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MicroRNAs and their potential involvement in HIV infection

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Abstract

Treatment and cure of human immunodeficiency virus-1(HIV-1) infection remains one of the greatest therapeutic challenges due to its persistent infection, which often leads to acquired immunodeficiency syndrome (AIDS). Although it has been 28 years since the discovery of the virus, the development of an effective vaccine is still years away. Relatively newly discovered microRNAs (miRNA) are a family of small non-coding RNAs that can regulate gene expression primarily by binding to the 3' untranslated region (UTR) of targeted transcripts. Understanding how HIV-1 infection affects the host miRNA pathway could generate new insights into the basic mechanisms underlying HIV-1-mediated pathologies and T-lymphocyte depletion. Here, we review literature related to the biogenesis of HIV-1 encoded miRNAs, cellular miRNAs that can directly target HIV-1 or essential cellular factors required for HIV-1 replication. We also discuss the feasibility of using miRNAs for HIV-1 therapy.

Keywords

microRNA; HIV

HIV-1 and AIDS

Human immunodeficiency virus (HIV) -1 is a lentivirus, and it is the primary cause of acquired immunodeficiency syndrome (AIDS) [1-2]. HIV-1 primarily infects CD4⁺ T memory cells, and its infection can be roughly divided into two phases: an acute phase (early phase), which lasts about one to two months after the initial infection, and a chronic infection phase, which can last for upwards of 10 to 20 years before the onset of AIDS [3-4]. HIV-1 is a relatively small RNA virus; its 9.7 kilobase (KB) RNA genome consists of several secondary structures (LTR, TAR, RRE, PE, SLIP, CRS, INS) and nine genes (gag, pol, env, tat, rev, nef, vif, vpr, vpu) encoding 19 proteins (Figure 1). There was optimism after the virus was initially discovered to be the cause of AIDS that within a few years a vaccine would be available to both treat and prevent infection. Despite numerous attempts by vaccine experts around the world, efforts to create an effective vaccine have failed. The failure of the Merck's STEP trial has brought HIV-1 vaccine research back to the starting point. The adenovirus serotype 5 vaccine used in the STEP trial was designed to make the

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body produce killer T-cells that could recognize and target Gag, Pol and Nef of HIV-1 subtype B, the predominant strain in America and Australia. This vaccine actually may have promoted, rather than prevented, HIV-1 infection [5-6].

The difficulties in creating an effective vaccine exemplify the need for more basic research into the host and viral mechanisms which support or ameliorate HIV-1 infection. Relatively newly discovered microRNAs (miRNA) are a family of small non-coding RNAs that can regulate gene expression primarily by binding to the 3'UTR of targeted transcripts. Research into HIV-1 infection affects the host miRNA pathway could improve understanding of the mechanisms underlying HIV-1-mediated pathologies and T-lymphocyte depletion. In this review, we examine the biogenesis of HIV-1 encoded miRNAs, cellular miRNAs that can directly target HIV-1 or essential cellular factors required for HIV-1 replication. We also discuss the feasibility of using miRNAs for HIV-1 therapy

MiRNAs biogenesis

MicroRNAs (miRNAs) are 21-23 nucleotide long regulatory, non-coding, small RNAs that repress target gene translation through base pairing to complementary sequences in the 3' untranslated region (3'UTR) of targeted transcripts [7]. Because microRNAs modulate many different types of viral infections, it was quickly speculated that miRNAs could directly target the HIV-1 viral RNA genome and play a role in modulating HIV-1 replication and infection [8].

The generation of miRNAs takes place through two major steps (Figure 2). First, the production of a precursor hairpin structure (pre-miRNA) is created from a primary miRNA transcript (pri-miRNA) by a microprocessor (formed by Drosha and DGCR8) in the nucleus. Next, the pre-miRNA is exported to the cytoplasm by Exportin5-RAN-GTP for further processing by Dicer [9-11]. Dicer excises the pre-miRNA to produce miRNA/miRNA* duplexes (guide strand/passenger strand). Dicer does not act alone; it needs one of the synonomous RNA binding proteins, TRBP or PACT to function as its partner [12-14]. The 'guide strand', is selected and incorporated into the RNA-induced-silencing-complex (RISC), and the other strand, the 'passenger strand', is usually degraded [15-16]. The guide strand is loaded onto Argonaute (Ago) to form the RISC. The human Argonaute family has four members, Ago1 through Ago4, which are closely related and are co-expressed in many cell types. However, endonuclease activity is exclusively associated with Ago2 [17-19]. It was found that miRNA-mediated repression was mostly dependent upon the seed sequence (the six nts of position 2 to 7 from the 5' end of a miRNA) Watson-Crick base pairing with its complementary target sequence, and G:U wobble pairing is generally not allowed [20]. Positions 13 to 16 can help the functional miRNA-target pairing and may compensate for weak seed region interactions [21]. RISC may direct miRNA repressed mRNAs to Processing bodies (P-bodies: small cytoplasmic granules for RNA degradation) for deadenylation, decapping and degradation, or alternatively for temporary storage and reuse of repressed transcripts [22-25]. P-bodies are especially of interest to the HIV-1 replicative cycle because they may serve as a storage compartment for HIV-1 genomes and could contribute to latency.

Studying the interactions of HIV-1 and miRNAs may generate new insights into the mechanism of HIV-1 infection. For instance, it would be useful to know if HIV-1 infection affects the miRNA pathways and if miRNA pathways target the HIV-1 genome to modulate infection. Below, we discuss evidence for each of these possibilities.

Perturbation of the microRNA biogenesis pathway by HIV-1 infection

An important concern is whether or not HIV-1 infection globally affects miRNA levels (affecting miRNA biogenesis through the miRNA processing pathway) or individually (affect the biogenesis of individual miRNAs through transcription perturbation or effects on miRNA maturation). To affect miRNA or siRNA pathways globally, HIV-1 would have to encode a suppressor of RNA silencing (SRS) protein. It appears that a common phenomenon in viral infections is that virally encoded proteins act as SRSs against the host RNA interference (RNAi) pathway components [26-34]. In plants and some lower organisms, the host RNAi pathway is the immediate defense response against viruses and other pathogens. The host can use RNAi to directly target viral genomes or transcripts to silence their expression and block viral replication. As part of the ongoing battle between host and virus, viruses may produce an SRS to block the host RNAi pathway.

There are several methods by which HIV-1 infection could globally affect miRNA pathways [35]. The HIV TAR (cis-acting, trans-activation response) element is an RNA structure that is used by the HIV-1 Tat protein to regulate HIV gene transcription. Functional TAR and Tat interactions require the participation of cellular factors including TRBP and P-TEFb, which promotes transcriptional activation from the stalled RNA polymerase complex on the LTR. Because Dicer and TRBP form the miRNA/miRNA* generation complex, the TAR structure in HIV-1 RNA could titrate TRBP and affect miRNA biogenesis [12-13, 36]. Other HIV-1 proteins, such as Tat, and Vpr may bind to Dicer or Drosha and affect the miRNA biogenesis pathway. To date, there are four publications supporting regulation of the RNAi pathway by the HIV-1 transcriptional activator Tat [37-40]. Tat was shown to interact with Dicer and acts as an SRS [37-38]. In contrast, a separate study showed that Tat, Tax (HTLV-1), and Tas (PFV-1) failed to inhibit RNAi in human cells, and the stable expression of physiological levels of Tat did not globally inhibit miRNA production or expression in infected human cells [41]. The latter finding was supported by a publication which demonstrated that both HIV-1 Tat and TAR expression do not reduce the efficacy of cellular RNA silencing [42]. However, there are data supporting Tat as a SRS [39-40]. Lastly, Coley *et al.* showed HIV-1 Vpr can suppress Dicer expression in monocytes [43]. The conflicting evidence concerning HIV-1 encoded SRSs may be attributable to the different systems used for the published studies. Time course comparisons of infection, evaluations of different ratios of cells to virus, and comparisons of different subtypes of HIV-1 in carefully controlled experiments are necessary to resolve this issue.

A different but equally important question is whether or not the host miRNA pathway affects HIV-1 infection. One publication showed both knockdown of Dicer or Drosha boosted HIV-1 infection and produced infectious virus [44]. By contrast, a different study reported that knockdown of Dicer or Drosha produced non-infectious virus. [45]. Yet another study found that TRBP contributes mainly to the enhancement of virus production and Dicer does not mediate HIV-1 restriction by RNAi [46]. Because Dicer or Drosha knockdown also depletes the cellular miRNA population, which in turn affects the normal metabolism of cells, it is not surprising that Dicer or Drosha knockdown would affect HIV-1 infection. Using RNAi to knock down an RNAi component may complicate such studies, thus making it difficult to determine whether these observations are due directly to RNAi on-target effects or are secondary or off-target effects.

Perturbation of microRNA expression profiles by HIV-1 infection

MiRNA profiles comparing HIV-1 infected samples versus uninfected samples can reveal how HIV-1 infection affects the miRNA pathway. Dysregulated miRNAs could have prognostic and diagnostic value for HIV-1 therapies. Yeung *et al.* first reported the RAKE

(RNA-primed Array-based Klenow Enzyme) miRNA microarray data of miRNAs prepared from pNL4-3 transfected cells versus untransfected cells and concluded that HIV-1 downregulates miRNAs [47]. This result is in contradiction with many later reports which show that HIV-1 infection upregulates the levels of some miRNAs. Using the same RAKE miRNA microarray platform and Northern blotting, Triboulet *et al.* reported that the polycistronic miRNA cluster miR-17/92 was downregulated following HIV-1 NL4-3 infection in Jurkat cells. They also showed that Dicer and Drosha expression inhibited virus replication both in peripheral blood mononuclear cells (PBMC) from HIV-1-infected donors and in latently infected cells. They concluded that the downregulated miR-17/92 cluster would result in upregulation of the target transcriptional co-activator P300/CBP-associated factor (PCAF), a histone acetyltransferase that can interact with Tat and synergize to activate Tat function for efficient viral replication. In contrast to the marked reduction of this cluster of miRNAs in the Northern blotting results described above, a recent study by Hayes *et al.* showed HIV-1 only downregulated the miR-17/92 cluster by 50% [40]. These results may be reflective of the different types of cells used for the two studies; in the former, Jurkat cells were used, and HEK293 were used in the latter.

Houzet *et al.* reported miRNA profiles in PBMCs from 36 HIV-1 seropositive individuals using the RAKE miRNA microarray. They categorized the 36 HIV-1 patients into four classes based upon their CD4+ T-cell counts and viral loads. They observed downregulation of miR-29a and 29b in HIV-1 patients and infected PBMCs, and downregulation of miR-29c, miR-26a, and miR-21 in HIV-1 patient samples. They proposed that specific miRNA signatures can be observed for various classes of HIV-1-positive individuals [48]. Part of their profiling data overlapped with data published by Hayes *et al.*, providing confidence that those results are reproducible [40]. To further support their conclusions, miRNA profiling data from large populations of HIV-1 infected individuals matched for stage of infection, treatment protocol and HIV subtypes are necessary. The mechanism for downregulation of miR-29a, 29b, 26a and 21 remains elusive. Nevertheless, if HIV-1 infection truly affects miRNA transcription and maturation in particular subsets of PBMCs, such knowledge could be used for development of HIV-1 therapies, diagnosis, prognosis and treatment response parameters. Differences in sites of HIV-1 integration may also cause discrepancies in profiling results. Other factors, such as transfection, virus infection, differences in virus titers, duration of infection, choice of cell types for analyses, and differences in strains of HIV-1 used for such analyses can complicate the profiling results.

Regulation of HIV-1 infection by host miRNAs

Understanding the role of host miRNAs in HIV-1 infection is complicated by the possibility that miRNAs could target cellular factors that suppress or activate HIV-1 replication. The situation becomes more complex if one includes the potential role of HIV-1 encoded miRNAs in the infectious cycle. MicroRNAs involved in HIV-1 infection could be defined as HIV-1 encoded or host-encoded according to their source of biogenesis; they could also be defined as suppressors or activators of HIV-1 infection by their function. MiRNAs that target HIV-1 can be further divided according to whether they directly target HIV-1 transcripts or indirectly affect HIV-1 by targeting host factors that are involved in the HIV-1 life cycle, or targeting both the HIV-1 RNA genome and host factors essential for HIV-1 infection. A single miRNA potentially can target over 100 transcripts. Most likely, miRNAs will target both suppressors and activators of HIV-1 infection simultaneously. This makes it difficult to classify one miRNA as a suppressor or an activator of HIV-1 infection without extended knowledge of their targets and effects on cell physiology.

It is of interest to determine if host miRNAs play a role in the regulation of HIV-1 gene expression, and whether or not any host miRNAs directly target the HIV-1 RNA genome.

Under normal conditions, miRNAs function as negative regulators of gene expression by binding to the target message 3'UTR [49-50]. Because the HIV-1 Nef sequence also serves as the 3'UTR for most viral transcripts, miRNA binding sites in this region may play a crucial role in HIV-1 infection [51] (Figure 1). Nef has been shown to play a positive role in viral replication and pathogenesis. HIV-1 strains with Nef gene deletions result in slower progression to AIDS (Reviewed in ref.[52]). Thus, miRNAs targeting the Nef region have the potential to affect HIV-1 pathogenesis. Several host miRNAs that target conserved regions of the HIV-1 genome were predicted *in silico* [53]. Using miRNA targets predicting program PITA (Probability of Interaction by Target Accessibility) [54], we predicted there are thousands of potential cellular miRNA seed sequence(6 nt to 8 nt) complementary sites in the Nef/3'LTR region (Table 1: lists miRNAs which have 7 nt to 8 nt seed sequence perfect matches to the HIV NL4-3 Nef/3'LTR region). Although not every target site is functional, simultaneous targeting by several miRNAs can be synergistic in downregulating target gene expression. It thus still remains an open question as to whether or not host miRNAs are functionally active in HIV replication.

Huang *et al.* compared the miRNA expression patterns in activated CD4+ T lymphocytes to resting CD4+ T lymphocytes [55]. They concluded that several host miRNAs (miR-125b, miR-150, miR-28, miR-223, and miR-382), which are highly expressed in resting CD4+ T lymphocytes, may target the Nef/3'LTR region and contribute to HIV-1 latency. Specific antagomirs (anti-micro RNA antisense) against these miRNAs substantially increased HIV-1 protein translation in resting CD4 cells [55]. They also proposed that application of pooled antagomirs targeting these miRNAs could possibly be used to purge latent state HIV-1 reservoirs [56]. Similar but somewhat controversial data have been reported for monocytes/macrophages [57-58]. The determination of the expression levels of those miRNAs enriched in resting CD4 cells relative to activated CD4 cells is difficult to evaluate because the absolute expression levels in CD4 cells are not very high according to published large scale miRNA cloning data [59-60]. Although it is difficult to perform experiments to profile miRNAs in HIV-1 infected CD4 cells versus resting CD4 cells, these types of experiments could provide valuable data supporting modulation of endogenous miRNAs for purging latently infected cells. It is also worth pointing out that highly expressed miRNAs may be important for normal cellular homeostasis. Thus, using pooled antagomirs to block miRNA functions to activate HIV-1 in latently infected cells could result in toxicities in uninfected cells.

It has been reported that miR-29a targets the HIV-1 Nef gene, and ectopic expression of this host miRNA resulted in the repression of Nef protein levels and a reduction in viral levels [61]. Nathans *et al.* also found that miR-29a can target HIV-1 and repress replication and infectivity. They also showed that miR-29a can directly target HIV-1 transcripts to P-bodies, and this could be a mechanism for maintaining HIV-1 in a latent state [45]. Other miR-29 family members can also target HIV-1 [53]. Interestingly, miR-29 family members are observed to be downregulated in both HIV-1 patients and infected PBMCs [40, 48]. Sung *et al.* reported that host miR-198 restricts HIV-1 replication in monocytes by repressing Cyclin T1 expression, thereby supporting functional roles for miRNAs that do not directly target HIV-1 but indirectly contribute to HIV-1 infection by targeting host factors essential for HIV-1 pathogenesis [62]. A study by Chable-Bessia *et al.* showed that the major components of P-bodies, such as RCK/p54 (an RNA helicase), GW182 (a P-body marker protein which interacts with Argonautes), LSm-1(an RNA binding protein) and XRN1 (a 5' to 3' exoribonuclease), negatively regulate HIV-1 gene expression by blocking viral mRNA association with polysomes, therefore supporting P-bodies as a place to sequester HIV-1 transcripts. Interestingly, their data also showed that knockdown of RCK/p54 or DGCR8 resulted in virus reactivation in PBMCs isolated from HIV-1 infected patients on highly active antiretroviral therapy (HAART) [63]. These results imply that HIV-1 RNAs can be

sequestered in P-bodies but can re-enter translation upon P-bodies disruption, thus creating a type of latent infection different from the gene silencing based latency.

HIV-1 encoded vmiRNAs

A common phenomenon in plants is that viral dsRNAs are processed to siRNAs which can act in post-transcriptional gene silencing, targeting the viral genomes for destruction and effectively preventing re-infection by the same virus [64]. In mammals, several virally encoded miRNAs (vmiRNAs) have been described and studied [30, 32, 65-84]. An example of a vmiRNA is one which is derived from Simian virus 40 (SV40) encoded miRNA-1. This vmiRNA accumulates late in SV40 infections, and its sequence is perfectly complementary to the early viral mRNAs transcript. Therefore, it can target these viral mRNAs for degradation [73]. It is still debatable as to whether or not HIV-1 encodes vmiRNAs because reproducible data for such vmiRNAs are still lacking [41, 85]. HIV-1 encoded candidate small RNAs and their cellular targets were described first from *in silico* studies [86]. Subsequently there have been several studies addressing HIV-1 encoded vmiRNAs. There are three miRBase documented HIV-1 encoded miRNAs: miR-H1-5p, miR-N367-3p and miR-TAR-5p/3p (Table 2).

The controversy in this area is highlighted by a systematic study which showed neither HIV-1 nor human T-cell leukemia virus type 1 (HTLV-1) expressed significant levels of either siRNAs or miRNAs in persistently infected T cells [41]. No repeat analyses for miR-N367, miR-H1 and vmiRNA1 have been published. The putative TAR miRNAs are exceptions. These have been reported by two independent groups, but the expression levels of the putative TAR-miRNAs are extremely low. Only *in vivo* processed TAR-3p can be detected by Northern blotting [91-94]. The cloned sequences from TAR-3p are 17 or 18 nt long, which makes them shorter than the normal 21 to 22 nt lengths of most Dicer processed miRNAs [94]. Pyro 454 sequencing of HIV-1 derived small RNAs yielded only about 100 reads which mapped to the HIV-1 genome [95]. All of these findings suggest that HIV-1 derived vmiRNAs are not a dominant miRNA group.

HIV-1 is a lentivirus that has an RNA genome. RNA viruses have been engineered to produce miRNAs that in essence sequester the cellular miRNA machinery while producing functional miRNAs during viral infection without impairing their own replication [96-98]. Therefore, it is possible that RNA virus-encoded miRNAs exist in nature. In the case of HIV-1, because of its sloppy replication mechanism, any encoded miRNAs would rapidly change and lose their target specificity. The exception is the TAR element for which there is a strong selective pressure to maintain the sequence composition. It therefore remains a possibility that the TAR 5p and or 3p miRNA-like sequences play a functional role in the HIV-1 life cycle, but the evidence to date is clearly less than compelling.

Concluding remarks

In summary, the question of whether or not miRNAs (host or viral) play an essential role in HIV-1 pathogenesis remains largely unanswered. The regulation of HIV-1 infection by host miRNAs that directly target the viral genome is possible, but it remains uncertain how effective these cellular miRNAs are in influencing HIV-1 infection. The regulation of HIV-1 infection via miRNA targeting of cellular factors is complicated because they affect the expression of genes that could facilitate or inhibit HIV infection. Finally, the question of whether or not HIV-1 infection produces sufficient amounts of HIV-derived small RNAs to regulate its infectious cycle also remains open. Given that cellular miRNAs are perturbed in HIV infection and the virally encoded TAR miRNA-like sequences are present in productive infections, it is conceivable that miRNAs could be used to monitor the pathogenic potential of viral isolates and could also be used as prognostic markers for treatment response.

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Figure. 1.

Diagram of HIV-1 NL4-3 genome.

HIV-1 genome contains two LTRs and nine major genes. The nine genes encode the structural or accessory proteins: Gag (p17,p24,p7,p6), Pol (RT, IN), Vif, Vpr, Vpu, Tat, Rev, Env (gp120, gp41) and Nef. HIV-1-vmiRNA-H1 and N367 are located in the U3 region of both LTRs. The Tar miRNA is also derived from the R region of both LTRs.

Figure 2.**MiRNA biogenesis pathway**

Celular miRNA processing involves a two-step process: 1) generation of pre-miRNAs in the nucleus by Drosha/DGCR8 complex, and 2) creation of mature, functional miRNAs in the cytoplasm by the Dicer/TRBP complex. The mature miRNAs will be loaded into the RNA induced silencing complex (RISC).

Table 1

PITA predicted miRNAs target HIV-1 Nef/3LTR (7 to 8 nt seed sequence)

microRNA	Position in Nef-3LTR	Position in NL4-3	Seed	microRNA	Position in Nef-3LTR	Position in NL4-3	Seed
hsa-miR-214	39	8825	8	hsa-miR-581	349	9135	7
hsa-miR-600	44	8830	7	hsa-miR-562	356	9142	7
hsa-miR-24	68	8854	7	hsa-miR-328	387	9173	7
hsa-miR-149	70	8856	7	hsa-miR-29a	420	9206	8
hsa-miR-137	136	8922	7	hsa-miR-29b	420	9206	8
hsa-miR-103	154	8940	8	hsa-miR-29c	420	9206	8
hsa-miR-107	154	8940	8	hsa-miR-149	445	9231	7
hsa-miR-15a	156	8942	7	hsa-miR-138	482	9268	8
hsa-miR-15b	156	8942	7	hsa-miR-484	503	9289	8
hsa-miR-16	156	8942	7	hsa-miR-942	532	9318	7
hsa-miR-195	156	8942	7	hsa-miR-203	569	9355	7
hsa-miR-424	156	8942	7	hsa-miR-623	636	9422	7
hsa-miR-497	156	8942	7	hsa-miR-103	708	9494	7
hsa-miR-218	176	8962	7	hsa-miR-107	708	9494	7
hsa-miR-1224-3p	193	8979	8	hsa-miR-646	722	9508	8
hsa-miR-134	205	8991	8	hsa-miR-24	766	9552	7
hsa-miR-616	232	9018	8	hsa-miR-484	767	9553	8
hsa-miR-22	246	9032	7	hsa-miR-603	844	9630	7
hsa-miR-147	345	9131	7	hsa-miR-377	860	9646	8

Table 2

HIV-1 encoded putative miRNAs/siRNAs and their proposed functions.

Name	Sequence (miRBase)	References	Function
miR-N367	acugaccuuuggauggugcucaa	[87-88]	Suppression of nef
miR-H1	ccaggaggcgugccugggc	[89-90]	Targets human cellular AATF
TAR-5p	ucucucugguuagaccagaucuga	[91-94]	Acts against apoptosis by specifically down-regulating cellular apoptosis genes ERCC1 and IER3; Chromatin remodeling of the viral LTR
TAR-3p	ucucuggcuaacuaggaacca	[91-94]	
vsiRNA1	uccuuggguucuuaggagc	[37]	Rescues Env RNA expression