THE CIRCULATING CELL-FREE DNA METHYLOME PREDICTS HIV POST-TREATMENT CONTROL

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BACKGROUND

- The development of HIV cure strategies depends on our capacity to predict HIV control when antiretroviral therapy (ART) is stopped.
- We performed multi-dimensional profiling of plasma cfDNA obtained from individuals who maintain HIV suppression following ART cessation termed post-treatment controllers (PTCs), to identify quantitative, genetic, and epigenetic signatures of PTC status.

METHODS

- Samples: cfDNA was extracted from 1 ml of plasma collected prior to ART cessation from 20 PTCs (HIV RNA maintained at < 400 copies/ml for >24 weeks post-ART) and 35 non-controllers (NCs) enrolled in AIDS Clinical Trials Group (ACTG) treatment interruption studies, and from 20 uninfected individuals enrolled in the SCOPE cohort.

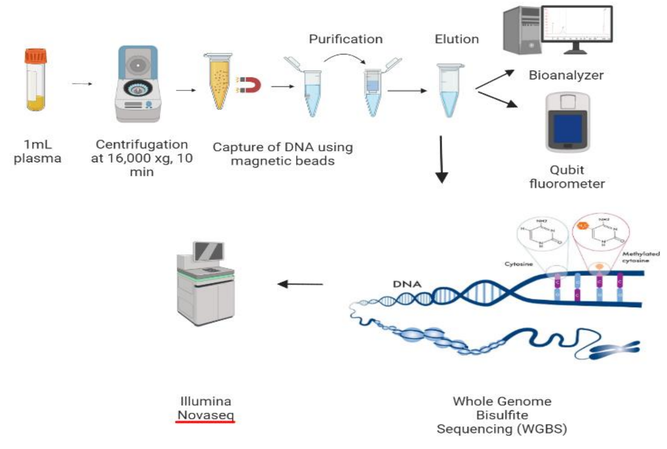


Figure 1. Schematic diagram of methodology

- Whole genome bisulfite sequencing (WGBS): cfDNA samples from PTCs (N=10), NCs (N=10), and uninfected individuals (N=9) were processed for WGBS using the IDT Xgen Methyl-Seq kit on the Illumina NovaSeq platform.

RESULTS

- Elevated levels of total plasma cell-free DNA and mitochondrial DNA (mtDNA) was observed in PTCs as compared to NCs and uninfected individuals (Fig. 2).
- cfDNA from PTCs exhibited significantly lower genome-wide cytosine methylation rates as compared to cfDNA from NCs (Fig. 2).

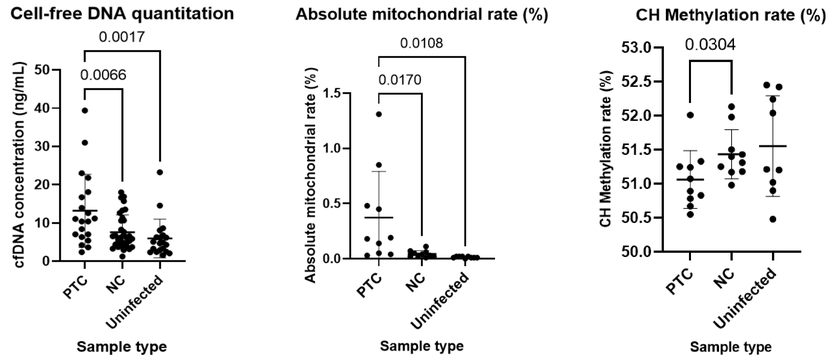


Figure 2. Plasma cell-free DNA levels and methylation rates are signatures of PTC status

HIV post-treatment controllers (PTCs) who maintain viral loads < 400 HIV RNA copies / ml following cessation of antiretroviral therapy (ART) exhibit elevated levels of total and mitochondrial cell-free DNA in plasma during ART. In addition, cytosine methylation, which regulates gene expression, is reduced in circulating cell-free DNA from PTCs. Cell-free DNA is a promising HIV cure biomarker.

- Numerous differentially methylated regions (DMRs) were identified in comparisons between PTCs, NCs and uninfected cfDNA samples (Fig. 3).

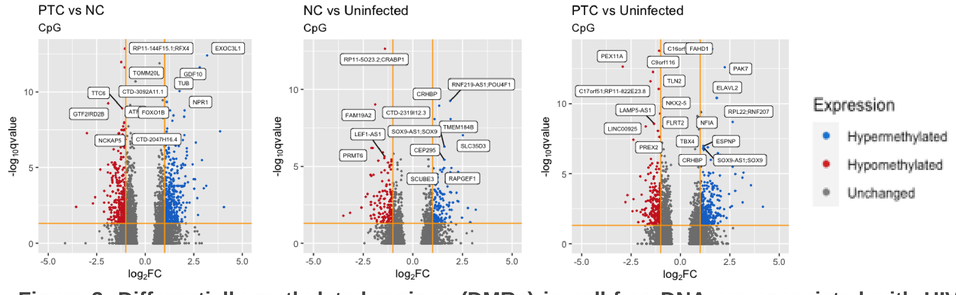


Figure 3. Differentially methylated regions (DMRs) in cell-free DNA are associated with HIV disease states

Upstream Regulator	Molecule Type	Predicted Activation State	p-value of overlap	Target Molecules in Dataset
PPP3CB	phosphatase	Inhibited	0.000099	CDKN1A,IRS1,NOX4,RBL2,RUNX1
DLX2	transcription regulator	Inhibited	0.000129	CDKN1A,FOXJ1,GAD1,MYC,TTI1,WNT1
TCF7L1	transcription regulator	Activated	0.00125	ATF3,EOMES,KLF4,NR5A2,PRDM14,SLC26A2
WNT3A	cytokine	Inhibited	0.00295	ATOH1,BCL2L11,BMP4,CCN1,EOMES,GATA4,HAS3,ID1,IGFBP5,IRS1
FOXP1	transcription regulator	Inhibited	0.00422	CDKN1A,NFIA,NR5A2,PCDH10,ST8SIA2,WNT1
VEGFA	growth factor	Inhibited	0.00632	ADAMTS1,BCAT2,BMP4,CCN1,CDKN1A,CTSB,EOMES,ID1,IGFBP5,ITGA4
APOE	transporter	Activated	0.00915	ANK3,ATF3,BMP4,CCN1,CTNND2,CTSB,F2RL1,FKBP4,GRIN2B,HSPA1A/HSPA1B
DKK1	growth factor	Activated	0.0186	BMP4,BMP6,CDKN1A,F2RL1,MYC,TTYH1,TWIST1
YBX1	transcription regulator	Inhibited	0.0247	CDKN1A,ERBB2,HES2,ID1,KLF4,MYC,PDGFB,PTPA,STC2
NPM1	transcription regulator	Inhibited	0.0295	ALK,CCN1,CDH11,COL6A2,EGLN3,HOXA7,IGFBP5,KLF4,MTSS1,NTRK2
MYC	transcription regulator	Inhibited	0.03	ABCB10,ACLY,ACTA1,ADAMTS1,ALCAM,ALDOA,ASCL1,ATOH1,AURKB,BCL2L11
SFTPA1	transporter	Activated	0.034	CCN1,ERN1,GUCY1A1,MYC,PLIN2,SH3PXD2A,TWIST1
DNMT1	enzyme	Inhibited	0.0343	CDKN1A,DAB1,DLC1,DMRT1,GATA4,MYC,NEIL1,WNT1
NDRG1	kinase	Activated	0.0345	ATF3,BCL2L11,CD82,ERBB2,MYC,SHH

Table. 1 CpG methylation hits translated into differential gene expression between PTCs and NCs

- Activation and inhibition of gene expression were predicted based on the concept that methylation at a given site inhibits gene expression, and absence of methylation enables gene expression (Table. 1).
- Principal component analysis (PCA) revealed pronounced segregation of cfDNA methylation patterns in PTCs, NCs and uninfected individuals (Fig. 4).

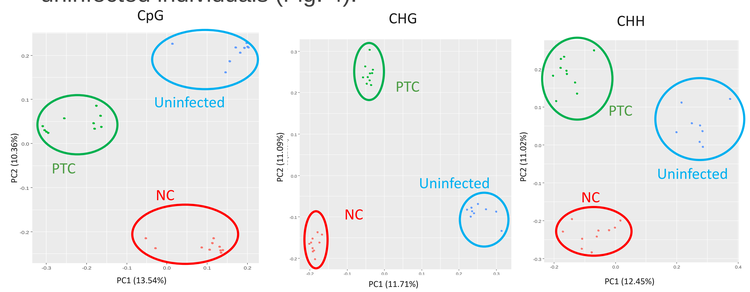


Figure 4. Principal component analysis (PCA) of cfDNA methylation at CpG, CHH and CHG sites

CONCLUSIONS

- Elevated circulating levels of cfDNA and mtDNA during ART are predictors of HIV post-treatment control.
- DMRs distinguishing PTCs from NCs further demonstrate the prognostic potential of cfDNA as a predictive biomarker of natural HIV control following ART cessation.
- Elevated levels of circulating hypomethylated DNA may reflect the death of infected cells and may promote innate immune control of HIV via innate DNA sensors including TLR-9.
- Our data warrant continued investigation into cfDNA as a marker and modulator of HIV persistence and control *in vivo.*

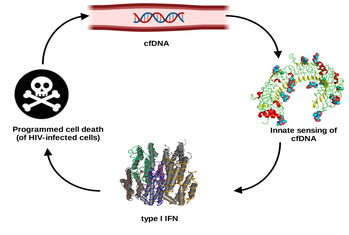


Figure 5. Hypothetical role of cfDNA in HIV persistence and control.

ADDITIONAL INFORMATION

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