Computational Identification of Dengue Virus MicroRNA-Like Structures and their Cellular Targets

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ABSTRACT: MicroRNAs (miRNAs) are small, noncoding RNA molecules that regulate transcriptional and posttranscriptional gene regulation of the cell. Experimental evidence shows that miRNAs have a direct role in different cellular processes, such as immune function, apoptosis, and tumorigenesis. In a viral infection context, miRNAs have been connected with the interplay between host and pathogen, occupying a major role in pathogenesis. While numerous viral miRNAs from DNA viruses have been identified, characterization of functional RNA virus-encoded miRNAs and their potential targets is still ongoing. Here, we used an in silico approach to analyze dengue Virus genome sequences. Pre-miRNAs were extracted through VMir software, and the identification of putative pre-miRNAs and mature miRNAs was accessed using Support Vector Machine web tools. The targets were scanned using miRanda software and functionally annotated using ClueGo. Via computational tools, eight putative miRNAs were found to hybridize with numerous targets of morphogenesis, differentiation, migration, and growth pathways that may play a major role in the interaction of the virus and its host. Future approaches will focus on experimental validation of their presence and target messenger RNA genes to further elucidate their biological functions in human and mosquito cells.

KEYWORDS: flavivirus, dengue virus, in silico screening, microRNA precursor, target prediction, functional annotation

Introduction
Dengue virus (DENV) is an enveloped, mosquito-borne virus with a single-stranded RNA genome of positive polarity and is a member of the Flaviviridae family, specifically belonging to the flavivirus genus. This genus contains nearly 80 viruses distributed worldwide and includes important human pathogens such as yellow fever virus, Japanese encephalitis virus, West Nile virus (WNV), and tick-borne encephalitis virus (TBEV).1,2 DENV is classified into four distinct antigenic types (DENV −1 to −4). In tropical and subtropical environments, DENV infection is an issue of critical importance for public health. Every year, millions of people are infected, producing mild to debilitating febrile illness or a more aggressive disease that can cause hemorrhagic episodes, vascular leakage, severe thrombocytopenia, and shock. Nevertheless, the molecular mechanisms involved in the pathogenesis of DENV remain poorly understood and there is no effective vaccine/drug available to reduce its symptoms or inhibit the infection based on any of the four serotypes. In recent years, due to anthropogenic intervention in natural habitats, the geographical range of DENV has been extended and more cases have been reported.3,4

DENV enters its target cells (primarily dendritic cells, monocytes, and macrophages) via receptor-mediated endocytosis...
in a clathrin-dependent manner. The viral genome consists of a single, long open reading frame (ORF) flanked by 5’ (100 nt) and 3’ (~400 nt) untranslated regions (UTRs). The genome has a positive–sense polarity and is translated at the rough endoplasmic reticulum, giving rise to a polypeptide. It is cotranslationally and posttranslationally cleaved into three structural proteins (C, pM, and E), constituting the building blocks of the virion, and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) that are involved in RNA replication, assembly, and modulation of the immune response of the host cell.\(^5\,6\,9\)

MicroRNAs (miRNAs) are small RNA molecules that are known for their important role as gene regulators in metazoans. In general, miRNAs are processed from structured RNAs into single-stranded molecules, 18–23 nt in length, which have been shown to control translation of messenger RNAs by cleaving or blocking the target. In the mammalian cytoplasmic context, miRNAs attach to their target with incomplete complementarity in association with cellular proteins commonly called the RNA-Induced Silencing Complex (RISC). Regulation mediated by miRNAs is important in an extensive range of biological processes, including growth, development, cellular division, and oncogenesis.\(^10\,12\)

In a viral infection panorama, miRNAs have been associated with the interplay between host and pathogen, playing a major role in viral pathogenesis. It is clear that studies on host–pathogen interactions at the miRNA level are still in their infancy and seem to be a promising field for the study of the pathogenicity of human viruses. Viral miRNAs can regulate both cellular and viral gene expressions through the regulation of cellular elements involved antiviral responses, simulating cellular miRNAs, or targeting their own viral messenger RNAs to modulate the viral replication cycle.\(^13\,15\)

Remarkably, most current knowledge about viral miRNAs is derived from DNA viruses, primarily from the Herpesviridae family. For cytoplasmic RNA viruses, miRNAs were hypothesized to be absent because of the potential degradation of the viral RNA genome during the excision of virus-encoded miRNA precursors (pre-miRNAs).\(^14\) However, it has been revealed that functional, virus-derived miRNAs can be processed by cytoplasmic RNA viruses when a pre-miRNA sequence is introduced into the virus genome. Good examples are the Sindbis virus (SINV) and TBEV.\(^16\,18\)

A special case is the WNV. This virus has a highly conserved stem-loop (SL) region in the viral 3’-UTR that serves as a source for the generation of a mature miRNA in infected mosquito cells. This miRNA, called KUN-miR-1, targets mosquito GATA4 mRNA that leads to its up-regulation in cells. Inhibition of KUN-miR-1 or depletion of GATA4 mRNA both led to reduced WNV RNA replication.\(^13\,19\)

Identification of animal and plant miRNAs is mostly based on homology methods; however, the same strategy cannot be followed with RNA viruses. The high mutation rates and the lack of validated miRNAs impede an adequate and accurate prediction. To increase the amount of information related to viral miRNA discovery in RNA virus, we methodically scanned pre-miRNAs derived from DENV genomes. Then, we filtered the results, validated the secondary structure and identified mature miRNAs. We also predicted the potential targets of those miRNAs and their structures and functions. Overall, we carefully produced a working methodology for identifying miRNAs and their potential cellular targets, which can be used with other flaviviruses or RNA viruses. The workflow of our investigation is shown in Figure 1.

**Methods**

**Viral sequences and the prediction of pre-miRNAs by an ab initio approach.** For this work, we used two different genome sequences of DENV serotype 2 downloaded from publicly available databases, which included the ORFs and the 5’ and 3’-UTRs of the viral RNA [GenBank accession numbers: JF357906.1 and FJ390389.1]. VMir was used to analyze the DENV2 sequences for possible pre-miRNA hairpin structures, using stringent filtering parameters as described in other publications.\(^13\) The VMir program (down-loadable online) uses several predefined but adjustable parameters. This *ab initio* program (ie, a platform that takes as input a genomic sequence without any other data and examines it for all possible pre-miRNAs occurring in the sequence) slides a window of adjustable size (500 nt) over the sequence of interest, advancing each window by a given step size (1 nt). The secondary structure of RNAs corresponding to each window is predicted using the RNAFold algorithm. Hairpins with a size above a certain threshold (50 nt) are then identified and scored.\(^13\,20\)

**Identification of putative pre-miRNA sequences and mature miRNAs.** To discriminate real pre-miRNAs from other hairpin structures with similar SLs (SL pseudo hairpins), we employed the ncRNA Feature Extraction and pre-miRNA Classification Web Tool (accessible at http://150.140.142.24:82/Prediction/Anon.aspx), which decides whether each hairpin is a pseudo-pre-miRNA-like hairpin or a real pre-miRNA using a Support Vector Machine classifier (SVM).\(^21\)

With the purpose of extracting mature miRNA:miRNA* duplexes from pre-miRNA hairpins, we used the MaturePred Web Tool (accessible at http://nclab.hit.edu.cn/maturepred/). This software also uses a model based on SVM that predicts the starting position of an miRNA by performing discriminant analysis against the query hairpin structure using various features of known real/pseudo miRNA:miRNA* duplexes as a training set (position-specific features, energy-related
Secondary structure validation. Pre-miRNA sequences were submitted to Mfold (accessible at http://mfold.rna.albany.edu) to check the fold-back secondary structure. The default parameters for Mfold were used, and all qualifiers were recorded, including the nucleotide length, position of the matching regions, the number of arms per structure, and the Minimal Folding Free Energy (MFE, $\Delta G$ kcal/mol). We also calculated the Minimal Folding Free Energy Index (MFEI) as previously described.

Prediction of potential targets and functional enrichment analysis. Human UTRs were downloaded from the UTRdb database. Subsequently, the potential 3'-UTR targets for the putative miRNAs were scanned with the assistance of the Linux-based miRanda software. This software operates thermodynamics and dynamic programming alignments, along with statistical parameters, for target prediction against the human genome. The parameters assigned for miRanda hybridization included a default alignment score of 140 and an MFE for an miRNA:miRNA* duplex of $\leq -35$ kcal/mol, and the other parameters were kept as default. The matched UTRs were submitted to the NCBI BLAST platform to visualize the genome context, and the biological function was annotated. The diverse steps involved in the identification and target prediction of the miRNAs from DENV are presented in Figure 1. In order to enrich the identified genes with connection to specific functional terms, the potential targets were analyzed using Cytoscape software and its plug-in: ClueGo, applying the Gene Ontology database (released January 2014). Ontologies were designated as biological processes, immune system processes, reactome, molecular function, and cellular component. Enrichment was executed by the right-sided hyper-geometric test and its probability value was corrected by the Bonferroni’s step-down method. For every procedure, the maximum stringency filters were utilized to minimize the noise and background results, thereby guaranteeing the fewest number of false-positive results.

Results and Discussion

Prediction of pre-miRNA in DENV. Many mature miRNAs are evolutionarily conserved from organism to organism, which simplifies the prediction of the existence of new miRNAs in other species. However, other approaches should be evaluated for fast-evolving biological entities such as viruses. Using an \textit{ab initio} approach and different depuration steps, we identified a total of eight DENV miRNAs that originate from viral genome sequences (Fig. 2). The different phases involved in miRNA prediction are shown in Figure 1. These eight miRNAs were found dispersed in several regions of the viral genome (both ORF and UTR sequences; all hairpins are shown in Supplementary File). The length of the pre-miRNAs varied from 62 to 157 nucleotides, with an average length of 102 nucleotides, which is in the same range as the
miRNA experimentally identified in the terminal 3′-SL of WNV (78 nucleotides). The diverse lengths of the identified miRNAs possibly suggest unique roles for modulation of miRNA biogenesis or gene expression. Interestingly, all the predicted miRNAs were encountered in the reverse strand of the viral mRNA (Table 1), which adds a new dimension to the search of structured noncoding RNAs in replication intermediaries. MFE is a central characteristic that defines the secondary structure of RNA. The thermodynamic stability of an RNA molecule increases as MFE values decrease. The MFEI for every pre-miRNA was calculated and the values ranged from 0.58 to 1.45, with an average value of 0.76, which is similar to that of other miRNAs. The G + C composition percentage was calculated. In DENV, the G + C content ranged from 43.31% to 59.68%, with an average value of 49.22%. Comparable nucleotide compositions have been detected in other organisms.

Mature miRNAs. Comparable to other biological systems, DENV miRNAs can be located on either of the two arms in the secondary hairpin structure (Fig. 2). Of the eight miRNAs identified in DENV2, three are located in the 5′-arm of the SL hairpin structure, while five are in the 3′-arm (Table 2). The existence of this feature is also present in animal miRNAs, where mature miRNA can be processed from either of the two arms of the SL hairpin secondary structure. The length of the mature miRNAs was the same because it was a controlled and recommended parameter for the prediction of sequences in non-plant organisms. Remarkably, just three mature miRNAs start with a 5′-terminal uridine residue, a typical characteristic of miRNAs recognized by the AGO1 protein. These findings indicate that the predicted miRNAs may be noncanonical.

Prediction of the potential targets for putative miRNAs in DENV and functional annotation. A better understanding of the dynamics between miRNAs and their targets will help to understand the complexity of biological regulation and other aspects of virus–host dynamics. In silico prediction of miRNA targets provides a good alternative for identifying potential
target sites based on their complete or partial complementarity with the miRNAs. Nevertheless, it is well known that in animal systems, miRNA targets are difficult to predict because miRNA and mRNA pairs frequently contain several mismatches, gaps, and G + U base pairs in many positions.26

In this study, pairwise comparison of whole human 3′-UTRs against eight mature miRNA of DENV has been conducted. The miRanda algorithm was used, which incorporates thermodynamic stability calculations of miRNA:mRNA* duplexes and alignment procedures for detecting the probable binding site on the 3′-UTRs. We observed 53 transcript targets for eight DENV miRNAs (Table 3). The predicted targets for a single miRNA vary from just one (DENV2-miR31, DENV2-miR6, DENV2-miR170, and DENV2-miR1) to >10 (DENV2-miR14, DENV2-miR15). While several miRNAs may regulate just one mRNA, these results demonstrate another type of regulation where a single miRNA can regulate numerous transcripts, elevating the complexity of cellular processes.

Gene ontology has become a very useful tool for the mining of gene/protein datasets and their functional annotations. The functional enrichment analysis was conducted using the ClueGo plug-in on the potential targets of the DENV miRNAs. The 53 genes were significantly enriched to two main functional clusters, i.e., anatomical structure formation involved in morphogenesis (10 terms) and cell projection morphogenesis (13 terms) (Fig. 3). Many of the terms obtained from the annotation were related to development, movement, differentiation and migration. Clearly, experimental confirmations are needed to validate these targets under infection conditions.

**Conclusions**

The importance of miRNAs in cellular systems is clear. However, understanding of the role for viral miRNAs is just in its infancy. Viral miRNAs can be extraordinary tools to modulate cellular or viral gene expression. Their nonimmunogenic nature, small size, high specificity, and capacity for multiple transcript regulation are reasons to expect their presence in RNA viruses. In addition, the picture becomes more complex when the search is extended to include viral replication intermediates. In this case, the presence of viral miRNAs in the negative-sense RNA used as a replication template for the viral genome may be a new source of information, which could explain pathogenesis-related phenomena.

The outcomes of the in silico predictions are useful to guide experimental design to achieve biological validation. The next logical step after these types of analyses is to scrutinize whether miRNAs can be processed in eukaryotic cells (mammalian and mosquito cell lines). In summary, we performed a complete scan of the DENV genome and its replication intermediates

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### Table 1. Predicted DENV2 pre-miRNA identified by VMir software.

| NO. | PREDICTED pre-miRNA | STRAND | SIZE pre-miRNA (NT) | SEGMENT ON GENOME | (G + C) % | Pre-miRNA MFES (∆G, KCAL/MOL) | MFEI |
|-----|---------------------|--------|---------------------|-------------------|----------|--------------------------|------|
| 1   | DENV2-miR31         | Reverse| 104                 | 10575–10678       | 51.92    | 78.2                     | 1.45 |
| 2   | DENV2-miR82         | Reverse| 108                 | 4798–4905         | 48.15    | 33.3                     | 0.64 |
| 3   | DENV2-miR6          | Reverse| 68                  | 2749–2816         | 48.53    | 23.5                     | 0.71 |
| 4   | DENV2-miR170        | Reverse| 62                  | 9872–9933         | 59.68    | 24.9                     | 0.67 |
| 5   | DENV2-miR14         | Reverse| 81                  | 5088–5168         | 51.92    | 24.9                     | 0.71 |
| 6   | DENV2-miR1          | Reverse| 157                 | 488–644           | 43.31    | 39.5                     | 0.58 |
| 7   | DENV2-miR15         | Reverse| 89                  | 5454–5542         | 49.44    | 28.8                     | 0.65 |
| 8   | DENV2-miR7          | Reverse| 153                 | 3317–3469         | 47.06    | 44.9                     | 0.62 |

### Table 2. Characteristics of DENV2 mature miRNAs.

| NO. | NEW miRNA NAME | LENGTH MATURE miRNA | miRNA START POSITION | LOCATION | PREDICTED MATURE SEQUENCE (5′–3′) |
|-----|----------------|---------------------|----------------------|----------|----------------------------------|
| 1   | DENV2-miR31    | 22                  | 68                   | 3        | AGCUUGAUCGCAUCUCAUGAGACG         |
| 2   | DENV2-miR82    | 22                  | 75                   | 3        | CCAAGUUCUCAAUGCCAGGCAUCU        |
| 3   | DENV2-miR6     | 22                  | 8                    | 5        | UGAGACUCUGUGAGAGCAUU            |
| 4   | DENV2-miR170   | 22                  | 10                   | 5        | ACGGCAUCUGCGAGCAAGAAU           |
| 5   | DENV2-miR14    | 22                  | 50                   | 3        | UUUCUCUCCUCUGGAUGAGGU           |
| 6   | DENV2-miR1     | 22                  | 88                   | 3        | UAUUGAGCAUGAGGGUACACU           |
| 7   | DENV2-miR15    | 22                  | 58                   | 3        | CUCUCCGGAGAGUGGCGUCUC           |
| 8   | DENV2-miR7     | 22                  | 56                   | 5        | ACCAGCAUCUCUCCCUCACCUCUC       |
| No. | Predicted miRNA | Targeted Protein | Protein Description | Target Gene ID Number |
|-----|----------------|-----------------|---------------------|-----------------------|
| 1   | DENV2-miR31    | AGTPBP1         | Cytosolic carboxypeptidase 1 | 23287     |
| 2   | DENV2-miR82    | DCLK2           | Doublecortin-like kinase 2 | 166614    |
|     |                | MCAM            | Melanoma cell adhesion molecule | 4162      |
|     |                | RAB3L1          | RAB3A interacting protein (rabin3)-like 1 | 5866      |
|     |                | RXRA            | Retinoid X receptor, alpha | 6256      |
| 3   | DENV2-miR6     | LRRC55          | Leucine rich repeat containing 55 | 219527    |
| 4   | DENV2-miR170   | FGFR2           | Fibroblast growth factor receptor 2 | 2263      |
| 5   | DENV2-miR14    | CBX2            | Chromobox homolog 2 | 84733     |
|     |                | CORO6           | Corin 6 | 84940      |
|     |                | DENND2A         | DENN/MAAD domain containing 2A | 27147     |
|     |                | EFNB3           | Ephrin-B3 | 1949      |
|     |                | FBF1            | Fas (TNFRSF6) binding factor 1 | 85320     |
|     |                | FGFRL1          | Fibroblast growth factor receptor-like 1 | 53834     |
|     |                | IDUA            | Iduronidase, alpha-L | 3425      |
|     |                | MOV10           | Moloney leukemia virus 10, homolog (mouse) | 4343      |
|     |                | SEMA5A          | Semaphorin-5A | 9037       |
|     |                | SPTB            | Beta-1 Spectrin | 6710       |
|     |                | SYNE2           | Spectrin repeat containing, nuclear envelope 2 | 23224     |
|     |                | TOP3A           | Topoisomerase (DNA) III alpha | 7156      |
|     |                | TYMP            | Thymidine phosphorylase | 1890      |
|     |                | VASP            | Vasodilator-stimulated phosphoprotein | 7408      |
| 6   | DENV2-miR1     | MKRN1           | Makorin ring finger protein 1 | 23608     |
| 7   | DENV2-miR15    | CAGE1           | Cancer-associated gene 1 protein | 285782    |
|     |                | AKAP10          | A kinase (PRKA) anchor protein 10 | 11216     |
|     |                | ATP8B3          | ATPase, aminophospholipid transporter, class I, type 8B, member 3 | 148229    |
|     |                | BBS5            | Bardet-Biedl syndrome 5 | 129880    |
|     |                | CACNA1C         | Calcium channel, voltage-dependent, L type, alpha 1C subunit | 775       |
|     |                | CDK5RAP1        | CDK5 regulatory subunit associated protein 1 | 51654     |
|     |                | CMBL            | Carboxymethylenebutanolidase homolog (Pseudomonas) | 134147    |
|     |                | DNAJC5          | DnaJ (Hsp40) homolog, subfamily C, member 5 | 80331     |
|     |                | FAM122C         | Family with sequence similarity 122C | 159091    |
|     |                | FAM131C         | Family with sequence similarity 131, member C | 348487    |
|     |                | GOSR1           | Golgi SNAP receptor complex member 1 | 9527      |
|     |                | HAND1           | Heart and neural crest derivatives expressed 1 | 9421      |
|     |                | IL16            | Interleukin 16 | 3603       |
|     |                | KLK10           | kalikrein-related peptidase 10 | 5655      |

(Continued)
Table 3. (Continued)

| NO. | PREDICTED miRNA | TARGETED PROTEIN | PROTEIN DESCRIPTION | TARGET GENE ID NUMBER |
|-----|----------------|-----------------|---------------------|----------------------|
|     | LRCH4          | Leucine-rich repeats and calponin homology (CH) domain containing 4 | Nervous system development | 4034 |
|     | NDOR1          | NADPH dependent diflavin oxidoreductase 1 | Electron transfer | 27158 |
|     | OCEL1          | Occludin/ELL domain containing 1 | Elongation factor | 79629 |
|     | PDZD4          | PDZ domain containing 4 | Cell cortex | 57595 |
|     | PLXNB1         | Plexin B1 | Axon guidance, invasive growth and cell migration | 5364 |
|     | PPP2RS5C       | Protein phosphatase 2, regulatory subunit B, gamma | Cell growth and division | 5527 |
|     | PRDM12         | PR domain containing 12 | Transcriptional regulation | 59335 |
|     | SIGIRR         | Single immunoglobulin and toll-interleukin 1 receptor (TIR) domain | Immunity | 59307 |
|     | SLC22A20       | Solute carrier family 22, member 20 | Organic ion transport | 440044 |
|     | TCEA3          | Transcription elongation factor A (SII), 3 | Regulation on transcription elongation | 6920 |
|     | OAZ3           | Ornithine decarboxylase antizyme 3 | Polyamine biosynthesis | 51686 |
|     | TRIM17         | Tripartite motif-containing 17 | Unknown | 51127 |
|     | WDR25          | WD repeat domain 25 | Unknown | 79446 |
| 8   | DENV2-miR7     | HLA-DOA | Major histocompatibility complex, class II, DO alpha | 3111 |
|     | IFITM10        | interferon induced transmembrane protein 10 | Cell membrane | 402778 |
|     | FTSJ2          | FtsJ methyltransferase domain containing 2 | mRNA processing | 23070 |
|     | IRF2BP1        | interferon regulatory factor 2 binding protein-like | Gene transcription | 64207 |

Figure 3. Functional enrichment analysis of the predicted targets of the Dengue Virus miRNAs.

Notes: *P < 0.05, **P < 0.01 are the statistical levels.

to obtain all putative pre-miRNAs for the entire sequence that were later filtered and matured by machine learning web tools. A total of eight miRNAs were identified, and these miRNAs share comparable features with other known miRNAs (animal miRNAs); however, other characteristics, such as the absence of a uridine at the 3’ end, reveal possible noncanonical processing and different biogenesis. In silico target prediction generated 53 genes. The potential targets are involved in different important biological processes as anatomical structure formation involved in morphogenesis and cell projection morphogenesis, suggesting that they could play an important role in the process of virus–host interactions during infection.

Author Contributions
MO carried out the experimental procedures and data analysis. MO, NC, JPF, and JCG contributed with the writing of
the manuscript. JCG designed the project from which this manuscript was planned. MO, NC, JPF, and JCG agreed with manuscript results and conclusions. All authors read and approved the final manuscript.

Supplementary Data

Supplementary File 1. VMir analysis of the Dengue Virus genome (A) isolate DENV-2/NI/BID-V3227/2008 and (B) New Guinea C derivative strain; shown are all hairpins that fold in a 500 nt window and achieved a VMir score of 115 or above.

REFERENCES

1. Silva PAGC, Pereira CF, Dalebout TJ, Spaan WJM, Breedenbeek PJ. An RNA pseudoknot is required for production of yellow fever virus subgenomic RNA by the host nuclease XRN1. J Virol. 2010;84(21):11395–406.
2. Lindenbach BD, Rice CM. The viruses and their replication. In Fields Virology. 5th ed. Philadelphia: 2007:1101–52.
3. Bente DA, Rico-Hesse R. Models of dengue virus infection. Inflammon Infect Dis. 2006;3(1):97-103.
4. Fischl W, Bartenschlager R. Exploitation of cellular pathways by dengue virus. Curr Opin Microbiol. 2003;3:5–41.
5. Chambers T, Monath T. The Flaviviruses: Structure, Replication and Evolution: a new role for microRNAs. Retrovirology. 2010;7:11395–406.
6. Chambers T, Monath T. The Flaviviruses: Detection, Diagnosis and Vaccine Development: Detection, Diagnosis and Vaccine Development: 61 (Advances in Virus Research). San Diego: Academic Press; 2003:3–8.
7. Friese P, Harris E. Interplay of RNA elements in the dengue virus 5′ and 3′ ends required for viral RNA replication. J Virol. 2010;84(12):6103–18.
8. Lindenbach BD, Rice CM. Molecular biology of flaviviruses. Adv Virus Res. 2003;59:23–61.
9. Fischl W, Bartenschlager R. Exploitation of cellular pathways by dengue virus. Curr Opin Microbiol. 2011;14(4):470–5.
10. Scaria V, Harirhan M, Matsu S, Pillai B, Brahmacari SK. Host-virus interaction: a new role for microRNAs. Retrovirology. 2006;3:68.
11. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.
12. Kroj J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2011;10(9):597–610.
13. Hussain M, Torres S, Schanerter E, et al. West Nile virus encodes a microRNA-like small RNA in the 3′ untranslated region which up-regulates GATA4 mRNA and facilitates virus replication in mosquito cells. Nucleic Acids Res. 2012;40(5):2210–23.
14. Skalsky RL, Cullen BR. Viruses, microRNAs, and host interactions. Annu Rev Microbiol. 2010;64(5):123–41.
15. Cullen BR. Viral RNAs: lessons from the enemy. Cell. 2009;136(4):592–7.
16. Rouha H, Thurner C, Mandl CW. Functional microRNA generated from a cytoplasmic virus RNA. Nucleic Acids Res. 2010;38(21):8328–37.
17. Shapira JS, Vaziri A, Pham AM, Tenover FR. Noncanonical cytoplasmic processing of viral microRNAs. RNA. 2010;16(11):2068–74.
18. Barrh S, Pichl T, Mamiani A, et al. Epstein–Barr virus-encoded microRNA miR-BART2 down-regulates the viral DNA polymerase Balf5. Nucleic Acids Res. 2008;36(2):666–75.
19. Usme-Ciro JA, Campillo-Pedroza N, Almazán F, Gallego-Gomez JC. Cytoplasmic virus RNA as potential vehicles for the delivery of therapeutic small RNAs. Viral J. 2013;10(1):185.
20. Grundhoff A, Sullivan CS, Ganem D. A combined computational and microarray-based approach identifies novel microRNAs encoded by human gammaherpesviruses. RNA. 2006;12(5):733–50.
21. Kleefroggiannis D, Theofillatos K, Papadimitriou S, Tsakalidis A, Likothanassis S, Mavroudi S. ncRNA-Class web tool: non-coding RNA feature extraction and pre-miRNA classification web tool. In: Iliaidis L, Maglioggiannis I, Papadopoulos H, Karatzas K, Sioutas S, eds. Artificial Intelligence Applications and Innovations. Vol. 382. Berlin, Heidelberg. Springer; 2012:632–41.
22. Xuan P, Guo M, Huang Y, Li W, Huang Y. MaturePred: efficient identification of microRNAs within novel plant pre-miRNAs. PLoS One. 2011;6(11):e27422.
23. Gong P, Xie F, Zhang B, Perkins EJ. In silico identification of conserved microRNAs and their target transcripts from expressed sequence tags of three earthworm species. Comp Biol Chem. 2010;34(5–6):313–9.
24. Wheeler BM, Heimberg AM, Moy VN, et al. The deep evolution of metazoan microRNAs. Nucleic Acids Res. 2005;33(1):84–93.
25. Silacci F, De Marchi E, Brancaccio S, et al. MicroRNA-like small RNA in the 3′ untranslated region which up-regulates GATA4 mRNA and facilitates virus replication in mosquito cells. Nucleic Acids Res. 2012;40(5):2210–23.