Chapter

Dengue Virus and the Relationship with MicroRNAs

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Abstract

Dengue is an acute febrile disease caused by a virus of the genus *Flavivirus*, family Flaviviridae, endemic in tropical regions of the globe. The agent is a virus with single-stranded RNA, classified into four distinct dengue virus (DENV) serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. The host's innate and adaptive immune responses play an essential role in determining the natural history of viral infections, especially in dengue. In this context, it has observed in recent years that the presence of RNA interference (RNAi) in viral infection processes is increasing, as well as immune defense. The context microRNAs (miRNAs) go for stood out, as their presence during viral infection, both in the replication of the virus and in the defense against these infections, becomes increasingly noticeable, therefore, making it increasingly necessary to better understand the role of these small RNAs within viral infection by DENV and what their consequences are in aggravating the consequences of patients affected by this disease.

Keywords: dengue, miRNA, genetics, immunology, *Flavivirus*

1. Introduction

RNA interference (RNAi) is understood as the mechanism of gene silencing through transcription or post-transcription. Post-transcription gene silencing (PTGS) operates through translational repression induced by microRNAs (miRNAs), from precursors transcribed in the nucleus [1].

The silencing machinery by microRNAs directs mRNA to the P bodies present in the cytoplasm. They are deprived of the translation machinery and conserve proteins involved in the degradation of the target mRNA [2].

The natural functions of RNAi and their related processes appear to be the protection of the genome against invasion by mobile genetic elements, such as viruses and transposons, as well as the functioning of eukaryotic organism development programs [3, 4].

These analyses led to the identification of proteins encoded by the host involved in gene silencing. Also, they revealed that several enzymes or essential factors are common to these processes. Some components have identified to serve as initiators. In contrast, others serve as effectors, amplifiers, and transmitters for the gene silencing process [5].

The RNAi is widely used to fight viruses, due to the exposure of their genetic material in the intracellular environment at various stages of their replication cycle [6, 7]. Due to the distinction between viral and cellular genomes, the

chances of cross-silencing are low. In contrast, mutational changes in the viral genome allow mechanisms to escape interference pathways [8, 9].

2. miRNA

The microRNAs are defined as small single-stranded RNA molecules with approximately 19–25 nucleotides, not protein encoders, that act as mediators for the regulation of the posttranscriptional gene expression [10].

The first miRNA was described in 1993 and related to the regulation of larval development in *Caenorhabditis elegans*. However, understand that the miRNA class is the largest class of gene regulators, with around 1000 miRNAs. Promoting regulation is necessary to bind the 3'untranslated region of the target mRNA [11].

There are more than 2500 miRNAs identified in the human genome. Although transcriptional targets are predicted, most of these have not been validated, which makes this area for investigation rich [6, 12]. Studies revealed that viruses encode miRNA, for example, Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), and human immunodeficiency virus type 1 (HIV-1) [13].

miRNAs play essential roles in the cell, such as proliferation, differentiation, apoptosis, stress response, and transcriptional regulation. Studies have shown that miRNAs change their expression in several pathologies, increasing their importance and the need for a better understanding of this process [14].

2.1 miRNA biogenesis and mechanism of action

The miRNA biogenesis process consists of transcription, export, processing, and maturation, at the cytoplasmic and cellular levels. As a fundamental element of this miRNA transcription, RNA polymerases II and III are found, with the function of transcribing gene encoding proteins [12].

Currently, most of the identified miRNAs are intragenic and processed from introns. Simultaneously, the rest are intergenic and transcribed independently of a host gene and regulated by their promoters [15]. Sometimes, miRNAs are transcribed as a long transcript called "clusters," which can have similar regions, and, in this case, are considered a family. miRNA biogenesis can be classified into two pathways: canonical and noncanonical (**Figure 1**) [5].

What defines the choice of these mechanisms is the complementarity between the bases of miRNA and mRNA. When there is perfect parity between the bases, degradation of the mRNAs will occur [16]. On the other hand, incomplete pairings generate inhibition of the translation of the target mRNA. Since miRNAs are small molecules, there is no need for complete pairing for binding. Thus, a miRNA can act in the regulation of several target mRNAs, or several miRNAs regulate a single mRNA [17].

2.1.1 Canonical pathway

The canonical pathway of biogenesis is the dominant pathway by which miRNAs are processed. In this way, pri-miRNAs are transcribed from their genes and processed into pre-miRNAs by the microprocessor complex, consisting of a protein that binds RNA to the critical region of DiGeorge syndrome 8 (DGCR8) and a ribonuclease III enzyme, Drosha [18]. DGCR8 recognizes an N6-methyladenylated GGAC and other motifs within the pri-miRNA [19]. At the same time, Drosha cleaves the duplex pri-miRNA based on the characteristic structure of the pri-miRNA, resulting in the formation of an excess of 2 nt 3′ in the pre-miRNA [20]. Once pre-miRNAs

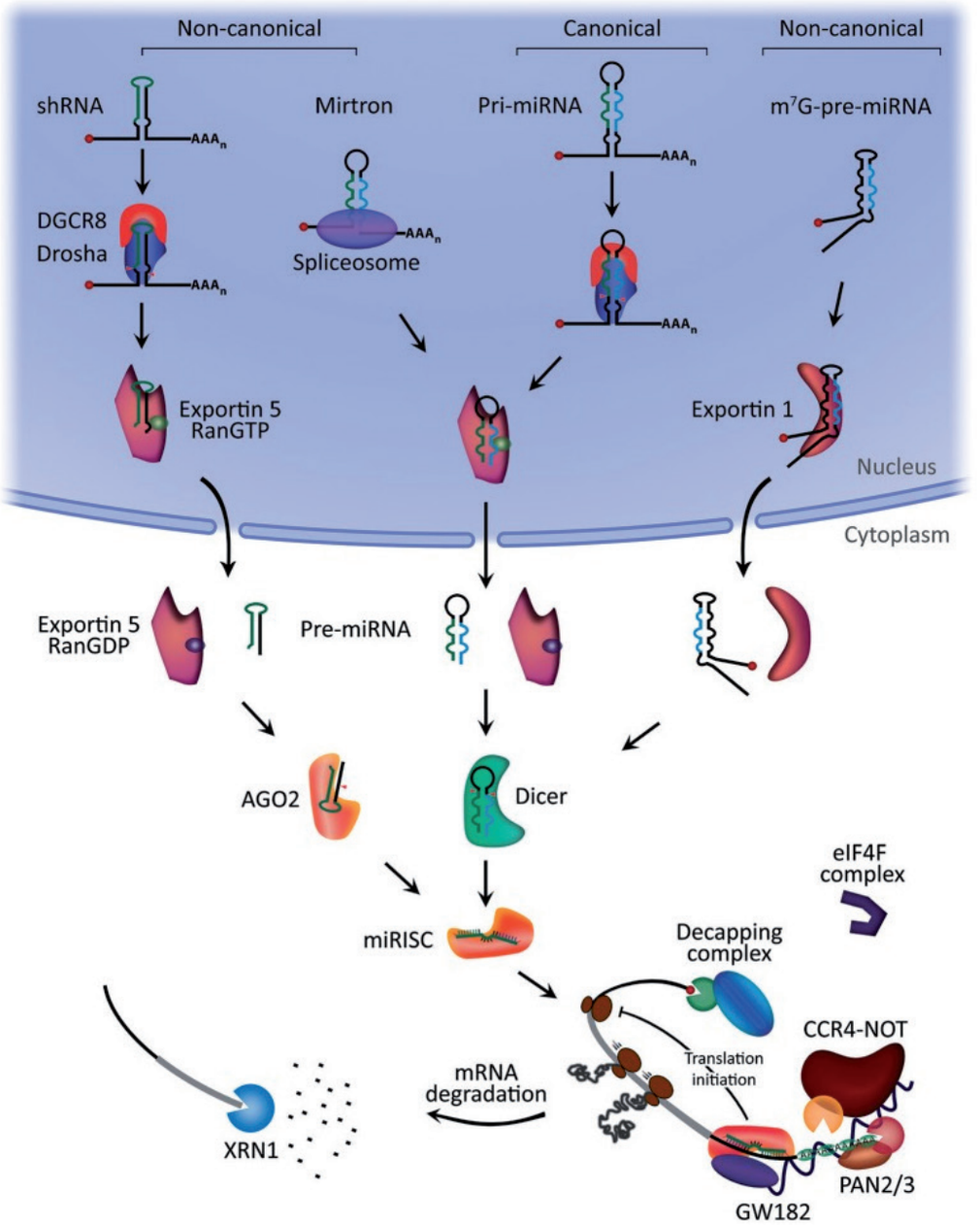

Figure 1.
MicroRNA biogenesis and mechanism of action. The canonical miRNA biogenesis begins with the generation of the pri-miRNA transcript passing through the microprocessor complex, composed of the critical region 8 of Drosha and DiGeorge syndrome (DGCR8), and cleaves the pri-miRNA to produce the precursor miRNA (pre-miRNA). The mature miRNA is associated with the Argonaute (AGO) protein family forming a miRNA-induced silencing complex (miRISC). In noncanonical pathways, small hairpin RNA (shRNA) is initially cleaved by the microprocessor complex and exported to the cytoplasm via exportin 5/RanGT, cleaved by AGO2, but this action is independent of Dicer (modified by Tanzer et al.) [21].

are generated, they are exported to the cytoplasm by an exportin 5 (XPO5)/RanGTP complex and processed by the RNase III Dicer endonuclease [5, 22].

This processing involves removing the loop from the terminal, resulting in a mature miRNA duplex. The directionality of the miRNA chain determines the name of the mature miRNA form. The 5p chain emerges from the 5′ end of the pre-miRNA hairpin, while the 3p chain originates from the 3′ end. Both chains derived from the mature miRNA duplex can carry in the Argonaute family of proteins. miRNA chains that do not contain incompatibilities are cleaved by AGO2 and degraded by cellular machinery that can produce a strong chain bias. Otherwise, miRNA duplexes with central mismatches or miRNA not loaded with AGO2 are passively unwound and degraded [23].

2.1.2 Noncanonical pathway

Several noncanonical biogenesis pathways in miRNA are elucidated (**Figure 1**). These pathways make use of combinations of proteins involved in the canonical pathway, mainly Drosha, Dicer, exportin 5, and AGO2. The noncanonical miRNA can be grouped into Drosha-/DGCR8-independent and Dicer-independent pathways [24, 25].

The pre-miRNAs produced by the Drosha-/DGCR8-independent pathway resemble Dicer products. On the other hand, Dicer-independent miRNAs are processed by Drosha from endogenous RNA transcripts of hairpins. These pre-miRNAs require AGO2 to complete their maturation in the cytoplasm. They are of insufficient length to be the substrates for Dicer. That, in turn, promotes the loading of the entire pre-miRNA in the AGO2 slicing [26, 27].

2.1.3 Argonaute and TNRC6 proteins

The proteins of the Argonaute family are related to the RISC complex, as a member of the machinery of the RNAi pathways [28]. The highly conserved between species and several organisms encode several members of the family. Usually found in the cytoplasm are concentrated close to the P bodies [29].

Such proteins, therefore, act with the transcriptional and posttranscriptional silencing pathways. The main stage of the interference mechanism is the cleavage of mRNAs; the Argonaute protein in the RISC complex catalyzes this process [28].

Argonautes are applied in transcriptional and posttranscriptional gene silencing, acting through the modulation of the degradation or inhibition of the translation of specific mRNAs, when associated with miRNAs [18].

The miRNAs associated with Argonaute proteins constitute a more massive complex called the miRNA-induced silencing complex, which will suppress the expression of mRNAs. In addition to interference at the translational level, it shows that miRNAs can induce poly(A) tail deadening [18, 19]. Studies suggest that proteins of the TNRC6 family are essential components when associated with miRISCs, for the location of cytoplasmic P bodies and the gene silencing of mRNAs [19, 20].

3. miRNA and dengue virus

The dengue virus is a virus composed of a single-stranded positive RNA belonging to the family Flaviviridae. DENV serotypes have been identified (DENV-1 to DENV-4). All serotypes are causing similar diseases and similar symptoms, without significant severity and serious diseases, such as dengue hemorrhagic fever and dengue shock syndrome. The DENV genome is approximately 11 kb in length that encodes a single polyprotein. This polyprotein is cleaved posttranslationally by the host and viral proteases into three structural proteins (capsid C; pre-membrane/membrane, prM/M; envelope, E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [30, 31].

The DENVs enter their target cells via receptor-mediated endocytosis in a clathrin-dependent manner. An acidified endosomal vesicle, virion, undergoes conformational changes that allow fusion and endosomal membrane and release RNA from the genome into the cytosol. After initial translation and cleavage of polyprotein, DENV triggers the formation of a replication complex in the perinuclear endoplasmic reticulum, and RNA replication and protein translation occur. Protein C then packages the newly synthesized positive RNA assembled into a virion, which

is covered with prM/E heterodimers. When the vesicles containing the immature virions move through the Golgi apparatus, the prM is cleaved by a furin protease. Finally, immature virions become mature or partially mature virions, which are secreted [32, 33].

3.1 Mosquitoes, miRNAs, and dengue

The functions that miRNA is involved in mosquitoes are related to posttranscriptional regulation of gene expression in physiological and immunological pathways and affect development, metabolism, host-pathogen interactions, and resistance to insecticides [34].

3.1.1 Development and metabolism

The specific expression of the miRNA stage in the four stages of development (eggs, larvae, pupae, and adults) was confirmed using sequencing. Understand the role of regulated miRNAs in the development of the mosquito and the action of knocking down the expressed miRNAs in a specific way carried out in *Ae. albopictus*. The knockdown of aal-miR-286b and aal-miR-2942 decreased the hatching of embryos and the hatching rate of larvae, respectively, compared to the knock-in groups. Reduced longevity and fertility (aal-miR-1891) were observed in the knockdown groups for miR-1891 compared to the knock-in and control groups in adults. Female mosquitoes require sugar for energy metabolism and a blood meal for egg development. Recent studies have indicated that blood supply leads to the differential expression of many genes, proteins, and miRNAs. The abundance of miRNA differs under sugar-fed and blood-fed conditions; ast-miR-2796-5p was observed exclusively in sugar-fed. The depletion of aae-miR-275 in *Ae. aegypti* females led to severe defects in blood digestion, fluid excretion, and egg development. aae-miR-1890 is induced after blood feeding and reaches a peak of 24 PMB. The systemic depletion of aae-miR-1890 resulted in less egg development and deposition, suggesting that miR-1890 may be the key to mosquito blood digestion. In contrast to the upregulated miRNAs after blood feeding, some miRNAs were downregulated. For example, reduced ast-miR-989 was observed 72 h after a blood meal. aga-let7 decreased in the midgut and other parts/leftovers, but most miRNAs increased after blood feeding [35–37].

3.1.2 Mosquitoes and dengue infection

The viruses of the *Flavivirus* genus are transmitted by mosquitoes and cause diseases, including dengue. It is observed that *Cx. quinquefasciatus* mosquitoes with West Nile virus (WNV) showed altered miR-92 and miR-989 expressions. In *Aedes albopictus*, the aae-miR-2940 miRNA is downregulated in response to WNV infection to restrict viral replication. In studies, the expressions of 35 miRNAs of mosquitoes modulating DENV infection in *Aedes aegypti* and more than 66 miRNAs were reported. *Ae. albopictus* is differentially expressed after DENV-2 infection. Therefore, aal-miR-34-5p and aal-miR-87 contribute to antipathogenic and immunological responses during DENV-2 infection. ae-miR-375 is the key to DENV replication, which can improve DENV-2 infection in an *Ae. aegypti* cell line. aae-miR-252 is three times more expressed after DENV-2 infection in *Ae. albopictus* cell line (C6/36); this inhibited DENV replication by suppressing the expression of the envelope protein of DENV. Regarding aal-miR-281, an abundant miRNA specific to the midgut, it was found that it facilitates the replication of DENV-2 in *Ae. albopictus* [36, 37].

4. miRNA impact on DENV infection

Studies have demonstrated the importance of miRNAs in viral infections. Mutations in the main catalytic components of the RNA interference pathway led to an increase in DENV replication in mammalian cells (**Figure 2**) [38]. Studies that analyzed the expression of miRNA in the blood of patients with dengue demonstrated a large number of miRNAs expressed differently in response to dengue infections [35]. About 348 miRNAs were described with different expressions in patients with dengue. Interestingly, studies have also identified 17 miRNAs that could use to distinguish between mild and severe dengue with complications [39].

The expressions of miR-24-1-5p, miR-512-5p, and miR-4640-3p were able to distinguish mild dengue from those with liver complications. At the same time, miR-383 was significantly more expressed in dengue with mild clinical status than those diagnosed with severe dengue and accumulation of body fluids [40, 41].

Studies have also shown 12 miRNAs with negative regulation and 41 with positive regulation in the serum of patients infected with DENV-1 when compared to the control group. Among these miRNAs we highlight hsa-miR-21-5p, hsa-miR-146a-5p, hsa-miR-590-5p, hsa-miR-188-5p, and hsa-miR-152-3p that were identified as promising invasive molecular markers for the detection of DENV infection [42, 43].

The microRNAs miR-21-5p and miR146a-5p are involved in inflammation and cell proliferation. They are expressed significantly concerning the control group, indicating their sensitivity and specificity as indicators of DENV infection. Besides, both miRNAs are correlated with the number of leukocytes and neutrophils. These findings suggested that some miRNAs could be used as diagnostic markers for DENV infections [44].

Effective disruption of host RNAi machinery is one of the pathogenic strategies of viruses to mitigate the host's response. Several viruses have reported producing protein suppressors through RNAi to cause silencing in infected cells, thereby

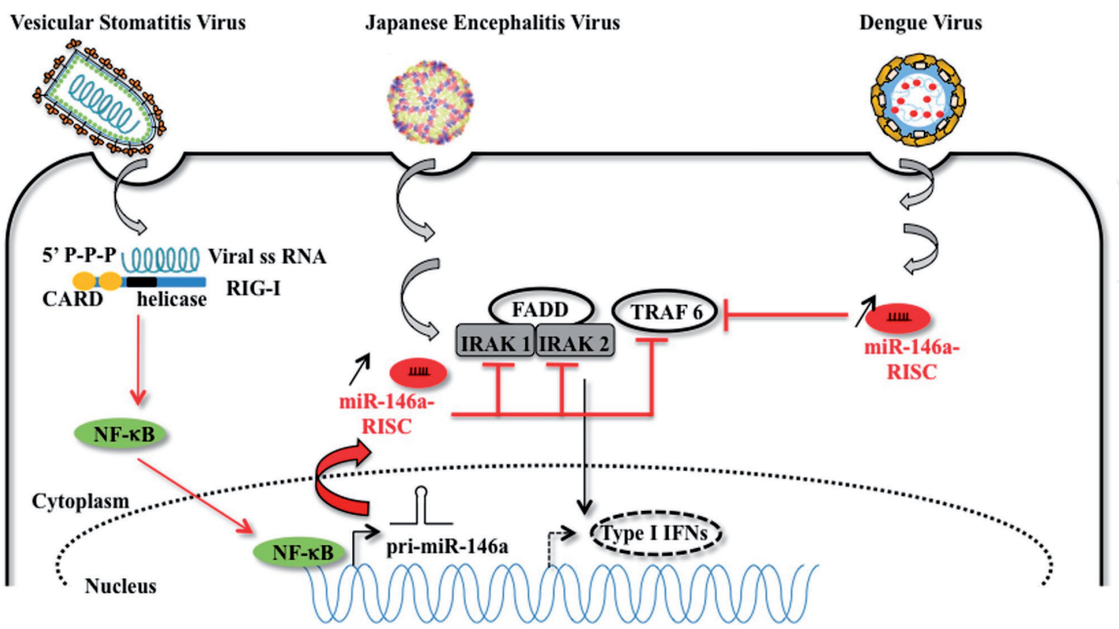

Figure 2.
An example of increased miRNA expression after the entry of vesicular stomatitis virus (VSV), Japanese encephalitis virus (JEV), and dengue virus in mammalian cells. miRNA expression increased in a RIG-I-dependent manner. The RIG-I protein interacts with viral RNA through its helicase domain, leading to nuclear transcription of the pri-miRNA by NF-kB. The reduction of target mRNAs explains the proviral function of this miRNA as the response of interferon type I (adapted from Bruscella et al.) [45].

interfering with RISC's loading or inhibiting the cutting activity of the AGO protein, a component of RISC and the biogenesis of miRNAs. DENV also has such suppressors that could neutralize the host's RNAi response. NS4B has reported suppressing host RNAi, interfering with the processing of Dicer, a key protein in miRNA biogenesis [38]. It has also reported that NS1 protein can interfere with apoptosis through miRNA-15 and miRNA-16 [5]. The NS3 protein has shown to interfere with the AGO1 protein [46].

5. miRNAs and the inhibition of viral replication

The miRNAs generally induce translational repression by binding to target mRNAs. Thus, it is not surprising that most of the miRNAs identified so far have DENV replication. The evidence points to the fact that miRNAs bind complementarily to the DENV 5'UTR or 3'UTR genome and thus inhibit DENV replication. The miR-548 g-3p represented the first evidence that a miRNA suppresses DENV multiplication by directly linking it to the viral genome. In the study, they showed that miR-548 g-3p was able to bind to the stem-loop A (SLA) promoter at 5'UTR, which is a crucial element for DENV RNA synthesis and replication, and uncontrolled replication of DENV-1. This inhibitory effect was proposed to arise from the binding of miR-548 g-3p to SLA, which could make it physically difficult, and thus attenuated the interaction between the SLA promoter and NS5. This DENV protein contains a C-dependent RNA-terminal RNA polymerase domain [45, 47].

Their hypothesis suggests that a functional miRNA has been preserved among all DENV serotypes and is present in the 3'UTR. The three dengue serotypes, miR-133a, miR-484, and miR-744, are involved in DENV replication, genome circularization, and viral viability. The overexpression of miR-133a, miR-484, and miR-744 in Vero cells had been experimentally validated to show the potencies of these three miRNAs in inhibiting DENV replication [48]. In another study, it found that miR-252 can inhibit DENV-2 replication in cell culture. The identification of protein E as a target for this miRNA is interesting since it has an indispensable role for viral entry [49, 50].

6. miRNAs that modulate host factors to inhibit or facilitate DENV replication

The DENV virus depends on its host's machinery for replication and infection, and it is not surprising that several miRNAs have shown to indirectly regulate DENV replication through modulation of host factors or the immune response. These indirect effects included modulation of the expression of a cell transcript that encodes a host factor necessary for one or a few stages of the viral cycle. The modulation of receptor expression can regulate virus entry, tropism, and essential cofactors for replication or translation that can impair or increase viral replication and the production of viral proteins. Also, miRNAs can increase or restrict cellular responses to viral infection, such as immune response or defense mechanisms [51].

The miRNA let-7c is highly expressed in Huh-7 cells and may be related to the protection of infected cells from oxidative stress and the response to inflammation after DENV infection. Let-7c has shown to bind directly to the basic transcription factor-1 of the leucine zipper (BACH1), a potent repressor of the anti-inflammatory and antioxidant protein heme oxygenase-1 (HO-1), and to dysregulate infection by DENV-2 and DENV-4. In this way, let-7c is probably able to protect the host from virus-induced infection [49].

In addition to modulating the host's immune response, miRNA may have antiviral actions. An example is miR-223, capable of inhibiting DENV-2 replication; its antiviral effect is probably associated with attenuated expression of the microtubule-destabilizing protein, stathmin 1 (STMN1), a key regulator of protein microtubules that controls microtubule dynamics [52]. The exact mechanism of how STMN1 affected DENV-2 replication is not yet known. However, studies have shown that an intact microtubule network involved in STMN1 sequestration was essential for HMCV to establish an infection. As such, it is highly likely that STMN1 is playing a similar role in establishing controlled microtubule dynamics in the context of DENV infection [53].

It is important to emphasize that despite all studies and the discovery of several miRNAs associated with cell regulation positively or negatively, better clarification of the processes involving miRNAs and DENV is still needed.

6.1 Perspective

6.1.1 Diagnostic using miRNA

As already reported in this chapter, several human diseases were induced due to differential miRNA expressions. Recently, several studies have confirmed the vibrant role of miRNAs in the successful regulation of various biological processes through the synergistic effects of the multiple miRNA networks, an integrated way to control an individual gene [17].

Also, several physiological functions (such as development, infection, immune response, inflammation, tumor genesis, and regulation of bone mass) have suggested being controlled by miRNAs. The miRNAs can regulate gene expression at the posttranscriptional level of more than 50% of the protein-coding genes in humans.

The miRNAs were found to play roles in helping and defending viruses. Mammalian miRNA genes generally exist in the noncoding region of the genes. However, they also occur both in the exonic regions of the gene encoding protein 16, and in alternative exon splicing, it can also regulate the expression of miRNA interionic genes. Study results show a role for unregulated autophagy in the pathogenesis of some RNA viruses. In this context, the positive regulation of hsa-miR-31 and the negative regulations of hsa-miR-493, hsa-miR-889, hsa-miR-655, hsa-miR-656, hsa-miR-26a-1, hsa-miR-154, hsa-miR-335, hsa-miR-1197, and hsa-miR-146a improve innate antiviral responses in cells infected by the virus [26].

Thus, this study aimed at the expression of these miRNAs during dengue infection; in this way, monitoring possible changes can be used as a complementary diagnostic method for faster interventions that can prevent more severe clinical conditions in patients infected with dengue.

6.1.2 Role of RNAi in dengue therapy

To date, miRNAs are used against some viruses that cause disease in humans, including influenza viruses, hepatitis C viruses, hepatitis B viruses, human immunodeficiency virus type 1, polio, and DENV. These viruses are characterized by the presence of ssRNA genomes, which are potential targets for RNAi in the cytoplasm. This functional interaction occurred during the removal and replication of viral RNA [15, 54].

Any changes in the miRNA pathway may shed light on why some mosquitoes are specific vectors for arthropod-borne virus infections (arboviruses), while others are not. The first evidence is the interference of Sindbis viruses that express the

recombinant part of the non-inconsequential unrelated RNA (DENV-2), with the replication of DENV-2 in mosquitoes (Egyptian Aedes) through a system such as the silencing mechanism in plants [55].

Potential evidence may be involved with the interaction of dsRNA or siRNA derived from the arbovirus genome. Mammals have thousands of Piwi-interacting RNA genes from producer types of microRNA regulation expression to control the various stages of cell development and physiology. The critical role of RNAi is a defense against viruses in primary organisms, but in mammals, it is the antiviral defense mechanism hitherto controversial. Currently, it is a conserved mechanism of RNAi in mammals, where its introduction to siRNA affects the silent replication of viruses. Currently, the most significant therapeutic attempt using miRNA is to block the "shutdown" actions of genes that end up facilitating viral replication [41, 42].

7. Conclusions

It is possible to conclude that miRNA has an important relationship during DENV infection, and this regulation can be positive for the virus, that is, facilitating the entry of the virus and helping in the replication process, but it is important to emphasize that there are miRNAs that can also have antiviral action, thereby blocking viral replication.

The miRNA studies demonstrate how important this small RNAi is for viral infections, whether in arthropod vectors like mosquitoes or mammals like humans.

One of the significant difficulties in the study of miRNA is the difficulty of understanding all of its relationships within the cell. Thus, further studies are needed to elucidate more forcefully what the functions of each miRNA are with the cell cycle and the viral replication cycle. Nevertheless, these RNAs have been bringing great perspectives both in treatment and as markers for DENV.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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References

[1] Wiesen JL, Tomasi TB. Dicer is regulated by cellular stresses and interferons. Molecular Immunology. 2010;**46**(6):1222-1228. DOI: 10.1016/j.molimm.2008.11.012

[2] Liu J, Rivas FV, Wohlschlegel J, Yates JR, Parker R, Hannon GJ. A role for the P-body component GW182 in microRNA function. Nature Cell Biology. 2005;**7**(12):1261-1266. DOI: 10.1038/ncb1333

[3] Almeida R, Allshire RC. RNA silencing and genome regulation. Trends in Cell Biology. 2005;**15**(5):251-258. DOI: 10.1016/j.tcb.2005.03.006

[4] Matskevich A, Jung JS, Schümann M, Cascallo M, Moelling K. Vero cells as a model to study the effects of adenoviral gene delivery vectors on the RNAi. Journal of Innate Immunity. 2009;**1**: 389-394. DOI: 10.1159/000191408

[5] Casseb SMM, Simith DB, Melo KFL, Mendonça MH, Santos ACM, Carvalho VL, et al. Drosha, DGCR8, and Dicer mRNAs are down-regulated in human cells infected with dengue virus 4, and play a role in viral pathogenesis. Genetics and Molecular Research [Internet]. 2016;**15**(2). DOI: 10.4238/gmr.15027891

[6] Ricarte Filho JCM, Kimura ET. MicroRNAs: Nova classe de reguladores gênicos envolvidos na função endócrina e câncer. Arquivos Brasileiros de Endocrinologia e Metabologia. 2006;**50**(6):1102-1107. DOI: 10.1590/S0004-27302006000600018

[7] Lin H-R, Ganem D. Viral microRNA target allows insight into the role of translation in governing microRNA target accessibility. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(13):5148-5153. DOI: 10.1073/pnas.1102033108

[8] Ooi YS, Stiles KM, Liu CY, Taylor GM, Kielian M. Genome-Wide RNAi screen identifies novel host proteins required for alphavirus entry. In: Dermody TS, editor. PLoS Pathogens [Internet]. Public Library of Science (PLoS). 19 December 2013;**9**(12):e1003835. DOI: 10.1371/journal.ppat.1003835

[9] Olson KE, Blair CD. Arbovirus-mosquito interactions: RNAi pathway. Current Opinion in Virology. 2015;**15**:119-126. DOI: 10.1016/j.coviro.2015.10.001

[10] Dalmay T, Itgb S. MicroRNAs and the hallmarks of cancer. Oncogene. 2006;**25**:6170-6175. DOI: 10.1038/sj.onc.1209911

[11] Brennecke J, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. PLoS Biology. 2005;**3**(3):85. DOI: 10.1371/journal.pbio.0030085

[12] Brandenstein MV, Richter C, Fries JWU. MicroRNAs: Small but amazing , and their association with endothelin. Life Sciences. 2012;**91**(13-14):475-489. DOI: 10.1016/j.lfs.2012.06.025

[13] Qi Y, Li Y, Zhang L, Huang J. microRNA expression profiling and bioinformatic analysis of dengue virus-infected peripheral blood mononuclear cells. Molecular Medicine Reports. 2013;**7**(3):791-798. DOI: 10.3892/mmr.2013.1288

[14] Zhang P, Zhang H. Autophagy modulates miRNA-mediated gene silencing and selectively degrades AIN-1/GW182 in *C. elegans*. EMBO Reports. 2013;**14**(6):568-576. DOI: 10.1038/embor.2013.53

[15] Yen P-S, Chen C-H, Sreenu V, Kohl A, Failloux A-B. Assessing the

Potential Interactions between Cellular miRNA and Arboviral Genomic RNA in the Yellow Fever Mosquito, Aedes aegypti. Viruses [Internet]. MDPI AG. 10 June 2019;**11**(6):540. DOI: 10.3390/v11060540

[16] Makarova JA, Ivanova SM, Tonevitsky AG, Grigoriev AI. New functions of small nucleolar RNAs. Biochemistry. 2013;**78**(6):638-650. DOI: 10.1134/S0006297913060096

[17] Taft RJ, Glazov EA, Lassmann T, Hayashizaki Y, Carninci P, Mattick JS. Small RNAs derived from snoRNAs. RNA. 2009;**15**(7):1233-1240. DOI: 10.1261/rna.1528909

[18] Höck J, Meister G. The Argonaute protein family. Genome Biology. 2008;**9**(2):210. DOI: 10.1186/gb-2008-9-2-210

[19] Baillat D, Shiekhattar R. Functional dissection of the human TNRC6 (GW182-related) family of proteins. Molecular and Cellular Biology. 2009;**29**(15):4144-4155. DOI: 10.1128/MCB.00380-09

[20] Castilla-llorente V, Spraggon L, Okamura M, Naseeruddin S, Adamow M, Qamar S. Mammalian GW220/TNGW1 is essential for the formation of GW/P bodies containing miRISC. The Journal of Cell Biology. 2012;**198**(4):529-544. DOI: 10.1083/jcb.201201153

[21] Tanzer A, Stadler PF. Molecular evolution of a microRNA cluster. Journal of Molecular Biology. 2004;**339**(2):327-335. DOI: 10.1016/j.jmb.2004.03.065

[22] Xiao C, Rajewsky K. MicroRNA control in the immune system: Basic principles. Cell. 2009;**136**(1):26-36. DOI: 10.1016/j.cell.2008.12.027

[23] Zheng GXY, Do BT, Webster DE, Khavari PA, Chang HY. Dicer-microRNA-Myc circuit promotes transcription of hundreds of long noncoding RNAs. Nature Structural & Molecular Biology. 2014;**21**(7):585-590. DOI: 10.1038/nsmb.2842

[24] Harris E, Holden KL, Edgil D, Polacek C, Clyde K. Molecular biology of flaviviruses. Novartis Found Symposium. 2006;**277**:23-39. DOI: 10.1002/0470058005.ch3

[25] Bernier A, Sagan S. The Diverse Roles of microRNAs at the Host–Virus Interface. Viruses [Internet]. MDPI AG; 19 August 2018;**10**(8):440. DOI: 10.3390/v10080440

[26] Al-Janabi O, Wach S, Nolte E, Weigelt K, Rau TT, Stöhr C, et al. Piwi-like 1 and 4 gene transcript levels are associated with clinicopathological parameters in renal cell carcinomas. Biochimica et Biophysica Acta. 2014;**1842**(5):686-690. DOI: 10.1016/j.bbadis.2014.01.014

[27] Stroynowska A, Agnieszka C, Krzyzosiak WJ. The panorama of miRNA-mediated mechanisms in mammalian cells. Cellular and Molecular Life Sciences. 2014;**71**:2253-2270. DOI: 10.1007/s00018-013-1551-6

[28] Faehnle CR, Joshua-Tor L. Argonautes confront new small RNAs. Current Opinion in Chemical Biology. 2008;**148**(4):825-832. DOI: 10.1038/jid.2014.371

[29] Zipprich JT, Bhattacharyya S, Mathys H, Filipowicz W. Importance of the C-terminal domain of the human GW182 protein TNRC6C for translational repression. RNA. 2009;**15**(5):781-793. DOI: 10.1261/rna.1448009

[30] Diniz DG, Fôro CAR, Turiel MCP, Sosthenes MC, Demachki S, Gomes GF, et al. Environmental influences on antibody-enhanced dengue disease outcomes. Memórias do Instituto Oswaldo Cruz [Internet]. FapUNIFESP (SciELO). Dec 2012;**107**(8):1021-1029. DOI: 10.1590/s0074-02762012000800010

[31] Huang YJS, Higgs S, Horne KM, Vanlandingham DL. Flavivirus-mosquito interactions. Viruses. 2014;**6**(11):4703-4730. DOI: 10.3390/v6114703

[32] Davis-Dusenbery BN, Hata A, Qi Y, Li Y, Zhang L, Huang J. Mechanisms of control of microRNA biogenesis. Molecular Medicine Reports. 2013;**148**(3):791-798. DOI: 10.3892/mmr.2013.1288

[33] Gubler DJ. Dengue and dengue hemorrhagic fever. Clinical Microbiology Reviews. 1998;**11**(3):480-496. DOI: 10.1016/S0140-6736(97)12483-7

[34] Smith JL, Jeng S, McWeeney SK, Hirsch AJ. A MicroRNA screen identifies the Wnt signaling pathway as a regulator of the interferon response during flavivirus infection. In: Diamond MS, editor. Journal of Virology [Internet]. American Society for Microbiology; 1 February 2017;**91**(8). DOI: 10.1128/jvi.02388-16

[35] Feng X, Zhou S, Wang J, Hu W. microRNA profiles and functions in mosquitoes. PLOS Neglected Tropical Diseases. 2018;**12**(5):6463. DOI: 10.1371/journal.pntd.0006463

[36] Asgari S. Role of microRNAs in Arbovirus/vector interactions. Viruses. 2014;**6**(9):3514-3534. DOI: 10.3390/v6093514

[37] Lima-Camara TN. Arboviroses emergentes e novos desafios para a saúde pública no Brasil. Rev Saúde Pública. 2016;**50**(36):1-7. DOI: 10.1590/S1518-8787.2016050006791

[38] Kakumani PK, Ponia SS, Rajgokul KS, Sood V, Chinnappan M, Banerjea AC, et al. Role of RNA interference (RNAi) in dengue virus replication and identification of NS4B as an RNAi suppressor. Journal of Virology. 2013;**87**(16):8870-8883. DOI: 10.1128/JVI.02774-12

[39] Tambyah PA, Ching CS, Sepramaniam S, Ali JM, Armugam A, Jeyaseelan K. microRNA expression in blood of dengue patients. Annals of Clinical Biochemistry. 2016 Jul;**53**(4):466-476. DOI: 10.1177/0004563215604001

[40] Pong LY, Parkkinen S, Dhanoa A, Gan HM, Wickremesinghe IAC, Syed HS. MicroRNA profiling of mouse liver in response to DENV-1 infection by deep sequencing. PeerJ. 2019;7(4):6697. DOI: 10.7717/peerj.6697

[41] Mishra R, Lata S, Ali A, Banerjea AC. Dengue haemorrhagic fever: A job done via exosomes? Emerging Microbes & Infections. 2019;**8**(1):1626-1635. DOI: 10.1080/22221751.2019.1685913

[42] Castillo JA, Castrillón JC, Diosa-Toro M, Betancur JG, St Laurent G, Smit JM, et al. Complex interaction between dengue virus replication and expression of miRNA-133a. BMC Infectious Diseases. 2016;**16**(1):29. DOI: 10.1186/s12879-016-1364-y

[43] Mishra R, Sood V, Banerjea AC. Dengue NS5 modulates expression of miR-590 to regulate ubiquitin-specific peptidase 42 in human microglia. FASEB BioAdvances. 2019;**1**(4):265-278. DOI: 10.1096/fba.2018-00047

[44] Ouyang X, Jiang X, Gu D, Zhang Y, Kong SK, Jiang C, et al. Dysregulated serum MiRNA profile and promising biomarkers in dengue-infected patients. International Journal of Medical Sciences. 2016;**13**(3):195-205. DOI: 10.7150/ijms.13996

[45] Bruscella P, Bottini S, Baudesson C, Pawlotsky JM, Feray C, Trabucchi M. Viruses and miRNAs: More friends than foes. Frontiers in Microbiology. 2017;**8**:1-11. DOI: 10.3389/fmicb.2017.00824

[46] Kakumani PK, Rajgokul KS, Ponia SS, Kaur I, Mahanty S, Medigeshi GR, et al. Dengue NS3, an RNAi suppressor, modulates the human miRNA pathways through its interacting partner. The Biochemical Journal. 2015;**471**(1):89-99. DOI: 10.1042/BJ20150445

[47] Wen W, He Z, Jing Q, Hu Y, Lin C, Zhou R, et al. Cellular microRNA-miR-548g-3p modulates the replication of dengue virus. The Journal of Infection. 2015;**70**(6):631-640. DOI: 10.1016/j.jinf.2014.12.001

[48] Castillo JA, Castrillón JC, Diosa-Toro M, Betancur JG, St. Laurent G, Smit JM, et al. Complex interaction between dengue virus replication and expression of miRNA-133a. BMC Infectious Diseases. 2015;**16**(1):29. DOI: 10.1186/s12879-016-1364-y

[49] Yan H, Zhou Y, Liu Y, Deng Y, Puthiyakunnon S, Chen X. miR-252 of the Asian tiger mosquito *Aedes albopictus* regulates dengue virus replication by suppressing the expression of the dengue virus envelope protein. Journal of Medical Virology. 2014;**86**(8):1428-1436. DOI: 10.1002/jmv.23815

[50] Castrillón-Betancur JC, Urcuqui-Inchima S. Overexpression of miR-484 and miR-744 in Vero cells alters dengue virus replication. Memórias do Instituto Oswaldo Cruz. 2017;**112**(4):281-291. DOI: 10.1590/0074-02760160404

[51] Girardi E, López P, Pfeffer S. On the importance of host microRNAs during viral infection. Frontiers in Genetics. 2018;**9**:1-17. DOI: 10.1186/s12929-019-0614-x

[52] Wu N, Gao N, Fan D, Wei J, Zhang J, An J. miR-223 inhibits dengue virus replication by negatively regulating the microtubule-destabilizing protein STMN1 in EAhy926 cells. Microbes and Infection. 2014;**16**(11):911-922. DOI: 10.1016/j.micinf.2014.08.011

[53] McCartney EM, Helbig KJ, Narayana SK, Eyre NS, Aloia AL, Beard MR. Signal transducer and activator of transcription 3 is a proviral host factor for hepatitis C virus. Hepatology. 2013;**58**(5):1558-1568. DOI: 10.1002/hep.26496

[54] Omoto S, Ito M, Tsutsumi Y, Ichikawa Y, Okuyama H, Brisibe EA, et al. HIV-1 nef suppression by virally encoded microRNA. Retrovirology. 2004;**1**:44. DOI: 10.1186/1742-4690-1-44

[55] Scaria V, Hariharan M, Maiti S, Pillai B, Brahmachari SK. Host-virus interaction: A new role for microRNAs. Retrovirology. 2006;**3**:68. DOI: 10.1186/1742-4690-3-68