Received: 10 April 2019 | Revised: 9 May 2019 | Accepted: 14 May 2019

DOI: 10.1002/jmv.25505

JOURNAL OF MEDICAL VIROLOGY WILEY

RESEARCH ARTICLE

In silico analysis revealed Zika virus miRNAs associated with viral pathogenesis through alteration of host genes involved in immune response and neurological functions

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Abstract

Background: The concurrent Zika Virus (ZIKV) outbreaks in the United States and Northeast Brazil have evoked global surveillance. Zika infection has been correlated with severe clinical symptoms, such as microcephaly, Guillain-Barré syndrome, and other congenital brain abnormalities. Recent data suggest that ZIKV predominantly targets neural progenitor cells leading to neurological impairment. Despite the clinical evidence, detailed experimental mechanism of ZIKV neurotropic pathogenesis has not been fully understood yet. Here we hypothesized that ZIKV produces miRNAs, which target essential host genes involved in various cellular pathways facilitating their survival through immune evasion and progression of disease during brain development.

Methods: From genome sequence information using several bioinformatic tools, we predicted pri-miRNAs, pre-miRNAs, and finally the mature miRNAs produced by ZIKV. We also identified their target genes and performed functional enrichment analysis to identify the biological processes associated with these genes. Finally, we analyzed a publicly available RNA-seq data set to determine the altered expression level of the targeted genes.

Results: From ZIKV genome sequence, we identified and validated 47 putative novel miRNAs. Functional enrichment of the targeted genes demonstrates the involvement of various biological pathways regulating cellular signaling, neurological functions, cancer, and fetal development. The expression analysis of these genes showed that ZIKV-produced miRNAs downregulate the key genes involved in these pathways, which in turn may lead to impaired brain development.

Conclusions: Our finding proposes novel ZIKV miRNAs and their targets, which upon experimental validation could help developing new therapeutics to combat ZIKV infection and minimize ZIKV-mediated pathologies.

KEYWORDS

functional annotation, immune response, miRNA, neurological functions, pathogenesis, Zika virus

Abbreviations: 3′-UTR, 3′-untranslated region; DAVID, Database for Annotation, Visualization and Integrated Discovery; EBV, Epstein-Barr virus; ER, Endoplasmic reticulum; GBS, Guillain-Barré syndrome; GO, Gene Ontology; GOBP, GO biological process; GOCL, GO cellular locations; GOMF, GO molecular functions; GSEA, Gene Set Enrichment Analysis; HCMV, Human Cytomegalovirus; hNPC, Human neural progenitor cell; KEGG, Kyoto Encyclopedia of Genes and Genomes; KSHV, Kaposi's Sarcoma-associated Herpesvirus; miRNA, microRNA; NCBI, National Center for Biological Information; ZIKV, Zika virus.

1 | INTRODUCTION

Zika virus (ZIKV) is a positive-sense RNA virus, which was first identified in the Zika forest, near Lake Victoria in Uganda in 1947[1] and isolated from the serum of a Rhesus monkey, *Macaca mulatta*. ZIKV is an emerging Arbovirus of the *Flavivirus* genus and *Flaviviridae* family,[2] and is closely related to Dengue, West Nile, and Japanese Encephalitis viruses[3] but exhibits uncommon health consequences. Like other Flaviviruses, ZIKV is enveloped and icosahedral and has a nonsegmented, single-stranded, 10 kilobases positive-sense RNA genome[4] that is transmitted by the day-time active *Aedis* mosquito, such as *A. aegypti* and *A. albopictus*,[2] although sexual and vertical transmissions have also been reported.[5]

The first case of Zika infection in human was reported in Uganda, 1952. The first noteworthy Zika epidemic was reported in 2007 on Yap Island,[6] infecting 75% of the population. Until then Zika remained obscure and was known to have limited and asymptomatic symptoms leading to acute febrile illness, fever, rash, malaise, joint and back pain, constipation, and diarrhea.[7] Later on, from 2013-2015 massive outbreaks were reported in French Polynesia, New Caledonia, Easter Island, and the Cook Islands indicating the fast spreading of the virus across Pacific region.[8] In May 2015, Brazil reported the first outbreak of ZIKV infection in America. The Brazil Ministry of Health estimated around 440 000-1 300 000 suspected cases of Zika infection in December 2015.[9] Since then, the infection has spread rapidly to several other countries, becoming a pandemic.

In 2015-2016, an increased number of congenital malformations including microcephaly, Guillain-Barré syndrome (GBS) and other neurological diseases in both newborns and adults were reported in Brazil.[10] In March 2016, the WHO reported that Zika virus was actively circulating in 38 countries and territories, 12 of which had reported an increase in GBS cases or laboratory evidence of Zika virus among patients with GBS.[10] As of June 2016, a total of 591 laboratory-confirmed travel-associated Zika virus infections have been reported in the United States, with none acquired via local vector-borne transmission. 11 cases have been transmitted sexually, and one case of associated GBS has been reported.[11] However, despite the experimental evidence the underlying mechanism showing how ZIKV causes the birth defect, still remains elusive. Unfortunately, no effective therapeutics are available till date, hence it is a crying need to study the underlying mechanism of ZIKV-mediated pathogenesis that would enrich our knowledge and accelerate the development of new therapeutics.

Micro-RNAs (miRNAs) are small (~22 nt) noncoding RNA molecules that posttranscriptionally downregulate the expression of target genes by RNA interference (RNAi) method. These RNAs are known to specifically recognize and bind to complementary sites of 3′-untranslated region (3′-UTR) of specific messenger RNAs (mRNA) leading to degradation or halting the translation of their target mRNAs. miRNAs play crucial roles in various biological processes, including the development of an organism,[12] cellular proliferation,[13] regulation of the immune system,[14] programmed cell death or apoptosis,[15] and oncogenesis.[16]

Previously human miRNAs were shown to subdue viral pathogenesis by targeting viral genes.[17] Further studies revealed the potentiality of viral miRNAs to target their host genes,[18] playing subtle roles in the survival and proliferation of viral particles through host immune system evasion,[19] establishing microenvironment for viral replication,[20] regulation of the innate immune system,[21] and differentiation of adaptive immune cells.[21] Several miRNAs were found to be encoded by Epstein-Barr virus,[22] Kaposi's Sarcoma-associated Herpesvirus (KSHV), Human Cytomegalovirus (HCMV), that belong to Herpesvirus family. Besides, viruses from Polyomavirus,[23] Ascovirus,[21] Iridovirus,[21] Baculovirus,[21] Adenovirus,[21] Retrovirus[23] families were also found to produce miRNAs. Interestingly, viral miRNAs encoded by EBV have been proposed to downregulate the expression of targeted host genes. miRNAs encoded by Herpes viruses might block the host immune response or interfere with the apoptosis of the infected cells, thereby facilitate the viral life cycle.

As described by Tang et al[24] ZIKV infection in human neural progenitor cells (hNPC) results in aberrant cell cycle and apoptosis while Rolfe et al[25] further showed the enrichment of numerous cytotoxic proinflammatory pathways in the CNS that could induce cell death. In this study, we hypothesized that like Herpes viruses, ZIKV-encoded miRNAs might modulate the host immune system and various physiological processes that would provide the virus selective advantages for prolonged refuge and disease pathogenesis within the host.

Using computational method, we identified novel ZIKV-encoded miRNAs. We demonstrate that ZIKV-encoded miRNAs downregulate host genes involved in neural functions, cellular and immune pathways. Viruses exploit host cells for their survival and replication, arguing the importance of repression of host genes to evade the host immune system. Genes targeted by these miRNAs that could provide insights into the affected pathways upon infection were identified next. Moreover, functional enrichment analysis of specific genes provided novel information about the functions that the miRNAs of ZIKV perform inside their hosts.

2 | MATERIALS AND METHODS

2.1 | Prediction of pre-miRNAs and mature miRNAs

The ZIKV genome sequence (Accession No. KU681082) was obtained from the National Center for Biological Information (NCBI).[26] To predict the presence and positions of the pre-miRNAs in the obtained sequence, miRNAFold,[27] and Vmir[28] tools using in-house Perl scripts with default parameters were used. Stem-loop secondary structure is one crucial feature to distinguish between pri-miRNA & pre-miRNAs. miRBase[29] (Release 21: June 2014) database was used to search for the stem-loop sequences within the ZIKV genome. In addition, Triplet SVM Classifier[30] tool was

used to find the true pre-miRNAs among a set of conserved stem-loops, which were further validated by miRdup.[31] The minimum bases for the stem-loop were set to 22 for the Triplet SVM Classifier[30] tool. In addition, using maturePred,[32] FOMmir[33] and matureBayes[34] the mature miRNAs were predicted from the pre-miRNA sequences utilizing the default parameters.

2.2 | Obtaining ZIKV miRNA target genes

RNAhybrid[35] was used to obtain the genes targeted by the predicted miRNAs. Gene symbols and Ensembl gene IDs of the targeted genes were extracted from Ensembl[36] (Ensembl Release 85). The annotated functions of all the genes were identified from the Gene Ontology Consortium (GO)[37] where the Biological Processes (GOBP), Cellular Locations (GOCL), and Molecular Functions (GOMF) of the annotated genes are provided. The pathways involved in the physiology of humans were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG)[38] (Release 74: April 2015). In both cases, the Ensembl Release 85 was used.

2.3 | Functional enrichment analysis

Gitools[39] (Version: 1.8.4) was used for functional enrichment analysis and to generate heatmaps for the targeted biological processes or pathways and the corresponding *P* values. The corrected right *P* value (False Discovery Rate [FDR]) 0.05 cutoff was used as the parameter for significantly enriched GO or KEGG pathway terms determination. Moreover, Gene Set Enrichment Analysis (GSEA)[40] provided the overlapped gene sets involved in specific biological processes and pathways. Later, Database for Annotation, Visualization and Integrated Discovery (DAVID)[41] was used to obtain the enriched biological processes and pathways involving the miRNA target genes. Specific processes related to similar functions were clustered together. In addition, STRING[42] (version: 10.5) was used to identify the interactions between the targeted proteins. Cytoscape (Version: 3.5.1)[43] was used to visualize the interactions. Then, enrichment analysis was done with these proteins using Gitools[39] (Version: 1.8.4) to identify important clusters of biological processes and pathways.

2.4 | Expression profile analysis

Gene Expression Omnibus (GEO)[44] is a curated, public reservoir of microarray gene expression data preserved at NCBI. To obtain the expression level of each of the target genes, the RNA-seq data set GSE78711 of ZIKV (Strain: MR766) infected human neural progenitor cells (hNPCs) using the platforms GPL15520 Illumina MiSeq (*Homo sapiens*) and GPL18573 Illumina NextSeq. 500 (*Homo sapiens*)[24] was used. Processing the data showed the Log2 fold changes in the expression level of the total human genes and the expression level of target genes was analyzed together with their significance levels.

3 | RESULTS

3.1 | ZIKV-encoded miRNAs target several host genes

A large number of human viruses are known to produce miRNAs such as EBV, KSHV, and HCMV, which are linked to the development of severe neurological birth defects. To identify if ZIKV also encodes miRNAs like these viruses, bioinformatics tools miRNAFold,[27] Vmir,[28] and miRBase[29] were utilized. These tools yielded 222, 1600, and 33 putative pre-miRNA candidates respectively (data not shown). Using Triplet SVM Classifier[30] 12 pre-miRNAs were identified to be truly positive, which were further validated by miRdup[31] (Supporting Data file 1). From these 12 pre-miRNAs, we obtained a total of 47 mature miRNA sequences by maturePred,[32] FOMmir,[33] and matureBayes[34] (Table 1). miRNA sequences obtained from these tools were then uploaded on the online tool RNAhybrid,[35] which provided ZIKV miRNAs targeted 40 human genes (Supporting Data file 2), among which 29 genes were unique (Table 2).

3.2 | ZIKV miRNAs targeted host genes that regulate cellular pathways associated with cell signaling and neurological functions

To better understand the functions of the ZIKV miRNA-targeted host genes and their associated pathways, functional enrichment analysis was performed using Gitools,[39] GSEA,[40] and DAVID,[41] which revealed a myriad of important cellular pathways associated with nucleocytoplasmic transport, cell cycle process, cell communication, immune system regulation, and development of central nervous system (Figure 1A). Pathways like anterior-posterior pattern formation, regionalization, transcription regulation, calcium ion binding were also found to be enriched (Figure 1B).

Three target genes such as *PRKCH*, *SH3GL2*, and *SPRN* are involved in regulating neurological functions. To explore the interaction partners of these genes and the pathways regulated by their interactions, protein-protein interaction studies were utilized to identify their first neighbors. Twenty-five genes were found to interact with the abovementioned target genes using the network analysis tool STRING[42] (version: 10.5; Table 3). Enrichment analysis of these 25 genes together with the 3 target genes by Gitools[39] revealed pathways regulating crucial signaling pathways and neurological function (Figure 1C, 1D, 1E).

Significant pathways like endocytosis, actin cytoskeleton regulation, calcium reabsorption, axon guidance (Figure 1C) were also found to be enriched. Other important pathways included leukocyte transendothelial migration, NK cell-mediated cytotoxicity, phagocytosis, and autophagy (Figure 1D), which are the major player of the host immune system. Moreover, pathways like the ErbB signaling pathway, Ras signaling pathway, PI3k-Akt signaling pathway were also significantly enriched (Figure 1E).

TABLE 1 List of mature miRNA sequences predicted to be produced by ZIKV

Serial	Name of the miRNA	Mature miRNA Sequence
1	Zika_mir-1	CUUGAAGAGGCUGCCAGCCGGA
2	Zika_mir-2	GCCCAUCAGGAUGGUCUUGGCGAU
3	Zika_mir-3	AGGCUGCCAGCCGGACUUCUGC
4	Zika_mir-4	UCAGGAUGGUCUUGGCGAUACU
5	Zika_mir-5	UUAGCAGCAGCUGUCAUCGCUU
6	Zika_mir-6	UAUCUGGUCAUGAUACUGCUGAU
7	Zika_mir-7	AGCUGUCAUCGCUUGGCUUUUG
8	Zika_mir-8	AAAGUCAUAUAUCUGGUCAUGA
9	Zika_mir-9	UUCGCGGAAAUGAACACUGGAG
10	Zika_mir-10	UAGCUCAUUUGGCGCUGAUAGC
11	Zika_mir-11	AUUUUGAUGGGUGCCACCUUCG
12	Zika_mir-12	AUGUAGCUCAUUUGGCGCUGAUAG
13	Zika_mir-13	UGGAGGGUUCGCCAAGGCGGAU
14	Zika_mir-14	CCAAGGCGGAUAUAGAGAUGGC
15	Zika_mir-15	CUGUUGGCCUGAUAUGCGCAUU
16	Zika_mir-16	UCCACCCAUGAGAGAGAUCAU
17	Zika_mir-17	UGGUGAUUUCUCCCUAGUGGAG
18	Zika_mir-18	GAUGAUGGUCCACCCAUGAGAG
19	Zika_mir-19	CACCCAUGAGAGAGAUCAUACU
20	Zika_mir-20	UCAGAUCAUGCGGCCCUGAAGU
21	Zika_mir-21	AUUCAAAGAGUUUGCCGCUGGG
22	Zika_mir-22	CGGCCUUUGGAGUGAUAGAAGC
23	Zika_mir-23	AUGGGACUUUGGAGUCCCGCUG
24	Zika_mir-24	CGCUGCUAAUGAUGGGUUGCUA
25	Zika_mir-25	GUUGUUUGGUAUGGGCAAAGGG
26	Zika_mir-26	UUGGUAUGGGCAAAGGGAUGCCAU
27	Zika_mir-27	AAGUUACUUGGCUGGAGCUUCU
28	Zika_mir-28	CUUCUCUAAUCUACACAGUAAC
29	Zika_mir-29	CAGCCACUUCACUGUGUAACAU
30	Zika_mir-30	UGUAACAUUUUUAGGGGAAGUUAC
31	Zika_mir-31	CUGGUUAUGGAAGGAGCUAGGC
32	Zika_mir-32	AGUCUGUACCAAAGAAGAGUUC
33	Zika_mir-33	GAGCAUGGUCUCUUCCUGGUUA
34	Zika_mir-34	GUGAUGAAGAAAAGUACAUGGA
35	Zika_mir-35	UACAUGGACUACCUAUCCACCC
36	Zika_mir-36	GGGUGAAGAAGGGUCCA
37	Zika_mir-37	ACAUGGACUACCUAUCCACCCA
38	Zika_mir-38	UGGGUGAAGAAGGGUCCACACC
39	Zika_mir-39	CAAUCUGGGGCCUGAACUGGAG
40	Zika_mir-40	GUGGAUCUCCAGAAGAGGGACUAG
41	Zika_mir-41	GGAGAUCAGCUGUGGAUCUCCA
42	Zika_mir-42	CAGAAGAGGGACUAGUGGUUAG
43	Zika_mir-43	UUUCCACCACGCUGGCCGCCAG
44	Zika_mir-44	CCGGUGUGGGGAAAUCCAUGGG
45	Zika_mir-45	CUCCAUGAGUUUCCACCACGCU
46	Zika_mir-46	CCAUGAGUUUCCACCACGCUGGCC
47	Zika_mir-47	GGCCGGUGUGGGGAAAUCCAUGG

Abbreviations: miRNA, microRNA; ZIKV, Zika virus.

3.3 | Expression analysis showed significant deregulation of several target genes

To investigate the expression level of the target genes of ZIKV infected host cells, the RNA-seq transcriptomic expression data set (GSE78711) was analyzed and the fold change values were calculated comparing the mock and ZIKV treated cells. Out of 29 targets, 17 targets of identified ZIKV miRNAs were highly differentially

TABLE 2 List of host genes targeted by the ZIKV miRNAs

Serial	Ensembl Gene ID	Gene Name
1	ENSG00000014164	*ZC3H3*
2	ENSG00000024422	*EHD2*
3	ENSG00000027075	*PRKCH*
4	ENSG00000068400	*GRIPAP1*
5	ENSG00000071655	*MBD3*
6	ENSG00000086159	*AQP6*
7	ENSG00000100417	*PMM1*
8	ENSG00000107295	*SH3GL2*
9	ENSG00000110987	*BCL7A*
10	ENSG00000120093	*HOXB3*
11	ENSG00000124151	*NCOA3*
12	ENSG00000125834	*STK35*
13	ENSG00000129226	*CD68*
14	ENSG00000133985	*TTC9*
15	ENSG00000143641	*GALNT2*
16	ENSG00000156675	*RAB11FIP1*
17	ENSG00000159388	*BTG2*
18	ENSG00000163703	*CRELD1*
19	ENSG00000164591	*MYOZ3*
20	ENSG00000168646	*AXIN2*
21	ENSG00000171246	*NPTX1*
22	ENSG00000172379	*ARNT2*
23	ENSG00000173894	*CBX2*
24	ENSG00000176907	*C8orf4*
25	ENSG00000178562	*CD28*
26	ENSG00000178585	*CTNNBIP1*
27	ENSG00000196776	*CD47*
28	ENSG00000203772	*SPRN*
29	ENSG00000235568	*NFAM1*

Abbreviations: miRNA, microRNA; ZIKV, Zika virus.

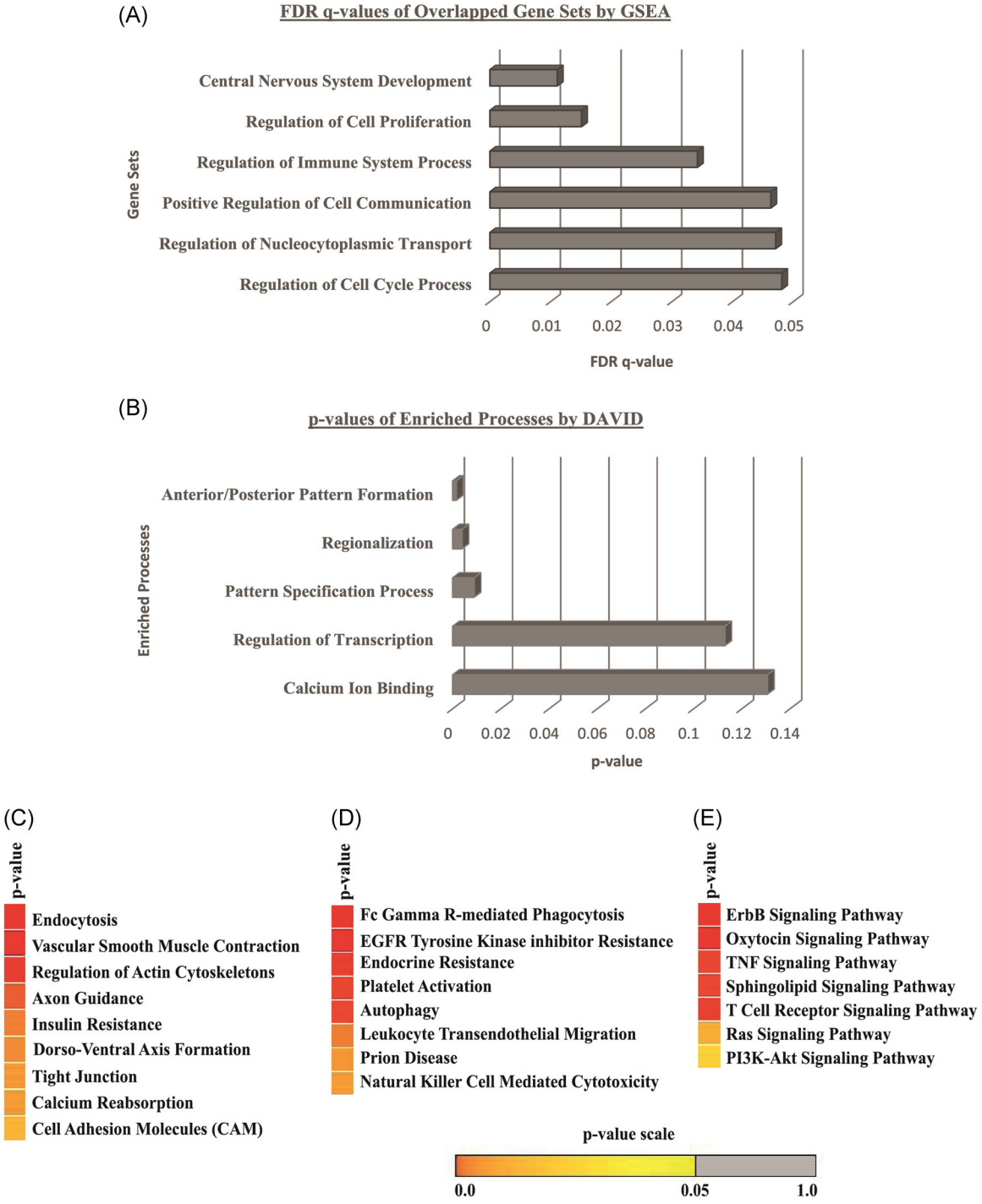

FIGURE 1 Enriched and overlapped gene sets of the biological processes and pathways provided by GSEA (A) and DAVID (B). The x-axes denote the FDR *q* values and *P* values, respectively, while the y-axes represent enriched terms and overlapped gene sets. Enriched heatmaps of the pathways associated with Development (C), Immune system regulation (D) and Signaling pathways (E) obtained by Gitools. The FDR *P* value cutoff value is set to 0.05, where the most significant pathways are colored as red. DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, False Discovery Rate; GSEA, Gene Set Enrichment Analysis

modulated compared with the control condition ($\chi^2 = 9.570$, *P* value = 0.002). Among them, 10 genes were significantly down-regulated ($\chi^2 = 4.50$, *P*-value = 0.034) (Supporting Data file 3), whereas the remaining 7 genes were significantly upregulated (Supporting Data file 4).

Expression level of the first neighbor genes of *PRKCH*, *SH3GL2*, and *SPRN* showed that 12 genes had significant changes in expression level compared with the control condition ($\chi^2 = 1.16$, *P*-value = 0.28) (Supporting Data file 3), where only 3 genes were significantly downregulated and the remaining 9 genes were significantly

TABLE 3 List of genes that are associated with neurological functions and their first neighbors

Serial	Ensembl ID	Associated Gene Name
1	ENSG00000078053	*AMPH*
2	ENSG00000110395	*CBL*
3	ENSG00000106976	*DNM1*
4	ENSG00000079805	*DNM2*
5	ENSG00000146648	*EGFR*
6	ENSG00000174775	*HRAS*
7	ENSG00000090339	*ICAM1*
8	ENSG00000100030	*MAPK1*
9	ENSG00000148824	*MTG1*
10	ENSG00000170248	*PDCD6IP*
11	ENSG00000163932	*PRKCD*
12	ENSG00000171132	*PRKCE*
13	ENSG00000027075	*PRKCH*
14	ENSG00000065675	*PRKCQ*
15	ENSG00000171864	*PRND*
16	ENSG00000171867	*PRNP*
17	ENSG00000101265	*RASSF2*
18	ENSG00000134318	*ROCK2*
19	ENSG00000007908	*SELE*
20	ENSG00000107295	*SH3GL2*
21	ENSG00000147010	*SH3KBP1*
22	ENSG00000203772	*SPRN*
23	ENSG00000197122	*SRC*
24	ENSG00000159082	*SYNJ1*
25	ENSG00000006638	*TBXA2R*
26	ENSG00000150991	*UBC*
27	ENSG00000162692	*VCAM1*
28	ENSG00000015171	*ZMYND11*

upregulated (Supporting Data file 4). Two different networks were prepared with their expression levels using the biomolecular interaction network generation software Cytoscape (Version: 3.5.1)[43] showing the interactions between miRNAs and their target genes (Figure 2).

In addition, the functions of the target genes with significant deregulation were identified using UniProt[45] protein database to better understand the mechanism of disease prognosis in human by ZIKV infection through miRNA production (Table 4).

4 | DISCUSSION

Over the past decade, ZIKV infection has become a global threat and has drawn much attention because of its association with microcephaly and congenital syndromes in newborns. Despite much ongoing research, the detailed mechanism illustrating the ZIKV pathogenesis causing ZIKV-related neuropathology is missing. In this study, identifying novel miRNAs encoded by ZIKV was the first aim. miRNAs have emerged as an important biomedical target in recent years because of their involvement in a number of biological phenomena. Studying virus-encoded miRNAs is therefore of remarkable importance to develop better therapeutic interventions.

It is already established through various experimental evidence that human miRNAs target viral genes[17] and function as antiviral mediators to suppress viral pathogenesis. By silencing the disease-causing genes of a virus, human miRNAs ensure the prevention of any deleterious events. In this context, one such experiment performed by Kozak et al[46] discovered through Next Generation Sequencing that the miRNAs in astrocytes are dysregulated followed by dysregulation of host mRNAs during the process of ZIKV infection. The experiment suggests that upon infection ZIKV induces global down-regulation of host miRNAs coupled with a few miRNAs being upregulated. This, in turn, dysregulate the host gene functions. Here, the primary focus was on the dysregulation of the host miRNAs after ZIKV infection. On the contrary, one of the most exciting aspects in virology comes from the virus-encoded miRNAs, although there is very little evidence available proving their role in effectively targeting and regulating host genes. Viruses usurp host cellular machinery to survive and establish a successful infection, which also activates the host immune system against the virus itself. As hosts have evolved to defend viruses, viruses have in turn evolved several strategies to circumvent host immune response, miRNA-mediated host gene silencing is one of such strategy. Silencing host genes can provide selective advantages to evade host defense system,[47] replicate within the host,[47] and avoid antiviral responses. To accentuate this event whether ZIKV can effectively target and control host genes we proceeded with several published works from different laboratories to gain insight into the role of ZIKV miRNAs in their pathogenesis.

Initially, 1600 putative pre-miRNAs were obtained using Vmir.[28] As the highest number of miRNAs produced by EBV[29] was reported 24, the results obtained from Vmir[28] were discarded to avoid a probable large number of false-positive cases. Some of the ZIKV miRNA target genes were found to be associated with pathways such as nucleocytoplasmic transport, cell cycle process, cell communication, immune system regulation and development of the central nervous system. Hijacking these processes could, therefore, facilitate viral transportation, immune evasion to establish successful pathogenesis. Additional miRNA-targeted genes were involved in anterior-posterior pattern formation, regionalization, transcription regulation, calcium ion binding, and alteration of which might affect the congenital brain development in the fetus upon ZIKV infection.

Tang et al[24] reported that ZIKV infects hNPCs with higher efficiency compared to neurons and embryonic stem cells, which results in abnormal cellular dynamics and apoptosis. In a recent study, Rolfe et al[25] analyzed the data set GSE78711 and predicted that ZIKV infection is associated with neuroinflammatory pathways. Correlation between ZIKV infection with alteration of other important signaling pathways involved in intracellular signaling, immune system, and neurological functions is shown in this study.

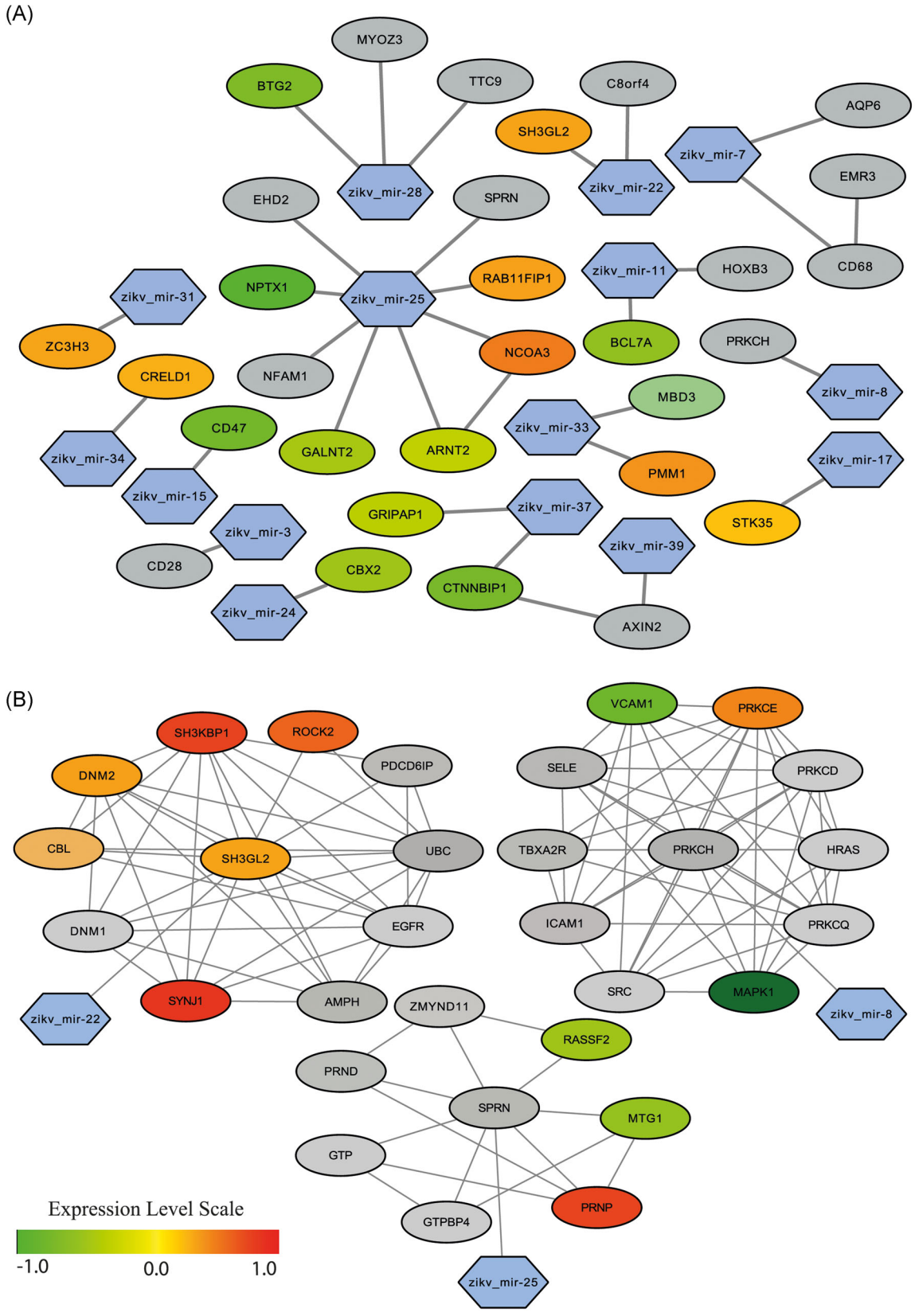

FIGURE 2 The network of interactions between ZIKV miRNAs and target genes (A) and between the first neighbor genes associated with neurological functions (B). The elliptical shapes represent the genes and the hexagonal shapes represent miRNAs. The expression levels of the genes are represented on a color-coded scale, where color towards green indicates downregulation, color towards red indicates upregulation and yellow indicates very little change in expression level. miRNAs are colored as blue and genes without any significant expression changes are colored as gray. miRNA, microRNA; ZIKV, Zika virus

TABLE 4 Functions of genes having significant fold changes of expression after ZIKV infection

Gene Name	Protein Name	Protein Function
ARNT2	Aryl hydrocarbon receptor nuclear translocator 2	Transcription factor that plays a role in the development of the hypothalamo-pituitary axis, postnatal brain growth, and visual and renal function.
BCL7A	B-cell CLL/lymphoma 7 protein family member A	Negative regulation of transcription
BTG2	Protein BTG2	Antiproliferative protein. Involved in cell cycle regulation. Could be involved in the growth arrest and differentiation of the neuronal precursors.
CBX2	Chromobox protein homolog 2	Involved in sexual development, acting as an activator of the NR5A1 expression
CD47	Leukocyte surface antigen CD47	Has a role in both cell adhesion and in the modulation of integrins. Plays an important role in memory formation and synaptic plasticity in the hippocampus. Prevents maturation of immature dendritic cells and inhibits cytokine production by mature dendritic cells.
CRELD1	Cysteine-rich with EGF-like domain protein 1	Calcium ion binding, cardiac septum development, endocardial cushion development
CTNNBIP1	Catenin beta-1	Key downstream component of the canonical Wnt signaling pathway. Involved in the regulation of cell adhesion. Blocks anoikis of malignant kidney and intestinal epithelial cells and promotes their anchorage-independent growth by downregulating DAPK2.
GALNT2	Polypeptide N-acetylgalactosaminyltransferase 2	Catalyzes the initial reaction in O-linked oligosaccharide biosynthesis, the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor.
GRIPAP1	GRIP1-associated protein 1	Guanine nucleotide exchange factor for the Ras family of G proteins (RasGEF). Interacts in a complex with Glutamate receptor interacting protein 1 (GRIP1) and plays a role in the regulation of AMPA receptor function
MBD3	Methyl-CpG-binding domain protein 3	Acts as a transcriptional repressor and plays a role in gene silencing. Recruits histone deacetylases and DNA methyltransferases
NCOA3	Nuclear receptor coactivator 3	Nuclear receptor coactivator that directly binds nuclear receptors and stimulates the transcriptional activities in the remodeling of chromatin. Displays histone acetyltransferase activity. Also involved in the coactivation of the NF-kappa-B pathway via its interaction with the NFKB1 subunit
NPTX1	Neuronal pentraxin-1	May be involved in mediating uptake of synaptic material during synapse remodeling
PMM1	Phosphomannomutase 1	Involved in the synthesis of the GDP-mannose. In addition, may be responsible for the degradation of glucose-1,6-bisphosphate in ischemic brain
RAB11FIP1	Rab11 family-interacting protein 1	Involved in the endosomal recycling process. Also involved in controlling membrane trafficking along the phagocytic pathway and in phagocytosis
SH3GL2	Endophilin-A1	Implicated in synaptic vesicle endocytosis.
STK35	Serine/threonine-protein kinase 35	Phosphorylates specific proteins
ZC3H3	Zinc finger CCCH domain-containing protein 3	Required for the export of polyadenylated mRNAs from the nucleus. Enhances ACVR1B-induced SMAD-dependent transcription. Involved in RNA cleavage

Pathways, such as ErbB signaling pathway, Ras signaling pathway, PI3k-Akt signaling pathway, are involved in controlling cell cycle,[48] intracellular signaling,[49] and apoptosis.[48] The ErbB signaling pathway is known to associate with the development of neurodegenerative diseases.[50] ZIKV miRNAs may downregulate the genes associated with these pathways to alter cell cycle and cellular metabolism, ensuring pathogenesis. Besides, targeted genes involved in pathways including endocytosis, actin cytoskeleton regulation, calcium reabsorption, and axon guidance may facilitate internalization of the virus inside the cell and also alter the structure of the neurons, which may result in the abnormal structure of the brain during development. Moreover, ZIKV miRNAs target genes associated with leukocyte transendothelial migration, NK cell-mediated cytotoxicity, phagocytosis, and autophagy, which typically provide an immune response against virus-infected cells to prevent pathogenesis. Downregulation of these genes by the ZIKV miRNAs might ensure prolonged refuge for the virus inside the host cells and thus increase their pathogenicity.

By analyzing the RNA-seq transcriptomic expression data set (GSE78711), fold change values of the ZIKV miRNAs targeted genes were obtained, among them 9 genes were significantly downregulated and 8 genes were significantly upregulated. Moreover, among the 28 genes associated with neurological functions, 3 genes were significantly downregulated, and 9 genes were significantly upregulated. In conclusion, we propose that the downregulated genes are directly targeted by the ZIKV-encoded miRNAs, whereas,

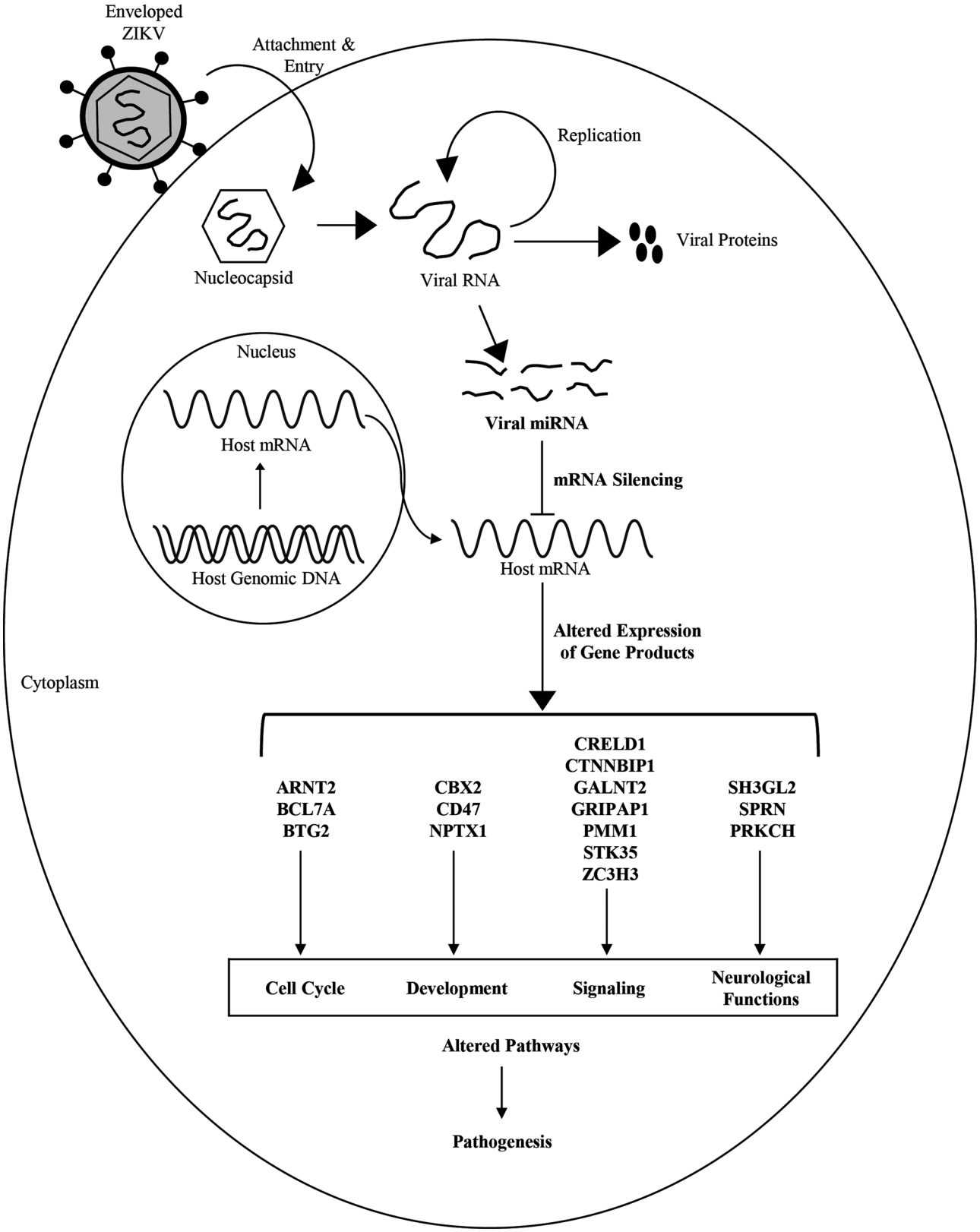

FIGURE 3 Schematic diagram illustrating the mechanism of ZIKV miRNAs driven pathogenesis. After entering into the host cell ZIKV releases its genomic RNA, which is translated into proteins necessary for further RNA synthesis. Some of the RNAs are converted to miRNAs that target specific host mRNAs, thereby alter the expression level of the genes as well as the pathways associated with them. This results in viral pathogenesis. miRNA, microRNA; ZIKV, Zika virus

the upregulated genes may be associated with pathways that are altered by the miRNA target genes.

Further functional analysis revealed that *ARNT2*, *BCL7A*, *BTG2*, *NPTX1* genes are involved with cell cycle progression and development. Moreover, *CRELD1*, *CTNNBIP1*, *GRIPAP1* are associated with intracellular signaling, which could explain the abnormal neuronal development and decreased brain volume causing microcephaly or other congenital diseases in ZIKV infected patients.

Based on these findings, we proposed a mechanism of ZIKV pathogenesis through miRNA-mediated gene silencing (Figure 3). After the attachment and entry into the cytoplasm of the host cell, ZIKV releases its genomic RNA, which then utilizes the host machinery for replication and translation. Probably the produced miRNAs target specific host mRNAs and mediate silencing, which might lead to the alteration of the associated pathways in the host cells, accelerating pathogenesis.

5 | CONCLUSIONS

In this study, we propose a mechanism, which portrays that ZIKV pathogenesis might be mediated through its miRNAs that target and downregulate essential genes involved in immune surveillance and modulation, cellular signaling, and neurological functions. We predicted several novel miRNAs produced by ZIKV, and as expected, the genes targeted by these miRNAs are associated with cellular signaling and neurological functions. Our finding could explain the association of microcephaly or several other neurological defects in ZIKV infected patients. However, further experimental analysis of these miRNAs would enhance our understanding to better combat this virus in the future through novel therapeutic interventions.

ACKNOWLEDGMENTS

We acknowledge research support from Biotechnology Research Centre (BRC), University of Dhaka and University Grants Commission (UGC) Bangladesh and The World Academy of Sciences (TWAS).

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

ABMMKI conceived the project. MSI and MK collected the data. MSI and ABMMKI performed the analyses. MSI, MK, MWM, MAAKK, and ABMMKI wrote the manuscript. The manuscript has been read and approved by all authors.

DATA AVAILABILITY

All the RNA-Seq data used in this study are available in GEO data set.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Islam MS, Khan MA-A-K, Murad MW, Karim M, Islam ABMMK. In silico analysis revealed Zika virus miRNAs associated with viral pathogenesis through alteration of host genes involved in immune response and neurological functions. *J Med Virol*. 2019;91:1584-1594.
https://doi.org/10.1002/jmv.25505