Blockade of HIV Latency Reversal in CD4+ T cells From ART-Suppressed PLWH by the Antisense RNA AST

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BACKGROUND

CD4+ T cells latently infected with the human immunodeficiency virus 1 (HIV-1) persist despite treatment with antiretroviral agents and represent the major barrier to a cure. Cure strategies explored to date have not shown promising results. We are pursuing a new strategy seeking to force HIV-1 into a state of permanent latency.

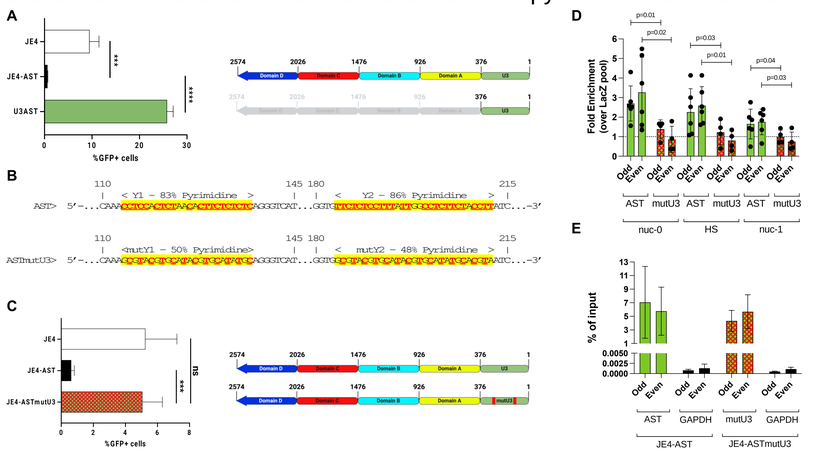
HIV-1 expresses an antisense transcript (AST) with protein-coding and noncoding roles. We showed that AST induces HIV-1 latency in cell line models by promoting a closed chromatin state at the 5'LTR. We used an in vitro latency model to study the mechanisms and AST domains that underlie these effects. Ectopic expression of AST in CD4+ T cells from ART-suppressed people living with HIV-1 (PLWH) blocks latency reversal in response to latency reversal agents and TCR stimulation, enforcing HIV-1 transcriptional silencing.

METHODS

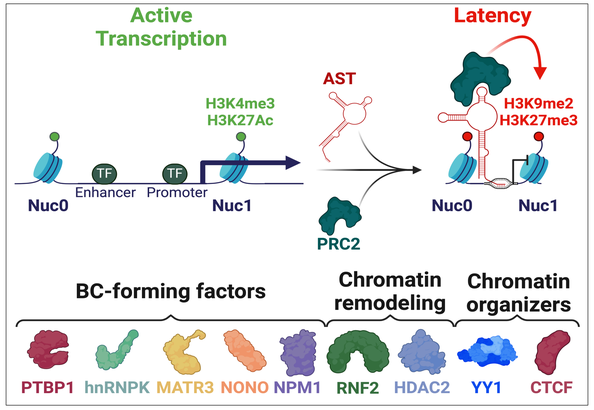
Flow cytometry was used to identify AST structural domains and functional motifs in the Jurkat E4 latency model. RIP and ChIRP assays were used to study the interaction of AST with chromatin factors and the 5'LTR, respectively. ChIP assays were used to assess epigenetic modifications at the 5'LTR. Affinity-Mass spectrometry was used to identify new AST interactors. AST was expressed by nucleofection in cells from PLWH under suppressive ART. Quantitative RT-qPCR assays were used to measure HIV-1 gag RNA expression after latency reversal.

RESULTS

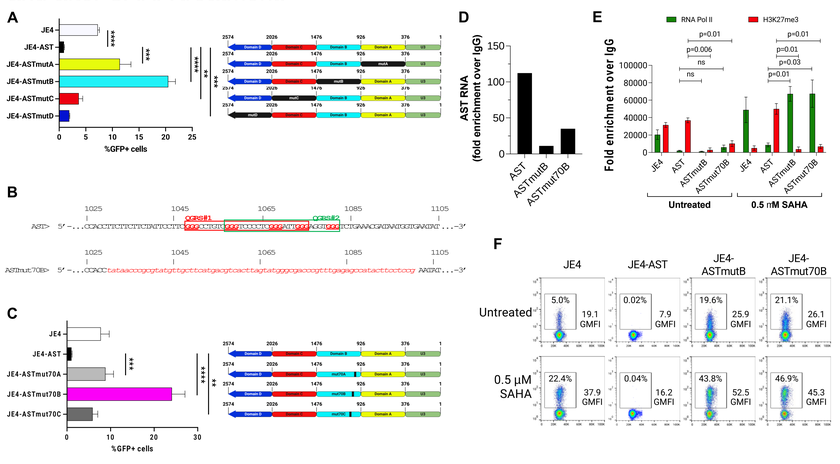
1. The 5' terminus of AST interacts with the 5' LTR in the HIV-1 genome. (A) Stable expression of the U3AST leads to latency reversal. **(B)** Nucleotide substitutions within the pyrimidine-rich motifs reduced pyrimidine content. **(C)** Stable expression of the ASTmutU3 leads to latency reversal. **(D)&(E)** Chromatin isolation by RNA precipitation (ChIRP) assays were performed to assess the interaction between AST and 5'LTR via pyrimidine-rich stretches.



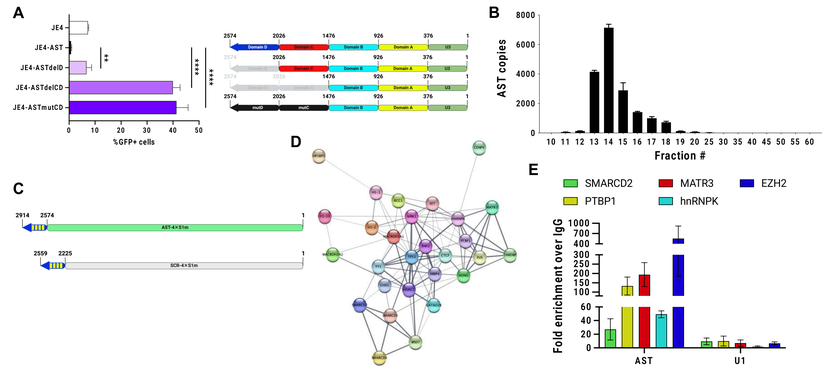
We found that the HIV-1 antisense transcript (**AST**) induces **epigenetic modifications at the HIV-1 promoter** causing a closed chromatin state that suppresses viral transcription. These properties require **sequence homology** between **AST** and **HIV-1**, and therefore they are highly specific with no off-target effects. We performed an unbiased **proteomics screen** of **AST** interactors, which revealed that **AST** binds previously known and potential new HIV-1-suppressive factors. The latter include **host factors** involved in the formation of **biomolecular condensates**, membrane-less compartments with regulatory function that are formed via liquid-liquid phase separation. Ectopic **AST** expression in CD4+ T-cells from people living with HIV-1 **blocks latency reversal** in response to pharmacologic and T-cell receptor stimulation, **enforcing transcriptional silencing**.



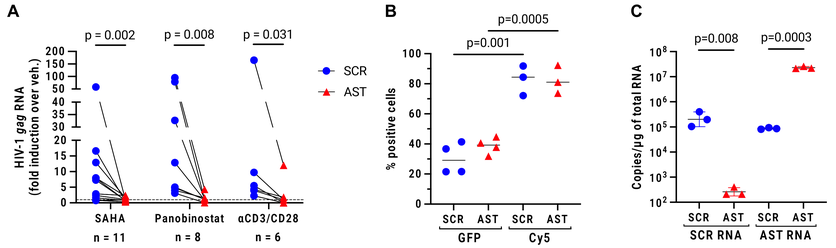
2. The domain B of AST binds PRC2. (A) Stable expression of the ASTmutB leads to latency reversal. **(B)** substitution of the 70-nt sequence encompassing both QGRS motifs. **(C)** Stable expression of the ASTmut70B leads to latency reversal. **(D)** RIP-RT-qPCR assays measured physical interaction between AST and EZH2. **(E)** ChIP-qPCR assays probing H3K27me3 and RNA polymerase II (RNA Pol II) levels at Nuc-1. **(F)** ASTmutB and ASTmut70B show markedly increased GFP expression both at baseline and after SAHA treatment.



3. The domains C and D of AST recruit additional host factors. (A) Stable expression of the ASTmutCD or ASTdelCD leads to latency reversal. **(B)** RT-qPCR assays measuring AST levels in size exclusion chromatography fractions from of JE4-AST lysates. **(C)** Schema of AST and SCR molecules with the streptavidin-binding S1m RNA aptamer fused at the 3' terminus. **(D)** STRING network of selected AST interactors. **(E)** RIP-RT-qPCR assays validating interaction between AST and the four new interactors, SMARCD2, PTBP1, MATR3, and hnRNPK.



4. Ectopic expression of AST in unstimulated CD4+ T cells from PLWH blocks latency reversal. (A) Levels of HIV-1 gag RNA were analyzed by semi-nested RT-qPCR to quantify latency reversal. **(B)** Flow cytometry analysis measuring efficiency of nucleofection by GFP% and Cy5-labeling. **(C)** Quantitative RT-qPCR assays measuring AST and SCR RNA expression levels in nucleofected CD4+ T-cells.



CONCLUSIONS

AST functions as a scaffold for the assembly of biomolecular condensates at the 5' LTR that suppress HIV-1 transcription. Our studies identify AST as a first-in-class biological molecule capable of blocking latency reversal and enforcing HIV-1 transcriptional silencing in CD4+ T cells from PLWH, showing that AST has potential applications in cure strategies.

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