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MicroRNAs and HIV Latency: a Complex and Promising Relationship

Maria G. Detsika, Alexandros Psarris and Dimitrios Paraskevis
National Retrovirus Reference Centre, Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece

Abstract

MicroRNAs are small RNA molecules of 20-22 nucleotides, initially discovered in* Caenorhabditis elegans*, and involved in the regulation of various biological processes in plant and mammalian systems. Essentially, they are key gene regulators as they may inhibit gene expression by mRNA degradation or inhibiting mRNA translation. The identification of microRNAs in plant and human viruses raised important questions regarding their function and potential use as antiviral targets. Reports have described microRNAs encoded by HIV and also the involvement of cellular mRNA in the course of HIV infection. This review investigates the potential use of microRNAs in therapeutic strategies against HIV infection and their role for the eradication of viral reservoirs. **(AIDS Rev. 2012;14:188-94)**

Corresponding author: Dimitrios Paraskevis, dparask@med.uoa.gr

Key words

MicroRNAs. HIV. Transcription. Cellular. Host.

Introduction

MicroRNAs (miRNAs) are small RNA molecules, 18-25 nucleotides (nt) in length, widely known as key regulators of post-transcriptional eukaryotic gene expression. MicroRNAs are expressed in fungi, plants, and animals[1,2] as well as in human viruses[3]. They were initially discovered in 1993 in the nematode *Caenorhabditis elegans* and were implicated in developmental timing[4]. The discovery of highly conserved miRNA molecules, such as the let7 in *C. elegans*, demonstrated the urgency to comprehend how miRNAs mediate gene expression and led to a flow of discovery of miRNA molecules, reaching more than 15,000 miRNA gene *loci* in over 140 species listed today[5].

Correspondence to:
Dimitrios Paraskevis
National Retrovirus Reference Centre
Department of Hygiene, Epidemiology and Medical Statistics
University of Athens Medical School
75 Mikras Asias
11527 Athens, Greece
E-mail: dparask@med.uoa.gr

MicroRNA maturation

MicroRNAs were thought to be transcribed from intergenic regions of the genome, where they may be clustered and share similar expression patterns[6] and may therefore be transcribed as polycistronic transcripts[7]. Other studies, however, identified miRNA encoding ***loci*** within protein encoding introns or within the introns or exons of non-coding RNA[8].

MicroRNAs are initially transcribed by RNA polymerase II as primary miRNAs, the length of which varies from hundreds to thousands of nucleotides[9] (Fig. 1). The yield of mature miRNAs from primary miRNAs is based on two steps, both involving a ribonuclease III (RNAse III) enzyme. The first step requires the process of primary miRNAs in the nucleus by the microprocessor complex into precursor miRNA molecules, which are stem loop structures about 70 nt in length. The microprocessor complex includes the Drosha and the Di George syndrome critical region 8 (DGCR8) proteins in mammals[10]. Precursor miRNA molecules have a distinct pattern as a result of Drosha enzyme processing, which defines either the 3' or the 5' end of the molecule, leaving characteristic 5' phosphate and 3' hydroxy termini and overhanging nucleotide 3' single-stranded ends[9]. Precursor miRNAs are then exported

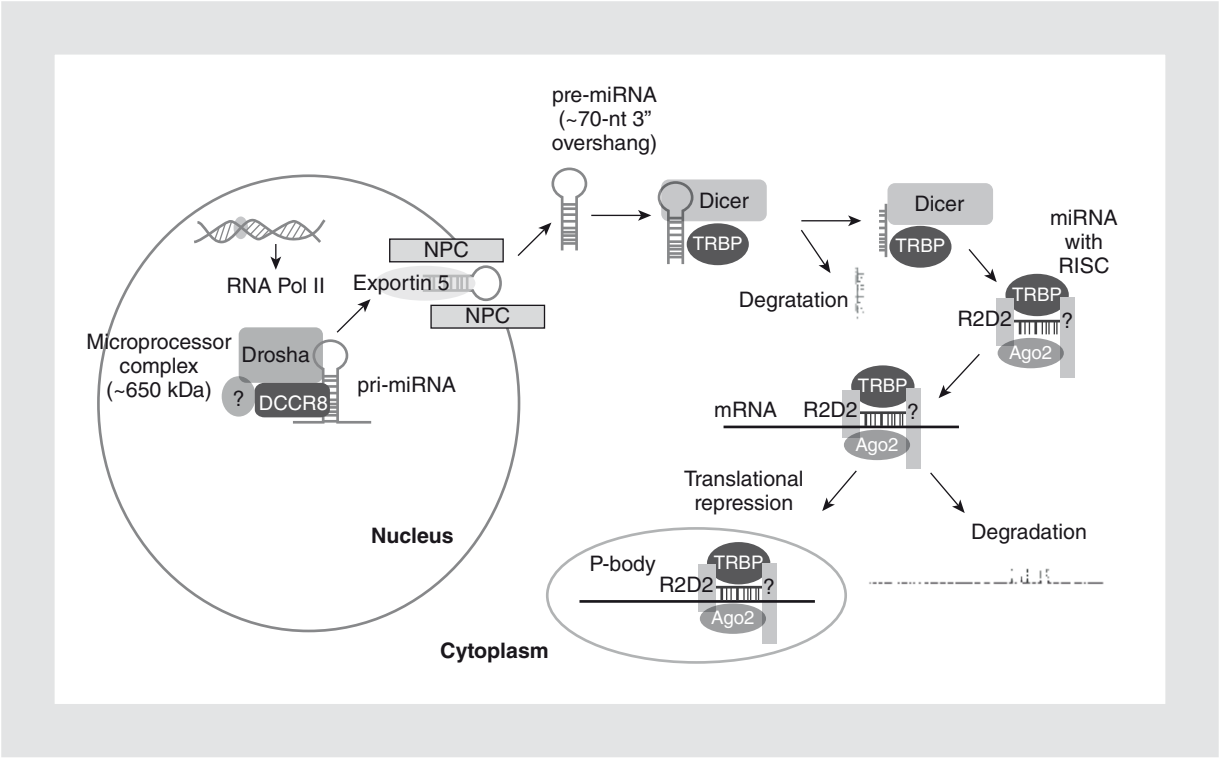

Figure 1. *Overview of the mechanism of action of microRNAs. MiRNAs are initially transcribed by RNA polymerase II as primary miRNAs in the nucleus. The primary miRNAs are processed in the nucleus by the microprocessor complex into precursor miRNAs. Precursor miRNAs are exported into the cytoplasm and processed by a complex formed by the Dicer and the transactivating response RNA-binding protein. The Dicer cleaves the loop regions of the pre-miRNAs at the base of the loop generating miRNA:miRNA duplexes. One of the miRNA strands of the duplex is incorporated into the RNA-induced silencing complex, while the other one is targeted for degradation. Gene silencing by miRNAs is achieved via two different mechanisms: degradation or translational repression. NPC: nuclear pore complex; pri-miRNAs: primary miRNAs; pre-miRNAs: precursor miRNAs; TRBP: transactivating response RNA-binding protein; RISC: RNA-induced silencing complex.*

into the cytoplasm with the aid of exportin 5 protein, which recognizes the specific structure of the precursor miRNAs[11]. In the second step of miRNA maturation, loop regions of precursor miRNAs are cleaved at the base of the loop by the RNAse III Dicer, which in mammalian systems forms a complex with the transactivating response RNA-binding protein enzyme to generate ~21 nt miRNA:miRNA* duplexes[12-15]. The miRNA strand of the duplex, which is characterized by a less stable 5' end referred to as the guide miRNA, is then incorporated into the RNA-induced silencing complex[15], following a separation and selection step based on the thermodynamic stability of the complex[16], and the other one is targeted for degradation[17] (Fig. 1).

MicroRNA-mediated gene silencing mechanisms

The RNA-induced silencing complex functions as a small RNA-directed gene-silencing mediator and its main component is the Ago2 protein[15]. Gene silencing by miRNAs may be achieved via two different mechanisms: cleavage of target mRNA and translational repression, depending on the complementarity of the miRNA and its target. In the first mechanism, the miRNA guides the complex to perfectly complementary target sequences and, upon binding, the target mRNA is cleaved by a member of the Argonaute family of proteins, which acts as an endonuclease[18-20]. This mechanism is most commonly observed in plants, but a number of animal[21,22] and viral[23] miRNAs have also been reported to act in this manner.

When the miRNA guides the complex to a complementary but not perfectly matching target region in the 3'UTR of the mRNA, then protein translation is repressed without degrading the mRNA[18,24,25]. Binding of the miRNA to its target relies on a very short segment of the 5' region of the miRNA, also known as the seed region. The short size of the binding region implies great target variation and therefore regulation of a large number of genes[9,26] by a single miRNA molecule. These processes most likely take place in cytoplasmic

foci called processing bodies (P-bodies), also known as GW-bodies as they have been shown to be concentrated with Argonaute proteins and miRNAs[27] (Fig. 1).

Another possibility is the incorporation of the RNA in the RNA-induced initiation of transcription silencing complex, which is guided by the miRNA to complementary regions of chromosomal DNA recruiting factors involved in chromatin remodeling, thus inducing transcriptional silencing[28-30].

MicroRNAs functions and roles

MicroRNAs are implicated in the regulation of a large spectrum of biological functions. Initial experiments on *C. elegans, Arabidopsis*, and mice implied their involvement in developmental timing[4,31-33]. Studies are now suggesting miRNAs are mediators of signal transduction, apoptosis, cell proliferation, and oncogenesis[26,34,35].

MicroRNAs have been implicated in viral oncogenesis as it has been shown that viral integration may lead to the upregulation of oncogenic miRNAs[36,37] and loss of miRNA function[38]. Moreover, viral infection triggers antiviral mechanisms such as the toll-like receptor pathway, which leads to the activation of transcriptional regulators such as nuclear factor kappa B (NFκB), which regulate the expression of oncogenic miRNAs[39].

Recent findings show that cellular miRNAs may be part of antiviral mechanisms of the host cell. It has been reported that miR-32, a cellular miRNA, suppressed infection by the primate foamy virus 1[40] and cellular miRNAs, miR-323, miR-491 and miR-654, have been reported to inhibit H1N1 influenza A virus infection[41]. Furthermore, a number of viruses such as herpes virus, HIV-1, and Epstein-Barr virus have been shown to encode miRNAs[23,42,43], the role of which is implicated in the regulation of cellular gene expression and latency. Primate foamy virus has also been reported to express Tas protein, which inhibits cellular miRNA functions and therefore infection was increased in Tas-expressing cells[40].

MicroRNAs potentially encoded by HIV

HIV genome

HIV is a retrovirus, the genome of which consists of two identical single-stranded RNA molecules[44]. The genome of HIV can form various complex and dynamic secondary structures involved in a number of processes such as transcription by RNA polymerase II, transport of spliced and unspliced molecules, and reverse transcription[45,46]. The transactivation response element (TAR) and the Rev responsive element (RRE) are two examples of such structures within the HIV genome. They both form loop regions and therefore resemble primary miRNA precursors, which can be targeted by the RNAi machinery. Furthermore, they are both involved in viral replication; TAR is the Tat-binding region, which leads to efficient transcription by RNA polymerase II[47,48], and RRE mediates the nuclear export of singly spliced or unspliced RNA through its interaction with Rev[49].

HIV-encoded microRNAs

The HIV-1 genome stem loop regions mentioned above harbor a number of precursor miRNA structures and, according to computer modeling studies, up to 10 mature viral miRNAs in various regions of the HIV-1 genome, including the TAR, capsid gag, the gag-pol frameshift, the nef gene and the 3' long terminal repeat (LTR), may be encoded[50]. Data also indicate that miRNAs regulating the production of CD28, CTL4, and various interleukins may also be encoded[51].

Recent studies have described HIV-encoded miRNAs (Table 1). The miR-N367 is a 25 nt nucleotide derived from the nef/LTR overlapping region[52] and HIV TAR miRNA is derived from the TAR loop region. Both miR-TAR-5p and mir-TAR-3p have also been identified in the TAR region[53], and miR-H1 is a miRNA molecule identified downstream from the NFκB sites of the LTR promoter region[54].

However, the production of miRNAs by HIV still remains a controversial matter as another study reported non-significant levels of miRNAs in persistently infected T-cells[55]. Furthermore, the TAR miRNAs have been described by two different groups, but with very low expression levels, and no reproducible data exist for miR-H1 and miR-N367.

MicroRNAs and HIV latency

A general property of all retroviruses and HIV-1 is latency, allowing persistence of HIV-1 in the setting of effective, or virally suppressive, HAART[56]. Retroviral latency is defined as integrated provirus with no active transcription. Cultivable virus has been shown to be recovered from HIV-1 patients at all stages of the disease[57]. However, studies have also shown that in patients, some cells may carry proviral DNA but express very little or no RNA and therefore produce very low amounts of virions[58,59]. Although virus replication is not

Table 1. Cellular microRNAs interacting with HIV and putative HIV-encoded microRNAs and their proposed role

MicroRNA		Role
Cellular		
miR-28, 125b, 150, 223, 382		Influence of viral latency[64]
miR-198		Repression of Cyclin T expression levels[63]
miR-29a and miR-29b		Suppression of expression of HIV-1 Nef protein; viral replication[66]
Cluster miR-17/92 encodes: miR-17-(5p-3p), 18,19a, 20a, 19b-1, 92-1.		Affect viral replication[67]
Viral	**Region**	
miR-N367	Nef-LTR	Suppression of HIV-1 Nef expression[68]
miR-H1	LTR	Cleavage of apoptosis antagonizing transcription factor and degradation of gene products[54]
miR-TAR	TAR	Apoptosis protection by downregulation of excision repair protein (ERCC1) and intermediate early response 3 (IER3)[43]
miR-TAR-5p	TAR	Regulation of gene expression[53]
miR-TAR-3p	TAR	Regulation of gene expression[53]

LTR: long terminal repeat; TAR: transactivation response element.

known to occur in CD4 T-cells of patients under suppressive HAART, multiply spliced or unspliced viral RNA as well as proviral DNA has been detected in cells targeted by HIV-1[60,61]. The mechanisms by which latency is established and maintained in the different cell types is not yet defined, and the complex role of miRNAs in gene expression and post-translational mechanisms has identified them as a plausible mediator of HIV latency.

Cellular microRNAs and latency

Cellular miRNAs are abundant in resting $CD4^+$ T-cells and may contribute to HIV latency. Several potential binding sites for cellular miRNAs have been identified on the 3' untranslated region of HIV RNA of different viral strains and may inhibit viral protein expression. The expression levels of five miRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) were found to be upregulated in resting T-cells compared to activated T-cells. Inhibition of these miRNAs by specific antisense inhibitors resulted in an increase of virus particle production in both resting T-cell lines and resting T-cells from HAART-experienced HIV-infected individuals, suggesting that they are highly likely to play an important role in viral latency and that their inhibitors counteract their effects.

The role of the same miRNAs was implicated in HIV-1 infectivity of monocytes/macrophages[62]. Increased levels of miRNAs-28, 150, 223, and 382 were detected in monocytes isolated from peripheral blood mononuclear cells (PBMC), which were reduced according to the stage of their differentiation. The lowest levels of these miRNAs were found in macrophages. Furthermore, reduction of the level of these miRNAs in PBMC monocytes increased their infectivity by HIV-1 and upregulation of the miRNAs in macrophages decreased their infectivity[62].

Another cellular miRNA reported to be downregulated during monocyte to macrophage differentiation is miR-198. MiR-198 is thought to repress expression levels of the protein Cyclin T1, levels of which are regulated during monocyte-macrophage differentiation, and a known Tat cofactor, the suppression of which might reduce viral gene expression and replication[63]. Furthermore, Cyclin T1 is a component of positive transcription elongation factor (P-TEFb), a general RNA polymerase II elongation factor, and therefore miR-198 could potentially affect expression of Cyclin T1-dependent genes[63].

The specific region targeted by these miRNAs (3' untranslated region) encodes for Tat and Rev, which are essential proteins for viral RNA transcription and translocation[64]. The potential effect of Tat on viral latency was also shown by another study in which a mouse

model was used to demonstrate that Tat activates latent proviruses from their PBMC[65].

The hsa-miR-29a and 29b cellular miRNAs have been reported to suppress expression of HIV-1 Nef protein and therefore viral replication. Furthermore a miR-29a inhibitor resulted in an increase in virus production in transfected cells[66].

Type III RNase Dicer has also been implicated in the processing of miRNAs viral replication control. HIV-1 was shown to replicate better in Dicer-knockout cells, suggesting that the effect of Dicer on the expression of miRNAs mediates viral replication. Furthermore, the finding also allows speculation on the direct effect of Dicer on viral RNA itself.

Finally, investigation of the effect of specific miRNAs on HIV-1 replication identified a polycistronic cluster of miRNAs miR-17/92, the levels of which were decreased upon HIV-1 infection. This cluster encodes miR-17-(5p/3p), miR-18, miR-19a, miR-20a, miR-19b-1 and miR-92-1 molecules, which are thought to act on host cellular factors rather than on the viral genome as the HIV genome was not their direct target[67]. Both miR-17-5p and miR-20a were found to target the histone acetyltransferase P300/CBP-associated factor (PCAF), which is a Tat cofactor and has potential target sites in its 3' UTR for these miRNAs, and HIV-1 production was enhanced in transfected cells with antisense inhibitors of miR-17-5p and miR-20a[67].

The above studies suggest that host miRNAs might be involved in HIV-1 latency in various ways such as regulating the transition from latency to activation, the clearance of latent reservoirs, and the reduction virus production.

Viral microRNAs and latency

The possibility of HIV encoding miRNAs raises the question of their involvement in latency. Initially it was demonstrated that HIV-1 TAR is processed by Dicer enzyme and yields a viral miRNA[43]. Further investigation on HIV-1-infected cell lines resulted in the identification of an HIV-1 miRNA derived from the 5' portion of the TAR element. This HIV-1 TAR miRNA was linked to the inhibition of LTR-driven gene expression and therefore transcriptional silencing of HIV-1, and may have a protective role against apoptosis[43]. Furthermore, another two miRNAs derived from the two arms of TAR in HIV-1-infected cell lines have been described. Mir-TAR-5p originating from the left arm of TAR and mir-TAR-3p originating from the right arm of TAR were found to be involved in the downregulation of the TAR miRNA sensor activity by processing through a miRNA-guided RNA silencing machinery[53].

Mir-N367, a miRNA generated by the ***nef*** region[68], may regulate the expression of ***nef in vivo,*** resulting in the blocking of HIV transcription, and could therefore have a role in HIV latency. Further investigation of the role of miR-N367 suggests it may regulate HIV-1 transcription through the U3 region negative responsive element[52].

MicroRNAs and potential therapeutic options for HIV infection

A number of studies have already reported the employment of RNA interference by either transient transfection with small interfering RNA (siRNA)[69] or the stable expression of vectors containing short hairpin RNA (shRNA)[70] in order to eliminate HIV infection, but have shown short-term success in reducing viral inhibition. Due to its high mutational rate and the fact that it may encode siRNA suppressors, HIV may evade the RNA interference process. The use of multiple siRNA, which target various parts of the genome, was shown to overcome viral escaping and have a stronger effect on the inhibition of infection[71]. Similar results were reported by another study, in which multiple expressions of siRNA from a single polycistronic miRNA molecule was employed[72].

However, the putative use of miRNAs in therapy is accompanied by various issues that need to be overcome for successful therapy options. The main issue is targeting the tissue of interest without affecting other tissues or organs, and therefore the delivery approach becomes highly important. Moreover, limiting the off-target effects is a major concern as a single miRNA molecule may target multiple proteins. Various delivery systems have been described so far and these may be divided in specific and non-selective methods. The former include the use of nanoparticles, aptamers, or antibody fragments, whereas the latter involve the use of lipids or polymers, and some combination approaches have also been described. Another approach for the delivery of shRNA involves use of lentiviral vectors, which may carry the molecule to the target cells. However, in the STEP trial, a recent attempt to use an adenoviral system for the delivery of HIV antigens to negative hosts in order to generate an immune response, the outcomes were not successful, indicating the need for further investigation[73].

The most promising example in the field of miRNAs in therapeutics is the case of Miravirsen. Miravirsen is an inhibitor of miR-122, a cellular miRNA that is essential for viral replication. Recent data from phase II

clinical trials demonstrate that administration of Miravirsen to hepatitis C virus patients resulted in viral load decrease to undetectable levels[74].

Conclusions

MicroRNAs hold a great potential for unraveling the complex virus-host interactions in order to comprehend infection mechanisms and unveil viral mechanisms of persistence in patients. Further investigation is needed in order to know the specific role of miRNAs in HIV-1 latency, a multifactorial and complex process, which remains to be elucidated in order to eradicate viral reservoirs. The identification of the possible involvement of miRNAs in different viral properties may point towards novel and promising therapeutic strategies.

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