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INVITED REVIEW

Do Opioids Activate Latent HIV-1 by Down-Regulating Anti-HIV microRNAs?

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Abstract Researchers have recently demonstrated the presence of anti-HIV-1 microRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) in monocytes, macrophages, and CD4+ T cells, which are the primary targets of HIV infection. These miRNAs appear to regulate the level of infectivity of HIV-1 in the target cells, and thus have an impact on HIV-1 latency. The levels of these miRNAs are significantly higher in resting CD4+ T cells than those in active CD4+ T cells, whereas HIV-1 infectivity is greater in active than in resting CD4+ T cells. Similarly, the levels of these miRNAs are significantly higher in monocytes than in macrophages, whereas HIV-1 infectivity is greater in macrophages than in monocytes. Down-regulation or inhibition of the activity of these miRNAs can promote replication of latent HIV-1 in resting CD4+ T cells and in monocytes. Recently, morphine was shown to down regulate the expression of anti-HIV miRNAs (miRNA-28, 125b, 150, and 382) in cultured human monocytes and this effect of morphine was mediated via activation of mu opioid receptors (MOR). In addition, levels of these anti-HIV miRNAs were significantly lower in the peripheral blood mononuclear cells (PBMCs) isolated from heroin-dependent subjects than those from control subjects. These findings raise an important question: Does morphine have potential to activate latent HIV-1 in resting CD4+ T cells and macrophages, including microglia of human subjects maintained on highly active antiretroviral therapy (HAART)? Further research is required to answer this question.

Keywords Anti-HIV-1 microRNAs · Morphine · CD4+T cells · Monocytes/macrophages · HIV latency

Introduction

MicroRNAs (miRNAs) are short non-coding regulatory RNAs, approximately 22 nucleotides in length, processed from short stem–loop precursors that are encoded in genomes of metazoans and viruses. miRNAs have the ability to bind to the 3′UTRs of messenger RNAs (mRNAs) and interfere with their translation, thus contributing to a significant post-transcriptional regulatory step in gene expression. They have been shown to play important roles in a number of organisms at the level of development, apoptosis, and establishment of cell lineage (Bartel 2004). Hariharan et al. (2005) were the first to discover the presence of miRNAs of human origin which have targets in critical genes of the HIV-1 genome. These targets include *nef*, *vpr*, *vpf*, and *vpu,* which correspond to the entire set of accessory genes of HIV-1. Since miRNAs interfere with the translational role of mRNA, these findings suggest a regulatory role of miRNAs on HIV-1gene expression/translation. In this report, we discuss the following aspects of anti-HIV miRNAs in relation to HIV infection: 1) the role of anti-HIV miRNAs in CD4+ T cell HIV infection; 2) the role of anti-HIV miRNAs in monocyte/macrophage HIV infection; and 3) the effect of morphine on anti-HIV miRNAs expression in monocytes. Since morphine has been shown to down-regulate the expression of anti-HIV-1 miRNAs in monocytes (Wang et al. 2011), we posed the question of whether morphine has a role in the activation of latent HIV-1 in the resting cells.

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Role of anti-HIV miRNAs in CD4+ T cell HIV infection

It is well established that viral replication does not occur in resting primary CD4+ T cells of HIV-1-infected individuals receiving highly active antiretroviral therapy (HAART), despite the fact that proviral DNA, integrated into host genome, is present in a latent form in these cells (Furtado et al. 1999; Chun et al. 2003; Lassen et al. 2006; Zhang et al. 1999; Patterson et al. 2001). This latency of HIV-1 in resting primary CD4+ T cells is a major barrier preventing the eradication of HIV-1 in the AIDS patients maintained on suppressive HAART. Since several host miRNAs have been identified that potentially target a set of accessory genes of HIV-1 genome (Hariharan et al. 2005), Huang and collaborators (2007) investigated whether anti-HIV miRNAs have a role in the repression of HIV-1 genes in resting CD4+ T cells. Huang et al. (2007) first compared the expression of anti-HIV miRNAs in resting vs. active CD4+ T cells in vitro. Of the miRNAs expressed in CD4+ T cells, 31 were at least 2 fold more abundant in resting than in activated CD4+ T cells. In addition, the levels of five host-coded anti-HIV miRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) were significantly higher in resting than in activated CD4+ T cells. They also demonstrated that the 3′ ends of HIV-1 messenger RNAs are targeted by these miRNAs.

Huang et al. (2007) further examined whether miRNAs have a direct role in the repression of HIV-1 gene expression in the resting CD4+ T cells isolated from PBMCs of normal human donors. Neutralization of the effects of these five miRNAs by their corresponding 2′-*O*-methyl-oligoribonucleotide antisense inhibitors resulted in increased HIV-1 production from pNL4-3 (an HIV-1 infectious clone)–transfected cells. Although individual inhibitors of anti-HIV-1 miRNAs only modestly increased viral production, the combination of the five inhibitors resulted in a substantial increase in HIV-1 production (11.3 times higher than the control) in resting $CD4^+$ T cells, but not in activated $CD4^+$ T cells. Additionally, the viral particles in the supernatants of resting $CD4^+$ T cells treated with the five miRNA inhibitors were infectious. Fluorescence Activated Cell Sorting (FACS) data indicated that these miRNA inhibitors do not affect cellular proliferation status. Moreover, transfection with the combined five miRNA inhibitors could rescue the viral production of several different HIV-1 strains. Importantly, transfection of these five miRNA inhibitors into resting $CD4^+$ T cells did not substantially alter the amounts of spliced and unspliced HIV-1 mRNA, but it did increase the expression of various HIV-1 proteins, suggesting a translational regulatory role for these miRNAs.

Finally, they examined the effect of these combined miRNA inhibitors on HIV-1 latency in resting $CD4^+$ T cells directly isolated from HIV-1–infected individuals receiving suppressive HAART. Post-integration HIV-1 latency in these cells was confirmed by detection of integrated HIV-1 proviral DNA in the chromosomal DNA via Alu-PCR. Stimulation of the cells with phytohemagglutinin (PHA) induced the production of a large number of viral particles, and serial passaging experiments further indicated that these viruses were replication competent. These data suggest that the cells harboring proviral HIV-1 DNA are indeed latently infected. After transfection with the combined miRNA inhibitors, these resting $CD4^+$ T cells generated at least ten-fold more HIV-1 particles than did the cells treated with a negative control inhibitor.

These results suggest that: 1) Cellular miRNAs can contribute to post-integration HIV latency through their interactions with the 3′ ends of HIV-1 mRNA in HIV-1–infected individuals receiving suppressive HAART. This action of miRNAs can control the translation of viral RNAs into proteins; 2) miRNA inhibitors could be used to activate latent HIV-1 in individuals receiving suppressive HAART; and 3) manipulation of cellular (host-coded) miRNAs could be a novel approach for purging the HIV-1 reservoir.

Role of anti-HIV miRNAs in monocyte/macrophage HIV infection

Monocytes and macrophages are the initial targets of HIV infection and they contribute to the disease pathogenesis throughout the course of infection. While freshly isolated monocytes from blood are difficult to infect with HIV-1, tissue macrophages are readily susceptible to this viral infection (Kedzierska and Crowe 2002; Rich et al. 1992; Sonza et al. 1996; Wang et al. 2009). Wang et al. (2009) investigated the modulatory effect of host-encoded anti-HIV miRNAs expression on the magnitude of HIV-1 infection of monocytes and macrophages. They showed that monocytes were refractory to HIV-1 infection, while donor-matched monocyte-derived macrophages were highly susceptible to HIV-1 infection. They also observed that freshly isolated monocytes had significantly higher levels of anti-HIV-1 miRNAs (miRNA-28, miRNA-150, miRNA-223, and miRNA-382) than donor-matched monocyte-derived macrophages. Thus, the level of infectivity was inversely correlated with the level of miRNA expression. Importantly, these investigators were able to reverse the severity of HIV-1 infection by modulating the expression of anti-HIV miRNAs. Thus, suppression of these anti-HIV miRNAs in monocytes enhanced HIV-1 infectivity, whereas increasing the expression of these miRNAs in macrophages suppressed HIV-1 infection/replication. Taken together, these findings suggest that intracellular anti-HIV miRNAs may play a role in protecting monocytes/macrophages from HIV infection, and down-regulation of these miRNAs may increase HIV infection by promoting viral replication.

Effect of morphine on anti-HIV miRNA expression in monocytes

Intravenous drug use (IVDU) is a significant risk factor for HIV-1 infection where heroin is the most commonly used drug. Opioids are widely prescribed and also abused drugs in the United States and all over the world. Several studies from different laboratories indicate that opioid use may promote HIV disease progression by enhancing HIV replication and infection (Peterson et al. 1990; Schweitzer et al. 1991; Suzuki et al. 2002; Guo et al. 2002; Li et al. 2003; Ho et al. 2003; Wang et al. 2005). Opoids, especially morphine, has been shown to promote HIV replication in many cell types through various mechanisms which are described below.

CC chemokine receptor 5 (CCR5) is a co-receptor required for the R5 tropic HIV entry into macrophages and lymphocytes. Morphine enhanced the expression of CCR5 in blood monocyte-derived macrophages (Guo et al. 2002; Li et al. 2003), in lymphoblasts and monocytes (Steele et al. 2003), in human CEMx174 lymphocytes (Miyagi et al. 2000), and in murine microglia (Bokhari et al. 2009). In addition, morphine-induced increased expression of CCR5 was associated with increased HIV-1 infection of macrophages (Li et al. 2003; Steele et al. 2003). These results suggest that morphine may promote HIV infection of monocytes/macrophages and lymphocytes and consequently viral replication by facilitating viral entry into the cells via increasing CCR5 expression. On the other hand, morphine has been shown to down-regulate the production or expression of CCR5 beta chemokine ligands (MIP-1alpha, Mip-1beta, or RANTES) in human macrophages (Guo et al. 2002) and human astrocytes (Mahajan et al. 2005). This way morphine may facilitate the entry of HIV into macrophages by down-regulating the expression of "competing" CCR5 receptor ligands that may promote HIV infection and subsequent viral replication.

CXC chemokine receptor 4 (CXCR4) is a co-receptor required for the X4 tropic HIV entry into CD4+ T cells which are the primary target of viral infection. Morphine upregulated the expression of CXCR4 on lymphoblasts and monocytes and this was associated with increased infection of X4-tropic HIV-1 (Steele et al. 2003). Thus, morphine may facilitate the entry of HIV into CD+ T cells by up-regulating the expression of CXCR4 receptors, which may result in increased viral replication.

Morphine may also promote HIV replication via modulating the production of cytokines such as tumor necrosis factor-alpha (TNF-alpha) or interferon gamma (IFN-gamma). In one study, morphine amplified HIV-1 expression in the chronically infected promonocytic clone U1 when cocultured with lipopolysaccharide-stimulated human fetal brain cells, and this effect of morphine involved enhanced production of TNF-alpha by microglial cells (Peterson et al. 1994). In another study, morphine inhibited CD8+ T cell-mediated, noncytolytic, anti-HIV activity in latently infected immune cells, and this effect of morphine appeared to be mediated via compromising anti-HIV activity of IFN-gamma (Wang et al. 2005). Finally, morphine may promote HIV replication by inducing transactivation of HIV-1 LTR (Squinto et al. 1990).

Although it is known that opioids (morphine and methadone) can up-regulate HIV in latently infected cells, it has not been shown that this effect is through modulation of miRNA. Wang et al. (2011) recently examined whether action of morphine on HIV replication is mediated via its effect on anti-HIV-1 miRNAs. They demonstrated that morphine significantly suppressed the expression of four anti-HIV miRNAs (miRNA-28, miRNA-125b, miRNA-150, and miRNA-382) in monocytes, which was associated with enhanced HIV-1 infectivity in the cells as shown by enhanced viral replication. Pretreatment of monocytes with pan-opioid antagonist (naltrexone) or mu opioid receptor (MOR) specific antagonist (CTAP) completely abrogated the effect of morphine on the expression of anti-HIV miRNAs and HIV-1 replication, suggesting that the morphine actions were mediated through MOR. To corroborate their in vitro data on the suppression of anti-miRNAs by morphine, Wang et al. (2011) measured the levels of these miRNAs in the PBMCs of heroin-dependent subjects. Interestingly, these subjects exhibited significantly lower levels of anti-HIV miRNAs (miRNA-28, miRNA-125b, miRNA-150, and miRNA-382) in their PBMCs when compared to normal subjects.

Type I interferons (IFN-α and IFN-β) play a crucial role in the control of viral replication because they have the ability to induce several antiviral cellular factors, including miRNAs (Pedersen et al. 2007; Scagnolari et al. 2010; Pan

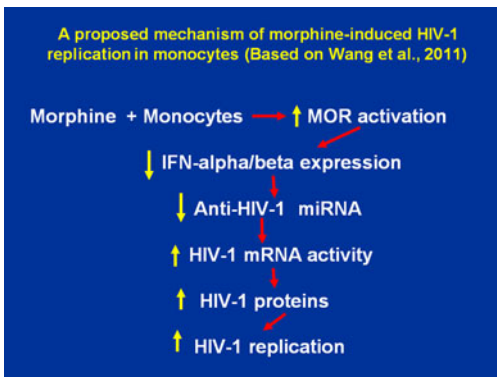

Fig. 1 Morphine can activate mu opioid receptors (MOR) on monocytes, leading to down-regulation of IFN-alpha and IFN-beta and subsequently down-regulation of anti-HIV-1 miRNAs. This may result in increased HIV-1 mRNA activity, increased HIV-1 protein synthesis, and increased HIV-1 replication

et al. 2011). Wang et al. (2011) demonstrated that IFN-α or IFN-β treatment of monocytes increased the expression of antiHIV-1 miRNAs (miRNA-28, miRNA-125b, miRNA-150, miRNA-382). However, pre-treatment of monocytes with morphine significantly inhibited IFN-mediated induction of the miRNAs. Since morphine has been shown to inhibit intracellular expression of IFN-α in liver (Li et al. 2007) and neuronal cells (Wan et al. 2008), it is possible that the adverse effect of morphine on anti-HIV miRNAs may be mediated through its negative impact on endogenous IFN-α/β expression in monocytes. Further studies are required to confirm the role of IFN- α/β in mediating the effect of morphine on the expression of anti-HIV miRNAs.

Taken together, these results indicate that morphine can promote HIV replication in monocytes by down-regulating the expression of anti-HIV miRNAs, which appears to be mediated through activation of MOR and possibly via inhibition of endogenous IFN-α/β expression (see Fig. 1).

Discussion

HAART is primarily composed of reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs). HAART is effective in suppressing viral load and with this therapy patients live longer than those without HAART. However, HAART cannot eradicate the latent virus integrated into the host DNA of the resting CD4+ T cells and resting tissue macrophages such as microglia. The latent HIV-1 present in resting primary CD4+ T cells and microglia is a major barrier for the eradication of the viruses in the patients maintained on suppressive HAART. One strategy to eradicate or purge the latent virus from the system is to activate the latent HIV, thus promoting its replication in reservoir cells (CD4+ T cells, microglia). Since some of the miRNAs play an important role in maintaining HIV latency in resting CD4+ T cells (Huang et al. 2007) and monocytes (Wang et al. 2009), suppressing the functions and/or production of these miRNAs may be a strategy to purge latent HIV from the reservoir cells in HIV-infected subjects treated with HAART.

Increasing evidence suggests that morphine treatment can modulate the expression of various miRNAs (Wu et al. 2009; Dave and Khalili 2010; Sanchez-Simon et al. 2010). Since morphine down regulates the expression of anti-HIV miRNAs in monocytes in vitro and heroin addicts have lower levels of these miRNAs in PBMCs (Wang et al. 2011), and down-regulation of these miRNAs can promote replication of latent HIV-1 in resting CD4+ T cells (Huang et al. 2007) and in monocytes (Wang et al. 2009), morphine could have the potential to activate latent HIV-1 in resting CD4+ T cells and resting macrophages such as microglia obtained from human subjects maintained on HAART. To investigate this hypothesis, further research is required in the following areas:

- Determine dose-dependent effect of morphine or MOR agonists on the expression of anti-HIV-1miRNAs in resting CD4+ T cells, monocytes, and resting macrophages such as microglia
- Ascertain whether morphine or MOR agonists can activate latent HIV-1 in CD4+ T cells and monocytes cultured in presence of protease inhibitors and/or reverse transcriptase inhibitors.
- Evaluate whether morphine or MOR agonists can activate latent HIV-1 in CD4+ T cells and monocytes obtained from AIDS patients maintained on HAART.
- Investigate the role of morphine and MOR agonists on anti-HIV-1 miRNAs expression and viral replication in humanized mouse model and monkey model (simian immunodeficient virus) of HIV/AIDS maintained on HAART

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