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MiRNA expression profiling in HIV pathogenesis, disease progression, and response to treatment: a systematic review

Running head: **The expression of miRNAs in HIV**

Abstract

Aim

We conducted a systematic review to identify the association of miRNAs expression with HIV pathogenesis, progression, and treatment.

Methods

Search of articles was conducted in MEDLINE®, Cochrane Central Register of Controlled Trials, Global Health.

Results

35 articles were included. Due to the heterogeneity of HIV phenotypes, a harmonization based on key progression parameters was proposed. The hsa-miR-29 family, -146b-5p, and -150-5p are the most frequently differently expressed in HIV. Direct comparison of studies was not possible due to heterogeneity exists for biological samples, and miRNA analysis techniques.

Conclusions

It is the first attempt to systematically identify the miRNAs different expression in well-defined compared patient phenotypes and could represent a very helpful way to increase general learnings on this field.

Lay Abstract

miRNAs, a novel class of small RNA molecules that play important roles in the regulation of gene expression, are involved in various physiological processes. Dysregulation of their function can lead to human diseases including cancer, cardiovascular and metabolic diseases, liver conditions and

immune dysfunction. Therefore, the analysis of their expression can provide valuable information for disease management.

The aim of our work is to systematically analyse the current scientific literature to identify miRNAs linked to the pathogenesis, progression and treatment of HIV.

We included 35 articles and highlighted the miRNAs that were found with significantly different levels in compared groups of subjects (e.g., subjects with HIV vs. healthy persons, persons able to limit the disease progression without therapy vs. those whose immune system is already compromised by HIV). The most frequently reported miRNAs are: hsa-miR-29 family, hsa-miR-146b-5p, and hsa-miR-150-5p.

To our knowledge, this is the first attempt to systematically identify the miRNAs associated with HIV and could be a useful contribution to increasing general knowledge in this field.

Keywords: microRNA, HIV-1, AIDS, pathogenesis, disease progression, Highly Active Antiretroviral Therapy

Introduction

The human immunodeficiency virus (HIV) is a retrovirus responsible for acquired immunodeficiency syndrome (AIDS), which leads to failure of the immune system [1] and is associated with several comorbidities [2]. Approximately 38 million people were living with HIV worldwide and around 690,000 subjects died of HIV/AIDS-related illness in 2019 [3].

Highly Active Antiretroviral Therapy (HAART) is the pharmacological treatment typically composed of a combination of three or more antiretroviral drugs for people infected with HIV. There are six main classes of HAART agents (Nucleoside/Nucleotide Reverse Transcriptase Inhibitors, Non-nucleoside Reverse Transcriptase Inhibitors, Protease inhibitors, Integrase Strand Transfer Inhibitors, Fusion inhibitors, Chemokine Receptor Antagonists) that target different stages in the life cycle of virus [4]. Since the HAART regimen was first used in 1996, it has significantly reduced HIV morbidity, and mortality [5]. Global scale-up of HAART contributed to a 59.4% reduction in AIDS-related mortality between 2005 (the peak) and 2019, with 25.4 millions of subjects on HAART treatment in 2019 [3]. Nevertheless, HAART is not a cure [6] and can lead to severe toxic effects [7,8]. Without a cure, HIV will remain a chronic infection with the potential to spread and cause a lethal disease. As a consequence, continuous efforts are needed to search for critical factors able to either modulate or mark onset, progression, and treatment.

There is evidence regarding the involvement of microribonucleic acids (miRNAs), a class of small RNAs of 18–25 nucleotides, in several human illnesses like cancer, neurodegenerative, inflammatory and autoimmune diseases, and their association with lifestyle factors and with exposure to environmental contaminants related to human health [9–14]. miRNAs regulate biological processes such as proliferation, differentiation, development, and apoptosis [15–19] and have a role in regulating host responses during viral infections; they can either directly affect viral replication or modulate the expression of host genes and pathways essential for it [20,21]. The variation of miRNAs in their abundance level is likely to be associated with the onset and progression of diseases [22]. In

a recent narrative review, miRNAs are indicated as important players in HIV pathogenesis with potential diagnostic applications as biomarkers in HIV infection [23].

Within this perspective, the present work systematically reviews the available evidence on the association of miRNA expression profiling with HIV pathogenesis, disease progression, and response to treatment.

Methods

Search strategy and selection criteria

This systematic review is based on papers published up to October 2020. Several bibliographic databases were systematically searched: Global Health (Ovid) - dedicated to public health and including subjects such as Diagnosis and therapy of infectious diseases and public health emergencies, MEDLINE (Ovid) - the National Library of Medicine's premier bibliographic database focused on biomedicine and health that also includes aspects of biology, Cochrane Central Register of Controlled Trials – a highly concentrated source of reports of randomized and quasi-randomized controlled trials. The research strategy combined the following terms: AIDS, HIV, CD4, Viral Load, HAART, and miRNA (full search in File S1, applied to all the DBs). Only articles published in English and in Italian were considered. Citation searches were also performed on references and forward citations of selected studies.

Paper selection and assessment have been carried out by a multidisciplinary team composed of members with scientific and methodological expertise. The team members worked either in plenary session meetings or in parallel tasks, and any disputes were resolved by discussion. In order to reduce the risk of having missed some relevant papers, several measures have been taken. In particular, after a pilot phase which involved all the reviewers for training purposes, two different reviewers were assigned to each abstract. In this phase all the abstracts with an undefined or conflicting judgment were reviewed in plenary sessions by all the reviewers. The same approach was adopted for full paper screening. Preliminary screening was performed using Abstrackr [24] based on title and abstract. The selected articles were stored and shared with the working team through the Alfresco Enterprise

Content Management platform [25]. These papers were assessed based on the full text and were excluded with reasons when appropriate. Articles were excluded if there was no: a) reporting on the effect of miRNAs dysregulation on HIV1+ infection, progression, response to treatment, or collateral effects of HAART; b) reporting on verified functional effects through experimental evaluation; c) all subjects were co-infected or with co-morbidities not caused by HIV; d) considering qRT-PCR, RT-PCR based array, nanoString, or sequencing (these molecular approaches, compared to microarray, give more reliable results because of their detection sensitivity, reproducibility, and large dynamic range) [22]; e) statistical analysis presented in research findings.

Data extraction

A data collection form was developed and filled in by each reviewer independently and finally agreed by consensus. As a preliminary methodological action, we determined how to coherently report the several existing sub-classes of HIV subjects. In fact, as reported by Gurdasani [26], there is no harmonized definition of phenotypes of HIV control and progression. We then classified HIV populations as reported in Table 1, which considers several HIV/AIDS key progression factors such as the history of seroconversion (HSC), the viral load (VL), the CD4+ T Cells count (CD4), the opportunistic infections (OI), and the HAART regimen. The table was refined based on the peculiarities of the selected studies.

[Table 1]

The specificity of descriptions increases from left to right (indentation in the first column), and phenotypes are reported according to disease severity (from up to down). Specific sub-classes are not intended to define the whole possibilities within a more general scenario but describe only relevant situations. As an example, the phenotype of HIV+ asymptomatic (*HIV+|As)* subjects theoretically would contain two major sub-phenotypes: HIV+ long-term nonprogressor (*HIV+|LTNP)* subjects and *HIV+|unknown future* subjects, i.e., the ones with a short HSC that, once reached the high threshold (HT) regarding the time since infection, could be classified either LTNP or progressing syndrome (PS). The latter class (*HIV+|unknown future*) is not reported and subjects in this situation are reported in the more general class of *HIV+|As*. Some populations may be characterised by a not univocal definition of some of the key parameters (e.g., included subjects could have a CD4 count spanning from 250 to 700 cells/mm^3, hence falling in part in the progressing syndrome and in part in the asymptomatic class). In such cases, the not univocally definable values are noted as *NU* in the parent class. Highly variable disease progression rates between individuals are well-recognized, with progression categorized as rapid, regular, or LTNP. LTNP have differing magnitudes of VL and are, hence, further divided into elite controller, viremic controller, and non-viremic controller [27]. As HIV infection progresses, the number of CD4 cells declines. When an HIV-infected individual has a CD4 count below 200 cells/μl or one of the defining illnesses – opportunistic infections (e.g., *Pneumocystis jiroveci*, Candidiasis, Cryptococcosis, Herpes simplex, Hodgkin's disease, Non-Hodgkin's lymphoma, lymphocytic leukemia, or a genetic immunodeficiency syndrome atypical of HIV infection, such as one involving hypogammaglobulinemia), the subject will get diagnosed with AIDS. It is possible also to have subjects with AIDS with no opportunistic infection and CD4 count over 200 cells/μl in case their health status is determined by an effective therapeutic regimen after the AIDS diagnosis. Regarding the HAART, a further classification is possible between responding and not responding subjects.

An additional problem of nomenclature heterogeneity is related to the name of the miRNAs, which is subject to constant official updates due to the numerous discoveries of new miRNAs that are often

linked to those already known. To allow comparisons, miRNAs were reported adopting the most recent official version adopted in *miRBase* [28–33].

The following data were collected from each study: populations compared, biological sample and methodology for miRNA counting, dysregulated miRNAs, details on HAART if applicable. Details on study objective were collected as a complementary information.

Data were reported in tabular form highlighting the miRNAs differently expressed between the compared groups.

This systematic review conforms to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [34].

Bioinformatic analysis

To identify miRNA target genes, we used TargetScanVert and miRDB, accessed through miRBase (http://www.mirbase.org/, accessed on 15 September 2021) [28] and miRWalk (http://mirwalk.umm.uni-heidelberg.de/ accessed on 15 September 2021) [35]. InteractiVenn (http://www.interactivenn.net/ , accessed on 15 September 2021) [36] was used as a tool to retrieve the predicted miRNA targets that are in common for the selected databases. Interactions between miRNA targets are visualized with Cytoscape software (version 3.7.1; https://cytoscape.org/ , accessed on 15 September 2021) [37].

Results

Included studies

The results of the search strategy and the selection process are shown in Figure 1. From 1,591 potentially relevant reports initially identified after eliminating duplicates, 35 papers were selected for inclusion.

[Fig. 1]

The selected works are reported in Table 2 with a description of their aim and the considered populations. Populations are reported according to the classification described in methodologies (Table 1), which allowed us to manage the heterogeneity of HIV categories.

The correspondence with populations described by authors of selected works is reported in Table S1.

[Table 2]

As reported in Table 2, the number of subjects ranges from eight [54] to 199 [46]. All but two works [60,71] enrolled subjects without HIV, with Bignami F et al. [40] considering Multiple Exposed but Uninfected subjects (MEU). Ten papers [40,42–44,53,61,63,67,69,70] consider LTNP, four [43,51,52,57] AS, six [51,52,54,57,58,64] AIDS and fourteen [39,42–44,46–48,51–53,55,59,60,62] HAART regimen.

A conversion table specifying the names of miRNAs used in the papers and the one adopted in the *miRBase* is provided in Table S2. Table 3 reports differently expressed miRNAs in HIV subjects vs. several typologies of HIV negative subjects: Healthy Subjects (HS), persons without the HIV, but potentially having other diseases (HIV-), and MEU. The biological sample (Peripheral blood mononuclear cells, PBMC, the most adopted, followed by Plasma) and the methodology of analysis (mainly qRT-PCR, followed by RT-PCR based array) are reported too. Some of the miRNAs mentioned in the papers are non-human according to the release 22.1 (October 2018) of miRBase and have not been reported in our work. The hsa-miR-29 family, hsa-miR-150-5p, hsa-miR-155-5p and hsa-miR-223-3p were the most reported differently expressed miRNAs between HIV positive and HIV negative subjects.

[Table 3]

With the same scheme of Table 3, the other analyses reported in the Tables S3 – S8, compare different phenotypes of seropositive subjects: differently expressed miRNAs in LTNP vs. other HIV categories (Table S3), asymptomatic vs. other HIV populations (Table S4), AIDS subjects when compared with other HIV categories with a lesser degree of progression (Table S5), HIV/AIDS populations under HAART when compared with categories without HAART or resistant to HAART (Table S6), HIV subjects differing only in terms of CD4 (Table S7), and HIV populations differing in terms of OIs (Table S8).

Table 4 reports the correlations (either positive or negative) between miRNAs and HIV-related parameters (CD4, VL, CD4/CD8 ratio) in specific populations. The hsa-miR-29 family and hsa-miR-150-5p have been reported as positively correlated with CD4 [48,60,67] [48,52,67]. The hsa-miR-29a-3p is negatively correlated with VL and positively with CD4/CD8 ratio in the study of Rosca [60]. With regard to the latter parameter, it is used as a prognostic marker of HIV disease progression with lower values associated with heightened immunosenescence and increased risk of mortality [73].

[Table 4]

An overall reading of the proposed Tables (3, S3-8, and 4) allows for the identification of the most relevant miRNAs with respect to pathogenesis, progression, and response to therapy. The hsa-miR-29 family is by far the most represented with statistically significant dysregulations reported in all the considered scenarios, with the only exception of the one related to the OIs, and with correlations with CD4 and VL. Even hsa-miR-146b-5p and hsa-miR-150-5p are reported in all the scenarios, with the same exception reported above on OIs, and are correlated with CD4.

miRNA Targets

miRNA targets analysis is performed on miRNAs that are most often reported in the included papers (i.e., hsa-miR-29a-3p, hsa-miR-29c-3p, hsa-miR-146b-5p and hsa-miR-150-5p). Adopting the methodology above described, we obtained: hsa-miR-29a-3p: 440 targets; hsa-miR-29c-3p: 340 targets; hsa-miR-146b-5p:61 targets; hsa-miR-150-5p: 127 targets. Interactions between miRNAs and their predicted targets are presented in Figure 2.

[Figure 2]

Discussion

Some general considerations on the methodologies adopted in the retrieved studies are needed before commenting on the results.

Concerning the biological samples adopted for the analyses, PBMCs, the mostly reported in the studies, are easily accessible and include the major circulating targets of HIV-1 infection [74]. Unfortunately, PBMCs have a low specificity as it is not possible to conclude which cellular subset is responsible for the differentially expressed miRNAs [75]. More HIV-related cell types (CD4+, CD8+, CD14+ cells, tonsil tissue, abdominal subcutaneous adipose tissue, cerebrospinal fluid, tumor tissue, brain tissue) have only been reported in some studies, and mainly to compare HIV positive subjects with seronegative. More specific comparisons of HIV classes (see Tables S3 to S8) and correlations of miRNAs with HIV-related parameters are only reported in two works using CD4+, CD14+ cells, and brain tissue. Caution is recommended in the generalization of results.

The detection of miRNAs is also influenced by analytical methods. The most frequently used technique is qRT-PCR (28 papers), often to validate previous observations on microarrays. qRT-PCR is currently the most adopted assay for miRNAs detection as it is specific, relatively quick, and simple to use but the reaction requires precise temperature control and expensive equipment [76]. Other adopted methodologies in the analysed papers are RT-PCR based arrays, NanoString and Sequencing. All these methods differ in accuracy, sensitivity, complexity, attainable throughput, and dynamic range [77]. RT-PCR based arrays, such as TaqMan low-density microRNA array, are easy to perform and a convenient way to screen in parallel, a large number of miRNAs [78]. NanoString is a hybridization-based technology that can detect specific nucleic acid molecules from low amounts of starting material without the need for amplification and it can discriminate similar mature miRNAs with accuracy. Nevertheless, this platform is quite expensive, and the instrumentation may not be as easily accessible as for other technologies, including qRT-PCR [79]. Next-generation sequencing allows the simultaneous discovery of new miRNAs and confirmation of known miRNAs. But, as for NanoString, the main limitation is its high cost and complexity [76]. Despite the good analytical

performance offered by these methods, there are non-negligible defects that in the last years have led to develop new tools for miRNA detection [80]. Electrochemical detection based on enzymatic signal amplification is a low-cost method that allows miRNA detection to have high sensitivity and high specificity, but needs to be greatly improved due to the difficulty of designing sequences for template amplification and for high background signals [81]. The analysis of miRNA by ligation-based identification has high detection sensitivity and specificity and requires reduced influence of temperature but has low throughput [80]. Finally, the technology of nanoparticle signal amplification has high sensitivity, high throughput, and high specificity that enables instant detection, but nanoparticles are susceptible to environmental factors [80]. Compared to qRT-PCR, microarray and NanoString, these new methods in addition to the Next-generation sequencing are widely used in the prediction of new miRNAs, screening of differentially expressed functional genes, prediction of miRNA target genes due to their properties of high productivity, high quality, high precision and repeatability [80].

The great heterogeneity in sample sources, miRNAs expression analysis (different platforms, analysis software, and normalization strategies used), and study populations make it not possible for direct comparison among studies.

When doing an overall reading of the proposed results (Tables 3, S3-S8, and 4) to identify the most relevant miRNAs associated with pathogenesis, progression, and response to therapy, our review shows that, in several studies, the most differentially expressed miRNAs for HIV are the hsa-miR-29 family, hsa-miR-146b-5p, and hsa-miR-150-5p. The same miRNAs are also correlated with the immunological and virological parameters of HIV.

The hsa-miR-29 family has potential target sites in Nef transcripts, hence all its members downregulate the expression of this protein and suppress viral replication. Consequently, the inhibition of the hsa-miR-29a-3p and hsa-miR-29c-3p significantly enhanced HIV-1 infection [60]. Previous studies have suggested that specific inhibitors of these “anti-HIV” miRNAs were shown to increase HIV-1 production; therefore, their manipulation could be used to purge latent state HIV-1

reservoirs [82]. Therefore, hsa-miR-29a-3p and -29c-3p could be potentially considered as mediators of HIV disease.

The association of hsa-miR-29a-3p with HAART has been positively investigated by Rosca [60], where it was overexpressed in subjects with a better response to treatment. Moreover, a multinomial regression analysis that included hsa-miR-29a-3p expression level, total exposure time to HAART, nadir CD4 cell count, CDC clinical stage, and zenith viral load showed that increased expression levels of this miRNA were linked with higher odds of treatment success, marking it as a potential marker for therapy response. Again, in the study by Liu [48], HAART failed to restore hsa-miR-29a-3p level, indicating that the suppressed miRNA may be attributed to the virus latency during HAART. These studies are too different to be compared and additional evidence is needed to understand whether hsa-miR-29a-3p can be used as a biomarker of HAART response.

The hsa-miR-146b-5p has been reported to have a role as a modulator of HIV infection [43]. The co-activation of this miRNA during the inflammatory response results in transcriptional activation of NF-kB target genes that encode various mediators of inflammation, such as cytokines and acute-phase proteins. Moreover, this miRNA, together with hsa-miR-155, targets mRNAs in the signalling cascade downstream of the toll-like receptor (TLR) and bolstered the link with NFκB-regulated innate immunity which led to a model in which these two miRNAs facilitated a negative-feedback loop that may protect from an excessive TLR [83].

Even hsa-miR-150 is a mediator of HIV infection. It may affect HIV replication by targeting a cellular cofactor (cyclin T1) via c-Myb, a transcription factor that controls multiple steps of lymphocyte development [48]. Cyclin T1 is essential for the efficient transcription of the provirus and HIV-1 replication is severely impaired in its absence [84]. Thus, an up-regulation of this miRNA leads to an inhibition of cyclin and, consequently, inhibition of HIV-1 replication. This miRNA also has a role in virus latency [67]. It targets the 3'UTR of HIV-1 transcripts, potentially rendering productive infection into latency in resting CD4+ T lymphocytes [85]. Conversely, the suppression of hsa-miR-150 might facilitate HIV-1 infection because it acts as a regulator of immune cell differentiation and

activation [43].Though literature reports that some miRNAs can be differently expressed in samples from males or females [86,87], only in one of the selected papers [58] the authors made this comparison. Based on their analysis, only hsa-miR-21-5p, hsa-miR-125b-5p and hsa-miR-146a-5p were more expressed in plasma men than plasma women in the group of HIV patients with Cerebral Toxoplasmosis, suggesting that gender could be an important factor of disease-associated miRNAs.

With regard to the bioinformatic analyses, the identified miRNA targets play a role in different cellular functions and cell signaling. For example, it is observed that serine/threonine kinase AKT plays a central role in many biological processes involved in HIV-1 pathogenesis [88] and, as a key regulator in the phosphoinositide 3-kinase pathway, it impacts cell survival, metabolism, growth, and proliferation [89]. Another target is cell division control protein 42 homolog (Cdc42), a member of the Rho GTPase family regulating multiple cellular processes involving the activation of signaling pathways [90]. It is involved in various events in the HIV-1 replication cycle; thanks to its energy function, it can be sequestered by HIV-1 to allow the intracellular transport of viral components within the host cell, leading to immune evasion [91].

Finally, although some studies make a general consideration on the type of therapy received by patients, none classifies them by type of HAART regimen, so no consideration can be made on the change in miRNA expression in relation to the specific treatment. In this review, a limitation is the great heterogeneity in the definition of the study populations, miRNAs' name, body samples, methodology for miRNAs expression analysis (different platforms, analysis software, and normalization strategies used), which complicates the direct comparison among the studies. To overcome this, we firstly have introduced a taxonomy to describe the different phases of HIV that we adopted to describe populations in the selected works and updated miRNAs' name according to the most recent version reported in miRBase. Furthermore, based on well-defined phenotypes such as LTNPs, AS, AIDS, on HAART, with different CD4+ T-Cells levels and with OIs, we performed several sub-analyses, in table format, on homogeneous study populations.

Conclusions

To our knowledge, this review is the first attempt to systematically identify the miRNA different expression in well-defined compared patient phenotypes and could represent a very helpful way to increase general learnings on these molecules in HIV.

A harmonization of HIV classes based on the history of seroconversion, HIV replication, impact of HIV virus on the subject, presence of OIs, HSC, and HAART regimen is proposed as a way to address the heterogeneity in the definition of the HIV populations.

The analysis across studies of the identified discriminating properties of some miRNAs and the correlation of their expression with relevant HIV progression parameters allowed us to identify the miRNAs with a potential role in the regulation of HIV infection and disease progression (i.e., hsa-miR-29 family, hsa-miR-146b-5p and hsa-miR-150-5p).

Future perspectives

Investigating the potential role of miRNAs also as biomarkers in HIV is a very promising field of study that could provide a novel public health approach to slow the negative impact of the virus in the host. Further studies are still needed to explore the potentiality of the identified relevant miRNAs, the molecular mechanisms behind the complex cross-talks between miRNA targets and HIV infection and their applicability in the management of this condition.

Summary point

- HIV remains a critical problem despite highly active antiretroviral therapy dramatically reduced its morbidity and mortality.
- Recent research is investigating the role of miRNAs in viral infections and disease progression, including in subjects with HIV.
- A systematic review was conducted to identify the most frequently reported miRNAs that are expressed differently in well-defined compared patient phenotypes.
- We included and processed 35 papers.

- To overcome the current heterogeneity in the definition of HIV phenotypes, a harmonized taxonomy was proposed for the description of the different stages of disease progression in HIV infected individuals.
- The hsa-miR-29 family, hsa-miR-146b-5p, and hsa-miR-150-5p have been identified as the most frequently differently expressed in the compared HIV phenotypes.
- The applicability of miRNAs as biomarkers for HIV management is a very promising field of study that could provide a novel public health approach but further research is still needed.

Financial & competing interest disclosure

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