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METHYLATION PROFILES OF HIV-1 PROVIRAL DNA IN ART-SUPPRESSED INDIVIDUALS

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HIV Dynamics and Replication Program

Introduction

The latent HIV-1 reservoir is populated with clones of cells infected with stably integrated, intact, but transcriptionally-silent proviruses. Previously, we described the integration site of one such clone harboring a replication-competent provirus called AMBI-1. Given that methylation of CpG bases in endogenous proviruses is a known mechanism of silencing, we hypothesized that the silencing of HIV-1 gene expression from this and other clones was due to DNA methylation of the 5'LTR promoter. To address this question, we investigated methylation at the single-proviral level in known CpG islands in the HIV-1 proviral genome, including one in the 5' LTR promotor region.

Methods

Using a bisulfite-based, methylation-specific single-genome-sequencing (ms-SGS) assay, we measured the levels of methylation in 4 CpG islands from HIV genomic DNA (gDNA) from 4 ART-suppressed, chronically-infected individuals, including the donor with the AMBI-1 clone.

- Tissues: PBMC and Lymph Node Mononuclear Cells (LN).
- HIV gene regions:
  1. 5'LTR , upstream of the mRNA start site and into gag.
  2. GAG leader (3' of mRNA start site).
  3. Integrase (transcription factor binding site).
  4. gp41 2nd exon of Tat (*env-tat-rev* overlapping reading frame).
- Obtained at least 20 SGS from non-bisulfite treated proviral DNA from each region and each tissue (~640 SGS) to define CpG sites.
- Treated proviral DNA with bisulfite to convert non-methyl C to U.
- Obtained at least 20 SGS from bisulfite treated proviral DNA from each region and each tissue (~640 ms-SGS)
- Using the Quantification tool for Methylation Analysis (QUMA) program, aligned, visualized and quantified sequence data for CpG methylation patterns in each genome compared to the consensus of untreated SGS from the same gDNA.
- Recorded the background of unconverted CpHs not in CpG sites in each genome.
- Performed control bisulfite reactions amplifying CCR5 gene and HIV5'LTR from same extraction of gDNA from donor 1 to test background unconverted CpHs.

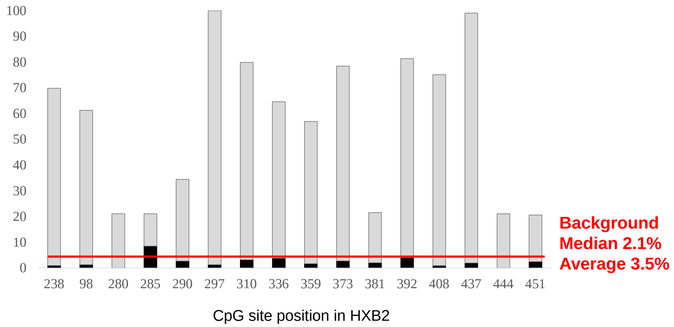
Results

- % methylation at CpG sites across all donors
- % of CpG sites present in untreated genomes across all donors

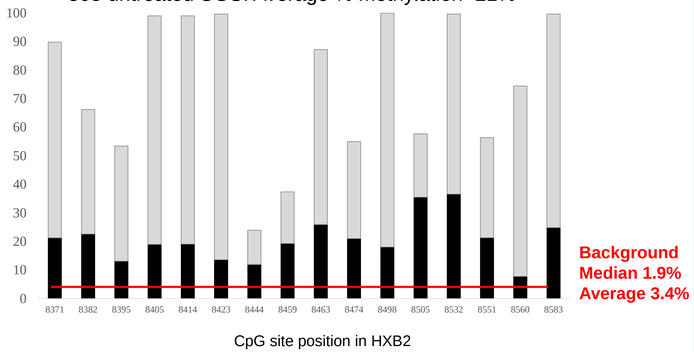
Background Control Experiment:

Gene	CCR5	HIV 5'LTR
Average % unconverted CpHs	4.30%	5.9%
Median % unconverted CpHs	4.60%	4.1%

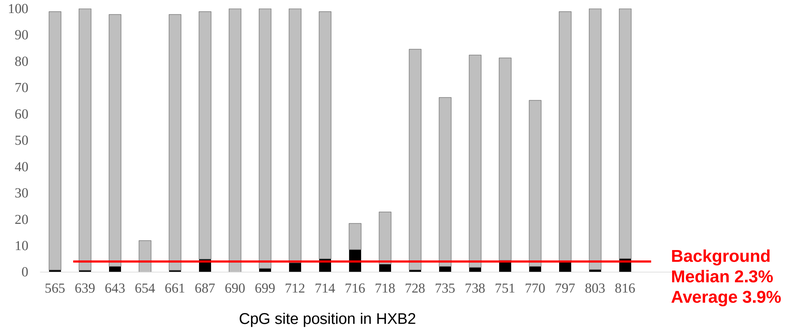
5' LTR :
163 Bisulfite treated SGS from 4 donors compared to 240 untreated SGS. Average % methylation=2.4%



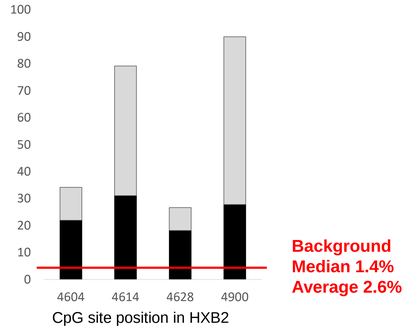
gp41:
182 Bisulfite treated SGS from 4 donors compared to 305 untreated SGS. Average % methylation=21%



Gag Leader:
162 Bisulfite treated SGS from 4 donors compared to 134 untreated SGS. Average % methylation=3%



Integrase:
169 Bisulfite treated SGS from 4 donors compared to 160 untreated SGS. Average% methylation=26%



Conclusion

Surprisingly, we did not find evidence that methylation of the 5'LTR promotor maintains HIV-1 latency *in vivo*, including LTRs of proviruses that are known to be intact and latent, as in the case of the AMBI-1 clone. However, significant levels of methylation, (p=<0.03), were found in CpG islands in integrase and gp41 but their role, if any, in transcriptional silencing is unknown. Since it is well known that methylation of transcriptional enhancers located many kilobases from mRNA start sites can result in gene silencing, it is important to determine if the identified methylated CpG islands have any function in HIV-1 latency *in vivo*.

Acknowledgments

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