Enhancing or Antagonizing HIV-1 Latency through Depletion of Select SR Kinases

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Abstract

Control of RNA processing plays a central role in the expression and replication of HIV-1. From a single transcript, over 69 viral mRNAs are generated through the process of alternative RNA splicing. Disrupting the balance of HIV-1 RNA processing inhibits virus replication. Control is mediated in part through the action of host SR proteins whose activity, in turn, is regulated by multiple SR kinases (CLK1-4, SRPKs). Our studies demonstrate that SR kinases play disparate roles in modulating HIV-1 gene expression. Depletion of CLK1 enhanced HIV-1 gene expression, loss of CLK2 or SRPK1 suppressed it, while CLK3 depletion had a modest impact. Altered HIV-1 protein expression reflected changes in viral RNA accumulation. The opposing effects of CLK1 vs CLK2 depletion were due to action at distinct steps; loss of CLK1 increasing HIV-1 promoter function while depletion of CLK2 affected steps post-initiation. Loss of CLK1 also enhanced the response to several latency reversing agents, in part, by increasing the frequency of responding cells, consistent with a role in regulating provirus latency. To determine if modulation of SR kinase function by small molecules could be used to control HIV-1 replication, we screened the GSK library of kinase inhibitors and identified two compounds that suppress HIV-1 gene expression/replication with EC_{50}~ 50 nM. The compounds resulted in dramatic suppression of HIV-1 proteins and viral RNA accumulation with minimal impact on cell viability. The compounds inhibited CLK1 and CLK2 but not CLK3 function and altered expression/activity *in cellulo*. These findings demonstrate the unique roles individual SR kinases play in regulating HIV-1 gene expression and validate the targeting of these functions by small molecules for therapeutic benefit to enhance latency reversal, essential for "Kick-and-Kill" strategies, or to silence HIV protein expression for "Block-and-Lock" strategies.

Experimental Design

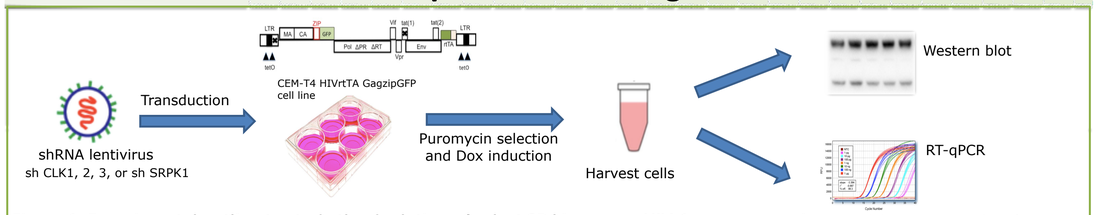

Figure 1. Experimental outline to study the depletion of select SR kinases on HIV-1 gene expression. Lentiviruses expressing shRNAs to individual CLKs or SRPK1 were transduced in CEM-T4 HIV-rtTA GagzipGFP (CEM-HIV*) cell line containing dox inducible HIV-1 provirus. After puromycin selection and doxycycline (Dox) induction for the expression of viral genes, lysates were harvested for western blotting and RNA analysis.

Results

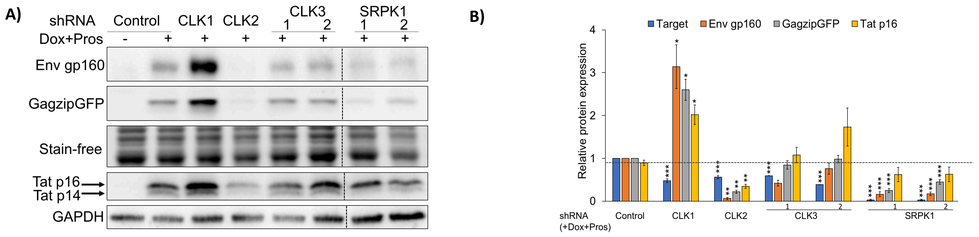

Figure 2. Depletion of select SR kinases has differential effects on HIV-1 protein levels. CEM-HIV* cells were infected with shRNA lentivirus against CLK1-3, or SRPK1 and selected with puromycin for 72 h. Follwing induction of HIV-1 provirus, cells harvested for western blotting. Shown in **(A)** are representative western blots for HIV-1 Env, Gag, and Tat. **(B)** Represents quantitation of the blots relative to Dox+Pros induced sh control and normalization to stain-free or GAPDH using Bio-Rad ImageLab software. Data are indicated as mean ± SEM, N ≥ 4 independent experiments, *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001.

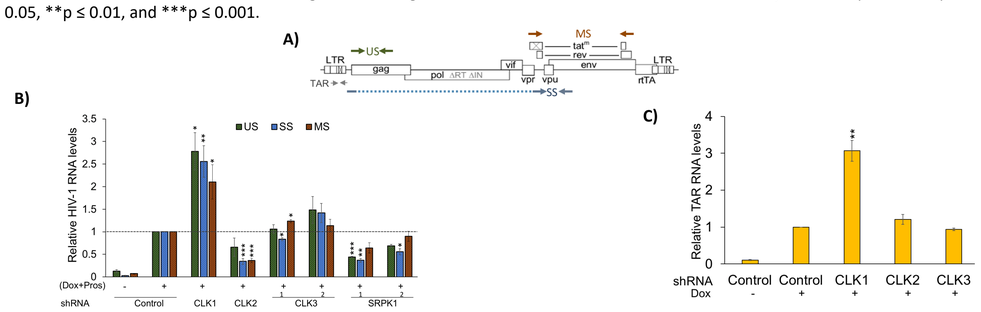

Figure 3. Depleting select SR kinases alter accumulation of HIV-1 RNA levels. (A) Schematic of the HIV-1 proviral construct showing the positions of the primers used for RT-qPCR analysis. **(B)** Quantification of viral unspliced (US), singly spliced (SS), and multiply spliced (MS) RNA levels in CEM-HIV* cells depleted of individual SR kinases. Viral mRNA levels were normalized to ß-actin and the mean mRNA levels expressed relative to sh control.**(C)** Quantification of TAR RNA levels in CLK-depleted RNA samples. Relative quantification was performed using comparative cycle threshold (CT) values. PUM1 was used as a reference gene to normalize the CT value and the fold changes were calculated using $2^{-\Delta\Delta CT}$ method. Data are indicated as mean ± SEM, N= 3 independent experiments, *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001.

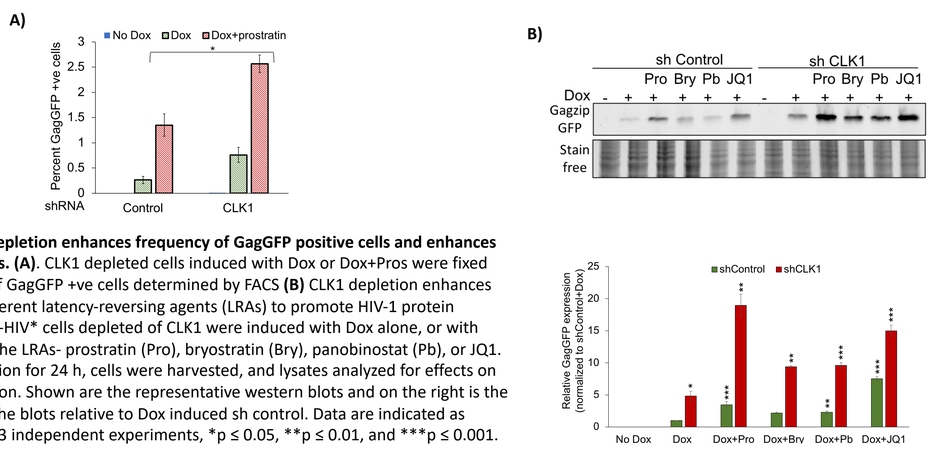

Figure 4. CLK1 depletion enhances frequency of GagGFP positive cells and enhances response to LRAs. (A). CLK1 depleted cells induced with Dox or Dox+Pros were fixed and frequency of GagGFP +ve cells determined by FACS **(B)** CLK1 depletion enhances the ability of different latency-reversing agents (LRAs) to promote HIV-1 protein expression. CEM-HIV* cells depleted of CLK1 were induced with Dox alone, or with Dox and one of the LRAs- prostratin (Pro), bryostratin (Bry), panobinostat (Pb), or JQ1. Following induction for 24 h, cells were harvested, and lysates analyzed for effects on GagGFP expression. Shown are the representative western blots and on the right is the quantitation of the blots relative to Dox induced sh control. Data are indicated as mean ± SEM, N=3 independent experiments, *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001.

Results

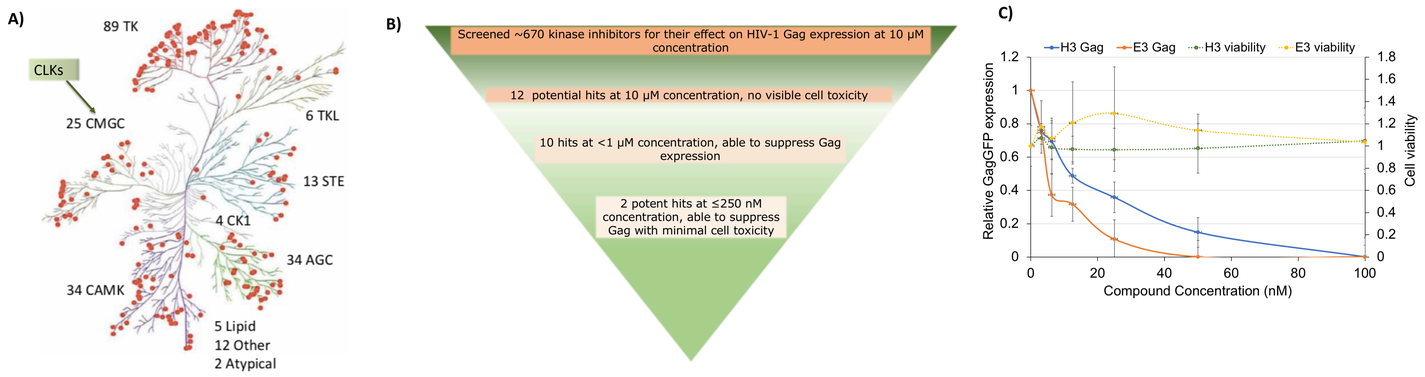

Figure 5. Identification of inhibitors of HIV-1 Gag expression from GSK PKIS library. (A) Kinome tree indicating kinase targets of PKIS. Red dots represent kinases targeted by inhibitors in the library. **(B)** Hits from the PKIS. Cell-based screen of GSK PKIS using Hela cell line containing GagGFP reporter inducible upon addition of Dox (HeLa C7 cells). **(C)** HeLa C7 cells were incubated with compounds 1H3 or 2E3 at increasing dose and HIV-1 gene expression induced with Dox for 24 h. Cells grown with or without Dox at 1% final concentration of DMSO served as positive and negative controls, respectively. Dose response on HIV-1 virion production in culture supernatant was measured by GagGFP levels in DMSO-treated samples. Effects of compounds on cell viability were assessed using alamarBlue assay. N>3 independent experiments.

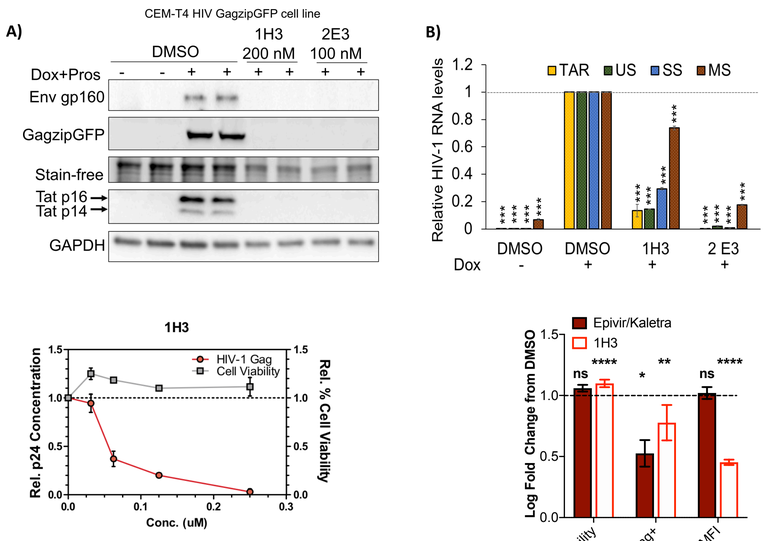

Figure 6. 1H3 and 2E3 inhibit HIV-1 gene expression in cell line. CEM-HIV* cells were treated with 1H3 (200 nM) or 2E3 (100 nM) and HIV-1 induced with dox+pros. After 24 h, cells were harvested for HIV-1 protein and RNA analyses. **(A)** Shown are representative HIV-1 Gag, Env, and Tat blots. **(B)** Quantification of viral TAR, US, SS, and MS RNA levels in cells treated with compounds relative to induced DMSO control. Data are indicated as mean ± SEM, N=4 independent experiments, *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001.

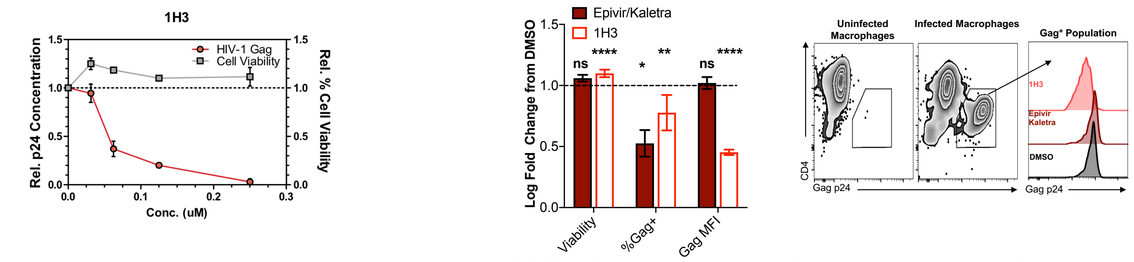

Figure 7. 1H3 inhibits HIV-1 replication in PBMCs and macrophages. (A) Effect of increasing concentration of 1H3 on HIV-1 BaL (MOI < 0.01) virion production in PBMCs. Indicated doses of 1H3 were added following infection, media harvested after 6 d, and virus replication measured by p24 ELISA. Values are expressed relative to p24 levels in DMSO-treated cultures at day 6. Cell viability was measured by trypan blue assay. **(B)** Effect of 1H3 on HIV-1 replication in human macrophages. Monocyte-derived macrophages from healthy donors were infected with HIV-1 89.6, treated with DMSO, a combined antiretroviral drug Epivir/Kaletra (1.5 μM/53 nM) or 1H3 (300 nM). After 10 d, cells fixed, stained to detect intracellular levels of Gag, and analyzed by flow cytometry to assess changes in %Gag positive cells and Gag MFI. Shown are the representative flow cytometry plots of uninfected and infected macrophages and histograms to show shifts in the Gag MFI with treatment. For the bar graph, summary data shown are expressed relative to DMSO treated samples (n=8 from 5 independent experiments, *p<0.05, **p<0.01, and ****p<0.0001.

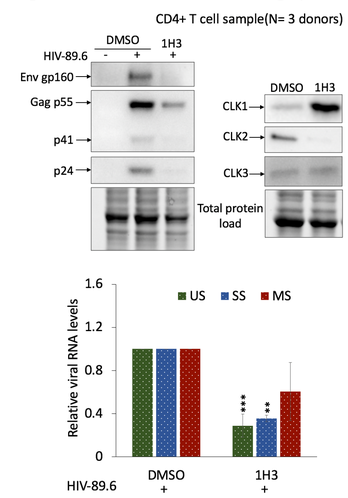

Figure 8. 1H3 and 2E3 inhibit HIV-1 gene expression in CD4+ T cells. CD4+ T cells from 3 different donors were infected with HIV-1 89.6 and treated with DMSO or 1H3 (300 nM). Cells were harvested 7 dpi for western and RNA analyses. **(A)** Shown are representative HIV-1 Gag, Env, and Tat blots. Shown on the right are CLK expression levels with 1H3 treatment relative to DMSO. **(B)** Quantification of viral US, SS, and MS RNA levels in 1H3-treated cells relative to DMSO. Data are indicated as mean ± SEM, N=4 independent experiments, *p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001.

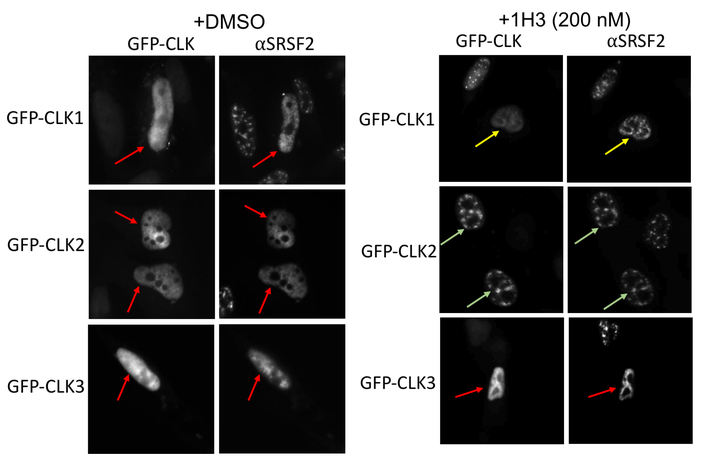

Figure 9. 1H3 inhibits CLK1 and CLK2 but not CLK3 function. Effect of 1H3 on SRSF2 subcellular distribution upon overexpression of CLKs in HeLa cells containing Dox inducible HIV-1 provirus. Cells were transfected with indicated GFP-CLK expression vectors and treated with DMSO or 200 nM 1H3 for 24 h and processed for immunofluorescence. Cells were stained with anti-SRSF2 antibody, a marker for nuclear speckles. Shown are the representative images of SRSF2 localization upon overexpression of CLK1, CLK2, or CLK3. Red arrows indicate loss of nuclear speckles due to CLK overexpression in DMSO treated or cells or 1H3-treated cells upon CLK3 overexpression. Green arrows indicate restoration of nuclear speckles in 1H3-treated cells upon CLK1 or CLK2 overexpression.

Summary

- Opposing effects of CLK1 and CLK2 on HIV-1 gene expression with CLK1 knockdown enhancing HIV-1 gene expression, while knocking down CLK2 reducing the same.
- Changes in viral protein levels in general correlate with viral RNA levels with CLK1 depletion increasing all classes of viral RNAs, while CLK2 depletion reducing the same.
- Depletion of CLK1 but not CLK2/3 alters HIV-1 transcription initiation as observed with increased TAR RNA levels i.e., initiated transcripts suggesting CLK1 acting to suppress the use of HIV-1 promoter while CLK2 promoting steps post-initiation.
- 1H3, identified from PKIS screening, inhibits HIV-1 gene expression in all cell systems analyzed, including primary CD4+ T cells and modulates CLK1/2 function and abundance.
- Elucidating changes in host protein phosphorylation affected by CLK depletion or 1H3/2E3 treatment will provide a framework to investigate novel factors regulating HIV-1 gene expression.

Acknowledgements

Molecular Genetics
UNIVERSITY OF TORONTO

CANADIAN FOUNDATION FOR AIDS RESEARCH

CIHR IRSC