Micro-RNAs and Their Role in HIV Non-progressive Diseases

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INTRODUCTION

UNAIDS estimates that 34 million people around the globe are currently HIV positive. Although Southern Africa amounts to half the deaths from AIDS and related causes – the developing countries are showing an increase in new infections. Despite considerable effort, there are no vaccines for controlling HIV and, as a consequence, the search for new generation of therapeutics and new targets has been intensified for achieving cure to HIV/AIDS.

The host gene and protein expression is intrinsically regulated by miRNAs, which play a vital role in the modulation of HIV pathogenesis in the infected host. How these small miRNAs regulate the host gene and protein expression and these miRNAs modulate HIV disease stages is not clearly known, primarily because of the complexity surrounding regulation of host gene expression by miRNAs. Now, through several studies there is clear evidence showing that HIV is able to subvert and manipulate host mRNA and miRNA machinery, therefore a clear understanding of complex aspects of the human genome and its regulation by miRNA harbors immense potential for not only developing new generation of biomarkers and therapeutic targets to control HIV, but also to delineate mechanisms regulating non-progressive HIV disease in Elite controllers in the absence of antiretroviral therapy.

MicroRNAs (miRNAs) are small (21-22nt), non-coding RNA fragments found in many organisms, from plants to humans, which function to negatively regulate gene expression. It has been shown that several cellular microRNAs can target conserved regions of HIV-1 genome (Harirhan et al., 2005). Moreover, evidence has shown that HIV-1 can also encode miRNAs (Cai et al., 2005), but this has remained controversial. In addition, HIV-1 Tat has been shown to down-regulate the host miRNA pathway (Bennasser et al., 2005; Bennasser and Jeang, 2006), implying role of viral genes in modulating miRNA expression and possible function. Several miRNAs have also been reported in the context of HIV-1 infection (Triboulet et al., 2007a), HIV latency (Huang et al., 2007a) and are involved in directing HIV-1 RNAs into P bodies (Nathans et al., 2009), respectively. Thus, virus-host interactions through host and virus-encoded miRNAs have considerably expanded our appreciation of these small molecules in functional regulation and dysregulation of host gene expression. Although the mechanisms by which miRNAs regulate gene and protein expression remain unclear, it is believed that a profound understanding of the genomic cross-talk between the host and virally-encoded
miRNAs are bound to further enhance our understanding of the molecular basis of HIV infection paving the way for the development of new generation therapeutic strategies to fight HIV infection.

This review attempts to summarize findings regarding miRNAs’s and their role in HIV disease progression, with special emphasis to their contribution to the non-progressive HIV disease, which is seen in about 1% of HIV-infected therapy naïve individuals, termed non-progressors or Elite Controllers, who remain virtually disease free by virtue of the strength of their immune system. Although several mechanisms have been proposed, the role of host gene expression and its regulation by miRNAs in progressive and non-progressive HIV disease remains poorly understood. Thus, it is essential to understand this regulation process governed by miRNA and its dysregulation by HIV in the infected host. Understanding the role of miRNAs in LTNPs is vital and it will not only unravel the underlying mechanisms critical in natural control of HIV-infected non-progressors, but will also provide much needed clues to targeting dysregulated miRNAs as possible antiviral targets. It is encouraging to see that the first miRNA- the miR122 in HCV infection for which an antagonist Miraversen has been designed and the clinical Phase-I/IIb trial is currently underway (Wahid et al., 2010).

**Dysregulation of host miRNA in relation to HIV Infection, viremia and elite suppression**

HIV is known to alter host miRNA expression profile in vitro and in vivo. In 2008, Houzet et al. showed that the vast majority of host miRNAs are downregulated in HIV infection of the peripheral blood mononuclear cells (PBMCs). Furthermore, Triboulet et al. (2007) showed that HIV-1 infection leads to suppression of host PBMC miRNAs miR-17-5p and miR-20 (Triboulet et al., 2007a). Moreover, recently Sun et al., (2012) have shown that the microRNA-223 levels were significantly enriched in HIV-1-infected CD4+CD8− PBMCs while microRNA-29a/b, microRNA-155 and microRNA-21 levels were significantly reduced. Furthermore, a recent study by Witwer et al. has shown that the elite suppressors (ES) and uninfected controls can be clearly discriminated from their viremic HIV-1+ counterparts based on miRNA profiles (Witwer et al., 2012). Interestingly, in this study, there was correlation between miRNA profiles and CD4+ T-cell count and plasma viral load, however ECs and viremic patients showed similar expression patterns for some miRNAs in comparison to HIV negative controls, suggesting that these miRNAs are specific to HIV infection. Witwer’s work also identified several miRNAs that have not been previously described in the context of HIV infection, including miR-31, which discriminated controls and ECs from viremic individuals. MiR-31 is known to regulate a protein functionally important in T-cell differentiation. Although this study has also shown that HIV-1-positive ECs are characterised by a PBMC miRNA profile that in general resembles that of uninfected individuals, they also reiterate that the ECs, on the basis of miRNA expression, are a heterogenous group and this concurs with the previous observations. Overall, these data suggest that different mechanisms, shaped or marked by different miRNA expression patterns, underlie sustained and durable control in therapy naïve HIV-infected individuals, stressing the need to study larger cohorts of HIV+ and HIV- individuals to address miRNA specific to different stages of HIV disease and explain the underlying genomic basis of natural control of HIV in therapy naïve elite controllers. In this context it is important to iterate that HIV proteins play a significant role in altering miRNA profiles. For instance, HIV-encoded RNA silencing suppressor Tat can alter host miRNA expression profile to prevent inhibition of HIV-1 replication by host cells and attenuate the cellular response to HIV infection. Deactivating mutations in the Tat region of the HIV-1 genome reduces HIV-1 down-regulation of twenty-two cellular miRNAs in lymphocytes (Hayes et al., 2011).

**Protective role of host miRNAs against HIV**

Although host miRNAs are dysregulated by HIV infection, the host miRNAs also play a significant role in countering viral infections. Hariharan et al., (2005) showed that some host miRNAs expressed in human T-cells show complementarity to the miRNA of HIV-1 accessory proteins in highly conserved regions (Hariharan et al., 2005). Using a consensus scoring approach and four well-established target predication softwares, high scoring miRNA-target pairs were selected. The 3‘ ends of HIV-1 miRNAs were targeted by a group of cellular miRNAs including miR-28, miR-125b, miR-150, miR-223 and miR-382. Effective inhibition by these miRNAs would affect viral infectivity, integration of the provirus into the genome, viral gene expression, cell cycle arrest and apoptosis and viral particle production (Hariharan et al., 2005). For instance, it has also been shown that host microRNAs, including microRNA-29 family, could directly bind to HIV-1 nef gene and affect its expression through binding on microRNA binding sites in a conserved sequence of the Nef-3’-LTR specifically inhibiting the action of these miRNAs altering viral replication (Huang et al., 2007a). Their work also showed that the cellular miRNA namely hsa-miR-29a, hsa-miR-20a, hsa-miR-330-5p, and hsa-miR-191 suppressed HIV-1 replication, while hsa-miR-511 enhanced viral antigen production. Supporting this, immunoblotting analysis has
shown that that hsa-miR-29a directly binds to HIV-1 Nef mRNA, while miR-20a, miR-330-5p, miR-191, and miR-511 bind to cellular factors that indirectly influence HIV-1 replication (Tan Gana et al., 2012). This further goes to show the role of miRNAs in latency, and that targeting the dysregulated miRNAs is a possible tool for eradicating HIV, which may be relevant in cure strategies for HIV. When miR-29a is inhibited, viral replication is enhanced, but when miRNA is enhanced, viral replication is suppressed (Nathans et al., 2009). Recently, Gana et al., at the recently concluded IAS Meeting in Washington DC, concurred with these findings (Zhu et al., 2012). All of these miRNAs are enriched in resting T-cells compared to active T-cells and act to enforce latency and inhibit viral replication. Most interestingly, significantly higher levels of anti–HIV-1 microRNAs (miRNA-28, miRNA-150, miRNA-223, and miRNA-382) have been found in freshly isolated monocytes from peripheral blood compared to their monocyte-derived-macrophage counterparts. These findings provide evidence at the molecular level to explain the refractory nature of monocytes to HIV-1 infection and 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 (Wang et al., 2009a). Moreover, it also explains the fact that peripheral blood monocytes are infrequently infected by HIV-1, and rarely harbour virus and act as latent reservoirs (Kedzierska and Crowe, 2002).

However, the same analysis could not demonstrate that low-level expression of both CCR5 and CD4 is not the more significant factor in explaining their relative resistance to infection, despite higher levels of anti-HIV miRNAs in monocytes. Supporting these findings, opioid treatment of monocytes has been shown to reduce their intrinsic anti-HIV-1 miRNA levels. This mirrors the finding that heroin-dependent HIV-infected individuals have lower levels of anti-HIV miRNAs in their peripheral blood monocytes, increasing susceptibility of the cells to HIV infection (Wang et al., 2011a). Furthermore, miR-17-5p/3p, miR-18, miR-19a, miR-20a, miR-19b-1 and miR-92-1 have also been reported to impact on HIV-1 replication indirectly (Triboulet et al., 2007a).

Previously, differentially expressed miRNAs have been shown to correlate to HIV-1 latency (Huang et al., 2007a), including miR-28, miR-125b, miR-150, miR-223 and miR-382. All of them were significantly up-regulated in the resting T-cells compared to activated T cells (Detsika et al., 2012). They were also shown to be up-regulated in monocytes isolated from peripheral blood mononuclear cells (PBMC) with a reduction following the stage of the differentiation (Wang et al., 2009a). Interestingly, in both cell types, the levels of those miRNAs negatively correlated with HIV infectivity, miR-198, miR-29a and miR-29b are potential cellular miRNAs that might be involved in HIV latency as well. Most of those miRNAs targeted specific regions encoding in the Tat and Rev, which have been reported to contribute to HIV latency before (Lin et al., 2003), while miR29a and 29b have been documented to suppress HIV-1 Nef protein expression and therefore viral replication (Ahluwalia et al., 2008). In 2007, Huang et al. showed over expression of host miRNAs in resting T-cells that target sequences in the 3’ end of HIV-1 RNA, silencing viral mRNA and enforcing latency (Huang et al., 2007a).

Overall, these findings provide strong evidence at the molecular level to explain the refractory nature of HIV-1 infection and to support the notion that the complimentarity between host miRNAs and HIV and the dysregulation of intracellular anti–HIV-1 miRNA-mediated innate immunity may have a key role in protecting monocytes/macrophages from HIV-1 infection (Wang et al., 2009b). Further, these analyses open doors to analyzing miRNAs specifically in cells refractory to HIV infection and the cells known to act as latent viral reservoirs, which may explain the role of host miRNAs regulating the protective function during HIV infection.

**HIV miRNAs and their regulation of infection**

HIV infection triggers antiviral miRNA defence in human cells. To combat this, HIV derives its own miRNA to target large numbers of cell transcripts or dicer and account for the gene landscape changes seen in HIV-1 infection. It has been suggested that HIV-derived miRNAs can play a significant role in RNA Interference (RNAi) in the regulation of HIV-1 gene expression (Weinberg and Morris, 2006). Currently, there are two pre-miRNA known to be HIV encoded. The HIV trans-activation response (TAR) element is a pre-miRNA, which can produce mature miRNAs from both stands of its stem-loop (Ouellet et al., 2008). TAR is required for the transactivation and virus replication. It has also been reported to prevent apoptosis of HIV infected cells by being processed into miRNAs, which can down regulate the apoptosis genes ERCC1 and IER3 (Klase et al., 2009). The HIV-1 Rev response element (RRE) contains 5 stem-loop structures, which resemble pri-miRNAs, and can be targeted by RNAi machinery. It is involved in the transition between early phase to the late phase of HIV life cycle via interacting with Rev protein (Ouellet et al., 2009). The TAR miRNA is a 50nt hairpin structure encoded at the 5’ end of the viral mRNA. TAR-miRNA is capable of binding both TRBP and Dicer. Loss of TRBP leads to loss of RNA silencing capability (Gatignol et al., 1991).

Apart from harbouring precursor miRNA structure, up to 10 mature viral miRNAs from different regions of the HIV-1 genome have been reported being encoded, including the miR-N367, miR-H1, miR-TAR, miR-TAR-5p and miR-TAR-3p (Detsika et al., 2012). Other miRNAs might also be encoded, such as those regulating the production of CD28, CTL4, etc. The TAR miRNA has been shown to down-regulate viral gene expression and affect host cell cycle. It also down-regulates host cell machinery involved in replication, receptor signalling, repair, apoptosis and mitochondrial function, protecting the infected cell from stress induced cell death and prolonging its lifespan (Klase et al., 2007;Klase et al., 2009). Nef miRNA has already been shown in HIV-infected cells, and functions to down-regulate HIV-1 transcription and Nef protein expression (Yamamoto et al., 2002;Omoto et al., 2004;Omoto and Fujii, 2005), contributing to long-term non-progression (LTNP) status. MiR-H1 is an 81nt stem loop downstream of two NFκB sites.
Potential of miRNAs in antiviral therapy

SiRNA, shRNA and miRNA structures have all been shown capable of inducing the RNA interference (RNAi) pathway in human cells (Elbashir et al., 2001) and as such RNAi treatment using any of these constructs is of great interest in the potential future genetic treatment of HIV infection. Furthermore, it is possible that a potential antiviral therapy might also be achieved by targeting host miRNAs involved in the infection and propagation processes directly using antagonists or inhibitors. Kulkarni et al. studied how miRNAs regulate HLA-C expression. The HLA-C locus has been shown to be associated with HIV disease progression. Variation in the HLA class I loci of the genome appears to have a stronger influence on the outcome of HIV disease than any other locus in the host genome. HIV+ individuals with high expression of HLA-C loci appear to progress slower to AIDS accompanied by better control of viremia than HIV+ individuals with low expression of HLA-C loci (Kerrigan, 2011; Kulkarni et al., 2011). By sequencing and analyzing many different variants of the HLA-C locus they found that a particular miRNA (miR-148a) does not bind to its target site in some variants, resulting in high HLA-C expression. Further, using different naturally occurring and artificially created molecular variant constructs of the HLA-C mRNA at the miR-148a binding site and nearby regions, they were able to demonstrate that there is differentially regulated HLA-C expression attributed to nucleotide variations in the region. This team of researchers found the first evidence in favour of miR-148-HLA-C binding in direct determination of expression of HLA-C. As stated by Kerrigan, a clear understanding of these intricate details will help in developing new strategies for targeted drug development for treating AIDS.

Various pharmaceutical companies are now vying for creating and finding viable therapeutic candidates/antagonists that target miRNA through appropriate inhibitors and miRNA mimetics to treat diverse diseases, viral infections, neurological disorders, cancers and cardiovascular/metabolic disorders (Janet and Amir, 2008). Thus, miRNAs are making their way as the most attractive therapeutic, diagnostic and prognostic targets. Further, a number of pre-clinical and clinical trials are being currently conducted using miRNA as targets, two of which relate to viral infections (Wahid et al., 2010) and an unspecified miRNA therapy for HIV by Rosetta Genomics, Columbia University-CUMC, USA., whereas all others are in the area of cancers (leukemia, lymphoma, carcinoma melanoma, etc) and metabolic disorders (cardiovascular diseases, hypercholesterolemia, hypertension, etc) (Wahid et al., 2010).

Conclusion and future outlook

miRNAs are small molecules with bigger functional impact, as single miRNA can target hundreds of mRNAs at any given time in regulating gene function. Thus, miRNA-mediated regulation of host gene expression underlies complex relationship that controls host-virus interactions. Several viruses are also known to encode miRNAs, which play a vital role not only in the viral life cycle, but also in determining disease course in the host through host-virus interactions and subversion and manipulation of the host genome. Collectively, this may have important implications in immune evasion by inhibiting immune surveillance and extending the life of the infected host cell. Thus, the determination of such targets and their interaction during the disease course may provide a clear view of this host-virus interaction and may lead to the development of new therapeutic strategies. Similarly, the identification of dysregulated miRNAs during HIV infection may yield attractive targets for anti-HIV therapy. In this context, it is important to mention that because of the stability of miRNAs in human tissues and fluids, they are emerging as a distinct class of biomarkers in diagnostics and prognostics.

Not much is known about the role of miRNAs in HIV disease staging. Given that HIV cure strategies are gaining momentum, analysing the role of miRNA in modulating the host gene expression in guiding HIV disease staging (progression or non-progression) is timely. As can be noted that most studies discussed in this review are small-scale studies, data from larger HIV cohorts is needed to unambiguously identify key miRNA candidates that statistically relate to natural control of HIV in elite controllers. The identification of such miRNAs will have profound implications at the level of treatment and also targeting dysregulated miRNAs with antagonists as therapeutic targets, as we have seen in case of Miraversen-an miR-122 antagonist for treating HCV infection.
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REFERENCES


