Cellular microRNA Expression Correlates with Susceptibility of Monocytes/Macrophages to HIV-1 Infection

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Abstract

Although both monocytes and macrophages possess essential requirements for HIV-1 entry, peripheral blood monocytes are infrequently infected with HIV-1 in vivo and in vitro. In contrast, tissue macrophages and monocyte-derived macrophages in vitro are highly susceptible to infection with HIV-1 R5 tropic strains. We investigated intracellular anti-HIV-1 factors that contribute to differential susceptibility of monocytes/macrophages to HIV-1 infection. Freshly isolated monocytes from peripheral blood had significantly higher levels of the anti-HIV-1 microRNAs (miRNA, miRNA-28, miRNA-150, miRNA-223, and miRNA-382) than monocyte-derived macrophages. The suppression of these anti-HIV-1 miRNAs in monocytes facilitates HIV-1 infectivity, while increase of the anti-HIV-1 miRNA expression in macrophages inhibited HIV-1 replication. These findings provide compelling and direct evidence at the molecular level to support the notion that intracellular anti-HIV-1 miRNA-mediated innate immunity may have a key role in protecting monocytes/macrophages from HIV-1 infection.
Introduction

Cells of the macrophage lineage play a crucial role in initial HIV-1 infection and contribute to the pathogenesis of the disease throughout the course of infection. Both monocytes and macrophages are considered as major cell targets for HIV-1 infection.\textsuperscript{1-3} However, peripheral blood monocytes are infrequently infected with HIV-1 \textit{in vivo} and \textit{in vitro} and only a very small proportion of them (0.001\%-1\%) harbor HIV-1 at any given time throughout the course of infection.\textsuperscript{2-4} In contrast, tissue macrophages are readily susceptible to HIV-1 infection \textit{in vivo}.\textsuperscript{3,5-9} These \textit{in vivo} findings are supported by the \textit{in vitro} observations, showing that peripheral blood isolated-monocytes acquire the ability to support productive HIV-1 infection only after their differentiation to macrophages\textsuperscript{7,10,11} and that HIV-1 replication in monocytes is generally enhanced by factors that facilitate differentiation.\textsuperscript{12-14} These studies suggest that host innate immunity plays a key role in governing HIV-1 infection of monocyte/macrophages.

Since HIV-1 R5 strains utilize CCR5 as a primary co-receptor for entry into macrophages, the demonstration\textsuperscript{10,15} of differential expression of the CCR5 receptor on monocytes and macrophages has been suggested as an explanation for the differential susceptibility of monocytes and macrophages to HIV-1 infection. However, this observation does not account fully for differences in HIV-1 infectivity in monocytes and macrophages.\textsuperscript{10,15,16} Recent studies\textsuperscript{16,17} showed that several members of APOBEC family members (APOBEC3G/3F), the newly identified HIV-1 restriction factors,\textsuperscript{18-21} are differentially expressed in immature monocytes and differentiated macrophages. While monocytes express high levels of functional APOBEC3G proteins, APOBEC3G in macrophages is sequestered in an inactive ribonucleoprotein complex, which increases macrophages permissibility to HIV-1 infection.\textsuperscript{18-21}
Among a growing list of innate cellular factors that impair various early steps of the HIV-1 life cycle, several cellular microRNAs (miRNA) have recently been identified to potentially target a set of accessory genes of HIV-1. For instance, human miRNA-28, miRNA-125b, miRNA-150, miRNA-223, and miRNA-382 can target the 3’UTR of HIV-1 transcripts, potentially rendering productive infection into latency in resting CD4+ T lymphocytes. In addition to their direct effect on HIV-1 replication, the miRNAs also have crucial roles in regulating the host innate immune defense that relies on phagocytes, such as macrophages. Thus, there is a need to examine the role of the anti-HIV-1 miRNAs in protecting monocytes/macrophages from HIV-1 infection. In this study, we investigated expression patterns of the innate anti-HIV-1 miRNAs in the course of monocyte differentiation to macrophages. We also determined the correlation between the levels of the anti-HIV-1 miRNAs and HIV-1 infectivity in monocytes and macrophages.

**Methods and Materials**

*Healthy Donor and Cells Culture*

Peripheral blood was purchased from University of Pennsylvania, Center for AIDS Research (CFAR). The protocol used to isolate blood from donors, purify the blood components and distribute this material to the University of Pennsylvania was approved by the IRB of the Center for AIDS Research. The donors gave informed consent in accordance with the Declaration of Helsinki for their blood to be used in this manner. The samples was screened for the common blood-born pathogens and certified to be pathogen-free. Monocytes were purified from peripheral blood of three healthy adult donors according to our previously described technique. Freshly isolated monocytes were cultured in 96-well culture plates at a density of 5 X 10^4 cells/well in Dulbecco’s Modified Eagle’s medium (DMEM) containing 10% fetal calf serum. Macrophages refer to seven-day-cultured monocytes *in vitro.*
miRNA Real-Time RT PCR

Total cellular RNA, including miRNA, was extracted from cells using miRNeasy Mini Kit from QIAGEN (Valencia, CA). Total RNA (1μg) was reverse-transcribed with miScript Reverse Transcription Kit from QIAGEN. The real-time RT PCR for the quantification of a subset of miRNAs (miRNA-28, miRNA-150, miRNA-223, and miRNA-382) was carried out with miScript Primer Assays and miScript SYBR Green PCR Kit from QIAGEN.

Transfection of miRNA mimics and miRNA inhibitors and HIV-1 Infection

The miRNA mimics and miRNA inhibitors for miRNA-28, miRNA-150, miRNA-223 or miRNA-382 were chemically synthesized by Dharmacon (Lafayette, Co). The design of miRNA mimic negative control 2 (miRNA Ctl) and miRNA inhibitor negative control 2 (Anti-miRNA Ctl) was previously described. D0 monocytes or D7 macrophages in 96-well plate were transfected with either 10 pmol mimic miRNA (for monocytes) or 10 pmol miRNA inhibitors (for macrophages) using the transfection reagents (Siport NeoFX, Ambion, Austin, TX or DharmaFECT 3, Dharmacon, Lafayette, CO). At 48 h post-transfection, monocytes or macrophages were infected with HIV-1 R5 strains (Bal or Yu2) at p24 protein concentration (30 ng/10⁶ cells) for 16 h. Both strains were obtained from the AIDS Research and Reference Reagent Program (National Institutes of Health). Culture supernatants were collected for HIV-1 RT activity at day 6, 8, 10 and 12 post-infection.

Results and Discussion

HIV-1 replication and the anti-HIV-1 miRNA expression in monocytes/macrophages
We first compared the susceptibility of D0 monocytes and D7-cultured macrophages to HIV-1 infection. Freshly isolated monocytes were either infected with HIV-1 Bal strain immediately after isolation (Day 0) or cultured for 7 days \textit{in vitro} when the majority of monocytes differentiated to macrophages (the insert in Fig. 1A). Macrophages were infected with HIV-1 Bal at the same titer as for donor-matched monocytes. As anticipated, HIV-1 Bal-infected D0 monocytes exhibited minimal evidence of HIV-1 replication, while donor-matched macrophages were highly permissive to HIV-1 infection as evidenced by a steady increase of RT activity during the period of cultures (Fig. 1A). In parallel, we also determined the expression of the miRNAs 28, 150, 223 and 382, the newly identified HIV-1 restriction factors, in monocytes/macrophages during the course of differentiation. Because very few monocytes harbor HIV-1 \textit{in vivo} and monocytes are poorly susceptible to HIV-1 infection \textit{in vitro}, we hypothesized that monocytes may have high levels of the anti-HIV-1 miRNAs, contributing to their refractory property to HIV-1 infection. This speculation is supported by the observation that D0 monocytes expressed the highest levels of intracellular miRNAs 28, 150, 223 and 382 than the cells cultured at later time points of the \textit{in vitro} differentiation (Fig. 1B). The lowest levels of these miRNAs were observed in the cells cultured for 7 days (Fig. 1B) when the majority of monocytes differentiate into macrophages (the insert in Fig. 1A). These observations demonstrated that although both monocytes and macrophages expressed the anti-HIV-1 miRNAs, the levels of these miRNAs in monocytes and macrophages significantly differ, which was correlated with the extent of cell’s permissibility to HIV-1 infection.

\textit{Modulation of anti-HIV-1 miRNA expression alters permissibility of cells to HIV-1 infection}

In order to demonstrate the crucial role of the anti-HIV-1 miRNAs in protecting of monocytes/macrophages from HIV-1 infection, we examined whether the modulation of the anti-HIV-1 miRNA levels in monocytes/macrophages could alter the cell’s susceptibility to HIV-1
infection. Monocytes transfected with the combined miRNA inhibitors showed higher levels of HIV-1 RT activity than untreated cells or control inhibitor-transfected monocytes (Fig. 2A). We then conducted second set of experiments to determine whether an increase of the anti-HIV-1 miRNA expression in macrophages could decrease the susceptibility of cells to HIV-1 infection. As shown in Fig. 2B, HIV-1 infectivity in macrophages transfected with the combined anti-HIV-1 miRNAs was significantly lower than that in untreated cells or in the cells transfected with the control miRNAs.

Although other studies have proposed several mechanisms, including the possibility that both extracellular and intracellular innate factors could be linked to myeloid differentiation and susceptibility to HIV-1 infection in vitro, the underlying molecular mechanisms remain to be determined. In an attempt to identify intracellular molecules that contribute to susceptibility of monocytes/macrophages to HIV-1, we examined the expression patterns of several anti-HIV-1 miRNAs in monocytes and macrophages. These miRNAs have been shown to be involved in HIV-1 latency in resting CD4+ T cells. Our observation that the levels of intracellular miRNAs were negatively associated with permissiveness of monocytes/macrophages to HIV-1 infection not only provides an additional explanation at the molecular level to address the fundamental question why differentiation of monocytes into macrophages is a prerequisite for productive HIV-1 infection, but also offers insight into the development of intracellular innate immunity-mediated therapy for HIV-1 infection and AIDS. Future studies will have to be done to reveal whether induction of anti-HIV-1 miRNAs in tissue macrophages might have therapeutic potential for purging the HIV-1 reservoir in vivo.
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Authorship

Contribution: X.W. carried out the experiments of the real-time PCR, HIV-1 infection, and RT assay with the support of Y.L., Y.Z. and W.H. Y-J Wang performed the experiments with monocytes isolation and cell culture. W-Z. H. and D.S.M directed and designed the entire study. All authors critically reviewed the manuscript.

Conflict of interest disclosure: The authors declare no competing financial interests.
References


Figure 1.  Differential HIV-1 infectivity and anti-HIV-1 miRNAs expression in monocytes and macrophages.  (A) HIV-1 infection of monocytes and macrophages. Cells were infected with HIV-1 Bal strain either immediately after isolation (D0 monocytes) or after having been cultured for 7 days (D7 macrophages).  Replication kinetics of HIV-1 Bal in cell culture was measured by RT activity in culture supernatants.  Culture supernatants were collected at the indicated time points post-infection.  The data shown are the mean ± SD of triplicate cultures, representative of three experiments using cells from three different donors.  The
inserts shown in Fig. 1A are the morphologies of freshly isolated monocytes (D0 monocytes) and 7-day cultured monocytes (D7 macrophages). (B) Anti-HIV-1 miRNAs expression during monocytes differentiation. Cells collected at the indicated time points were subjected RNA extraction for miRNA expression by real-time RT PCR. The data shown are the mean ± SD of triplicate cultures representative of three experiments using cells from three different donors.
Figure 2. Effect of modulation of the anti-HIV-1 miRNA expression on HIV-1 infection of monocytes and macrophages. (A) D0 monocytes were transfected with the combined microRNA (miRNA-28, 150, 223, and 382) inhibitors or the negative control inhibitor (Anti-miRNA Ctl) for 48h, and then infected with HIV-1 Bal or Yu2 strain. Culture supernatants were collected for HIV-1 RT activity at day 12 post-infection. (B) D7 macrophages were transfected with the combined miRNAs (miRNA-28, 150, 223, and 382) or negative control miRNAs (miRNA Ctl) for 48h, and then infected with HIV-1 Bal or Yu2 strain. Culture supernatants were
collected for HIV-1 RT activity at day 12 post-infection. The data shown are the mean ± SD of triplicate culture, representative of three experiments using cells from three different donors (*, p<0.05, **, p<0.01; combined miRNAs or combined anti-miRNAs vs miRNAsCtl or anti-miRNA Ctl).
Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection

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