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Review Article

Investigating the role of circulating microRNAs in human immunodeficiency virus infection: friends or foes?

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ABSTRACT

Introduction: Human immunodeficiency virus (HIV), which causes Acquired Immunodeficiency Syndrome, still affects millions of people worldwide. Despite recent advances in the understanding of biological mechanisms of viral replication, there are relevant gaps regarding the virus-host relationship. Unraveling these complexities may lead to the development of new therapeutic strategies and the establishment of new biomarkers useful for the diagnosis and prognosis of infection and its comorbidities. Therefore, in this study we discuss the main biological characteristics of microRNAs and the potential use of these nucleic acids in their free circulating form as indicators of risk or protection against HIV infection.

Methods: A narrative review of the literature was carried out in the following databases through keyword and/or health descriptor searches: i) Google Scholar; ii) CAPES periodicals portal; iii) United States National Library of Medicine (PubMed) and iv) Elsevier's Science Direct library. The keywords "microRNA; HIV infection; circulating microRNA; biomarkers" were used to search the databases as mentioned above.

Results: Circulating microRNAs (ci-miRNA) are closely related to numerous processes in the HIV infection pathophysiology. They are involved in viral latency, increased viremia, hepatic injury, heart dysfunction, pulmonary hypertension, immune response impairment, and participate in Kaposi's sarcoma pathology. Additionally, these molecules may indicate protection in elite controllers, reduce viral replication and load, and be useful markers of the infection's eclipse phase.

Conclusion: Ci-miRNA levels are altered levels in individuals with HIV, playing a dual role in infection. Advances in research have shown that ci-miRNAs could differentiate stages of HIV infection and diseases associated with a viral infection and serve as biomarkers for antiretroviral therapy's effectiveness through changes in their expression.

Keywords: *MicroRNAs; HIV; biomarkers; circulating miRNA*

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INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS), a disease of infectious and contagious etiology, is caused by the human immunodeficiency virus (HIV)[1]. The United Nations Program on HIV/AIDS estimated that by the end of 2018 there were 37.9 million people infected with HIV worldwide. About 8.1 million of these people did not know they were living with HIV. Gradually, the annual number of new HIV infections worldwide has continued to decline. Since 2010, it has fallen from 2.1 million to 1.7 million, a reduction of about 16%[2]. However, during the same period in Brazil, new cases of infection have increased by 21%[3]. At this rate, the decline will still fall short of the United Nations' goal of 500,000 new infections worldwide by 2020[4].

HIV can be contracted through sexual intercourse, vertical transmission[5], needlestick injuries, or blood transfusion[6]. Although activated $CD4^+$ T lymphocytes

are the main target of HIV, other cells, such as resting $CD4^+$ T cells, monocytes, macrophages, and dendritic cells may also be infected, particularly those expressing the CD4 receptor and the CCR5 and/or CXCR4 chemokine receptors, which enable the virus to fuse through its viral envelope glycoprotein, gp120[7].

After the retrovirus enters the host cell, RNA is reverse-transcribed to DNA, after which the viral DNA is integrated into the host genome. Protein synthesis and assembly follows, and the virus multiplies, leading to host cell death[8]. In the early stage of infection, although no obvious signs may appear, the number of $CD4^+$ T cells in the body decreases, suppressing the cell-mediated immune response and eventually causing increased susceptibility to opportunistic infections[9].

After the discovery of microRNAs (miRNAs), scientists directed their studies to the possibility of identifying the expression profiles of these molecules in different pathological contexts, especially HIV infection. Specific changes in miRNAs during the infectious process have been described, suggesting that miRNAs are involved in infection pathogenesis[10]. For instance, miRNA-mediated post-transcriptional blockade restricts viral translation in resting T cells[11].

miRNAs are of great importance in the regulation of gene expression, acting at the post-transcriptional level to degrade or repress messenger RNA (mRNA). They belong to the non-coding RNA family and have a small nucleotide sequence (between 18-25 nucleotides)[12], being involved in several physiological processes, such as apoptosis, regulation of cell division, differentiation, proliferation, stress response, transcriptional regulation, and growth[12-13].

Although they are found primarily in the cytoplasm[12], they are also exported and found in circulation. They can be measured in blood samples and have remarkable stability in serum and plasma[14]. Thus, circulating miRNAs (ci-miRNAs) have gained scientific interest due to their diagnostic and prognostic roles in human diseases[15].

Studies on diabetes have demonstrated the presence of specific miRNAs in pancreatic islets[16]. Changes in miRNA expression are related to cancer, since they can interfere in the genes involved in cell proliferation, apoptosis, DNA repair, invasion, and metastasis, promoting oncogenic effects and loss of tumor suppressor function[17]. miRNAs can be found in plasma samples from patients with cardiovascular diseases and are involved in the pathophysiology of acute myocardial infarction (let-7g-5p, miR-1, miR-30d-5p, miR-106a-5p, miR-125b-5p, miR-144-3p, miR-208a, miR-424-5p, miR-499, miR-660-5p), atrial fibrillation (miR-23a, miR-26a, miR-126, miR-150, miR-483-5p), atherosclerosis (miR-29a, miR126, miR-212) and heart failure (miR-126, miR182, miR-423-5p)[18].

Accurate laboratory diagnosis of HIV is essential for reducing the risk that individuals with HIV will transmit HIV-1 infection. Therefore, host-derived prognostic and predictive biomarkers should be added to current diagnostic strategies[14].

Little is known about the potential of ci-miRNAs as biomarkers in HIV infection. Furthermore, to the best of our knowledge, no study in the literature has reviewed this possibility. Thus, this article aimed to detail the role of ci-miRNAs in the viral pathogenesis of HIV infection and their role as potential biomarkers of risk or protection for virus acquisition.

METHODS

A narrative review of the literature was carried out in the following databases through keyword and/or health descriptor searches: i) Google Scholar; ii) CAPES periodicals portal; iii) United States National Library of Medicine (PubMed) and iv) Elsevier's Science Direct library. After providing a current state of the art on HIV infection and the biology of miRNAs, a review of the English-language literature will be presented. The keywords "microRNA; HIV infection; circulating microRNA; biomarkers" were used to search the aforementioned databases, and following analysis of the title, abstract, and methodology, 41 articles, one declaration, and 2 reports were selected for further investigation. Nine of these publications were found eligible for inclusion, based on the following criteria: i) original research of an experimental character that ii) detected ci-miRNAs in biological fluids of HIV-infected patients using a sensitive and specific method.

All publications dealing with cellular miRNA, non-circulating miRNA, or that detected miRNA in any form other than extracellular were excluded from the analysis.

RESULTS

MiRNA biology

The first miRNA was discovered thirty years ago in a nematode, *Caenorhabditis elegans*, in association with the developmental regulator *lin-4*[19]. *lin-4* was originally thought to be a gene that encoded a conventional protein. However, Ruvkun and Ambros made the surprising discovery that *lin-4* did not encode a protein, but a regulatory RNA 22 nucleotides in length[20]. They demonstrated that *lin-4* RNA could be complementary with the mRNA of another gene

in the *C. elegans* development network, *lin-14*, and controlled the production of the *lin-14* protein[21].

miRNAs are mostly intergenic (68%). Of the intragenic RNAs, most are intronic (12%). The remaining miRNAs are located in coding regions, in tandem, 3' non-translatable regions, and in genes that encode long non-coding RNAs[22].

Concerning the canonical pathway of their biogenesis, miRNAs are transcribed in the nucleus by RNA polymerase II, along with several transcriptional factors[23], forming the primary miRNAs transcripts (Figure 1). Primary miRNAs are cleaved by the microprocessor complex (consisting of the ribonuclease Drosha and its cofactor DGCR8) to release the shortest precursor. This precursor molecule is transported to the cytoplasm by the cytoplasmic transport protein exportin-5, where it is cleaved by the Dicer ribonuclease, which results in a double strand of mature miRNA. This results in the 5p and 3p chains. The generated miRNA is loaded into an Argonaute protein, which is a major component of the RNA-induced silencing complex. One of the strands is discarded, while the other mediates post-transcriptional regulation of the target RNAs[24].

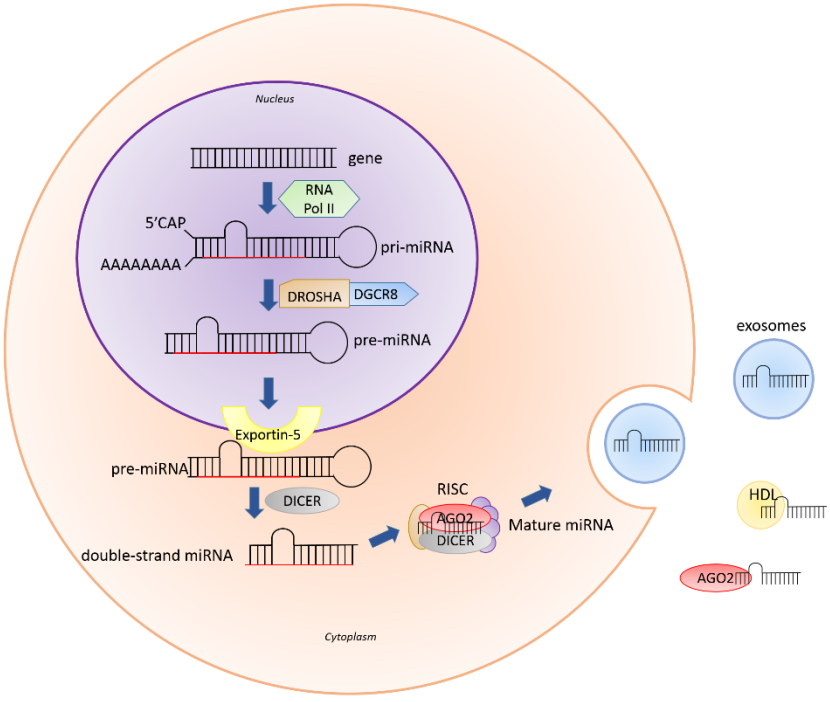

Figure 1: microRNA (miRNA) biogenesis. RNA Pol II: RNA polymerase II; pri-miRNA: primary microRNA; pre-miRNA: precursor-microRNA; AGO2: Argonaute 2 protein; HDL: high-density lipoprotein.

Therefore, miRNAs are highly complex with regard to gene regulation. mRNA transcripts are targets of their action, mainly due to their complementarity to the seed region, a heptameric sequence located between positions 2-7 of the 5' region of the mature miRNA. miRNAs mediate gene silencing, particularly the degradation of mRNA or, in the absence of complementarity, preventing its translation[25]. miRNAs are stable in body fluids such as serum, plasma[26], urine, saliva, sweat[27], and breast milk[28].

Exosomes are important vesicles attached to the cell membrane and are released from many types of cells in the extracellular space, being present in almost all biological fluids. Their release into the extracellular space occurs after fusion with the plasma membrane[29]. Several nucleic acids, including miRNAs, have been identified in the exosomal lumen. These may be absorbed by neighboring or distant cells and modulate gene expression and, consequently, the function of receptor cells (cells that absorb the exosome containing miRNAs)[30].

In addition to being wrapped in exosomes or microvesicles, extracellular miRNAs can be incorporated into high-density lipoproteins[31], which protects them from degradation and ensures their stability. Given the transportability of vesicles, the role of miRNAs in exosomes is gaining increasing attention. Transporting information through circulating vesicles is a third means of intercellular communication, considered

essential for signaling and cell-cell contact, as well as signaling via the transfer of soluble molecules[32].

miRNAs communicate information between cells by regulating gene expression at the post-transcriptional level. They are involved in almost every cellular process that has thus far been investigated, including regulation of viral infections and antiviral responses. It has been shown that miRNA-mediated changes in gene expression modulate viral replication, antiviral immune response, viral latency, and pathogenesis[33].

Since viruses often encode minimal genetic information, they depend on factors and molecular pathways in the cell to complete productive replication. Although there is evidence that host cells may express miRNAs with antiviral activity, this is not always the case. In fact, cellular miRNAs favor viral replication, associating with the virus or viral components to fight antiviral defenses[34].

Circulating microRNAs in HIV infection and associated clinical conditions

Chim et al.[34] were the first to identify the expression of miRNAs in human blood through quantitative real-time polymerase chain reaction (qRT-PCR). Since then, researchers have mainly investigated their expression in cancer, but also in infectious diseases of viral etiology, particularly HIV. The pleiotropic functions of ci-miRNAs in this context are summarized in Figure 2.

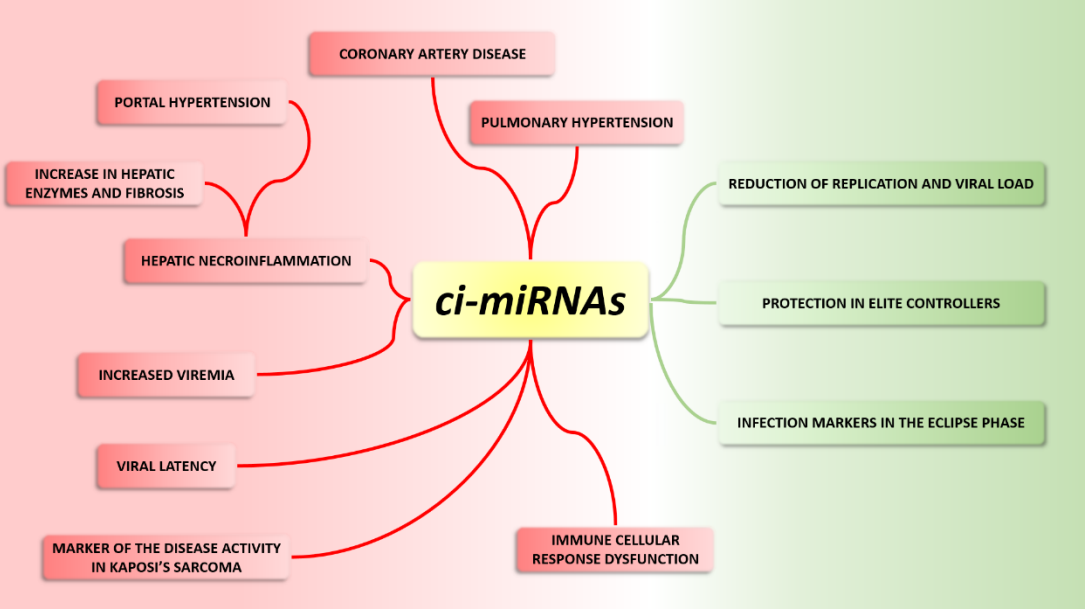

Figure 2: The dual role of ci-miRNAs in HIV infection.

Infographic representing the involvement of circulating microRNAs (ci-miRNAs) in HIV infection. ci-miRNAs are differentially expressed in HIV-infected patients and play a dual role in the pathophysiology of the infection and its comorbidities. Green branches represent situations in which ci-miRNAs are associated with a positive effect. Red branches represent situations in which ci-miRNAs are associated with a negative and/or pathological effect.

In an unprecedented study, Reynoso et al.[35] described ci-miRNA profiles in plasma samples from 27 individuals, who were divided into healthy controls, chronically infected HIV patients and elite controllers (i.e., individuals who maintain undetectable levels of viral load without having received treatment, including high levels of $CD4^+$ T cells). A large-scale molecular analysis was performed using the qRT-PCR technique. No significant differences were observed between elite controllers and healthy patients. However, there was a considerable difference between chronically infected HIV patients and healthy controls for 16 miRNAs. Higher levels of hsa-miR-29b-3p, hsa-miR-33a-5p, and hsa-miR-146a-5p were found in the plasma of elite controllers than chronically infected patients. Hsa-miR-29b-3p and hsa-miR-33a-5p expression was also investigated in an *in vitro* model, in which they were transfected into MT2 cells, a model for $CD4^+$ T cells. Overexpression of hsa-miR-29b-3p and hsa-miR-33a-5p significantly reduced viral production in MT2 cells and primary $CD4^+$ T cells. Therefore, the ci-miRNAs levels have diagnostic and prognostic value in HIV infection, and hsa-miR-29b-3p and miR-33a-5p are potential new therapeutic targets for new antiretroviral therapies.

Narla et al.[36] demonstrated the presence of ci-miRNAs in the plasma of 69 HIV-infected patients in a large-scale molecular analysis study using the qRT-PCR technique. The patients were divided into HIV negative (HIV-), HIV positive undergoing antiretroviral therapy (HIV+ART+), HIV positive not undergoing antiretroviral therapy (HIV+ART-), and elite controllers. Overall, 29 ci-miRNAs were differentially expressed between HIV+ and uninfected controls. Nineteen ci-miRNAs were differentially expressed between HIV+ART+, HIV+ART-, and elite controllers. The profile of overexpressed ci-miRNAs indicated that they are involved in the virus latency process, high levels of viremia, and low $CD4^+$ T cell counts. In contrast, miRNA-29c, 223, and 382 are involved in inhibiting HIV genome expression in resting cells, not in producing viral particles. This suggests that these miRNAs may be therapeutic targets, and increasing their expression might inhibit virus replication. In addition, HIV + individuals, regardless of the allocated group, had lower expression of miR-126, 145, and let7c. Patients with atherosclerosis also had lower expression of these miRNAs. Therefore, it is reasonable to suspect that these miRNAs are involved in HIV-associated coronary artery disease.

Hubert et al.[37] surveyed 43 patients infected with HIV-1 who were divided into 4 groups: 17 never treated with ART, 13 who were treated with ART and had a viral load <50 copies/mL of plasma, and 13 elite controllers, in addition to 16 healthy controls. Because the specific content of extracellular vesicles depends on the cells that secrete them, the researchers hypothesized that extracellular vesicles, including exosomes from HIV-1 patients, must contain miRNA. miRNAs were more abundant in the extracellular vesicles of patients who had not undergone ART (6 out of 8 patients for miR-155, 5 out of 8 for miR-223). This result suggests there higher miRNA levels in patients who have never undergone ART, although only miR-155 and miR-233 levels have been quantitatively correlated with the size of exosomes and extracellular vesicles. Moreover, larger exosomes were found in untreated patients than all other groups. miRNA levels were negatively correlated with $CD4^+$ T cell counts (i.e., the more miRNA, the fewer $CD4^+$ T cells). Thus, exosome and extracellular vesicle levels are associated with: i) T cell count, a classic marker of infection progression, and ii) induction of apoptosis in $CD4^+$ T cells and an increase in $CD8^+$ T cells, a mechanism that prevents an efficient antiviral immune response.

Four differentially expressed miRNAs may indicate early HIV-1 infection, namely miR-16-5p, miR-20b-5p, miR-195-5p, and miR-223-3p. These miRNAs have been called the miRNA panel P_{eHIV-1}. It was discovered that this panel could be used with 100% sensitivity and specificity in early HIV-1 detection, i.e., while the patient is still in the eclipse phase (the phase lasting approximately 10 days post-infection before the viral RNA is detected in the plasma). The miRNAs miR-16-5p, miR-206, let-7 g-3p, and miR-181c-3p can detect infection during the eclipse stage with 100% sensitivity and 95.8% specificity, demonstrating that miRNA panels are a potential biomarker for early and/or acute HIV-1 infection[14].

Isolated expression of miRNA-122 in HIV infection was investigated in a sample of 74 (31% female) patients with HIV/hepatitis C virus (HCV) co-infection. Serum levels of miRNA-122 were analyzed by qPCR. Circulating levels of miRNA-122 were elevated in HIV patients and showed a correlation with the severity of liver damage. Elevated miRNA-122 levels were associated with elevated liver injury markers, such as aspartate aminotransferase and alanine aminotransferase. Despite these results, the authors were unable to establish a relationship between miRNA-122 and portal hypertension, which was accurately assessed using the hepatic venous pressure gradient test. Nevertheless, miRNA-122 may still be associated with this condition[38].

Franco et al.[39] analyzed the ci-miRNA levels of 21 healthy controls and 54 patients with HIV-1 monoinfection using RT-PCR and large-scale sequencing. Compared to controls, patients with HIV-1 monoinfection had significantly altered levels of 25 miRNAs. It should be pointed out that most of these miRNAs correlated significantly and positively with aspartate aminotransferase and alanine aminotransferase levels and a higher stage of liver fibrosis. miR-122-3p and miR-193b-5p were also positively regulated (i.e., had increased levels) in HIV-1-positive patients with focal nodular hyperplasia.

Franco et al.[39] corroborated the findings of Jansen et al.[38] , revealing that HIV infection impacts circulating miRNA levels expressed in liver parenchyma, which highlights their potential as biomarkers for the progression of liver damage associated with infection.

A study by Murray et al.[40] involving 373 patients (82 co-infected with HIV/HCV and 291 infected with HIV-1) found greater expression of miR-122 and miR-200a in co-infected patients than monoinfected patients, as well as in HIV-1 infected individuals treated with antiretroviral therapy prior to developing fatal liver disease. In addition, serum levels of both miRNAs correlated modestly with aspartate aminotransferase and alanine aminotransferase liver enzymes, in addition to interleukin-6, a marker of systemic inflammation, which is an adverse outcome of ART. Moreover, it demonstrates that these miRNAs are promising new biomarkers for liver disease in HIV-1-infected patients undergoing ART.

Piano et al. investigated the potential of ci-miRNAs as biomarkers for active Kaposi's Sarcoma in AIDS

patients[41]. miR-375 was found in greater abundance in these patients than asymptomatic individuals infected with HIV/Human Herpesvirus type 8, which suggests that high levels of miR-375 may be an indicator of active Kaposi's Sarcoma. qRT-PCR was also used to obtain these results.

Parikh et al.[42] evaluated the expression of plasma miR-21 in HIV-infected patients, with and without pulmonary arterial hypertension (PAH) and HIV/HCV co-infection. There was greater expression of miR-21 in HIV and HIV/PAH patients than uninfected individuals, and HIV/HCV co-infection was correlated with even higher levels of miR-21 in the HIV-infected population. Taken together, these data provide clear evidence about the dysregulation of plasma miRNA-21 during HIV infection, PAH, and HIV/HCV co-infection, which indicates that there is a complex interaction between infection and comorbidities. However, the marker's prognostic properties, especially regarding infection, should be further investigated.

DISCUSSION

Numerous studies with *in vitro* and *in vivo* models have been developed to elucidate the molecular mechanisms of cellular miRNAs in the context of viral infection. HIV infection models have been extensively investigated, and it is now known that cellular miRNAs are differentially expressed, interact with host cell factors, and modulate viral replication, impairing or promoting it in different cells such as monocytes, macrophages, T cells, astrocytes, epithelial cells, and peripheral blood mononuclear cells[43]. The expression pattern of cellular miRNAs is complex and extensive. They are potentially useful as biomarkers of infection. However, an in-depth discussion is beyond the scope of this article.

Recently, further studies have focused on ci-miRNAs. In contrast to the large number of studies investigating cellular miRNAs, there has been little research into the actual involvement of ci-miRNAs in HIV infection. These nucleic acids have great potential as new biomarkers in different diseases, especially since they are reproducible and obtained non-invasively. The term "liquid biopsy" has been proposed in reference to ci-miRNAs, mainly due to the possibility of providing monitoring and individualized therapeutic strategies, facilitating the development of "personalized medicine"[44].

Although ci-miRNAs are promising biomarkers for many diseases due to their stability in biological fluids, several challenges must still be overcome before they can be used in clinical practice. Consistent and reproducible results remain elusive due to the difficulty of isolating and detecting miRNA in blood, serum or plasma samples. Thus, researchers are continually standardizing protocols for the cross-comparison of results. As these methods are refined, the utility of ci-miRNAs as biomarkers to predict disease risk, especially in HIV infection, could increase, although further investigation is required.

In this review, we outlined how miRNA acts to regulate gene expression in HIV infection, influencing viral entry and replication, as well as host cell response. Ci-miRNA levels are altered levels in individuals with HIV, playing a dual role in infection. Advances in research have shown that ci-miRNAs could differentiate stages of HIV infection and diseases associated with a viral infection and serve as biomarkers for antiretroviral therapy's effectiveness through changes in their expression. Understanding how ci-miRNAs function in HIV could lead to the discovery of new therapeutic targets.

Conflicts of Interest

The authors have nothing to disclose.

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