Zinc-Finger Nucleases Induced by HIV-1 Tat Excise HIV-1 from the Host Genome in Infected and Latently Infected Cells

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INTRODUCTION

Highly active anti-retroviral therapy (HAART) cannot clear infected cells harboring HIV-1 proviral DNA from HIV-1-infected patients. We previously demonstrated that zinc-finger nucleases (ZFNs) can specifically and efficiently excise HIV-1 proviral DNA from latently infected human T cells by targeting long terminal repeats (LTRs), a novel and alternative antiretroviral strategy for eradicating HIV-1 infection. To prevent unwanted off-target effects from constantly expressed ZFNs, in this study, we engineered the expression of ZFNs under the control of HIV-1 LTR, by which ZFN expression can be activated by the HIV-1 (Trans-Activator of Transcription) Tat protein. Our results show that functional expression of ZFNs induced by Tat excise the integrated proviral DNA of HIV-NL4-3-eGFP in approximately 30% of the population of HIV-1-infected cells. The results from HIV-1-infected human primary T cells and latently infected T cells treated with the inducible ZFNs further validated that proviral DNA can be excised. Taken together, positively regulated expression of ZFNs in the presence of HIV-1 Tat may provide a safer and novel implementation of genome-editing technology for eradicating HIV-1 proviral DNA from infected host cells.

RESULTS

Analysis of the Activity of the Promoter

The HIV-1 LTR promoter is responsible for viral transcription initiation, whereas Tat protein, an HIV-1-expressed protein, can recruit transcription elongation complexes to the TAR of the HIV-1 5’ LTR to promote transcription elongation.9–11 In our study, we first detected the effects of Tat protein on the LTR promoter by luciferase assay. Here we used one TAR and tandem TAR repeat (2 × TAR)
located in the LTR to promote reporter gene luciferase expression. The schematics of two luciferase gene expression cassettes driven by the LTR or LTR-2 × TAR in the presence or absence of Tat are shown in Figure 1A. We co-transfected HEK293T cells with a reporter gene vector comprising the luc gene driven by LTR (pLTR-luciferase) or LTR-2 × TAR-luciferase (pLTR-2 × TAR-luc) in the presence or absence of the Tat plasmid. The luciferase activity was measured after 72 hr transfection. The data were analyzed by normalizing the Tat-transfected group to the pcDNA3.1(-) transfected group. Data represented the mean ± SD of three independent experiments.

Analysis of Different Amounts of Tat-Induced Effects on Luciferase Expression
We further tested the dose-dependent effects of Tat on LTR or LTR-2 × TAR promoter activity. pLTR-luciferase was transfected with pCMV-Tat at the indicated amount into HEK293T cells. Data represent the mean ± SD of three independent experiments.

Analysis of the Activity of Inducible ZFNs by Transient Luciferase Assay
Previous study have suggested that ZFN expression plasmids targeting viral LTRs (ZFN-LTRs) can specifically and efficiently excise HIV-1 proviral DNA from infected and latently infected human T cells. In this study, we used previously reported ZFNs to target LTR under the control of a viral promoter. For this purpose, we constructed two sets of regulated ZFNs under the control of the HIV-1 LTR or LTR-2 × TAR promoter, termed pLTR-ZFN and pLTR-2 × TAR-ZFN (Figure S1) and then monitored the protein expression of the two sets of regulated ZFNs by western blot. Because the ZFN-LTR pair (ZFN-LTR-L and ZFN-LTR-R) under the control of the HIV-1 LTR or LTR-2 × TAR promoter was isolated by T2A "self-cleaving" peptide in both constructs, the result demonstrated two bands in both constructs after detection of the infused FLAG tag.
the percentage of EGFP+ cells to determine the activity of ZFNs. To this end, YA were left untransfected (mock) or nucleofected with C2 TAR-ZFN, or pLTR-2 T2A driven by elongation factor 1 vector into HEK293T cells. The plasmid expressing ZFNs isolated excision by luciferase assay. An HIV-1 pseudovirus vector carrying ure S2). Next, we examined the inducible ZFN-induced viral DNA infection, as studied previously. We could monitor the change in Cytotoxicity in HIV-1-Infected Cells

To determine whether ZFN treatment of HIV-1-infected cells could induce HIV-1 proviral DNA excision by the inducible ZFNs, we ligated the PCR product into the pLTR-ZFN vector, followed by detecting virus p24 antigen content in the culture supernatants at the indicated time by ELISA. We found that HIV-1 p24 production decreased up to ~40% in HIV-1-infected CD4+ T cells transfected with pLTR-ZFN or pLTR-2 × TAR-ZFN compared with the pcdNA3.1(−)-transfected group (Figure 6). Collectively, the data confirmed the excision of integrated HIV-1 proviral DNA mediated by ZFNs in HIV-1-infected human primary CD4+ T cells.

ZFN-Mediated Excision of Integrated HIV-1 Proviral DNA in Human Primary T Cells

To determine whether ZFNs could induce integrated HIV-1 proviral DNA excision in HIV-1-infected human primary CD4+ T cells, CD4+ T cells were infected with HIV-1 NL4-3 viruses and then nucleofected with pLTR-ZFN or pLTR-2 × TAR-ZFN vector, by following that the integrated HIV-1 5 LTR sites had been joined to the ZFN half target site at 3 LTR locus, indicating left and right ZFP protein expression in both cassettes (Figure S2). Next, we examined the inducible ZFN-induced viral DNA excision by luciferase assay. An HIV-1 pseudovirus vector carrying luc (pHIV-NL4-3-luc) was transfected with pcdNA3.1(−), pLTR-ZFN, or pLTR-2 × TAR-ZFN and the internal control pRL-SV40 vector into HEK293T cells. The plasmid expressing ZFNs isolated by T2A driven by elongation factor 1z (EF1z), named pEF1z-ZFN, was used as a positive control. After 72 hr transfection, cells were collected, lysed, and subjected to a luciferase assay. The results revealed a significant decrease in luciferase activity upon transfection of pLTR-ZFN or pLTR-2 × TAR-ZFN, up to 60% and 55%, respectively. No reduction was observed in cells transfected with pcdNA3.1(−) with ZFN empty vector pLTR or pLTR-2 × TAR (Figure 3), suggesting that the HIV-1 proviral DNA excision was induced by the inducible ZFNs.

Evaluation of the Biological Activity of ZFN Treatment and Cytotoxicity in HIV-1-Infected Cells

To determine whether ZFN treatment of HIV-1-infected cells could decrease viral gene expression, we used Jurkat-derived HIV-1 pseudo-virus-infected cells (YA), which express EGFP as a marker for viral infection, as studied previously. We could monitor the change in the percentage of EGFP+ cells to determine the activity of ZFNs. To this end, YA were left untransfected (mock) or nucleofected with pcDNA3.1(−), pLTR-ZFN, or pLTR-2 × TAR-ZFN at specified times. The percentage of EGFP+ cells was measured by flow cytometry at different time points. The results showed a loss of EGFP expression up to 20% after treatment with pLTR-ZFN or pLTR-2 × TAR-ZFN on day 5 post-transfection compared with pcdNA3.1(−)-treated cells (Figure 4). We further detected a loss of 30% of EGFP+ cells after 7-day transfection. However, no reduction of the percentage of EGFP+ cells was observed in the mock- and pcdNA3.1(−)-transfected group up to 7-day transfection, indicating that the inducible ZFNs could excise proviral DNA in HIV-1-infected cells. We also evaluated the effect of the inducible ZFNs on YA viability at the indicated times. The results revealed no effects on cell viability in cells transfected with the inducible ZFNs compared with the pcdNA3.1(−)-transfected group (Figure 5).

Analysis of ZFN Treatment-Induced Proviral DNA Deletion in HIV-1 Latently Infected Cells

To further validate that the treatment of the inducible ZFNs could excise proviral DNA from the host genome in latently infected cells, we performed a study in C11 cells, which have been found to carry a single integrated HIV-1 vector at position Ch16p13.3. To probe for the deletion of the proviral DNA, we used primers located at the outsiders of the integrated HIV-1 genome separated by 10.4 kb when the provirus is integrated into the host genome (Figure 7A). The PCR product size we observed after co-treatment with pLTR-ZFN or pLTR-2 × TAR-ZFN and pCMV-Tat was approximately 1 kb (Figure 7B), a band size expected if the DNA segments between the ZFN target sites were deleted from the chromosome. No 1-kb band in cells co-transfected with pLTR-ZFN or pLTR-2 × TAR-ZFN and pcdNA3.1(−) was observed (Figure 7B), indicating that HIV-1 proviral genome deletion occurred only in the presence of pLTR-ZFN or pLTR-2 × TAR-ZFN with pCMV-Tat. To ensure that the 1-kb band was generated by rejoining DNA at the breakpoint induced by ZFNs, we ligated the PCR product into the pMD18-T vector and then performed a sequence analysis. The results demonstrated that the ZFN half target site at the 5' LTR locus is indeed linked to the ZFN half target site at 3' LTR locus, suggesting that the integrated HIV-1 5' and 3' LTR sites had been joined and that the intervening 9.8-kb DNA segment had been deleted (Figure S3).
DISCUSSION

The viral trans-activator Tat binding to the trans-activating response element TAR on the LTR recruits intracellular transcription-related proteins to promote viral gene expression. These transcription factor complexes phosphorylate the positive transcription elongation factor b (P-TEFb) and RNA polymerase II, which, in turn, promotes the transcription of viral genes. Additionally, nuclear factor κB (NF-κB), p300 and CBP (CREB binding protein), and GCN5, which bind to LTR, interact with Tat to promote transcriptional elongation of the HIV-1 virus gene. Thus, Tat protein plays a vital role in the transcriptional regulation of HIV-1. Previous studies have shown that inhibition of Tat binding to TAR or blocking Tat binding to intracellular transcription factors by inhibitors suppressed viral replication. In this study, we used the transactivation of Tat protein to induce ZFNs-LTR and established a inducible method for deleting viral genes by ZFNs. To this end, we first tested the biological activity of the inducible promoter by the luciferase reporter assay system. The results showed that LTR or LTR-2 × TAR-regulated luciferase expression was significantly increased in the presence of Tat protein, indicating that the activation of LTR or LTR-2 × TAR promoter requires Tat protein.

After confirming the biological activity of the LTR or LTR-2 × TAR promoter, we constructed inducible ZFN expression cassettes under the control of the LTR or LTR-2 × TAR promoter. First we tested the biological activity of ZFNs by transient luciferase assay. The results showed that the ZFN-transfected group could mediate the decrease of luciferase expression, suggesting that ZFNs had biological activity. Next we demonstrated that inducible ZFNs could mediate the reduction of the number of EGFP+ cells to 30% in HIV-1-infected cells without affecting cell viability. We also performed a similar study to investigate inducible ZFN-mediated HIV-1 proviral DNA excision in human primary T cells. The results from this study revealed a significant suppression of p24 production (Figure 6). Moreover, the inducible ZFN-mediated HIV-1 provirus genome excision from HIV-1 latently infected cells was also validated, consistent with the results from Kaminski et al. using CRISPR/Cas9 for ablation of HIV-1.

However, there are still many unknown problems that need to be explored if this inducible ZFNs expression vector is to be used in the clinical treatment of HIV. Safe and effective gene delivery
methods are important issues in the field of HIV-1 gene therapy. At present, viral vectors are widely used in gene therapy; lentiviral vectors can achieve the sustained expression of genes, but the potential risk of random integration is under consideration. However, in our study, we constructed the inducible ZFNs expression vector to solve the problem of potential off-target risks by persistent expression of ZFNs. Therefore, a lentiviral vector was not suitable for this study; the problem of potential off-target risks by persistent expression of ZFNs. This technology could not only prevent the unwanted off-target but also provide a new direction for gene therapy of AIDS.

Taken together, we conducted antiviral activity studies by inducing ZFNs. This technology could not only prevent the unwanted off-target but also provide a new direction for gene therapy of AIDS.

MATERIALS AND METHODS

Cells

HEK293T cells purchased from the American Type Culture Collection (Manassas, VA, USA) were maintained in DMEM supplied with 10% (v/v) fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin at 37°C under 5% CO2. C11 cells are a type of HIV-1 latently infected cells constructed by our lab.8,27 YA were derived from Jurkat T cells infected with HIV-1 –”peptide in one plasmid under the control of the HIV-1 LTR or LTR-2 × TAR promoter.

Plasmid Constructs

To obtain the HIV-1 LTR-driven luciferase reporter gene expression plasmid (LTR-luc), a full-length LTR fragment was amplified from the HIV-1 NL4-3-EGFP backbone with the forward primer F-LTR (5'-CCGGGTACACTGGAAGGCTAATTCACTCCAAAGAAGACGA-3') and the reverse primer R-LTR (5'-CCGCTCGAGGAGGCTTAAGCAGTGGGTTCCTAG TTAGCC-3'), followed by purification, digestion by KpnI and XhoI, and ligation into the KpnI-XhoI clone site of the pGL3-basic plasmid (Promega, Madison, WI, USA). All expression plasmids were confirmed by sequencing.

In our study, the expression of one ZFN-LTR pair (ZFN-LTR-L and ZFN-LTR-R) was isolated by T2A "self-cleaving" peptide in one plasmid under the control of the HIV-1 LTR or LTR-2 × TAR promoter. The FLAG tag was introduced at the N terminus of the LTR or LTR-2 × TAR promoter (constructed by our lab) by KpnI and XhoI. Then the bGHpA fragment amplified from the pX260 vector (Addgene, 42229) was digested by EcoRI I and XhoI and ligated into the plasmid pLTR or pLTR-2 × TAR comprising the LTR or LTR-2 × TAR promoter. Next we synthesized T2A oligonucleotides and inserted them into the digested plasmid pZFN-LTR-L by BglII and SfuI sites.

Conclusively, the application of an adenovirus vector delivering the ZFN-LTR-L plasmid containing the T2A fragment (ZFN-LTR-L-T2A) was then used to amplify ZFN-LTR-L-T2A, including the ZFN-LTR-L fragment, digestion by KpnI and XhoI, and ligation into the KpnI-XhoI clone site of the pGL3-basic plasmid (Promega, Madison, WI, USA). All expression plasmids were confirmed by sequencing.
and reverse primer (5'-CATCGAATTCTTAGAAGTTGATCTCGCCGTTGTTGAACCTG-3') and then digested by Xho I and EcoR I. Finally, fragments ZFN-LTR-L-T2A and ZFN-LTR-R were both ligated into the vector cut by Cla I and EcoR I. The final positive clones were named pLTR-ZFN and pLTR-2 × TAR-ZFN.

Luciferase Reporter Assay
To examine LTR or LTR-2 × TAR promoter activity, pLTR-luc or pLTR-2 × TAR-luc (200 ng) with or without the Tat expression plasmid (40 ng) was co-transfected into HEK293T cells and then subjected to detection of relative luciferase activity using the Dual-Luciferase Reporter Assay system (Promega, USA). Each experiment was performed in triplicate.

Next we detected the effect of ZFNs on HIV-1 proviral DNA deletion by luciferase assay. The pLTR-ZFN (600 ng) or pLTR-2 × TAR-ZFN (600 ng) vector with pHIV-NL4-3-luc (200 ng) and pRL-SV40 (50 ng) were co-transfected into HEK293T cells. Cells transfected with pEF1α-ZFN (600 ng) with pHIV-NL4-3-luc (200 ng) and pRL-SV40 (50 ng) were used as controls. Each experiment was performed in triplicate.

Western Blot
HEK293 T cells were plated in a 6-well plate and then transfected with 1 μg of pLTR-ZFN or pLTR-2 × TAR-ZFN plasmid with or without Tat using ViaFect reagent (Promega). After 72 hr transfection, cells were harvested, lysed, and subjected to western blot. The membrane was detected by primary antibody against FLAG for detecting ZFN protein expression. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was used as a reference. The Immun-Star WesternC chemiluminescence Kit (Bio-Rad) was used to detect the aforementioned proteins, and the chemiluminescence signal was captured using the ChemiDoc XRS+ System and analyzed using Image-Lab software (Bio-Rad).

Nucleofection and Flow Cytometry Assay
To assess the change in EGFP+ cells number after ZFN-LTR treatment in HIV-1-infected cells, YA were nucleofected with 2 μg of pcDNA3.1(−) vector, pLTR-ZFN, or pLTR-2 × TAR-ZFN using the Amaxa Cell Line Nucleofector Kit V (Lonza, Gaithersburg, MD, USA). The percentage of EGFP-negative cells indicated the treatment of the regulated ZFNs. At the indicated time, each group of cells was collected and washed with PBS. EGFP expression was measured using a FACSscan flow cytometer (Becton Dickinson, Mountain View, CA, USA), and the data were analyzed using CellQuest software (Macintosh, Sunnyvale, CA, USA).

Cell Viability
Briefly, HIV-1-infected cells YA were seeded at approximately 1 × 10⁶ cells/well in a 6-well plate and then transfected with 2 μg of pLTR-ZFN or pLTR-2 × TAR-ZFN constructs the following day. The pcDNA3.1(−) transfected group was used as a control. 4 × 10⁵ cells in each group were reseeded into a 96-well plate after different times of transfection, and then we added 10 μL of CCK-8 solution to each well, followed by incubation for 4 hr at 37°C. The absorbance at 450 nm was measured using a microplate reader. Each experiment was performed independently in triplicate.
Isolation of Primary CD4⁺ T cells

Peripheral blood mononuclear cells (PBMCs) from two blood units (400 mL) isolated from healthy donors were purchased from the Shanghai Blood Center (Shanghai, China). CD4⁺ T cells were further purified from PBMCs by negative selection according to the manufacturer’s instructions (Miltenyi Biotec). The CD4⁺ T cells were maintained in RPMI 1640 medium containing 5 ng/mL interleukin-2 (IL-2) supplemented with 10% FBS (Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin and then activated by beads coated with anti-CD3 and CD28 (Gibco, Thermo Fisher Scientific, USA) for 72 hr, followed by washing off and then culture at 37°C under 5% CO₂.  

Virus Infection of Human Primary T Cells and ELISA Detection of Antigen p24 Levels

Replication-competent HIV-1 NL4-3 viruses (CXCR4 tropism) were kindly provided by Dr. Wang (Institute Pasteur of Shanghai, China). For infection, Beads coated with anti-CD3- and CD28-activated CD4⁺ T Cells were inoculated with 10 ng p24 of HIV-1 NL4-3 for 5 hr and then washed off cell-free viruses, followed by changing the medium. On day 3 and day 5 infection, we moved half of the volume of medium and then supplied the corresponding volume of medium. For nucleofection, 5 μg of pLTR-ZFN or pLTR-2 × TAR-ZFN plasmid using the Amaza Human T Cell Nucleofector Kit (Lonza, Gaithersburg, MD, USA). After 72 hr transfection, viral replication was monitored by quantifying the amounts of p24 produced in the culture supernatant by using the HIV-1 p24 Antigen ELISA Kit (XpressBio) according to the manufacturer’s instructions.

PCR and Sequencing Analysis

To detect HIV-1 genomic deletion in HIV-1 latently infected cells (C11) after treatment with pLTR-ZFN or pLTR-2 × TAR-ZFN, PCR analysis was performed. Briefly, genomic DNA was extracted using the Blood and Cell Culture DNA Midi Kit (QIAGEN, China) according to the manufacturer’s instructions and then subjected to PCR analysis. The primers F-HG (5' TGCCACCCGAAACTATTCACAAG-3') and R-HG (5' CGGGATGGATTCCAGTCTAG-3') were used for amplifying excised proviral DNA from C11 cells un-transfected or transfected with pLTR-ZFN or pLTR-2 × TAR-ZFN in the absence or presence of Tat. PCR products were analyzed by agarose gel electrophoresis and further sequencing.

Statistical Analysis

The Data represent the mean ± SD (SE) of three independent experiments in triplicate. Paired samples t tests were performed using SPSS version 13.0 (SPSS, Chicago); *p < 0.05, **p < 0.01, and ***p < 0.001 indicate statistical significance.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and can be found with this article online at https://doi.org/10.1016/j.omtn.2018.04.014.

AUTHOR CONTRIBUTIONS

H.Z. conceived and designed the experiments. H.J. carried out most experiments. P.L., B.L., X.Q., Y.W., Z.J., X.Y., Y.Z., H.Y., H.P., and L.Z. participated in some of the experiments. J.X. and H.L. kindly provided some suggestions for the revised manuscript. H.Z. directed and supervised the experiments and interpretation of data. The manuscript was prepared by H.J. and H.Z.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Liu, H., Ma, Y., Su, Y., Smith, M.K., Liu, Y., Jin, Y., Gu, H., Wu, J., Zhu, L., and Wang, N. (2014). Emerging trends of HIV drug resistance in Chinese HIV-infected patients receiving first-line highly active antiretroviral therapy: a systematic review and meta-analysis. Clin. Infect. Dis. 59, 1495–1502.
2. Sluis-Cremer, N. (2014). The emerging profile of cross-resistance among the nonnucleoside HIV-1 reverse transcriptase inhibitors. Viruses 6, 2960–2973.
3. Chun, T.W., Stuyver, L., Mizell, S.B., Ehler, L.A., Micen, J.A., Baseler, M., Lloyd, A.L., Nowak, M.A., and Fauci, A.S. (1997). Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc. Natl. Acad. Sci. USA 94, 13193–13197.
4. Trono, D., VanLint, C., Rouzioux, C., Verdin, E., Barré-Sinoussi, F., Chun, T.W., and Chomont, N. (2010). HIV persistence and the prospect of long-term drug-free remissions for HIV-infected individuals. Science 329, 174–180.
5. Deeks, S.G., Autran, B., Berkhour, B., Benkirane, M., Cairns, S., Chomont, N., Chun, T.W., Churchill, M., Di Mascio, M., Katlama, C., et al. International AIDS Society Scientific Working Group on HIV Cure (2012). Towards an HIV cure: a global scientific strategy. Nat. Rev. Immunol. 12, 607–614.
6. Sgarbanti, M., and Battistini, A. (2013). Therapeutics for HIV-1 reactivation from latency. Curr. Opin. Virol. 3, 394–401.
7. Xing, S., and Siliciano, R.F. (2013). Targeting HIV latency: pharmacologic strategies toward eradication. Drug Discov. Today 18, 541–551.
8. Qu, X., Wang, P., Ding, D., Li, L., Wang, H., Ma, L., Zhou, X., Liu, S., Lin, S., Wang, X., et al. (2013). Zinc-finger-nucleases mediate specific and efficient excision of HIV-1 proviral DNA from infected and latently infected human T cells. Nucleic Acids Res. 41, 7771–7782.
9. Marciniak, R.A., Calnan, B.J., Franklin, A.D., and Sharp, P.A. (1990). HIV-1 Tat protein trans-activates transcription in vitro. Cell 63, 791–802.
10. Wei, P., Garber, M.E., Fang, S.M., Fischer, W.H., and Jones, K.A. (1998). A novel CDR9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. Cell 92, 451–462.
11. Van Herreweghe, E., Egloff, S., Goiffon, I., Jady, B.E., Froment, C., Monsarrat, B., and Kiss, T. (2007). Dynamic remodelling of human 7SK snRNP controls the nuclear level of active P-TEFb. EMBO J. 26, 3570–3580.
12. Arya, S.K., Guo, C., Josephs, S.F., and Wong-Staal, F. (1985). Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science 229, 69–73.
13. Sodroski, J., Rosen, C., Wong-Staal, F., Salahuddin, S.Z., Popovic, M., Arya, S., Gallo, R.C., and Haseltine, W.A. (1985). Trans-acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. Science 227, 171–173.

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14. Mbonye, U., and Karn, J. (2014). Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. Virology 454–455, 328–339.

15. Taube, R., and Peterlin, M. (2013). Lost in transcription: molecular mechanisms that control HIV latency. Viruses 5, 902–927.

16. Taylor, J.P., and Khalili, K. (1994). Activation of HIV-1 transcription by Tat in cells derived from the CNS: evidence for the participation of NF-kappa B-a review. Adv. Neuroimmunol. 4, 291–303.

17. Col, E., Caron, C., Seigneurin-Berny, D., Gracia, J., Favier, A., and Khochbin, S. (2001). The histone acetyltransferase, hGCN5, interacts with and acetylates the HIV transactivator, Tat. J. Biol. Chem. 276, 28179–28184.

18. Kiernan, R.E., Vanhulle, C., Schlitz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K.T., et al. (1999). HIV-1 tat transcriptional activity is regulated by acetylation. EMBO J. 18, 6106–6118.

19. Ott, M., Schnolzer, M., Garnica, J., Fischle, W., Emiliani, S., Rackwitz, H.R., and Verdin, E. (1999). Acetylation of the HIV-1 Tat protein by p300 is important for its transcriptional activity. Curr. Biol. 9, 1489–1492.

20. Tabarini, O., Desantis, J., and Massari, S. (2016). Recent advances in the identification of Tat-mediated transcriptional inhibitors: progressing toward a functional cure of HIV. Future Med. Chem. 8, 421–442.

21. Kaminski, R., Chen, Y., Salkind, J., Bella, R., Young, W.B., Ferrante, P., Karn, J., Malcolm, T., Hu, W., and Khalili, K. (2016). Negative Feedback Regulation of HIV-1 by Gene Editing Strategy. Sci. Rep. 6, 31527.

22. Mohammadi, E.S., Ketner, E.A., Johns, D.C., and Ketner, G. (2004). Expression of the adenovirus E4 34k oncoprotein inhibits repair of double strand breaks in the cellular genome of a 293-based inducible cell line. Nucleic Acids Res. 32, 2652–2659.

23. Chen, H.Z., Wu, C.P., Chao, Y.C., and Liu, C.Y. (2011). Membrane penetrating peptides greatly enhance baculovirus transduction efficiency into mammalian cells. Biochem. Biophys. Res. Commun. 405, 297–302.

24. Sandig, V., Hofmann, C., Steinert, S., Jennings, G., Schlag, P., and Strauss, M. (1996). Gene transfer into hepatocytes and human liver tissue by baculovirus vectors. Hum. Gene Ther. 7, 1937–1945.

25. Apolonia, L., Waddington, S.N., Fernandes, C., Ward, N.J., Bouna, G., Blundell, M.P., Thrasher, A.J., Collins, M.K., and Philpott, N.J. (2007). Stable gene transfer to muscle using non-integrating lentiviral vectors. Mol. Ther. 15, 1947–1954.

26. Nightingale, S.J., Hollis, R.P., Pepper, K.A., Petersen, D., Yu, X.J., Yang, C., Bahner, I., and Kohn, D.B. (2006). Transient gene expression by nonintegrating lentiviral vectors. Mol. Ther. 13, 1121–1132.

27. Deng, J., Qu, X., Lu, P., Yang, X., Zhu, Y., Ji, H., Wang, Y., Jiang, Z., Li, X., Zhong, Y., et al. (2017). Specific and Stable Suppression of HIV Provirus Expression In Vitro by Chimeric Zinc Finger DNA Methyltransferase1. Mol. Ther. Nucleic Acids 6, 233–242.

28. Lu, P., Qu, X., Shen, Y., Jiang, Z., Wang, P., Zeng, H., Ji, H., Deng, J., Yang, X., Li, X., et al. (2016). The BET inhibitor OTX015 reactivates latent HIV-1 through P-TEFb. Sci. Rep. 6, 24100.

29. Ji, H., Jiang, Z., Lu, P., Ma, L., Li, C., Pan, H., Fu, Z., Qu, X., Wang, P., Deng, J., et al. (2016). Specific reactivation of latent HIV-1 by dCas9-SunTag VP64-mediated guide RNA targeting the HIV-1 promoter. Mol. Ther. 24, 508–521.

30. Wang, P., Qu, X., Zhou, X., Shen, Y., Ji, H., Fu, Z., Deng, J., Lu, P., Yu, W., Lu, H., and Zhu, H. (2015). Two cellular microRNAs, miR-196b and miR-1290, contribute to HIV-1 latency. Virology 486, 228–238.

31. Wang, P., Qu, X., Wang, X., Liu, Z., Zhu, X., Zeng, H., and Zhu, H. (2013). As2O3 synergistically reactivates latent HIV-1 by induction of NF-kB. Antiviral Res. 100, 688–697.

32. Wang, P., Lu, P., Qu, X., Shen, Y., Zeng, H., Zhu, X., Zhu, Y., Li, X., Wu, H., Xu, J., et al. (2017). Reactivation of HIV-1 from Latency by an Ingenol Derivative from Euphorbia Kansui. Sci. Rep. 7, 9451.

33. Hauber, I., Hofmann-Sieber, H., Chemnitz, J., Dubeau, D., Chusainow, J., Stucka, R., Hartjen, P., Schambach, A., Ziegler, P., Hackmann, K., et al. (2013). Highly significant antiviral activity of HIV-1 LTR-specific tre-recombinase in humanized mice. PLoS Pathog. 9, e1003587.

34. Dalal, A.R., Homey, S., and Balaji, M.Y. (2018). Third-Generation Human Epidermal Growth Factor Receptor 2 Chimeric Antigen Receptor Expression on Human T Cells Improves with Two-Signal Activation. Hum. Gene Ther., Published online March 20, 2018. https://doi.org/10.1089/hum.2017.244.

35. Meltzer, B., Dabbagh, D., Guo, J., Kashanchi, F., Tyagi, M., and Wu, Y. (2018). Tat controls transcriptional persistence of unintegrated HIV genome in primary human macrophages. Virology 518, 241–252.