ROLE OF UHRF1 IN HIV-1 TRANSCRIPTIONAL REGULATION

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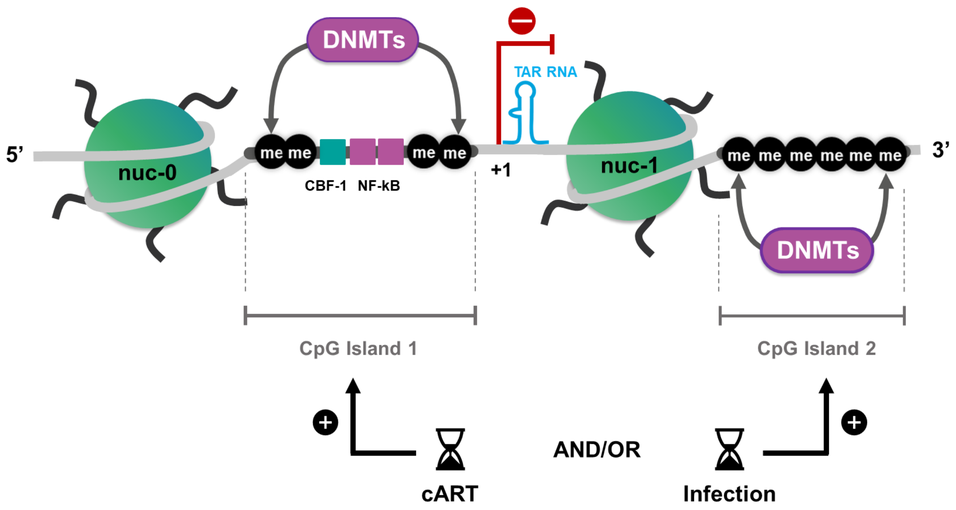
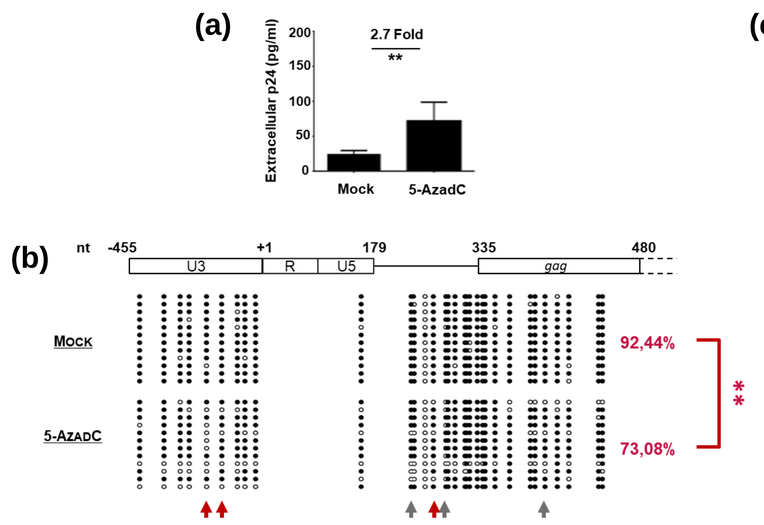
BACKGROUND



DNA methylation is one of the epigenetic mechanisms involved in HIV-1 latency. The latent 5'LTR methylation profile is heterogeneous in latency model cell lines and in patient cells, where DNA methylation accumulation is positively correlated with the duration of the treatment and/or of the infection. We have previously demonstrated that 5-Aza-2'-deoxycytidine (decitabine) treatment, an inhibitor of DNA methylation, resulted in variable levels of HIV-1 reactivation in latently infected T-cell lines and in *ex vivo* patient cell cultures. Nevertheless, the mechanisms mediating HIV-1 latency through DNA methylation remain unclear.

5-AZADC TREATMENT TARGETS SPECIFIC CpG POSITIONS IN THE HIV-1 5'LTR

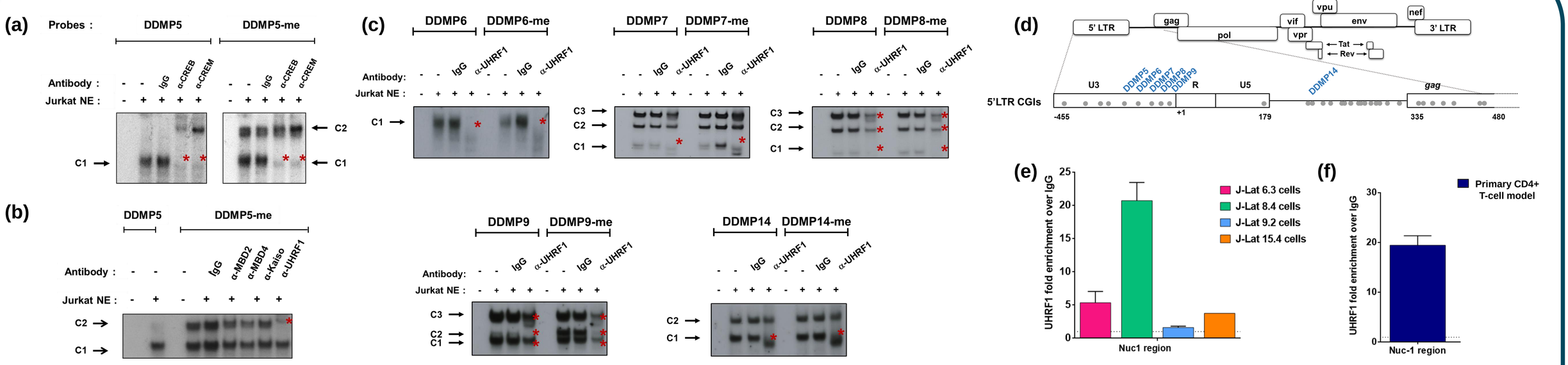


(c)

J-Lat	DDMP	Location	stat
6.3	DDMP6	NF-κB	*
	DDMP7	Sp1 I	*
	DDMP8	Sp1 II	*
	DDMP9	Sp1 III	*
	DDMP14	ISRE	*
8.4	DDMP5	CRE	**
	DDMP6	NF-κB	*
	DDMP14	ISRE	*
15.4	DDMP9	Sp1 III	*
	DDMP14	ISRE	*

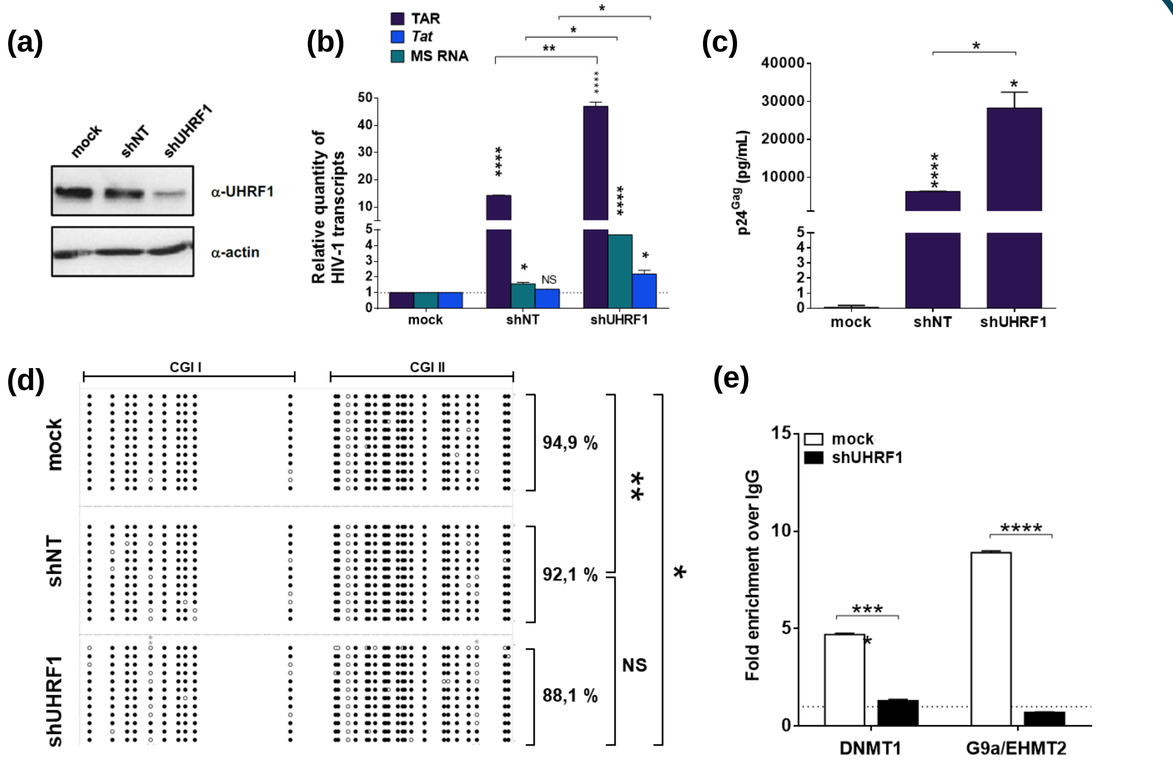
The HIV-1 latently-infected T-cell line J-Lat 8.4 was treated with 400nM of 5-AzadC for 72h. Following this, **(a)** reactivation of HIV-1 production was quantified by ELISA on $p24^{Gag}$ capsid protein in culture supernatants and **(b)** the 5'LTR DNA methylation profile was established by sodium bisulfite sequencing. Unmethylated and methylated CpG dinucleotides are respectively represented by open and closed circles, where each line corresponds to individual sequenced molecules. Arrows indicate 5-AzadC-induced Differentially DeMethylated Positions (DDMPs). Red arrows indicate DDMPs located in transcription factor binding sites. Statistical significance was determined by unpaired T-test. **(c)** Summary of the different DDMPs identified in several J-Lat clones.

UHRF1 IS RECRUITED TO THE LATENT HIV-1 PROMOTER



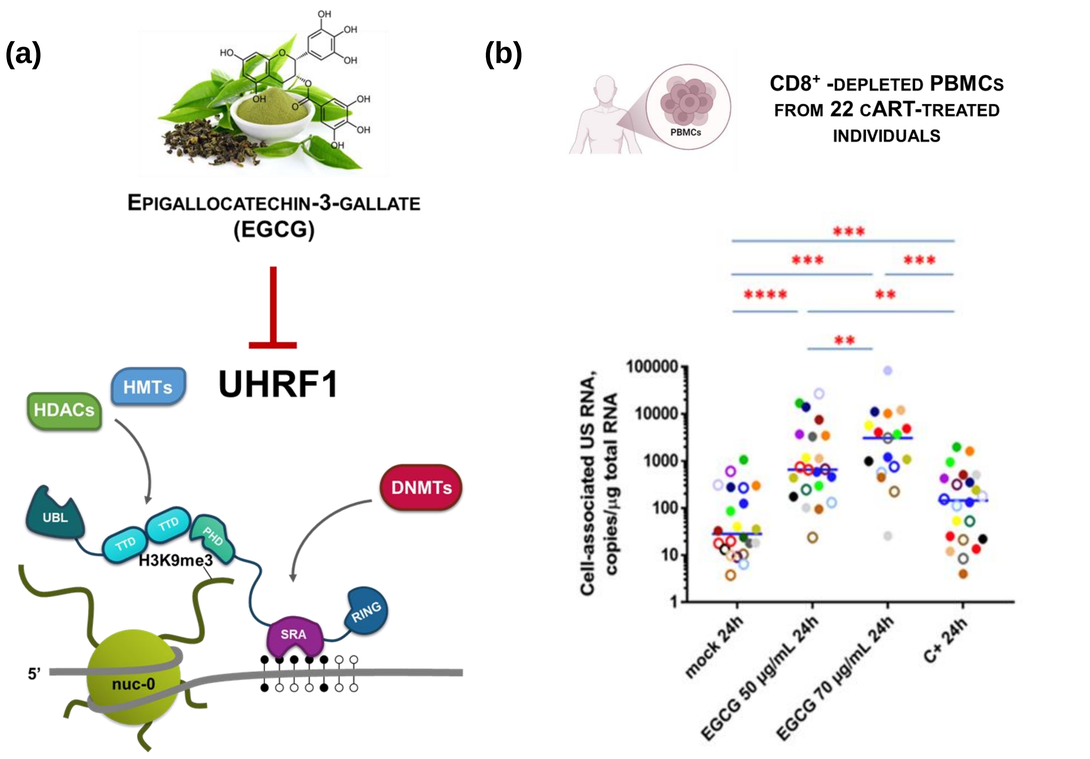
(a)-(c) Radiolabeled probes for either the unmethylated or the methylated HIV-1 DDMP5, 6, 7, 8, 9 or 14 (respectively indicated as "DDMP" and "DDMP-me") were incubated with 10µg of nuclear extracts from the Jurkat T cell line ("Jurkat NE") and with either a purified IgG as a negative control or a specific antibody directed against CREB/CREM family members, or several proteins known to bind methylated DNA including UHRF1.**(d)** Schematic representation of the two 5'LTR CpG islands. **(e)-(f)** Chromatin preparations of either J-Lat cell line models or a primary $CD4^+$ T-cell model for HIV-1 latency were immunoprecipitated with an anti-UHRF1 antibody or with purified IgG, serving as a negative control. qPCRs were performed with primers hybridizing specifically to the 5'LTR, in the Nuc-1 region.

UHRF1 REPRESSES HIV-1 GENE EXPRESSION



J-Lat 8.4 cells were either mock-transduced, shNT-transduced or shUHRF1-transduced. **(a)** UHRF1 and β-actin, serving as a loading control, protein levels were assessed by immunoblot in whole-cell lysates. **(b)** Total RNA preparations were reverse transcribed and HIV-1 transcripts were quantified by RTqPCR. **(c)** Reactivation of HIV-1 production was quantified by ELISA on p24Gag capsid protein in culture supernatants. **(d)** the 5'LTR DNA methylation profile was established by sodium bisulfite sequencing. **(e)** Chromatin Immunoprecipitations were performed using anti-DNMT1, anti-G9a or purified rabbit IgG, as a negative control.

EGCG IS A NOVEL HIV-1 LATENCY REVERSING AGENT



(a) Schematic representation of the UHRF1 inhibitor epigallocatechin-3-gallate**. (b)** *Ex vivo* cultures of CD8+-depleted PBMCs isolated from 22 cART-treated aviremic HIV+ individuals were mock-treated or treated for 24h with EGCG, at the indicated concentrations, or with anti-CD3+anti-CD28 antibodies serving as positive control stimulation. Total intracellular RNA was extracted and cell-associated HIV-1 US RNA was quantified. Medians are represented. Open circles depict undetectable values, censored to the assay detection limits.

TAKE HOME MESSAGE

- 5-AZADC INDUCES SPECIFIC DEMETHYLATION CpG SIGNATURES IN THE HIV-1 5'LTR
- UHRF1 IS RECRUITED TO THESE SIGNATURES
- UHRF1 REPRESSES HIV-1 TRANSCRIPTION THROUGH DNA AND HISTONE METHYLATIONS
- EGCG INHIBITS UHRF1 AND REVERSES HIV-1 LATENCY

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NIAID/NHLBI/NIDDK/NINDS/NIDA **UM1AI164562**

Presented at AIDS 2022 – The 24th International AIDS Conference

AIDS 2022 29 July – 2 August