HIV Reactivation Potential Evaluated by RNA-Seq and DNA Methylation Following LRA Stimulation

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BACKGROUND

Latency-reversing agents (LRAs) have shown promise in reactivating the HIV reservoir within "shock and kill" approaches. However, molecular mechanisms underlying HIV reactivation within CD4⁺ T cells, as well as predictors of LRAs efficacy, remain partially unknown. Here, we investigated the transcriptomic and whole genome DNA methylation profile of CD4⁺ T cells cultured *in vitro* with or without two LRAs and assessed for associations with HIV viral reservoir inducibility.

METHODS

Leukapheresis (2.5 - 9.4 x 10^9 PBMCs) were collected from 9 study participants (10 - 27 years old, 7 female) with perinatal HIV infection who initiated ART < 1 year of life and with long viral suppression (IQR: 13 - 22 years). The HIV reservoir was characterized by Flip-Seq, IPDA, and QVOA (**Table 1**). HIV inducibility was calculated as infectious units/10^6 cells divided by the number of intact proviruses/10^6 cells (**Fig. 1**).

Study participant	PBMCs assayed by FLIP-seq	HIV copies	Intacts	CD4⁺ assayed by QVOA	QVOA infectious units
S1	1000M	226	0	300M	0
S2	19,9M	48	10	47M	1
S3	222M	130	5	21M	4
S4	86M	645	8	69M	5
S5	989M	377	1	502M	0
S6	62,3M	342	47	25M	45
S7	30,7M	220	1	95M	77
S8	63,2M	278	1	62M	1
S9	119M	1353	7	430M	0

Table 1. HIV reservoir assessment

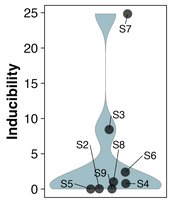
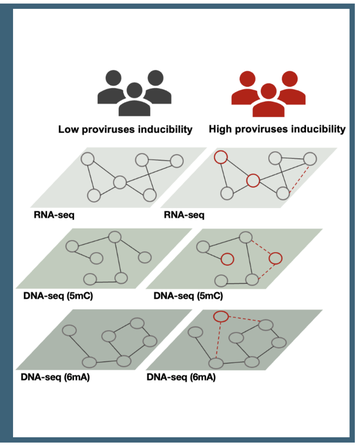


Figure 1. HIV inducibility distribution

Purified CD4⁺ T cells were cultured *in vitro* for 20h with AZT and two LRAs: fimepinostat (CUD, a PI3K and HDAC inhibitor) and Ingenol-3-angelate (PEP, a PKC activator), with PMA + ionomycin + IL2 as positive control (CTR) or left unstimulated (UNS). After the *in vitro* culture, RNA and DNA were extracted and sequenced using the long-read Oxford Nanopore platform. Both **5-methylcytosine (5mC)** and **N6-methyladenosine (6mA)** DNA methylations were quantified. Spearman's correlations were applied to identify genes and genomic sites potentially associated with HIV inducibility (|rho| > 0.75 & FDR < 0.05). Transcriptome and epigenome were integrated using a 54M of records regulome database built by joining 13 public databases of regulatory regions and their target genes (**Fig 2**).

Distinct 5mC and 6mA DNA methylation patterns, along with specific transcriptional profiles, are associated with a higher risk of provirus reactivation



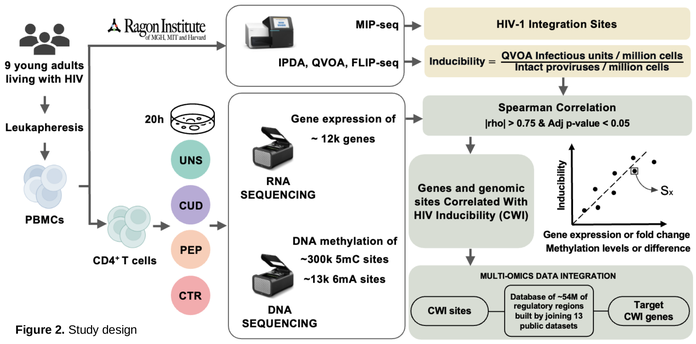


Figure 2. Study design

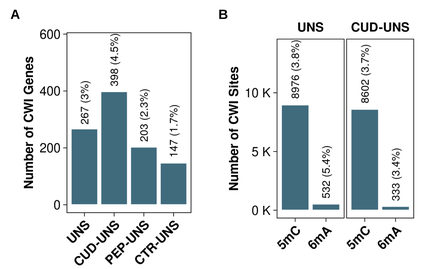


Figure 3. Number of Genes whose expression Correlates With Inducibility (CWI). The percentage is computed in relation to the number of quantified genes (A). Genomic sites whose 5mC and 6mA DNA methylations CWI. The percentage is computed in relation to the number of quantified sites (B).

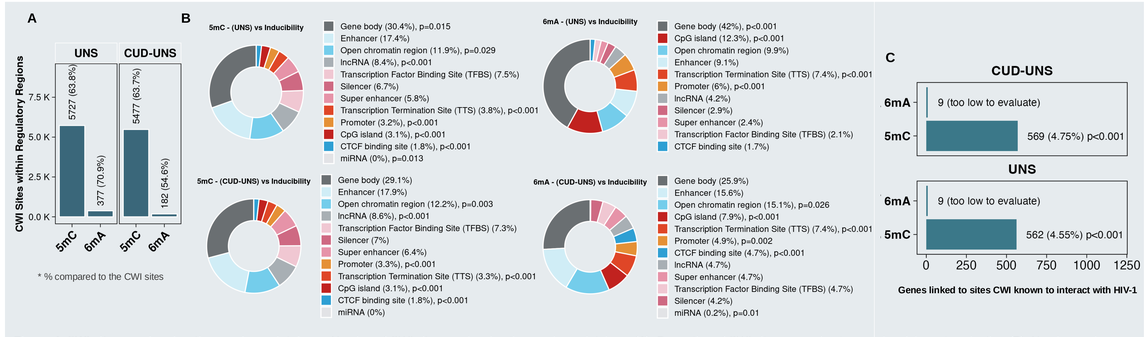


Figure 4. CWI sites overlapping the regulome database. Number of CWI sites overlapping the regulome database (A). Proportion of CWI sites per region type and enrichment evaluation (B). Number and enrichment evaluation of Genes linked to sites CWI overlapping the regulome database known to interact with HIV according to the HIV-1 Human Interaction Database (C). Top results from the Reactome pathway enrichment analysis of the genes linked to CWI sites by the regulome database (D).

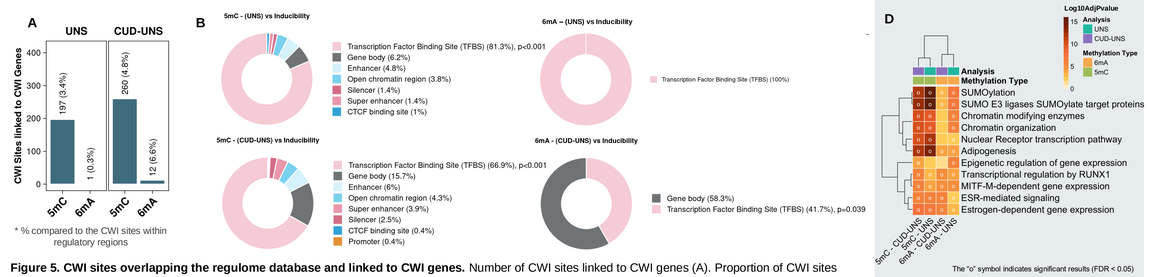


Figure 5. CWI sites overlapping the regulome database and linked to CWI genes. Number of CWI sites linked to CWI genes (A). Proportion of CWI sites linked to CWI gene per region type and enrichment evaluation (B).

RESULTS

The multi-omics analysis performed in four distinct *in vitro* conditions revealed 897 genes and 16616 genomic sites whose expression and DNA methylation Correlate With Inducibility (CWI) (**Fig. 3**). Between 54% and 71% of CWI sites overlap with gene bodies or regulatory regions, showing significant enrichments (FDR < 0.05) in **promoters**, **CpG islands**, and **transcription termination sites**, both for 5mC and 6mA sites at UNS and CUD. Genes linked to these sites via the regulome database are significantly enriched (FDR < 0.05) in HIV-1 interacting genes and transcriptional/post-transcriptional pathways, including **chromatin organization**, **PTEN and RUNX1 regulation**, and **SUMOylation**. Up to 6.6% of CWI sites in gene bodies and regulatory regions have associated genes CWI at the expression level (**Fig. 4**). Over 41% of these sites and genes correspond to Transcription Factor Binding Sites (TFBS) and their respective transcription factors (**Fig. 5**). Genes CWI at multi-omics layers include proteins implicated in HIV replication, such as NFATC1, ATF4, SMAD4, and CEBPD, potentially serving as in vitro correlates of HIV reactivation by LRAs.

CONCLUSIONS

Multi-omics analysis of CD4⁺ T cells cultured *in vitro* with or without LRAs identified genes and sites correlated to HIV inducibility in children and young adults with prolonged viral control and an extremely low reservoir. CWI sites were enriched in regulatory regions targeting genes involved in transcriptional and post-transcriptional mechanisms. These findings suggest that distinct 5mC and 6mA DNA methylation patterns, along with specific transcriptional profiles, contribute to a higher risk of provirus reactivation. Furthermore, these features provide insights into latency reversal mechanisms and may aid in identifying more effective LRAs.

ADDITIONAL KEY INFORMATION

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