Single-Genome Profiling of Promoter DNA Methylation Signatures in Intact HIV-1 Proviruses

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Xing Wang[1], Chenyang Jiang[1], Toong Seng Tan[1], Marie Armani-Tourret[1], Mathias Lichterfeld[1,2] and Xu G Yu[1,2],

[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA 02139, USA, [2]Infectious Disease Division, Brigham and Women's Hospital, Boston, MA 02115, USA

Intact HIV-1 proviruses exhibited substantially higher levels of promoter DNA methylation

BACKGROUND

DNA methylations located near promoter region and transcription start site of HIV proviral sequence silence proviral expression in in vitro latency models[1], but their roles for HIV persistence and expression in vivo remain elusive. Prior studies have shown that CpG sites within 5' LTR and Gag leader regions of proviral DNA exhibit low levels of methylation in total reservoir cells, which largely consist of defective proviruses[2]. However, methylation patterns in single intact proviruses that persist long-term during ART have not been well characterized.

METHODS

We used SEM-Seq (Single-genome Enzymatic methylation sequencing) to access the DNA methylation in promoter region of intact HIV-1 proviruses. gDNA from the PBMCs of study participants were fragmented with restriction endonucleases to keep the intactness of interested regions, followed by enzymatic conversion of unmethylated cytosines with TET and APOBEC and subsequent whole-genome amplification. DNA was subjected to nested PCR spanning the integration sites of previously-characterized intact proviruses. Amplicons were sequenced on Illumina MiSeq and compared with unconverted viral-host junction region sequences to map methylated CpGs.

RESULTS

We profiled the methylation of promoter regions in 8 clones of cells infected with intact proviruses. The average methylation levels range from 22.4% to 50.0% for each participant.

Participants	Cohort	Years on ART or elite control	Chromosome Location	Gene
P1	EC	13	Chr19:58194555 (-)	ZNF274
P2	LT-ART	20	Chr19:58194512 (-)	ZNF274
P3	LT-ART	16	Chr12:133105183 (-)	ZNF140
P4	LT-ART	23	Chr12:133054916 (-)	ZNF84
P5	LT-ART	18	Chr19:57981442 (+)	ZNF606
P6	LT-ART	23	Chr3:38084550 (-)	DLEC
P7	EC	18	ChrY:11543665 (-)	Satellite
P8	LT-ART	21	Chr15:22082341 (-)	RP11-69H

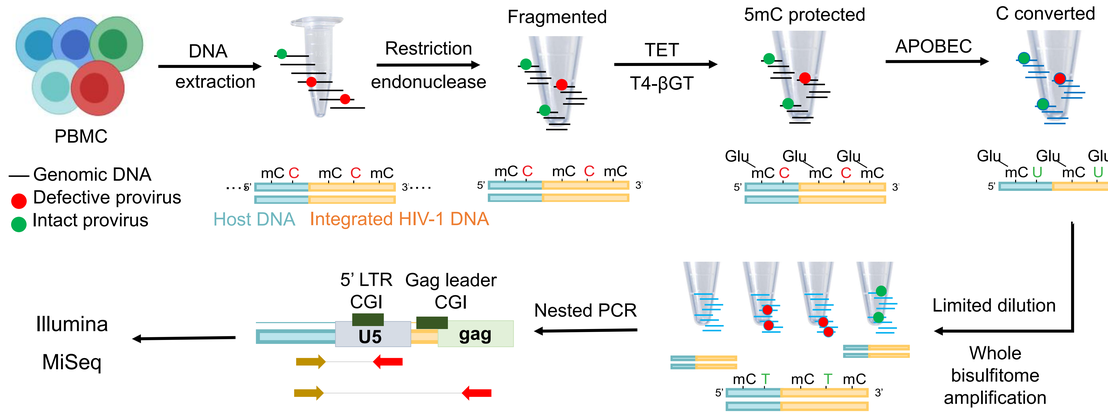
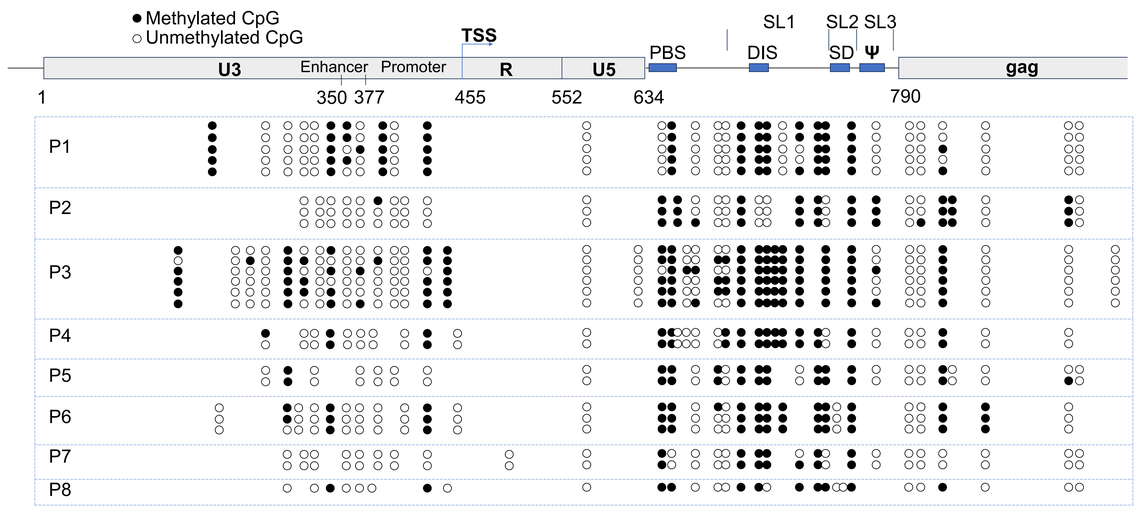


Figure 1. Schematic diagram of SEM-seq.



CpG sites (on HXB2, bp)	273	297	310	336	359	408	565	639	643	661	687	690	699	712	714	728	735	738	751	770	797	803	816
Conservativeness	6/8	7/8	7/8	7/8	8/8	8/8	8/8	8/8	7/8	8/8	8/8	8/8	8/8	8/8	8/8	7/8	7/8	8/8	8/8	6/8	8/8	8/8	8/8
Methylation (%)	57.9	0.0	75.0	13.6	12.5	66.7	0.0	75.0	90.4	12.5	25.0	20.8	100	87.5	83.3	76.2	100	72.7	100	25.0	0.0	4.2	79.2

Figure 2. Methylation profiles of proviral 5' LTR and gag leader CGIs.

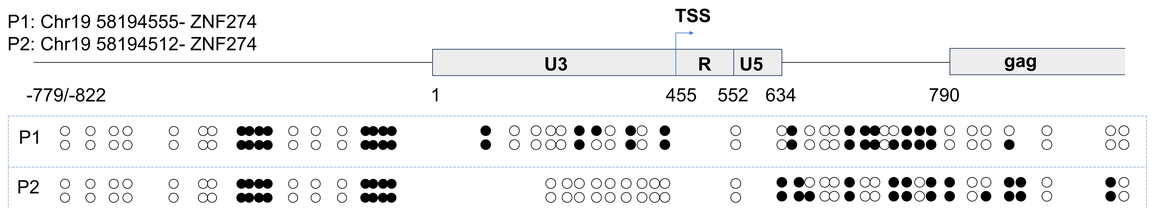


Figure 3. Methylation profiles of upstream host genome from participants 1 and 2

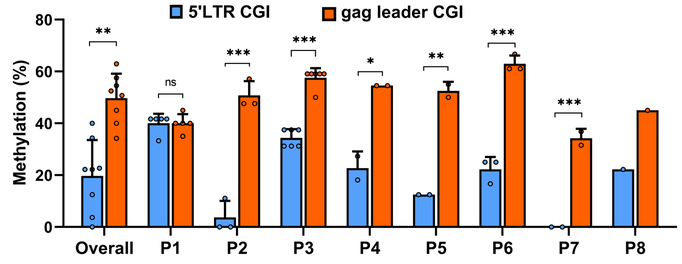


Figure 4. gag leader CGI exhibits superior methylation levels compared to 5'LTR CGI.
Overall and individual methylation level of 5'LTR CGI and gag leader CGI of the intact proviruses profiled from the 8 participants. *P* values were calculated by a Wilcoxon test.

CONCLUSIONS

- SEM-Seq enables detection of locus-specific proviral and genic DNA methylation, providing a framework to study epigenetic heterogeneity within the intact HIV reservoir.
- Intact proviruses in the participants showed higher levels of methylation in the 5' LTR CGI region than unselected total reservoir cells in prior studies.
- The gag leader CGI region is more CpG-dense, more conserved across participants, and exhibits substantially higher levels of methylation, suggesting a more prominent role in maintaining proviral latency.
- Host DNA upstream of the proviral integration sites potentially contained stably methylated CGIs.
- Additional studies will be needed to define the functional contribution of these methylation patterns.

REFERENCES

1. Roxane Verdikt et al, Epigenetic Mechanisms of HIV-1 Persistence, *Vaccines*. Vol. 17 (2021): 514.
2. Jana Blazkova et al, Paucity of HIV DNA Methylation in Latently Infected, Resting CD4+ T Cells from Infected Individuals Receiving Antiretroviral Therapy, *Journal of Virology*, Vol. 86 (2021): 5390-5392.

PLAIN LANGUAGE SUMMARY

By studying single copies of provirus in people living with HIV, we discovered that the virus's promoter region often carries methylations that may prevent it from activating. This strong and consistent DNA methylation may help explain how intact HIV remains silent yet survives for years despite therapy.