9.6	5.81
GJB2	Gap Junction Protein Beta 2	2.26E-06	0.012	0.929	-7.1	0.68
KCNJ2-AS1	KCNJ2 antisense RNA 1	2.49E-06	0.012	0.916	-8.4	5.20
AC034199.1	AC034199.1	2.88E-06	0.012	0.946	-5.4	0.28
CSF3	Colony Stimulating Factor 3 (G-CSF)	3.93E-06	0.013	0.925	-7.5	8.04
TNFAIP6	Tumor Necrosis Factor alpha induced protein 6	5.99E-06	0.016	0.924	-7.6	1.14
TLR7	Toll Like Receptor 7	6.59E-06	0.016	0.929	-7.1	0.60
MRAS	Muscle RAS oncogene homolog	1.07E-05	0.024	0.945	-5.5	0.17
TNFAIP9/ STEAP4	Tumor Necrosis Factor alpha induced protein 9	1.53E-05	0.031	0.931	-6.9	0.83
MIR3945HG	MIR3945 Host Gene	2.09E-05	0.038	0.942	-5.8	0.36
DAPK1-IT1	DAPK1 Intronic Transcript 1	2.72E-05	0.043	0.950	-5.0	1.11
OR2B11	Olfactory Receptor Family 2 Subfamily B Member 11	2.96E-05	0.043	0.939	-6.1	1.04
CXCL3	C-X-C Motif Chemokine Ligand 3	3.03E-05	0.043	0.928	-7.2	31.06
TNFAIP5 /PTX3 /TSFG14	TNF Alpha-Induced Protein 5 (TNFAIP5), Pentraxin-related protein (PTX3), Tumor Necrosis Factor-Inducible Protein TSG-14 (TSG14)	3.27E-05	0.043	0.941	-5.9	15.37
RRN3P4	RRN3 Pseudogene 4	3.97E-05	0.049	0.950	-5.0	0.83
CXCL10	C-X-C Motif Chemokine Ligand 10	4.21E-05	0.049	0.908	-9.2	5.79

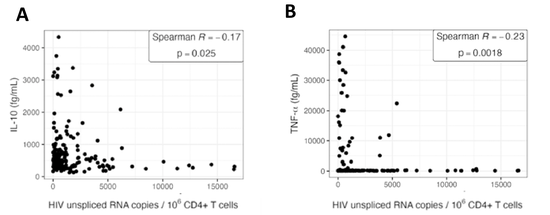
[a] p = two-sided p-value. [b] q = two-sided false discovery rate (FDR) Benjamini-Hochberg q-value. [c] FC = fold-change in host gene expression per two-fold change in copies of HIV from multivariate model adjusted for age, sex, nadir CD4+ T cell count, timing of ART initiation, ancestry (PCs), and residual variability (probabilistic estimation of expression residuals, PEERs). [d] % Change = percent change in host gene expression per two-fold change in copies of HIV. [e] Transcripts per million. Novel associations with HIV usRNA were observed with two membrane channel protein genes: *KCNJ2*, which encodes for inwardly rectifying potassium channel Kir2.1, previously associated with HIV-1 entry and release, and *GJB2*, which encodes for gap junction beta 2 protein (connexin 26), which plays a role in cell-cell communication.

Figure 4. Gene Set Enrichment Analysis (GSEA) showed that ↑ HIV unspliced RNA was associated with inflammatory, innate immune, and microbial translocation pathways



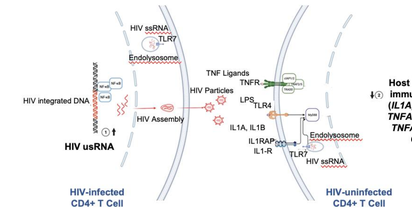
Network analysis of the top differentially expressed genes associated with HIV unspliced RNA. The top significant genes mapped to immunologic pathways involving bacterial translocation (e.g., TLR4 signaling, activated by bacterial lipopolysaccharide, LPS), pro-inflammatory responses (e.g., IL-1β signaling, NLRP3 inflammasome assembly) and Th2 cell cytokine production (e.g., IL-10). A Benjamini-Hochberg false discovery rate (FDR) of q<0.05 was used to generate nodes (circles) based on kappa scores ≥0.4. The size of the nodes reflects the enrichment significance of the terms, and the different colors represent distinct functional groups.

Figure 5. Protein validation using plasma cytokine assay showed ↑ HIV usRNA associated with ↓ IL-10 and TNF-α



Plasma protein levels were quantified from a subset of N=175 of the participants. Notably plasma IL-10 was also significant correlated with HIV total DNA (Spearman R = -0.18, p = 0.016).

Figure 6. Proposed model of the association between ↑ HIV usRNA and ↓ Inflammatory/Innate Immune Genes



Within bulk peripheral CD4+ T cells, higher transcriptional reservoir activity (HIV usRNA) from HIV+ cells (left) may chronically lead to downregulation of host proinflammatory gene expression in bystander cells (right) in an attempt to suppress persistent inflammation and innate immune activation. Created with BioRender.com

CONCLUSIONS

- We observed 17 host genes that were significantly associated with **HIV usRNA**, all of which demonstrated an inverse relationship with HIV usRNA and are highly interrelated inflammatory pathways (e.g., *IL-1A*, *IL-6*, *IL-10*, *TNFAIPs*, *TLR4*, *TLR7, NRLP3* inflammasome signaling).
- Two host tumor suppressor genes (*P3H3, NBL1*) were significantly (inversely) associated with **HIV total DNA**.
- We did not observe any host genes that were significantly associated with **HIV intact DNA**, but this may have been due to a large number of undetectable provirus in our population, potentially due to low sample input DNA concentrations.
- While we were able to validate some of these findings at the protein level (P3H3, IL-10, TNF-α), further studies are needed to confirm these findings in larger cohorts with longitudinal sampling, including underrepresented PWH in cure research
- Our findings suggest that host gene expression may vary in response to the transcriptionally active reservoir and that changes in cellular proliferation genes may influence the size of the HIV reservoir.

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