Virology 438 (2013) 1–4

Contents lists available at SciVerse ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

miR-132 enhances HIV-1 replication

Karen Chiang [a,b,1], Hongbing Liu [b], Andrew P. Rice [a,b,*]

[a] Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX 77030, United States
[b] Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, 77030, United States

ARTICLE INFO

Article history:
Received 14 December 2012
Returned to author for revisions
28 December 2012
Accepted 29 December 2012
Available online 26 January 2013

Keywords:
microRNA
miR-132
HIV
MeCP2

ABSTRACT

MicroRNAs upregulated during CD4$^+$ T cell activation may contribute to the increased efficiency of HIV-1 replication seen following perturbation of the resting state. We have found miR-132 to be highly upregulated following CD4$^+$ T cell activation, and show that miR-132 potentiates viral replication in the Jurkat CD4$^+$ T cell line. Knockdown of MeCP2, a previously identified target of miR-132, also increases HIV-1 replication. To the best of our knowledge, miR-132 is the first miRNA reported to enhance HIV-1 replication.

Introduction

Permissivity to HIV-1 replication is very low in resting CD4$^+$ T cells, but is greatly enhanced upon cell activation (Siliciano and Greene, 2011). This shift towards a favorable environment for viral replication is largely driven by widespread transcriptional changes, including those induced by altered microRNA (miRNA) expression. MiRNAs are short, non-coding RNAs which mediate post-transcriptional repression of gene expression, and are estimated to regulate over 50% of human protein-coding genes (Pasquinelli, 2012). Recent studies have shown that many microRNAs (miRNAs) downregulated following CD4$^+$ T cell activation can inhibit HIV-1 replication, either via targeting of the HIV-1 RNA itself (Huang et al., 2007; Nathans et al., 2009), or via the repression of cellular co-factors of HIV such as Tat partner Cyclin T1 (Triboulet et al., 2007; Sung and Rice, 2009; Chiang et al., 2012). We hypothesized that miRNAs which are upregulated following CD4$^+$ T cell activation may, on the converse, act to potentiate HIV-1 replication. We found that miR-132 fulfills both of these conditions, and as such, is, to the best of our knowledge, the first miRNA identified to increase HIV-1 replication.

Results and discussion

We previously performed miRNA expression profiling in resting and PHA-activated CD4$^+$ T cells isolated from four healthy donors (Chiang et al., 2012), and observed that miR-132 was very highly upregulated following activation (Fig. 1A). In order to confirm the microarray results, we measured miR-132 levels in five additional healthy donors, and found a two to five-fold increase in miR-132 levels after activation with anti-CD3/28 (Fig. 1B). MiR-132 levels also increased to comparable levels after PBMCs isolated from relatively young (age 25 and under) and elderly (60 and over) HIV-positive donors were treated with PHA (Fig. 1C), suggesting that activation-induced increases in miR-132 expression are unaffected by age or HIV status.

Transfection of synthetic miR-132 into Jurkat cells followed by infection with replication-competent NL4.3 virus resulted in increased viral replication (Fig. 2). MiR-132 also appeared to affect HIV-1 latency, as its over-expression reactivated virus in 2D10 cells (Fig. 3), a clonal Jurkat cell line latently infected with virus carrying attenuated Tat and destabilized GFP with a shortened half-life, which better reflects fluctuations in viral gene expression (Pearson et al., 2008). TNF-α treatment of the 2D10 cell line re-activates latent virus in a very high percentage of cells, with re-establishment of latency occurring over the course of a few days. MiR-132 transfection prior to TNF-α treatment of 2D10 cells was found to delay viral return into latency (data not shown).

Methyl-CpG binding protein 2 (MeCP2), a transcriptional regulatory protein (Guy et al., 2011), was previously shown to be a miR-132 target in the murine brain (Klein et al., 2007; Alvarez-Saavedra et al., 2011). Overexpression of miR-132 in

* Corresponding author at: One Baylor Plaza, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, 77030, United States. Fax: +1 713 798 3170.
E-mail address: arice@bcm.edu (A.P. Rice).
[1] Present address: 500 Gilman Drive, Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2012.12.016

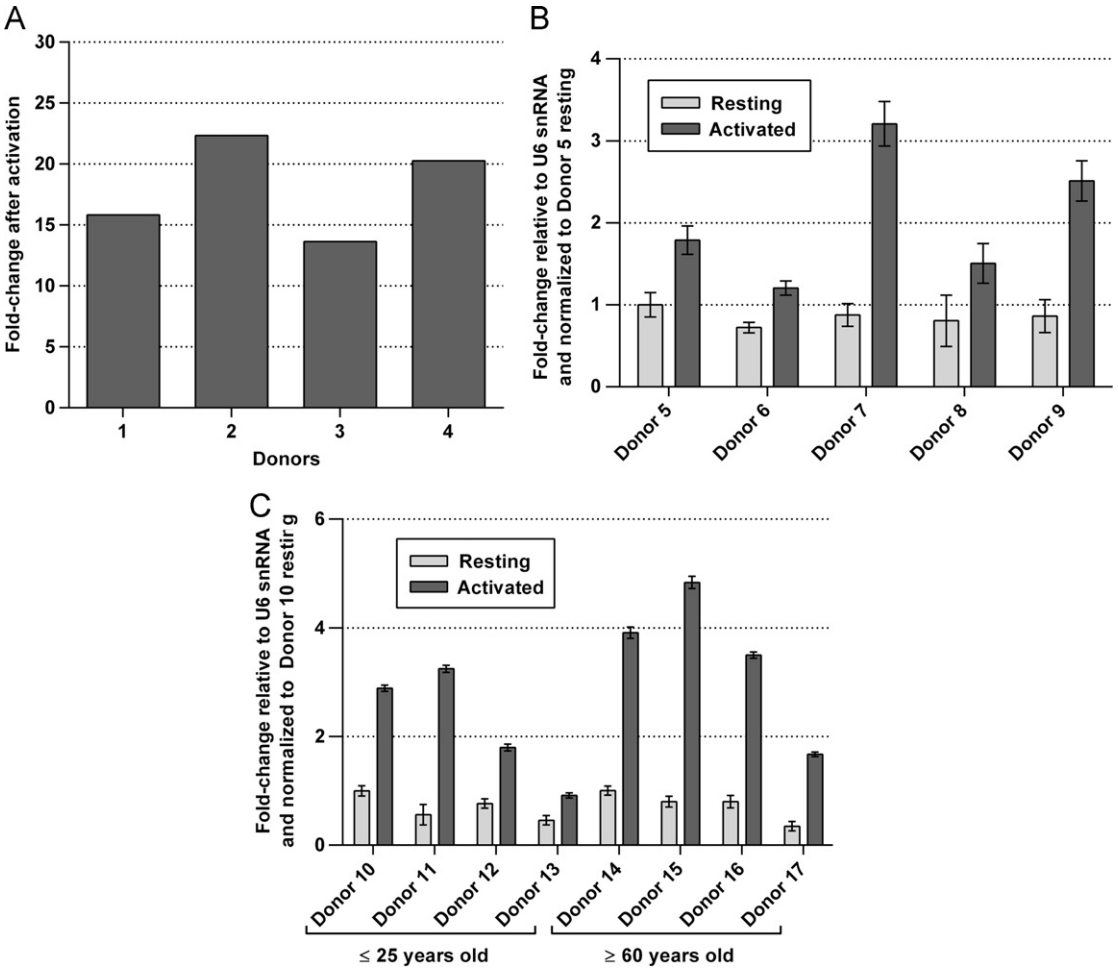

Fig. 1. MiR-132 is upregulated in activated CD4$^+$ T cells. (A) Resting CD4$^+$ T cells were isolated from four healthy blood donors, and a portion of the cells was activated with PHA. Activated CD4$^+$ T cells were harvested two days later, and total RNA was isolated for use in microRNA microarray analysis. Columns represent fold-change in miR-132 expression following activation. (B) Resting CD4$^+$ T cells were isolated from five healthy blood donors, and a portion of the cells was activated with anti-CD3/28 beads. Two days later, total RNA was isolated and quantitative real-time PCR was performed to measure miR-132 levels relative to U6 snRNA; values presented were normalized relative to miR-132 levels in the resting cells of Donor 5. MiR-132 levels were found to be significantly higher following CD4$^+$ T cell activation (Student's *t*-test, $p < 0.02$). (C) Relative miR-132 levels were measured as above in resting or PHA-activated PBMCs from HIV-positive, viremic donors; no significant difference in miR-132 levels was observed between activated cells from young versus elderly donors (Student's *t*-test, $p > 0.05$).

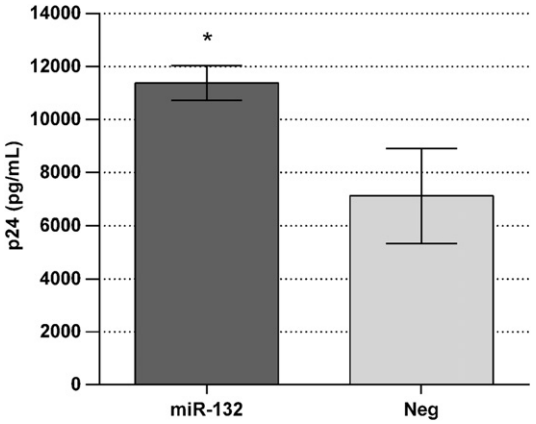

Fig. 2. MiR-132 increases NL4.3 replication in Jurkat cells. Jurkat cells were transfected with miR-132 or the negative control and infected two days later with NL4.3. Two days post-infection, supernatant was harvested and assayed by p24 ELISA. Error bars represent standard deviation of triplicate infections; $^*=p < 0.02$ by Student's *t*-test.

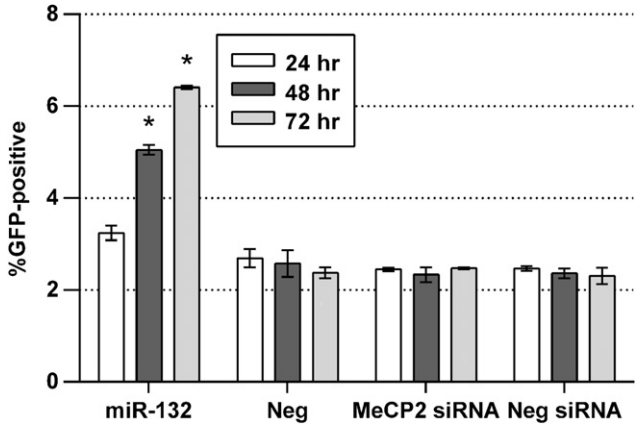

Fig. 3. MiR-132 activates latent viruses in 2D10 cells. MiR-132 and negative control miRNA, or MeCP2 and negative control siRNA, were transfected in triplicate into 2D10 cells, and GFP expression was analyzed by flow cytometry 24, 48, and 72 h post-transfection. $^*=p < 0.01$ by Student's *t*-test; error bars represent standard deviation of triplicate transfections.

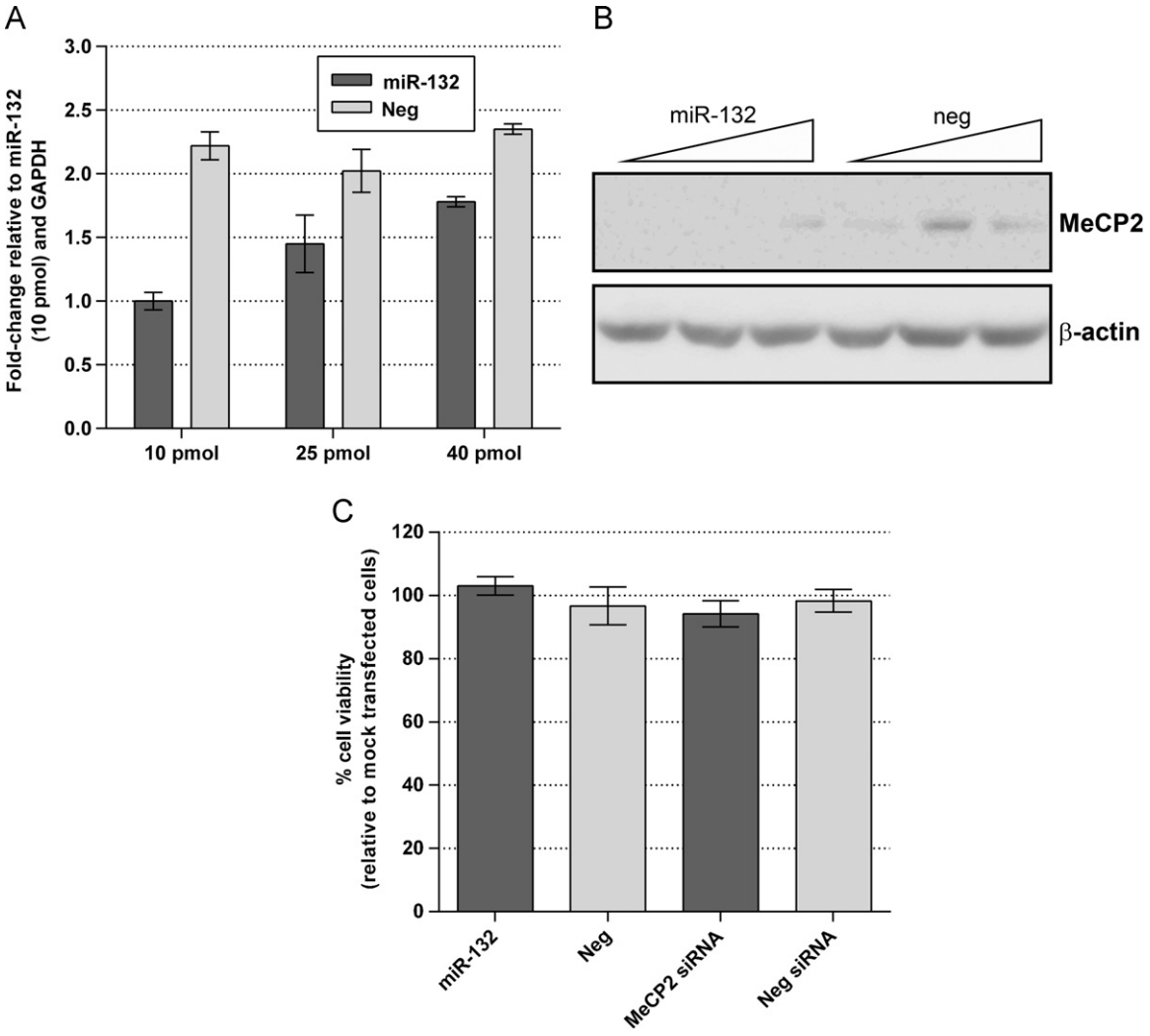

Fig. 4. MiR-132 decreases MeCP2 expression in Jurkat cells. (A) Jurkat cells were transfected with miR-132 or the negative control, and MeCP2 mRNA levels (relative to GAPDH) were measured by qPCR. MeCP2 mRNA was significantly decreased following transfection with miR-132 versus the negative control (Student's *t*-test, $p < 0.05$). (B) MeCP2 protein levels were analyzed by Western blot. (C) 2D10 cells were transfected with miR-132 or MeCP2 siRNA, and cell viability was assayed by measuring MTT production relative to mock transfected cells.

Jurkat cells led to a decrease in MeCP2 mRNA and protein levels (Fig. 4A and B), indicating that MeCP2 is also a target of miR-132 in T cells. Furthermore, we found that knockdown of MeCP2 also enhanced NL4.3 production in Jurkat cells, as measured by luciferase expression of the TZM-bl reporter line infected with the virus-containing supernatant (Fig. 5A). Neither transfection of MeCP2 siRNA nor miR-132 affected cell proliferation, as measured by an MTT assay (Fig. 4C). Unlike miR-132, MeCP2 siRNA was unable to re-activate latent virus in 2D10 cells (Fig. 3), indicating that additional targets of miR-132 may be contributing to its ability to disrupt viral latency.

These data suggest that miR-132 potentiates viral replication, in a manner which may be partially dependent on its ability to suppress MeCP2. It has previously been shown that miR-132 is highly upregulated following infection with Kaposi's sarcoma herpesvirus, herpes simplex virus-1, and human cytomegalovirus, and that miR-132 suppresses the host antiviral innate immune response by targeting the transcriptional co-activator p300 (Lagos et al., 2010). While p300 is a known co-factor of HIV-1 transcription (Nekhai and Jeang, 2006), it is also possible that modulation of p300 expression serves to regulate the innate immune response during HIV-1 infection. However, in the current study we did not test this hypothesis, nor the effects of other miR-132 targets on HIV-1 replication. Interestingly, along with being upregulated by $CD4^+$ T cell activation, miR-132 expression has also been shown to increase in murine brain tissue following cocaine administration (Hollander et al., 2010; Nudelman et al., 2010), and as cocaine has previously been shown to potentiate HIV-1 replication (Bagasra and Pomerantz, 1993; Peterson et al., 1991; Roth et al., 2002), this suggests that miR-132 may influence HIV-1 infection in cocaine abusers. While recent work has provided evidence that miR-217 can enhance Tat transactivation by down-regulating SIRT1 (Zhang et al., 2012), our findings comprise the first report of a miRNA which can enhance HIV-1 replication and re-activate latent virus.

Materials and methods

Resting $CD4^+$ T cells were isolated from healthy donor blood (Gulf Coast Regional Blood Center, Houston, TX) using the RosetteSep human $CD4^+$ T cell enrichment cocktail (STEMCELL); activated cells were removed using CD30 Microbeads (Miltenyi Biotec). Cells were activated with phytohemagglutinin (PHA) treatment (10 ng/mL) or CD3/28 Dynabeads (Invitrogen) and cultured in RPMI supplemented with 10% fetal bovine serum

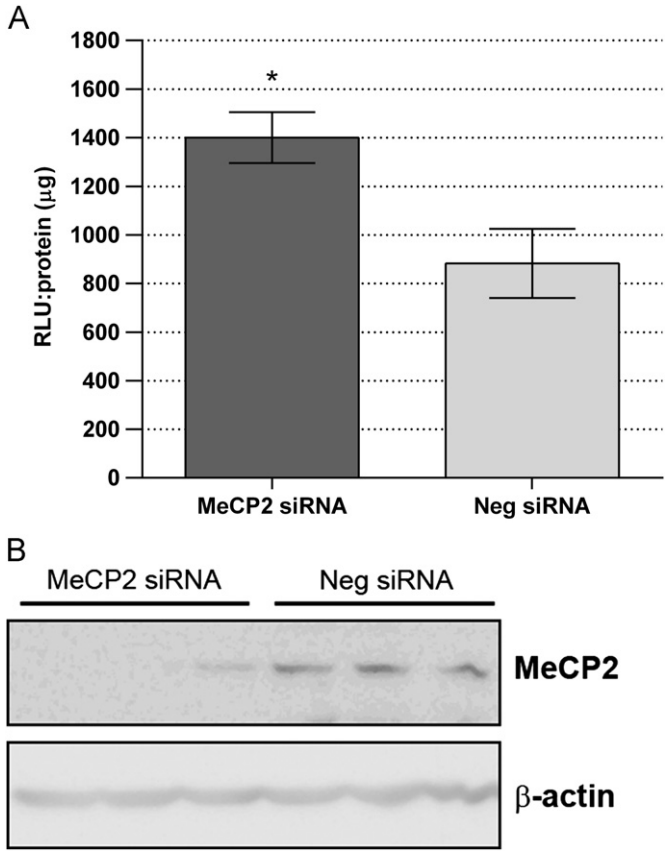

Fig. 5. MeCP2 siRNA increases NL4.3 replication. (A) Jurkat cells were transfected in triplicate with MeCP2 siRNA or negative control siRNA. Three days later, cells were infected with NL4.3 virus. Two days post-infection, supernatant was harvested and used to infect TZM-bl reporter cells in duplicate. Values represent averaged luciferase expression of TZM-bl cells two days post-infection normalized to protein concentration; $^{*}=p<0.05$ by Student's t-test. (B) Immunoblotting of cells treated with MeCP2 or negative control siRNA.

(FBS). Banked, frozen PBMCs from HIV-positive donors were thawed and half were left untreated, while the other half was treated with 1 μg/mL PHA; cells were harvested after two days. Total RNA was isolated using either the miRVana miRNA isolation kit (Applied Biosystems) or the miRNeasy Mini Kit (Qiagen), and qRT-PCR was performed using the TaqMan microRNA reverse transcription kit, TaqMan microRNA assays, and Universal PCR Master Mix (all from Applied Biosystems).

MiRNAs and siRNAs used were as follows: Pre-miR miR-132 Precursor or Pre-miR negative control ♯1 (Applied Biosystems), MeCP2 siRNA (5′-AAGCAUGAGCCCGUGCAGCCA-3′) (Bakker et al., 2002), and AllStars Negative Control siRNA (Qiagen). Jurkat cells were transfected with the indicated miRNAs and siRNAs using Lipofectamine RNAiMAX reagent (Invitrogen), followed by infection with NL4.3 virus (the NL4.3 plasmid was obtained from Malcolm Martin through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, and virus was produced via plasmid transfection into 293T cells). Cells were washed 24 h post-infection, and virus production was measured 48 h post-infection either by quantifying p24 levels in the supernatant via enzyme-linked immunosorbent assay (ELISA) (Zeptometrix) or by using virus-containing supernatant to infect TZM-bl cells, with luciferase expression measured using the Luciferase Assay System (Promega). 2D10 cells were transfected with miRNA as above, and GFP expression was monitored by flow cytometry. MTT assays were performed two days post-transfection using Cell Proliferation Kit I (Roche).

Acknowledgments

This study was supported by funding from the National Institute on Drug Abuse (Grant 5R21DA030166-03 to APR). This project was also supported by the BCM Cytometry and Cell Sorting Core with funding from the NIH (NCRR Grant S10RR024574, NIAID Grant AI036211, and NCI Grant P30CA125123). We thank Dr. Roberto Arduino for the patient PBMC samples.

References

Alvarez-Saavedra, M., Antoun, G., Yanagiya, A., Oliva-Hernandez, R., Cornejo-Palma, D., Perez-Iratxeta, C., et al., 2011. miRNA-132 orchestrates chromatin remodeling and translational control of the circadian clock. Hum. Mol. Genet. 20, 731–751.

Bagasra, O., Pomerantz, R.J., 1993. Human immunodeficiency virus type 1 replication in peripheral blood mononuclear cells in the presence of cocaine. J. Infect. Dis. 168, 1157–1164.

Bakker, J., Lin, X., Nelson, W.G., 2002. Methyl-CpG binding domain protein 2 represses transcription from hypermethylated pi-class glutathione S-transferase gene promoters in hepatocellular carcinoma cells. J. Biol. Chem. 277, 22573–22580.

Chiang, K., Sung, T.-L., Rice, A.P., 2012. Regulation of cyclin T1 and HIV-1 Replication by microRNAs in resting CD4+T lymphocytes. J. Virol. 86, 3244–3252.

Guy, J., Cheval, H., Selfridge, J., Bird, A., 2011. The role of MeCP2 in the brain. Annu. Rev. Cell Dev. Biol. 27, 631–652.

Hollander, J.A., Im, H.-I., Amelio, A.L., Kocerha, J., Bali, P., Lu, Q., et al., 2010. Striatal microRNA controls cocaine intake through CREB signalling. Nature 466, 197–202.

Huang, J., Wang, F., Argyris, E., Chen, K., Liang, Z., Tian, H., et al., 2007. Cellular microRNAs contribute to HIV-1 latency in resting primary CD4+T lymphocytes. Nat. Med. 13, 1241–1247.

Klein, M.E., Lioy, D.T., Ma, L., Impey, S., Mandel, G., Goodman, R.H., 2007. Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat. Neurosci. 10, 1513–1514.

Lagos, D., Pollara, G., Henderson, S., Gratrix, F., Fabani, M., Milne, R.S.B., et al., 2010. miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. Nat. Cell Biol. 12, 513–519.

Nathans, R., Chu, C.-Y., Serquina, A.K., Lu, C.-C., Cao, H., Rana, T.M., 2009. Cellular microRNA and P bodies modulate host-HIV-1 interactions. Mol. Cell 34, 696–709.

Nekhai, S., Jeang, K.-T., 2006. Transcriptional and post-transcriptional regulation of HIV-1 gene expression: role of cellular factors for Tat and Rev. Future Microbiol. 1, 417–426.

Nudelman, A.S., DiRocco, D.P., Lambert, T.J., Garelick, M.G., Le, J., Nathanson, N.M., et al., 2010. Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo. Hippocampus 20, 492–498.

Pasquinelli, A.E., 2012. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nat. Rev. Genet. 13, 271–282.

Pearson, R., Kim, Y.K., Hokello, J., Lassen, K., Friedman, J., Tyagi, M., et al., 2008. Epigenetic silencing of human immunodeficiency virus (HIV) transcription by formation of restrictive chromatin structures at the viral long terminal repeat drives the progressive entry of HIV into latency. J. Virol. 82, 12291–12303.

Peterson, P.K., Gekker, G., Chao, C.C., Schut, R., Molitor, T.W., Balfour, H.H., 1991. Cocaine potentiates HIV-1 replication in human peripheral blood mononuclear cell cocultures. Involvement of transforming growth factor-beta. J. Immunol. (Baltimore, Md.: 1950) 146, 81–84.

Roth, M.D., Tashkin, D.P., Choi, R., Jamieson, B.D., Zack, J.A., Baldwin, G.C., 2002. Cocaine enhances human immunodeficiency virus replication in a model of severe combined immunodeficient mice implanted with human peripheral blood leukocytes. J. Infect. Dis. 185, 701–705.

Siliciano, R.F., Greene, W.C., 2011. HIV Latency. Cold Spring Harbor Perspect. Med. 1, a007096.

Sung, T.-L., Rice, A.P., 2009. miR-198 inhibits HIV-1 gene expression and replication in monocytes and its mechanism of action appears to involve repression of cyclin T1. PLoS Pathogens 5, e1000263.

Triboulet, R., Mari, B., Lin, Y.-L., Chable-Bessia, C., Bennasser, Y., Lebrigand, K., et al., 2007. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. Science 315, 1579–1582.

Zhang, H.-S., Wu, T.-C., Sang, W.-W., Ruan, Z., 2012. MiR-217 is involved in Tat-induced HIV-1 long terminal repeat (LTR) transactivation by down-regulation of SIRT1. Biochim. Biophys. Acta 1823, 1017–1023.