# Histone deacetylase inhibitor Scriptaid reactivates latent HIV-1 promoter by inducing histone modification in *in vitro* latency cell lines

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**Abstract.** Human immunodeficiency virus type 1 (HIV-1) latency remains a major problem for the eradication of viruses in infected individuals undergoing highly active anti-retroviral therapy. By inhibiting HIV-1 gene expression and virus production, histone deacetylase (HDAC) may contribute to the quiescence of HIV-1 within resting $CD4^+$ T cells. A novel HDAC inhibitor, Scriptaid, has been found to have robust activity and lower toxicity compared to trichostatin A (TSA). We therefore investigated Scriptaid for its capability to reverse HIV-1 latency by inducing HIV-1 activation in the Jurkat T cell line containing latent HIV proviruses. We found that Scriptaid can activate HIV-1 gene expression in these latent infected cells by 2-15-fold over background levels, as analyzed by flow cytometry. Chromatin immunoprecipitation (ChIP) assays further revealed that the Scriptaid increased the acetylation level of histones H3 and H4 at the nucleosome 1 site of the HIV-1 long terminal repeat compared to mock treatment. In addition, Scriptaid can synergize with prostratin or tumor necrosis factor-α to activate the HIV-1 promoter, with relatively lower toxicity compared to TSA. These studies suggest the potential of Scriptaid in anti-latency therapies.

## Introduction

Human immunodeficiency virus type 1 (HIV-1) infection can now be treated effectively and become a chronic disease in many patients, due to the advent of the highly active anti-retroviral therapy (HAART). Unfortunately, whereas HAART regimens can significantly reduce levels of viral presence in plasma and lymphoid tissues, cessation of the therapies can quickly lead to the rebound of viral loads to pre-treatment levels, indicating that HAART treatment alone cannot completely eradicate HIV-1 (1,2). It is now widely believed that the presence of a long-lived, stable population of latently infected resting memory $CD4^+$ T cells is a major barrier to virus eradication (3-5). These latently infected cells can go dormant, and remain in the peripheral circulation and other tissues for years despite effective HAART (5). The decay rate of the HIV-1 latent reservoir has been found to be so low that their eradication during a human lifespan is unlikely (6,7). Therefore, any curative AIDS therapy should provide a solution to the HIV-1 latency hurdle (3,6). Under such a context, several 'shock and kill' strategies have been proposed to purge the viral reservoirs by deliberately forcing HIV-1 gene expression in latent infected cells, along with HAART treatment to prevent spreading the infection by newly synthesized viruses (8-10). Some treatments being considered are able to reduce the size of latent reservoirs by directly killing from the virus-aroused cytopathic action or the intervenience from the immune system.

Mechanisms that allow HIV-1 to establish and maintain latency can be multi-factoral. Interestingly, several recent lines of evidence suggest that histone deacetylases (HDACs) are critical regulators of HIV latency. Margolis and colleagues demonstrated that the transcription factor YY1 can act as a repressor of HIV transcription by recruiting HDAC-1 to the provirus (11). Later studies demonstrated that nuclear factor (NF)-κB p50 homodimers (12), AP-4 (13), CTIP2 (14), Sp1 and c-Myc (15), and CBF-1 (16) can participate in HDAC-1 recruitment. HDAC-2 and -3 can also associate with the HIV long terminal repeat (LTR), and play a role in the repression of LTR expression (14,17). It has been shown that the disruption of HDAC-1 recruitment to LTR, resulting from the inhibition of HDAC activity by global HDAC inhibitors, leads to LTR activation and the escape of viral expression in both cell line models and primary cells obtained from patients (10,18-26). Furthermore, HDAC inhibitor valproic acid (VPA) and suberoylanilide hydroxamic acid (SAHA; vorinostat) can induce viral outgrowth from resting CD4 T cells of aviremic patients undergoing HAART (27,28). These observations have led to the investigation of HDAC inhibition as a putative therapeutic approach to induce HIV from latency.

Scriptaid, the 6-[1,3-dioxo-1H,3H-benzo(de)isoquinolin-2-yl]-hexanoic acid hydroxyamide, was identified by screening a library of 16,320 compounds (DiverSet, Chembridge, San Diego, CA) using a platform that contains a stably integrated

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transcriptional reporter (29). Scriptaid has been reported to inhibit growth and induce differentiation and/or apoptosis in a variety of cancer cells such as breast, endometrial, ovarian cancers, squamous carcinoma and colorectal cancer cell line (29-33). The molecule is considered to be a novel HDAC inhibitor with robust activity and relatively lower toxicity than trichostatin A (TSA) (29). However, little is known about its effect in inducing HIV expression in latently infected cells. This study aimed to investigate the effect of Scriptaid on the epigenetic change at HIV-1 LTR and the expression induction of the latent viruses. The synergistical effect between Scriptaid and tumor necrosis factor (TNF)-α/prostratin/5-azacytidine (5-Aza) were analyzed in J-Lat Tat-GFP Clone A7 cells. In addition, the effects of Scriptaid on cell viability were also assessed in human embryonic kidney 293 cells and Jurkat cells. Our results suggest that Scriptaid induces reactivation of latent HIV-1 transcription, increases acetylation of histones H3 and H4 at the nucleosome 1 (nuc-1) site of the HIV-1 LTR, and synergizes with prostratin or TNF-α to activate the HIV-1 promoter in the Jurkat T cell line. As such, Scriptaid should be further explored as a viable candidate for anti-latency therapies.

## Materials and methods

*Chemical treatment and cell culture*. Scriptaid, the 6-(1,3-dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide, was purchased from Alexis Biochemicals (ALX-270-298). Recombinant human TNF-α was purchased from Chemicon International. 5-Aza and TSA, were purchased from Sigma (A1287, T8552). Prostratin was purchased from LC laboratories (P-4462). Scriptaid, TSA, 5-Aza and prostratin were dissolved in anhydrous dimethyl sulfoxide (DMSO) to a 100-mM stock solution.

A7 cells, a latently infected Jurkat cell line encoding the green florescence protein (GFP) as a marker for Tat-driven HIV LTR expression were kindly provided by the NIH AIDS Research and Reference Reagent Program (from Dr Eric Verdin) (34,35). A7 cells, as well as Jurkat cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin and 100 μg/ml of streptomycin (Invitrogen) at 37˚C under 5% $CO_2$. Human embryonic kidney 293 cells (HEK 293) were purchased from the American Type Culture Collection and were grown at 37˚C in Dulbecco's modified Eagle's medium (DMEM) (Gibco) with 10% fetal bovine serum.

*Visualization of GFP*. Expression of GFP as a marker for reactivation of HIV-1 promoter in A7 cells was observed by fluorescence microscopy. After treatment with Scriptaid or TSA with the indicated concentrations at the different times, A7 cells were viewed using a Nikon fluorescence microscope. All microscope samples were photographed using a Nikon E2 digital camera.

*Flow cytometry*. A7 cells were washed with phosphate-buffered saline (PBS) and incubated with the indicated concentration of Scriptaid at different time points. Cells were washed and resuspended in PBS containing 2% paraformaldehyde. GFP expression was measured by FACScan (Becton Dickinson FACScan Flow Cytometer), and FACS data were analyzed with FlowJo software (Tree Star, CA). Live cells were gated and two parameter analysis was used to differentiate GFP-associated fluorescence from background fluorescence. A total of 10,000 gated events were collected and data represent the percentage of GFP-expressing cells in total gated events.

*Cytotoxicity assay*. HEK 293, Jurkat cells, J-Lat Tat-GFP Clone A7 cells were treated with or without Scriptaid or TSA for 24 h, at of 25, 50, 100, 200 and 400 nM. To measure proliferation and viability in the presence of drugs, cells were subjected to an MTT assay (23). In brief, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was placed in solution with PBS (5 mg/ml) and used to measure cellular proliferation. Cells ($1x10^3$) were incubated in 100 μl of culture medium for 48 h in 96-well plates, and 10 μl of MTT solution was added. After 4 h incubation, 50 μl of solubilization solution (20% SDS) was added, and cells were then incubated at 37˚C for 16 h. In this assay, MTT was cleaved to an orange formazan dye by metabolically active cells. The dye was directly quantified using an enzyme-linked immunoabsorbent assay reader at 540 nm. The 50% cytotoxic concentration (CC50) was determined from the dose response curve. All experiments were performed independently at least three times in triplicate per experimental point.

*Western blotting*. To determine the expression of various proteins in A7 cells following various stimulations, Western blot analysis was performed as described previously (24). Briefly, cells were harvested by trypsinization and lysed in lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, 1 mM DTT, 0.1% SDS, 1% Nonidet P-40, 1 mg/ml leupeptin and soybean trypsin inhibitor, 0.5 mM PMSF) for 30 min on ice. Approximately 50-150 mg of protein extracts were loaded on 12% polyacrylamide gel. Next, separated proteins were electroblotted from the gel onto nitrocellulose membrane and then blocked with a blocking buffer (5% non-fat dry milk in 1x TBST, i.e. 20 mM Tris-HCl, pH 7.6 containing 0.8% NaCl and 0.1% Tween-20) at room temperature for 1 h. The membrane was incubated with primary antibodies in blocking buffer, followed by incubation with second antibodies. Bands were visualized using the ECL Western blotting system. Proteins were electro-transferred onto nitrocellulose (GE Healthcare, USA), and subsequently immunoblotted with primary antibodies, rabbit anti-human anti-acetyl histone 3 (Ac-H3, Milipore), mouse monoclonal antibodies against actin (AC-74, Sigma, USA) and appropriate secondary antibodies, i.e., goat anti-mouse IgG (1:1,000) or goat anti-rabbit IgG (1:1,000). Afterward, proteins of interest were visualized using ECL chemiluminescence system (Santa Cruz Biotechnology, USA).

*Chromatin immunoprecipitation (ChIP) assay*. ChIP analyses were performed according to Milipore Company online protocol and a procedure described previously (26-28). Briefly, A7 cells ($1x10^7$ cells/100-mm dish) were treated with or without Scriptaid (200 nM) or TSA (200 nM) for 4 h, then crosslinked with formaldehyde to a final concentration of 1% for 10 min at 37˚C. Cells were washed in ice-cold PBS twice, resuspended in sodium dodecyl sulfate (SDS) lysis buffer and incubated for 20 min on ice. Lysates were sonicated to produce DNA fragments of an average length at 500-

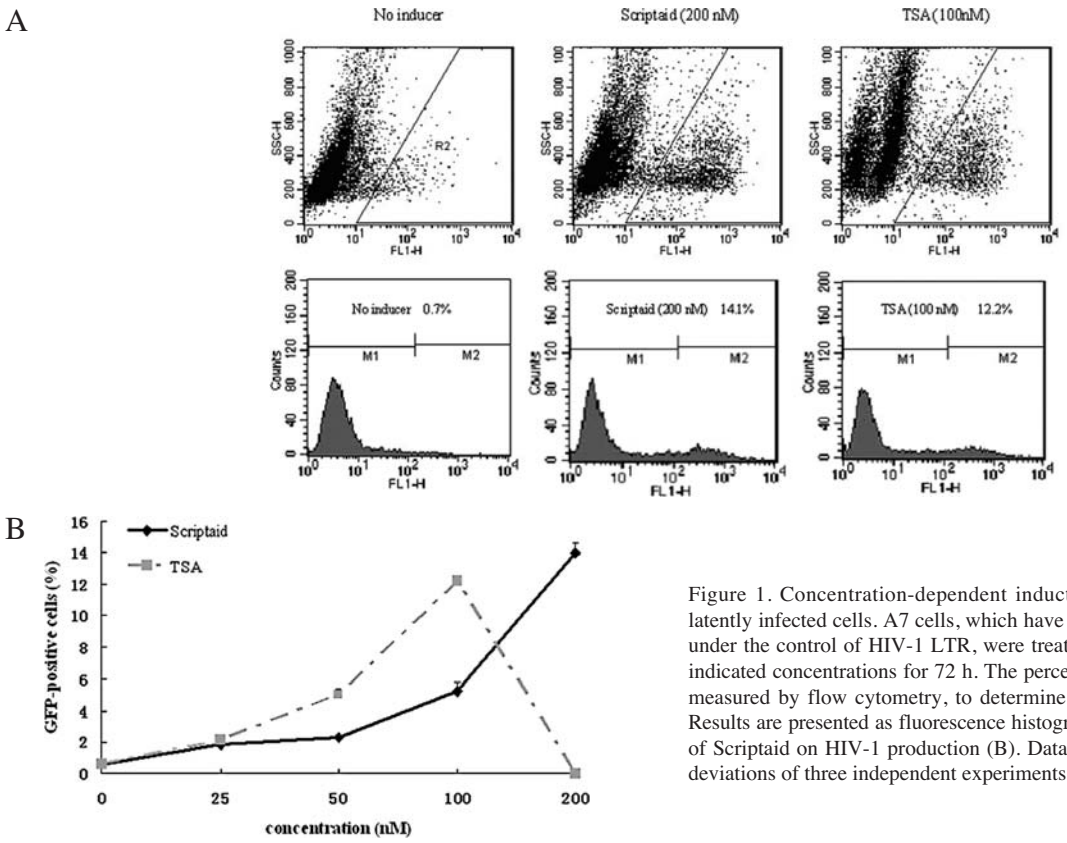


Figure 1. Concentration-dependent induction of the HIV-1 promoter in latently infected cells. A7 cells, which have an integrated GFP/Tat construct under the control of HIV-1 LTR, were treated with Scriptaid or TSA at the indicated concentrations for 72 h. The percentage of GFP-positive cells was measured by flow cytometry, to determine the level of HIV-1 expression. Results are presented as fluorescence histograms (A). A dose-response curve of Scriptaid on HIV-1 production (B). Data represent the means ± standard deviations of three independent experiments.

1,000 bp. Extracts were then diluted 10-fold with immunoprecipitation (IP) dilution buffer. Diluted sample (10%, 200 $\mu$l) was used as input control and 2 ml of diluted sonicated extract was used for IP. After pre-clearing with Protein G Agarose for 30 min at 4˚C with agitation, Ac-H3, Ac-H4 (Milipore) or rabbit preimmune immunoglobulin G (Milipore) were incubated overnight at 4˚C with rotation. To collect immune complexes, appropriate Protein G Agarose mixture was added to each reaction mixture and the mixture was rotated for 2 h at 4˚C. Beads were centrifuged and washed for 5 min at 4˚C with each of the following: low salt, high salt, LiCl, and Tris-EDTA buffer. Immune complexes were eluted by incubation in elution buffer, and supernatants were isolated and further incubated for 4 h at 65˚C to reverse cross-linking. Input controls were treated in the same manner at this point. After reverse cross-linking, proteinase K was added and the mixture was incubated for 1 h at 45˚C. DNA was deproteinized by phenol-chloroform extraction and ethanol precipitation in the presence of 20 $\mu$g of glycogen. DNA was washed in 70% ethanol, dried, and resuspended in 20 $\mu$l of TE. For a typical PCR, 2-5 $\mu$l of the 20 $\mu$l total DNA was amplified for 32-34 cycles and visualized by ethidium bromide staining of agarose gels. Primers for HIV-1 LTR, LTR-109 forward (5-TACAAGGGACTTTCCGCTGG-3) and LTR-82 reverse (5-AGCTTTATTGAGGCTTAAGC-3). DNA products of ChIP were quantitated by real-time PCR (ABI Prism 7900 Real-Time PCR System, USA). To calculate the relative acetylation levels, PhosphorImager data of the amounts of PCR product obtained for immunoprecipitated chromatin samples were normalized against the amount of PCR product obtained for input DNA. All values represent the average of at least three independent experiments.

## Results

*Scriptaid induced latent HIV-1 transcription*. The ability of Scriptaid to induce HIV-1 expression was investigated by measuring the percentage of GFP-expressing cells in A7 cells, a latently infected Jurkat cell line encoding the GFP as a marker for Tat-driven HIV LTR expression. The percentage of GFP-expressing cells was measured by flow cytometry. Three days after treatment with Scriptaid (200 nM), maximum expression of HIV-1 activity was obtained, with GFP expression being at background level in 14.1% of the cells (Fig. 1A), while 100 nM TSA induced GFP expression in 12.2% of the cells (Fig. 1A). As shown in Fig. 1B, addition of nanomolar concentrations of Scriptaid to the culture medium for 3 days increased the percentage of GFP-expressing cells by 2-15-fold over background levels. But induction by TSA was similar to that observed following Scriptaid treatment, TSA was found to be very toxic to A7 cells at a routinely used concentration of 200 nM. These results were confirmed by fluorescence microscopy (data not shown). Result shown in Fig. 1 confirmed that Scriptaid induced HIV LTR reactivation, indicating dose-dependent effects of Scriptaid on HIV-1 transcription.

We performed a kinetics experiment to analyse the kinetics of HIV LTR expression, in A7 cells with and without Scriptaid (200 nM) or TSA (100 nM) treatment for 1-4 days. At each time point, GFP-expressing cells were assayed by flow cytometry techniques. After A7 cells were treated with Scriptaid, the percentage of GFP-expressing cells increased with increasing time. Four days after treatment, we observed that the percentage of GFP-expressing cells were increased to 16.7% for Scriptaid, 12.6% for TSA (Fig. 2A). The kinetics

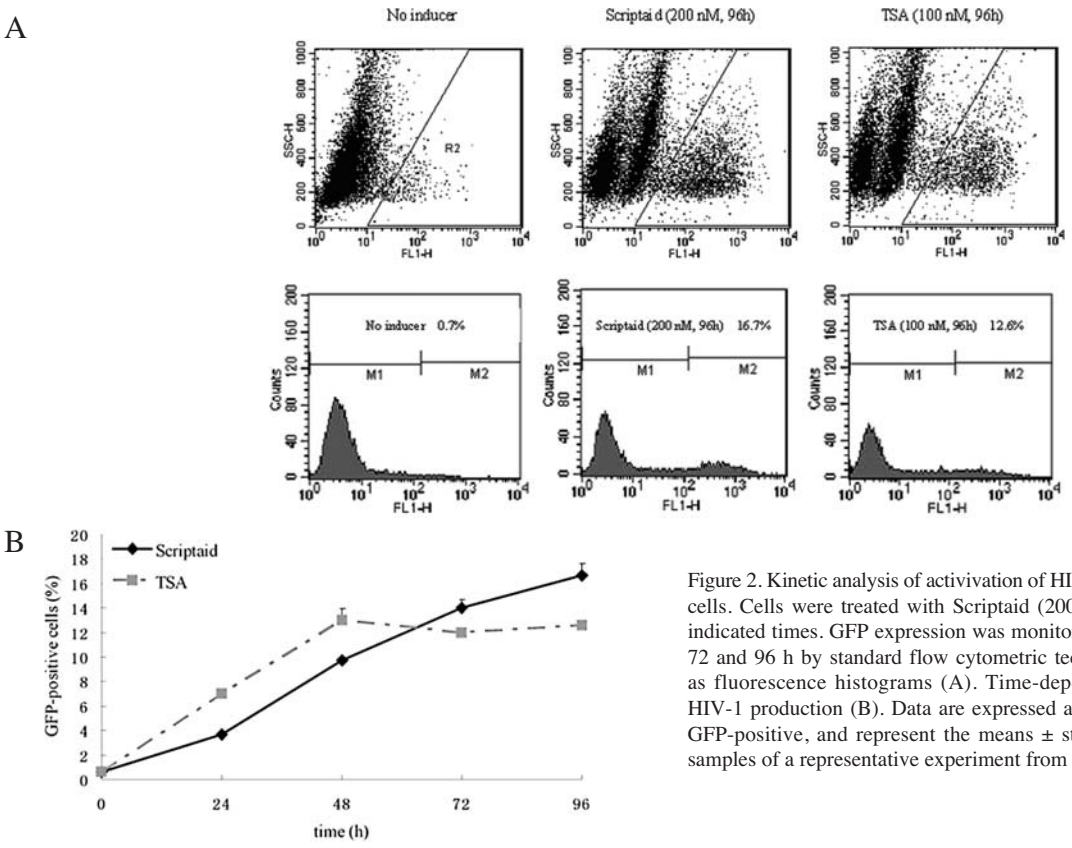


Figure 2. Kinetic analysis of activivation of HIV-1 promoter in latently infected cells. Cells were treated with Scriptaid (200 nM) or TSA (100 nM) at the indicated times. GFP expression was monitored in gated live cells at 24, 48, 72 and 96 h by standard flow cytometric techniques. Results are presented as fluorescence histograms (A). Time-dependent effects of Scriptaid on HIV-1 production (B). Data are expressed as percentage of cells becoming GFP-positive, and represent the means ± standard deviations of triplicate samples of a representative experiment from two independent experiments.

of HIV LTR expression showed a rapid increase the first two days and plateaued by day 4 (Fig. 2B). These results indicate time-dependent effects of Scriptaid on HIV-1 transcription.

*Scriptaid synergizes with prostratin or TNF-α to activate the HIV-1 promoter in the Jurkat T cell line*. To test if synergistic activation exists when Scriptaid is combined with 5-Aza, TNF-α, and prostratin, latent HIV-infected cells were teated with single or combined drugs for 48 h, and assayed by flow cytometry. As shown in Fig. 3, in the absence of stimulation, A7 cells expressed almost no GFP, indicating a blockage of viral transcription. After stimulation of A7 cells with single or combined drugs, the results revealed the percentage of GFP-expressing cells, 2.3% in Scriptaid (50 nM), 34.1% in TNF-α (10 ng/ml), 3.01% in prostratin (100 nM), 1.2% in 5-Aza (10 ng/ml), 37.4% in Scriptaid/TNF-α, 6.4% in Scriptaid/prostratin, 2.82% in Scriptaid/5-Aza. The percentage of GFP-expressing positive cells of two groups (Scriptaid/TNF-α or Scriptaid/prostratin) are more than the sum of the single drug treatment. Our data indicate that Scriptaid synergizes with prostratin or TNF-α to activate the HIV-1 promoter in the Jurkat T cell line.

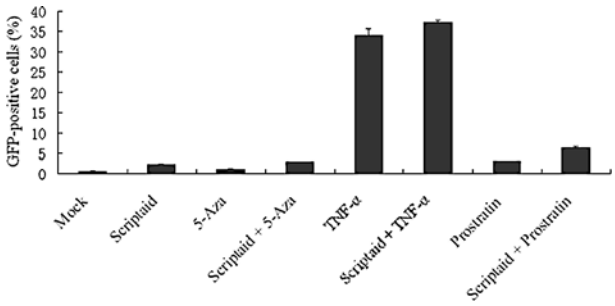


Figure 3. Synergistic activation of HIV-1 promoter by Scriptaid and 5-Aza, TNF-α and prostratin. A7 cells were mock treated or treated with Scriptaid (50 nM), TNF-α (10 ng/ml), 5-Aza (100 nM), prostratin (100 nM), Scriptaid + TNF-α, Scriptaid + 5-Aza or Scriptaid + prostratin for 48 h. The effect of synergistic activation of HIV-1 promoter was determined by quantifying the GFP-positive cells using flow cytometry 72 h after treatment. Summary of synergistic activation assays are presented as histograms. Data represent the means ± standard deviations of three independent experiments.

*Scriptaid has low toxicity compared to TSA in vitro*. To measure viability of HEK 293, Jurkat and A7 cells treated with or without Scriptaid or TSA for 24 h, normal human cells, in the presence of drugs, were subjected to an MTT assay. We found a significant correlation between the concentration of HDAC inhibitors and MTT expression in the HEK 293, Jurkat and A7 cell lines (Fig. 4). The CC50 in the HEK 293, Jurkat and A7 cell lines for Scriptaid, was 371, 186 and 161 nM, respectively. The CC50 in the HEK 293, Jurkat and A7 cell lines for TSA, was 181, 76 and 72 nM, respectively. This result indicates that Scriptaid has relatively low toxicity compared to TSA.

*Scriptaid increases acetylation of histone*. To determine whether Scriptaid induces acetylation of histones, we treated A7 cells with 100, 200 and 400 nM Scriptaid for 8 h and performed immunoblot analysis using antibodies for acetylated histone H3. As shown in Fig. 5, exposure of cells to 100-400 nM Scriptaid resulted in a significant increase in acetylated histone.

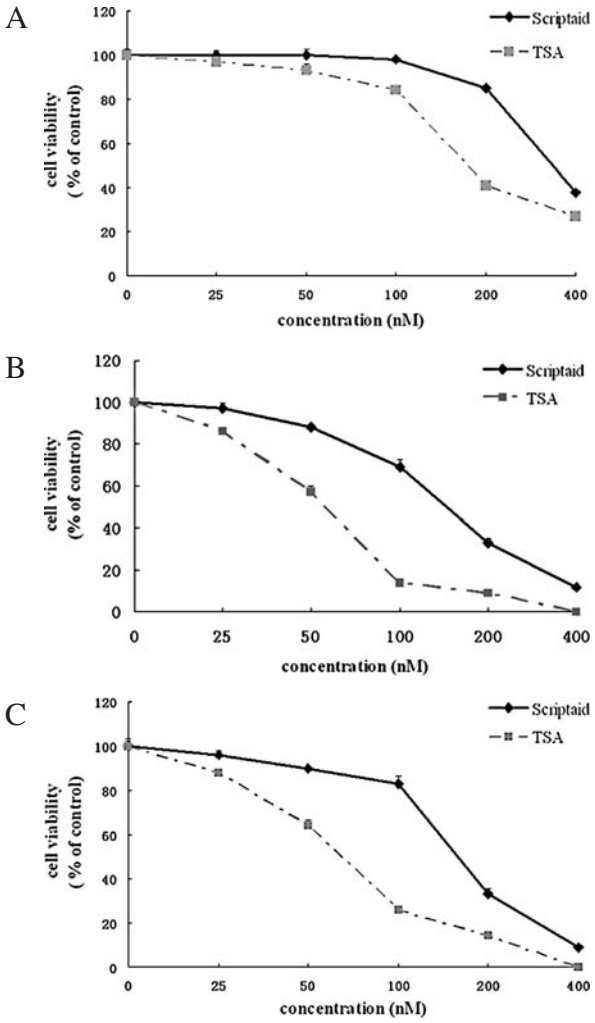


Figure 4. Summary of cell viability assays using Scriptaid and TSA. HEK-293 (A), A7 cells (B) and Jurkat T cells (C) were treated with Scriptaid or TSA at the indicated concentrations for 24 h, and measured by the methyl tetrazolium (MTT) method. Results are presented as a percentage of the O.D. (P=550) of untreated controls subtracted from background. Data represent the means ± standard deviations of three independent experiments.

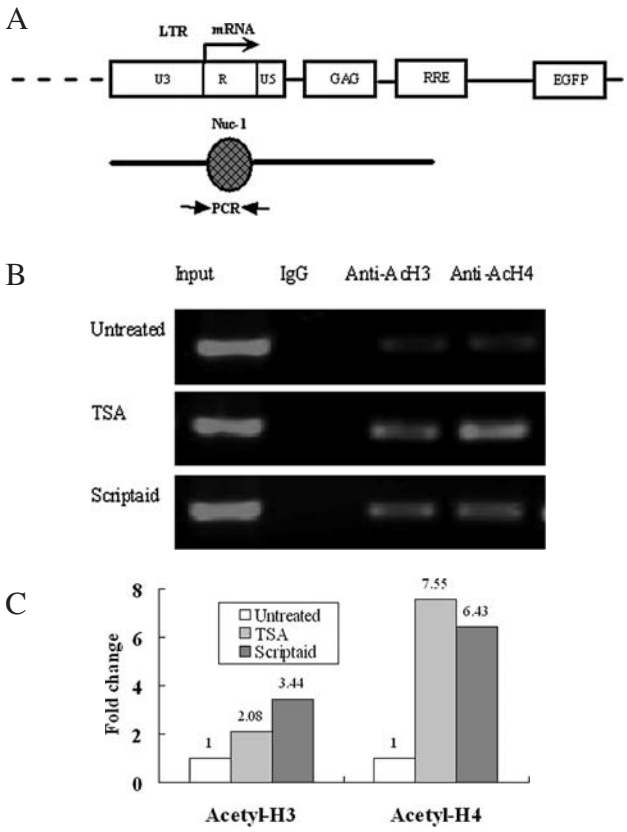


Figure 6. Scriptaid exposure acetylates histones at nuc-1 of the HIV LTR. (A) Diagram of the LTR promoter in the integrated HIV-derived vector. The positions of nucleosomes are indicated with respect to the HIV promoter, including nuc-1, which is remodeled upon HIV transcriptional activation. Positions of primers used for PCR amplification are indicated. (B) Chromatin fragments from A7 cells cultured with or without Scriptaid or TSA for 4 h were immunoprecipitated with antibody to acetylated (Ac) histones H3 or H4 or control normal rabbit serum (IgG). PCR primers for the LTR promoters were used to amplify the DNA isolated from the immunoprecipitated chromatin as described in Materials and methods. (C) Each ChIP experiment was repeated three times to confirm reproducibility of results and data shown are an average of relative amounts of accumulated histones at LTR promoter compared to the control.

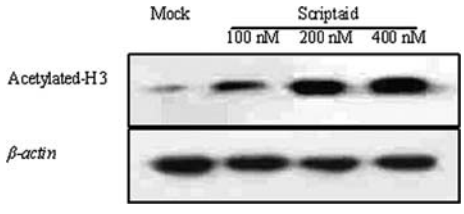


Figure 5. Effect of Scriptaid on the acetylation of histones in latent infected cells, as measured by Western blot analysis. A7 cells were treated without (mock) or with Scriptaid (100, 200, 400 nM), and cell lysates were harvested after 8 h. Western blot analysis was performed with antibodies acetylated histone H3. The amount of protein was normalized by comparison to levels of ß-actin.

*Scriptaid induces histone modifications at the nuc-1 region of HIV-1 LTR*. To examine whether histone acetylation modification occurred at the LTR promoter after Scriptaid treatment, we employed the ChIP assay. Chromatin fragments from A7 cells cultured with or without Scriptaid (200 nM) or TSA (200 nM) for 4 h were immunoprecipitated with antibodies to acetylated histones H3 or H4 or rabbit preimmune immunoglobulin G. DNA from the immunoprecipitates was isolated, and PCR was performed using HIV promoter primers spanning the nuc-1 region of LTR (Fig. 6A). We observed that the amount of acetylated histone proteins, Ac-H3 and Ac-H4, bound to the core promoter region within HIV-1 LTR were increased by treatment of cells with Scriptaid or TSA (Fig. 6B). Normal immunoglobulin control showed no specific 190-bp fragment (Fig. 6B). The percentage of input for each immunoprecipitation was calculated and the relative fold occupancy of acetylated histones reported. Fold increase in immunoprecipitation over mock antibody immunoprecipitation is shown in Fig. 6C. We found that Scriptaid treatment increased the acetylation of H3 (3.44-fold) and H4 (6.43-fold) within nuc-1 in A7 cells, while TSA treatment increased the acetylation of H3 (2.08-fold) and H4 (7.55-fold) within nuc-1.

## Discussion

Established early during infection, the long-lived pool of latently infected resting memory T cells is incapable of being

removed by both the host immune system and the most potent antiretrovirals. Treatments targeting this persistent reservoir are likewise essential for the eradication of HIV infection. One molecular mechanism involved in the maintenance of proviral quiescence is the activity of HDACs as the viral promoter. Therefore, the blockage of HDACs has become an attractive approach for inducing reactivation of HIV-1 reservoirs (36). HDAC inhibitors present several advantages for HIV-1 purging strategies (37). First, HDAC inhibitors do not induce proliferation or activation of T cells (38-40), which meets the criteria of an ideal adjuvant agent that can induce expression of HIV-1 without inducing global T-cell activation to prevent the generation of new target cells for the newly synthesized viruses. Indeed, recent data suggest that HDAC inhibitors inhibit $CD4^+$ T-cell proliferation in a dose-dependent manner (41). Second, although infected resting $CD4^+$ T cells represent the major long-term HIV-1 reservoirs, other cell types (in the central nervous system or other tissues) may also contribute to the persistence of HIV-1 under HAART (42,43). The broad spectrum of cell types that HDAC inhibitors can act on renders them another layer as an advantage, in contrast to agents that can only specifically induce T cells. Thirdly, HDAC inhibitors are relatively non-toxic compared to normal cells, as reflected in some novel cancer therapy studies (44). In fact, >12 different HDAC inhibitors are undergoing clinical trials (44), and more recently, SAHA (marketed as Vorinostat), a selective for the Class I and some activity against the Class II HDAC inhibitor was approved by the Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma (45). Moreover, another HDAC inhibitor, valproic acid, has already been applied to HIV-positive patients for the treatment of neurological pathologies (46). Studies have demonstrated the ability of valproic acid to cross the blood-brain barrier and the possibility of using HDAC inhibitors as adjuvants to HAART.

Scriptaid is a newly identified HDAC inhibitor with strong potency. In this study, we investigated the ability of Scriptaid to induce HIV-1 expression in A7 cells. A7 cells constitute an *in vitro* model of HIV-1 latency in which expression of integrated provirus can be induced by activation, and quiescence can be restored once the activating stimulus is withdrawn (34,35). We used TSA as a reference standard for comparison of results. TSA is a non-specific inhibitor of both classes of HDACs when used in the upper-nanomolar range of concentrations. Our results showed that the Scriptaid was not only effective in inducing HIV-1 LTR expression in A7 cells, but also with lower toxicity than TSA in various human cells, indicating that Scriptaid has the potential of a drug candidate for HIV-1 eradication when used in combination with HAART.

The establishment of specific restrictive chromatin structures along the HIV LTR is believed to have an important role in reducing Tat levels during the establishment of latency (47,48). Verdin *et al* showed that the chromatin structure of the HIV LTR contained two well-ordered nucleosomes called nuc-0 and nuc-1 (48). Nuc-0 is positioned immediately upstream of the enhancer (-415 to -255), while nuc-1 is very close to the viral RNA start site. Reactivation of HIV transcription requires histone acetylation and remodeling of the critical nuc-1 by SWI/SNF (49-51). To document changes induced by Scriptaid in histone H3 and H4 acetylation at nuc-1 in A7 cells, ChIP assay was performed in A7 cells containing a single integrated HIV genome. Our results showed that Scriptaid increased acetylation of histone H3 and H4 at the nuc-1 region of HIV-1 LTR, which is associated with HIV transcription in A7 cells. This is consistent with a number of studies reporting that reactivation of HIV transcription requires histone acetylation and remodeling of the critical nuc-1 (27,52,53). These observations suggest that the acetylation level of histone in the nuc-1 region of HIV-1 LTR is a key element regulating HIV-1 transcription.

In order to maximize the activation of latently infected cells and to reduce cell toxicity, we examined the presence of synergy between Scriptaid/TNF-α, Scriptaid/prostratin (non-tumor-promoting phorbol ester that lacks tumor promotion and coupled with its ability to block viral spread yet induce latent proviral expression) (54-56), and Scriptaid/5-Aza in A7 cells. Our results showed that under the co-treatment of Scriptaid/prostratin or Scriptaid/TNF-α, HIV-1 expression was induced with higher proportions 6.4 or 37.4% of A7 cells than being treated by the Scriptaid alone, indicating that Scriptaid synergistically reactivates HIV-1 production with prostratin or TNF-α in the latency cell line model. These results are similar to other studies (26) in which the proportion of J-Lat cells displaying GFP epifluorescence was increased by prostratin + HDACI cotreatments, compared to treatments with the compounds alone. However, we did not find a synergistic activation in the induction with Scriptaid/5-Aza. These observations are consistent with the reports from Jordan *et al* that treatment with 5-axa-2-deoxycytidine, an inhibitor of DNA methylation, had little effect on the fraction of cells induced to transcribe HIV alone or in combination with a histone deacetylase inhibitor (34). Further experiments are needed to elaborate on the molecular mechanisms of these two different types of stimulants.

Collectively, our results show that the novel HDAC inhibitor Scriptaid induces the reactivation of HIV-1 expression by increasing the acetylation level of histone H3 and H4 at the nuc-1 region of HIV-1 LTR in A7 cells. We also found that the Scriptaid can synergize with prostratin or TNF-α to activate the HIV-1 promoter with relatively lower toxicity compared to TSA. These results indicate the candidacy of Scriptaid to be further explored in anti-latency therapies.

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