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HIV transcriptional regulation in the CNS of ART-suppressed people with HIV

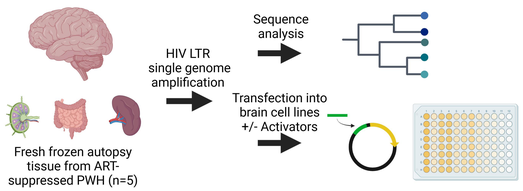
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BACKGROUND

- We and others have demonstrated that intact, transcriptionally competent HIV genomes persist within the brain of ART-suppressed people with HIV (PWH)[1,2].
- HIV promotors isolated from the brain of non-ART suppressed PWH are genetically distinct from those isolated in matched peripheral tissue and have a reduced ability to drive transcription and respond to select latency reversing agents (LRAs) in brain cell lines [3,4].
- **Whether promotors isolated from the brain of ART-suppressed PWH are functional, genetically distinct and responsive to LRAs is unclear.**

STUDY DESIGN



RESULTS

Table 1. Cohort Table

Participant ID	Age	Viral load	Viral suppression (years)[a]	CD4+ T cells	ARV (at autopsy)	CPE score[b]	IPDA: copies/10^6 cells Intact	3' defective	5' defective	Total
P1	62	UD	3.93	172	DTG, TRU	8	25	4.2	182.5	211.7
P2	59	UD	2.47	328	RGV, TRU	7	58.4	105.9	181.0	345.3
P3	64	UD	2.89	537	ZDV, EFV, CBV, 3TC	7	65.5	12.5	20.4	98.4
P4	62	UD	7.75	N/A	NVP, TRU	8	16.2	0	49.7	65. 9
P5	52	UD	6.04	417	EFV, KTA, TZV, ATR	7	0	24.7	17.6	42.4
Median	62		3.93	372.5						

TC: lamivudine, ARV: antiretroviral, ATR: atripla, CBV: combivir (zidovudine/lamivudine), CPE: CNS penetrance score, DTG: dolutegravir, EFV: efavirenz, KTA: biktarvy (bictegravir/emtricitabine/tenofovir alafenamide), NVP: nevirapine, RGV: raltegravir, TRU: truvada, TZV:trizivir, UD: undetectable, ZDV: zidovudine (emtricitabine/tenofovir), [a] defined by >2 years undetectable viral load, [b] CPE score ≤5 (low penetration), 6-8 (medium penetration) and ≥ 9 (high penetration) calculated as per (5)

- 68 promotor sequences were isolated from brain (frontal lobe) and peripheral (spleen, lymph node, gut) tissue from 5 ART-suppressed PWH (undetectable pVL for a median of 3.93 years).
- HIV DNA was detected in brain tissue from all participants, as measured by the intact proviral DNA assay (Table 1).

RESULTS

1. HIV promotors from ART-suppressed PWH do not compartmentalise based on tissue origin

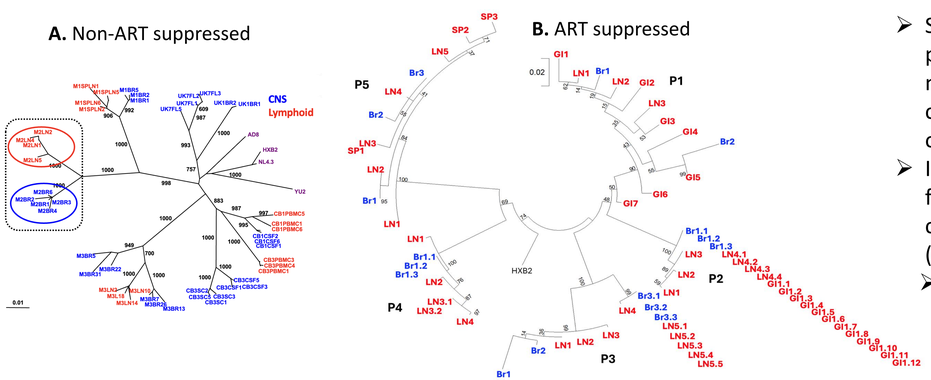


Figure 1. Maximum likelihood tree of HIV promotors isolated from brain and matched peripheral tissue of (A) non-ART suppressed and (B) ART suppressed PWH. Blue represents CNS derived isolates; red represents non-CNS derived isolates. Numbers associated with each branch are bootstrap values obtained from 1,000 replicates.

- Similar to previous findings[3,4], HIV promotor sequences derived from non-ART suppressed PWH compartmentalised based on tissue origin (Fig. 1A).
- In contrast, promotor sequences from ART-suppressed PWH do not compartmentalise based on tissue (Fig. 1B).
- Promoters with the same sequence were observed in multiple tissue compartments (Fig 1B; P2 and P3).

2. HIV promotors from ART-suppressed PWH are intact

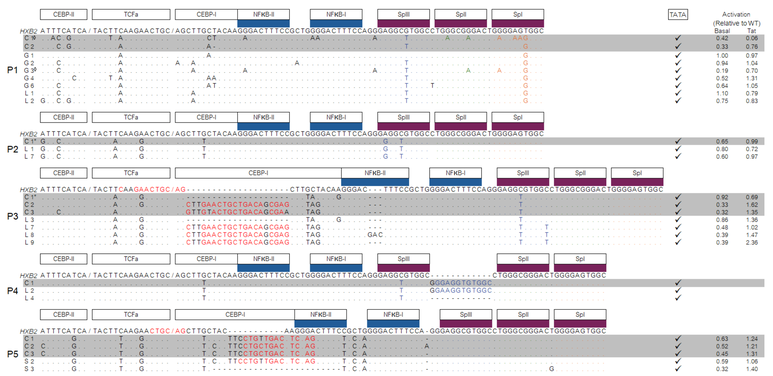


Figure 2. Multiple sequence alignment of HIV promotor

- Unlike previous findings in non-ART suppressed PWH[3,4,6], no brain specific mutations within the promotors of ART-suppressed PWH were observed.
- Promoters from both brain and peripheral compartments of ART-suppressed PWH have intact Sp1, NF-κB and TATA motifs with predicted high Sp1 binding affinities.

RESULTS

3. HIV promotors from ART-suppressed PWH are functional *ex vivo*

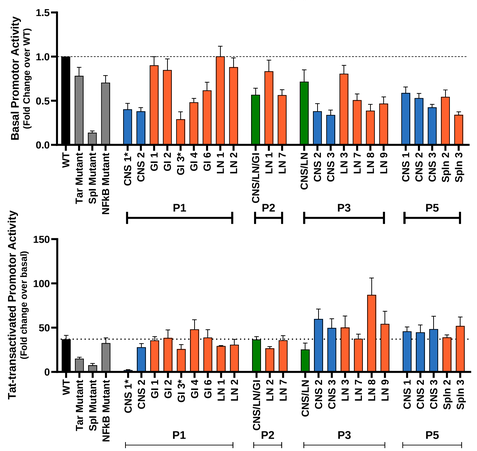


Figure 3. HIV promotors were cloned into a luciferase reporter vector and transfected into the microglial cell line HMC3s to determine their ability to drive basal (top) and tat-transactivated (bottom) transcription *ex vivo*.

- We have previously shown that in non-ART suppressed PWH, brain derived promoters exhibit a lower transcriptional activity than those isolated from the periphery of the same PWH[3].
- Here, we show that HIV promotors from ART-suppressed PWH are:
  - Functional and able to drive transcription (Fig. 3)
  - Display a heterogenous basal and tat-transactivated transcriptional activity, independent of tissue compartment (Fig. 3)

4. HIV promoters isolated from brain and peripheral tissue of ART-suppressed PWH respond similarly to activators

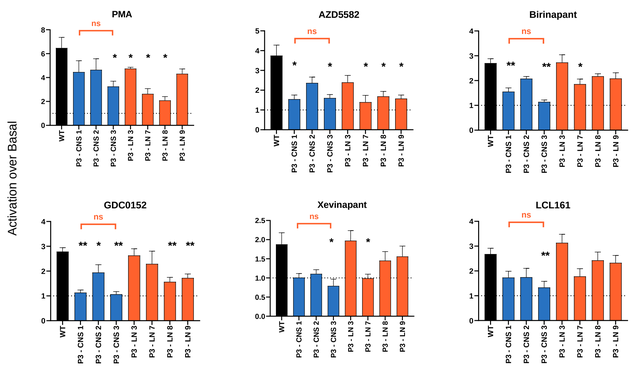


Figure 4. HIV promotors from participant 3 were transfected into the HMC3 microglial cell line and treated with 100nM of each activator for 48 hours prior to cell lysis. Black values: significance of isolates relative to a wild type T-cell-derived consensus sequence (HXB2). Red values: significance of CNS-derived isolates relative to lymphoid-derived isolates from the same PWH. *P<0.05, **P<0.01

- We have previously shown that brain derived promoters from non-ART suppressed PWH exhibit a reduced response to LRAs [3].
- Here, we show that HIV promotors from ART-suppressed PWH:
  - Do not respond to SMAC mimetics as well as wild type
  - Show a similar response across brain and peripheral tissue

CONCLUSION

- HIV promotors from virally suppressed PWH are intact, with functional Sp1, NF-κB and TATA sites.
- CNS-derived HIV promotors from virally suppressed PWH are functional *ex vivo* and display a heterogenous basal and activated transcriptional activity.

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