Computational Biology and Chemistry 92 (2021) 107462

Contents lists available at ScienceDirect

Computational Biology and Chemistry

journal homepage: www.elsevier.com/locate/cbac

Computational analysis revealed miRNAs produced by Chikungunya virus target genes associated with antiviral immune responses and cell cycle regulation

Md. Sajedul Islam [a,1,*], Md. Abdullah-Al-Kamran Khan [b,1]

[a] Department of Biochemistry & Biotechnology, University of Barishal, Barishal, 8254, Bangladesh
[b] Department of Mathematics and Natural Sciences, BRAC University, Dhaka, Bangladesh

ARTICLE INFO

Keywords:
CHIKV
miRNA
Virus-host interaction
Immune escape
Cell cycle
Viral pathogenesis

ABSTRACT

Chikungunya virus (CHIKV) that causes chikungunya fever, is an alphavirus that belongs to the *Togaviridae* family containing a single-stranded RNA genome. Mosquitoes of the *Aedes* species act as the vectors for this virus and can be found in the blood, which can be passed from an infected person to a mosquito through mosquito bites. CHIKV has drawn much attention recently because of its potential of causing an epidemic. As the detailed mechanism of its pathogenesis inside the host system is still lacking, in this *in silico* research we have hypothesized that CHIKV might create miRNAs, which would target the genes associated with host cellular regulatory pathways, thereby providing the virus with prolonged refuge. Using bioinformatics approaches we found several putative miRNAs produced by CHIKV. Then we predicted the genes of the host targeted by these miRNAs. Functional enrichment analysis of these targeted genes shows the involvement of several biological pathways regulating antiviral immune stimulation, cellular proliferation, and cell cycle, thereby provide themselves with prolonged refuge and facilitate their pathogenesis, which in turn may lead to disease conditions. Finally, we analyzed a publicly available microarray dataset (GSE49985) to determine the altered expression levels of the targeted genes and found genes associated with pathways such as cell differentiation, phagocytosis, T-cell activation, response to cytokine, autophagy, Toll-like receptor signaling, RIG-I like receptor signaling and apoptosis. Our finding presents novel miRNAs and their targeted genes, which upon experimental validation could facilitate in developing new therapeutics to combat CHIKV infection and minimize CHIKV mediated diseases.

1. Introduction

Chikungunya virus (CHIKV) is a single-stranded, positive-sense RNA virus (Rougeron et al., 2015) that causes a tropical disease called chikungunya fever which, in 1952, occurred first in Tanzania (Ross, 1956). It contains an icosahedral capsid, which is covered by a lipid layer (Thiberville et al., 2013). CHIKV is an alphavirus that belongs to the *Togaviridae* family (Thiberville et al., 2013) and mosquitoes of the *Aedes* species act as the vectors for this virus (Ross, 1956). CHIKV can be found in the blood and can pass from an infected person to a mosquito through mosquito bites during the first week of infection. Specific cell types that are particularly susceptible to infection include human epithelial and endothelial cells, monocyte-derived macrophages, and primary fibroblasts (Matusali et al., 2019). Following CHIKV infection, RNA and proteins of CHIKV have been found in synovial tissue and fluids, with synovial fibroblasts and macrophages susceptible to the infection (Couderc et al., 2008; Hoarau et al., 2010; Zhang et al., 2018). Infected macrophages are the preferred site for viral replication of CHIKV, contributing to viral persistence and chronic symptoms (Hoarau et al., 2010). Despite several kinds of research on this virus, the pathogenesis of persistent manifestations after CHIKV infection is still unclear. Proteins of chikungunya virus have been detected in macrophages and muscle cells of patients with relapse of chronic pain, suggesting that low replicative viruses or non-replicative CHIKV debris may persist. *in vitro* infection of human cells has exhibited the susceptibility of microglial cells, neuroblastoma cells, and glial cells, such as astrocytes (Abere

* Corresponding author.
E-mail address: sajedtuhin2203@gmail.com (Md.S. Islam).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.compbiolchem.2021.107462
Received 11 October 2020; Received in revised form 11 February 2021; Accepted 17 February 2021
Available online 19 February 2021

et al., 2012; Dhanwani et al., 2012; Abraham et al., 2013). Yet, it is still unclear if the pathogenesis of the nervous system is directly connected with the infection of the neurons and glial cells or is circuitously connected triggering the immune-mediated effects.

MicroRNAs (miRNAs) are novel ideal models in the field of the molecular regulation of gene expressions. It is turning into a magnificent research topic day by day for different researchers engaged with molecular biology. miRNAs are ~22 nucleotide, brief, non-coding RNAs that are available in the vertebrates, invertebrates, plants, and in a wide range of viruses (Lim et al., 2003; Ding and Voinnet, 2007). The essential capacity of miRNAs is to regulate the expression of genes post-transcriptionally, through the base-pair formation with the 3′-untranslated region (3′-UTR) of distinct messenger RNAs (mRNA). miRNAs assume indispensable jobs in different biological processes, including the development of an organism, regulation of the immune system, cell proliferation, oncogenesis, customized cell passing or apoptosis, and so on (Wienholds et al., 2003; Manni et al., 2009; Lu and Liston, 2009; Wang and Lee, 2009; Cho, 2007). Previously, human miRNAs were accounted for quelling viral pathogenesis by targeting their genes (Hariharan et al., 2005). Additional examinations uncovered the possibility of viral miRNAs targeting their host genes (Ghosh et al., 2009; Grundhoff and Sullivan, 2011), assuming unobtrusive jobs in the endurance and proliferation of viral particles through host immune system evasion, building up microenvironment for viral replication, regulation of the innate immune system, differentiation of versatile immune cells (Stern-Ginossar et al., 2007; Skalsky and Cullen, 2010; Kincaid and Sullivan, 2012; Islam et al., 2019). Mishra et al. illuminated the potential roles of viral miRNAs in different DNA and RNA virus infections and pathogenesis through altering the host immune response (Mishra et al., 2020). Previously, miRNAs produced by RNA viruses such as HIV-1, H5N1 influenza virus, West Nile virus, and Ebola virus were reported (Li and Zou, 2019). More importantly, a mosquito-borne Dengue virus has also been reported to encode miRNA like small viral RNAs (Hussain and Asgari, 2014). In this context, to explore the mechanism of action of CHIKV mediated pathogenesis, we hypothesize that CHIKV-encoded miRNAs modulate host immune system and various physiological functions that provide the viruses selective advantages for prolonged refuge and disease pathogenesis within the host.

2. Materials and methods

2.1. Prediction of pre-miRNAs & mature miRNAs

From the National Center for Biotechnology Information (NCBI) (Pruitt and Maglott, 2001) we obtained the complete genome sequence of CHIKV (NCBI Reference Sequence: NC_004162.2). To predict the presence and positions of the pre-miRNAs in the genome sequence we used the miRNAFold (Tempel and Tahi (2012); Tav et al. (2016)) tool with default parameters. Stem-loop secondary structure is one crucial feature to distinguish between pri-miRNA & pre-miRNAs. We used the Triplet SVM Classifier (Xue et al., 2005) tool to find the true pre-miRNAs among a set of conserved stem-loops. The minimum bases for the stem-loop were set to 22 for the Triplet SVM Classifier (Xue et al., 2005) tool. In addition to this, using a fixed-order Markov model-based algorithm, FOMmir (Shen et al., 2012) and a SVM-based tool, iMiRNA-SSF (Chen et al., 2016), we identified the true precursor miRNAs from the initially predicted pre-miRNAs. We utilized the default parameters to predict these sequences. Furthermore, we checked the stable secondary structures within these pre-miRNAs using miRNAfold (Tav et al., 2016) tool. Then we took only those pre-miRNAs which satisfy all these filtering to reduce the false negative results. Finally, utilizing a Naive Bayes classifier, matureBayes (Gkirtzou et al., 2010), we predicted the mature miRNAs from the pre-miRNA sequences.

2.2. Prediction of miRNA target genes

Firstly, we obtained the 3'-UTR sequences of the human protein coding genes from Ensembl biomart (Kinsella et al., 2011). We utilized three different tools, namely RNAhybrid (Kruger and Rehmsmeier, 2006) (with default parameters and targets were filtered using a cutoff of MFE $\leq$ -27 kcal/mol and p-value $<$ 0.05), IntaRNA 2.0 (Mann et al., 2017) (with parameters –mode=H –model = X, –outMode = C, $\Delta\Delta G \leq -10$ kcal/mol, with seed 2–8 allowing G:U base pairs), and psRNA-Target (Dai et al., 2018) (with Schema V2 parameters) to obtain the genes targeted by the predicted miRNAs. We then took only those targets which were predicted by at least two of the three prediction tools in order to get the high confidence targets only.

2.3. Functional enrichment analysis

We employed the standalone functional enrichment analysis tool Gitools (Perez-Llamas and Lopez-Bigas, 2011) v1.8.4 using Gene Ontology Biological Process (GOBP) (Gene, 2015) module and Kyoto encyclopedia of genes and genomes (KEGG) (Kanehisa et al., 2016) to obtain the enriched biological processes and pathways involving the miRNA target genes. We adjusted the resulting p-values for multiple testing using the Benjamin and Hochberg's method of False Discovery Rate (FDR) (Benjamini and Hochberg, 1995). A cut-off of adjusted p-value $<$ 0.05 was utilized.

2.4. Expression profile analysis

Gene Expression Omnibus (GEO) (Barrett et al., 2013) is a public reservoir of microarray datasets. To obtain the expression level of each of the target genes the microarray dataset GSE49985 of CHIKV infected HEK293 T cells using the platform GPL15207 Affymetrix Human Gene Expression Array was used (Saxena et al., 2013). We utilized Limma (Ritchie et al., 2015) R package to find out the significant differentially expressed genes from this experiment. We performed the analysis assigning the replicates of uninfected cells (GSM1211129 and GSM1211130) as controls; and did the analysis twice, one for the replicates of 12 -h infected samples (GSM1211131 and GSM1211132) and another one for the 24 -h infected samples (GSM1211133 and GSM1211134). Processing the data showed the Log2 fold changes in the expression level of the total human genes and the expression levels of target genes were analyzed together with their significance levels (Adjusted p-value $<$ 0.05 using Benjamini & Hochberg's FDR (Benjamini and Hochberg, 1995). We called those genes to be significantly differentially expressed if a gene had Log2FC value of $\pm$0.5 with an adjusted p-value $<$ 0.05, while those with negative logFC values are termed as "Downregulated" and the genes with positive LogFC values are termed as "Upregulated".

We then performed another round of functional enrichment analysis for the downregulated target genes of the predicted miRNAs using Gitools (Perez-Llamas and Lopez-Bigas, 2011) software utilizing GOBP and KEGG pathway module. A schematic diagram depicting the complete methodological strategy of this study is provided in Fig. 1.

3. Results

3.1. Putative miRNAs produced by CHIKV

To identify whether CHIKV produces any miRNAs, we used the tool miRNAFold that yielded 200 putative pre-miRNA candidates (data not shown). Triplet SVM Classifier predicted all of them to be truly positive. Using FOMmir, iMiRNA-SSF and RNAFold, we found a set of 7 true pre-miRNAs which can yield functional mature miRNAs. Finally, matureBayes tools predicted 14 putative mature miRNA sequences from the CHIKV genome (Supplementary File 1).

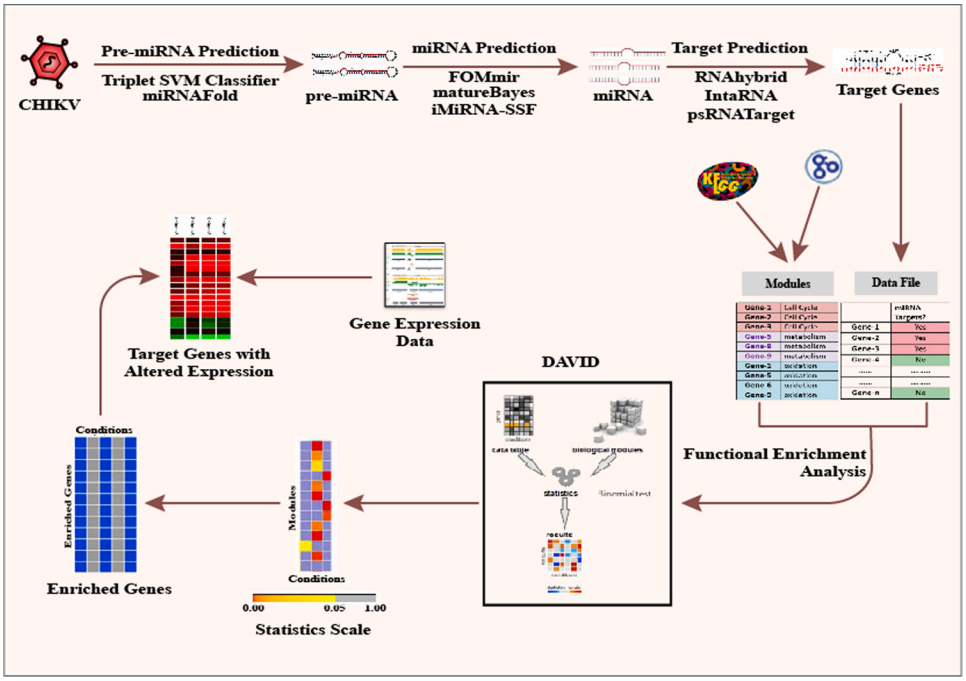

Fig. 1. Schematic diagram summarizing the study.

3.2. Genes targeted by putative CHIKV miRNAs

After obtaining the putative miRNAs, we utilized three different target prediction tools, RNAhybrid tool provided a total of 239 genes, while IntaRNA and psRNATarget tools predicted 18,193 and 780 targets, respectively. Among all these predicted targets, we took only 979 targets which were predicted by at least two of the target prediction algorithms. We considered these 979 genes for further analyses. A table containing the putative target genes of the predicted CHIKV miRNAs is provided in the Supplementary File 2.

3.3. Functional enrichment analysis

To understand the functions of the genes targeted by the putative miRNAs produced by CHIKV we used the exhaustive functional enrichment analysis tool "Gitools" that provided us with the functionally enriched biological processes and pathways. Functional enrichment analysis revealed a myriad of important biological processes and pathways associated with the regulation of immune signaling, cell cycle arrest, and cell proliferation (Fig. 2A, 2B). Pathways such as autophagy, T cell receptor signaling, B cell receptor signaling, phagocytosis, interferon-gamma-mediated signaling, Natural killer cell mediated signaling, RIG-I-like receptor signaling, Toll-like receptor signaling were observed enriched for the target genes (Fig. 2A, 2B). Dampening these host antiviral immune signaling pathways might provide the virus a competitive edge in the virus-host tug-of-war.

3.4. Expression profile analysis

To investigate the differential gene expression level of the targeted genes we used the microarray dataset GSE49985 of CHIKV infected HEK293 T cells. Fold change values of gene expression were calculated comparing the uninfected control replicates to the CHIKV infected replicates of HEK293 T cells. For the 12 -h infected samples, 8188 genes were found deregulated (3559 genes were downregulated and 4629 genes were upregulated); and for the 24 -h infected samples. 7152 genes were found deregulated (4083 genes were downregulated and 3069 genes were upregulated) (Supplementary file 3). While comparing the target genes with these downregulated genes obtained from analyzing this dataset, we observed that 104 of the initially predicted targets were deregulated in both 12 -h and 24 -h CHIKV infection models (Supplementary file 4).

We then sought to elucidate the functional implications of these downregulated target genes of the predicted CHIKV miRNAs. We then performed another round of functional enrichment analysis using these 104 downregulated target genes. From the enrichment analysis, we detected that the downregulated targets are involved in important biological processes and pathways such as cellular growth and differentiation, phagocytosis, viral entry into host, T cell activation, response to cytokine, apoptosis, PI3K-Akt signaling (Fig. 2C, 2D). Furthermore, we constructed a gene-pathway network using Cytoscape (Kohl et al., 2011) software using the enriched pathways and associated target genes (Fig. 3, Supplementary Fig. 1) to illustrate the important pathways which the CHIKV might target using its encoded miRNAs.

4. Discussion

CHIKV has become a global health concern for its potential of causing epidemic and thus has drawn much attention recently. Though a lot of research has been done or currently ongoing, the detailed mechanism of its pathogenesis inside the host system is still wanting. In this *in silico* research we have mainly focused on the possibility that CHIKV might create miRNAs, which would target the genes associated with host cellular regulatory pathways, thereby providing the virus with prolonged refuge.

It is known already that miRNAs produced by humans target viral genes so that they can prevent potential viral pathogenesis (Hariharan et al., 2005). A previous study on the Zika virus (ZIKV) was also performed by us based on similar strategy (Islam et al., 2019). By switching the disease-causing genes of a virus off the host system ensures its disease suppression. Pathogenic viruses cause several diseases in human and human defense machinery to continuously encounter and remove these pathogenic viruses from the system. To evade these host defense molecules viruses might have further evolved to produce miRNAs to silence host genes. This silencing can provide them with various selective advantages including host defense evasion, viral replication, and

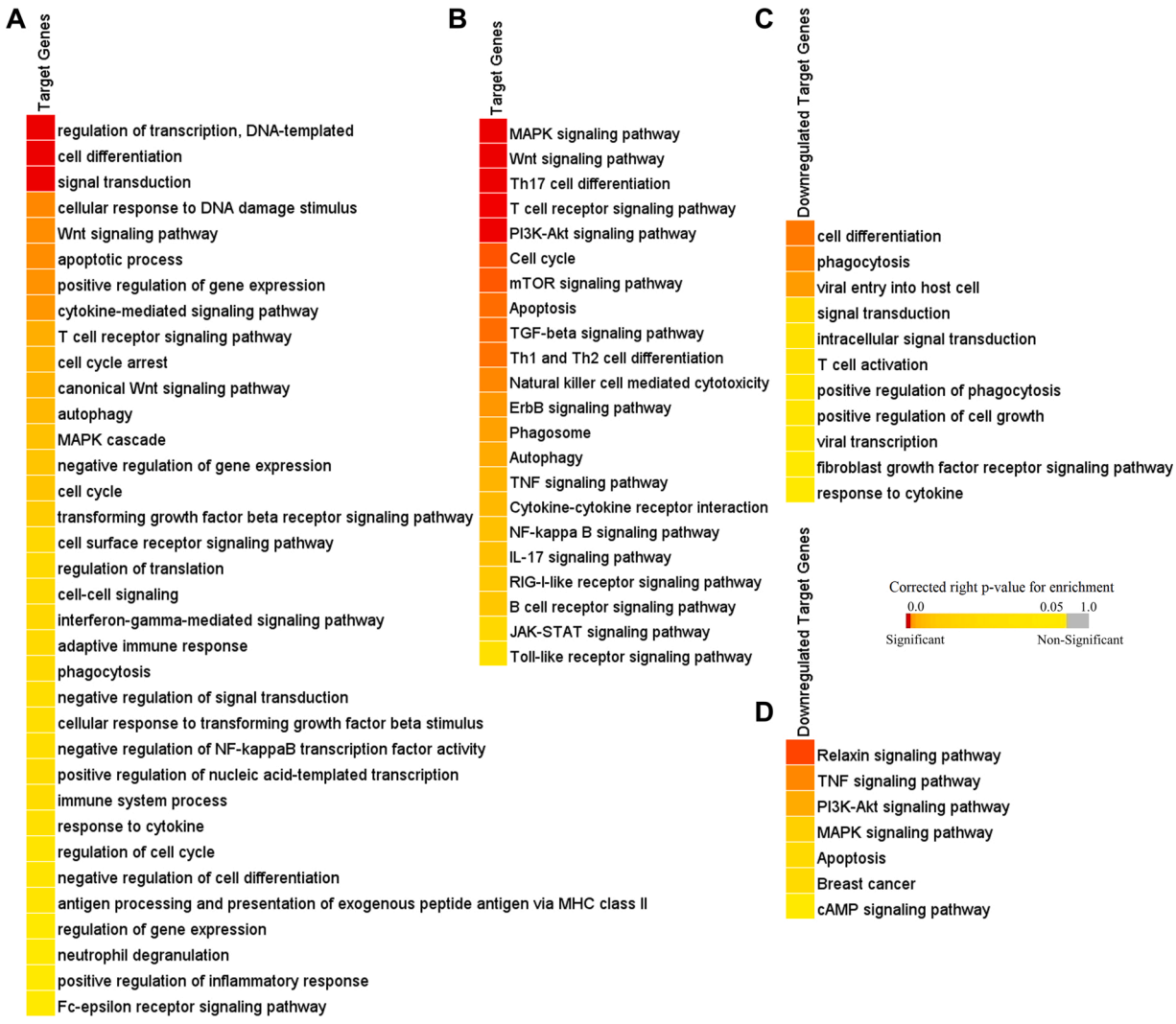

Fig. 2. Functional enrichment analysis of the target genes of predicted CHIKV miRNAs. Color-coded heatmaps showing the enriched pathways of the target genes using **A.** GOBP and **B.** KEGG pathway modules. Color-coded heatmaps of the enriched pathways of the downregulated target genes using **C.** GOBP and **D.** KEGG pathway modules. FDR p-values are represented as a color-coded scale, color towards red meaning more significant while color towards grey means non-significant.

diminishing antiviral responses (Nukui et al., 2014). To accentuate this event whether CHIKV effectively targets and controls host genes we proceeded with several scientific works from different laboratories and gained insight into the role of CHIKV miRNAs in their pathogenesis.

From the enrichment analysis, it was evident that the miRNAs might be associated with preventing cellular proliferation. From this observation it can be inferred that miRNAs of CHIKV target those genes that are associated with regulating the cellular proliferation and cell cycle, thus ensuring their prolonged refuge while inside the host system. Additionally, some target genes function in the viral transcription and translation. Viral miRNAs might target these genes to facilitate their own replication, transcription, and/or translation. These findings substantiate our hypothesis that CHIKV miRNAs may target the host genes associated with cell cycle regulation.

Host pathways such as autophagy, apoptosis, interferon signaling, and cytokine signaling play immense antiviral roles in different viral infections (Ahmad et al., 2018; Barber, 2001; Mogensen and Paludan, 2001), all of these were observed targeted by the putative CHIKV encoded miRNAs which could facilitate the immune escape of CHIKV. Host RIG-I signaling (Chan and Gack, 2015) and Toll-like receptor signaling (Lester and Li, 2014) can work to eliminate viral threats, so CHIKV might use its miRNAs in regulating this pathway to evade these responses. Moreover, MAPK signaling cascades (Mohanta et al., 2020), PI3K-Akt signaling (Wen-Tsai and Hung, 2008), and Natural killer cell mediated cytotoxicity (Brandstadter and Yang, 2011) pathways have roles in extinguishing the surge of viral propagation and pathogenesis, thus, CHIKV miRNAs might help the virus to hijack these pathways for the successful completion of its life cycle. Based on these findings we propose a mechanism of CHIKV pathogenesis through miRNA-mediated gene silencing (Fig. 4).

Though this study suggested promising results in decoding the CHIKV pathogenesis from the context of small viral non-coding RNAs, taking cue from our results, a more targeted experimental design using patient-derived samples will be useful in elucidating more functional insights.

5. Conclusion

In this study, we propose a mechanism, which portrays that CHIKV may progress its pathogenesis through producing miRNAs that target and downregulate essential genes involved in regulating cellular proliferation and cell cycle. We predicted several novel miRNAs, which may be produced by CHIKV, and interestingly, the genes targeted by these miRNAs are associated with immune regulation, regulation of cell cycle as well as cellular proliferation. This study will serve as an important pathfinder for the researchers in identifying the pathogenic pathways

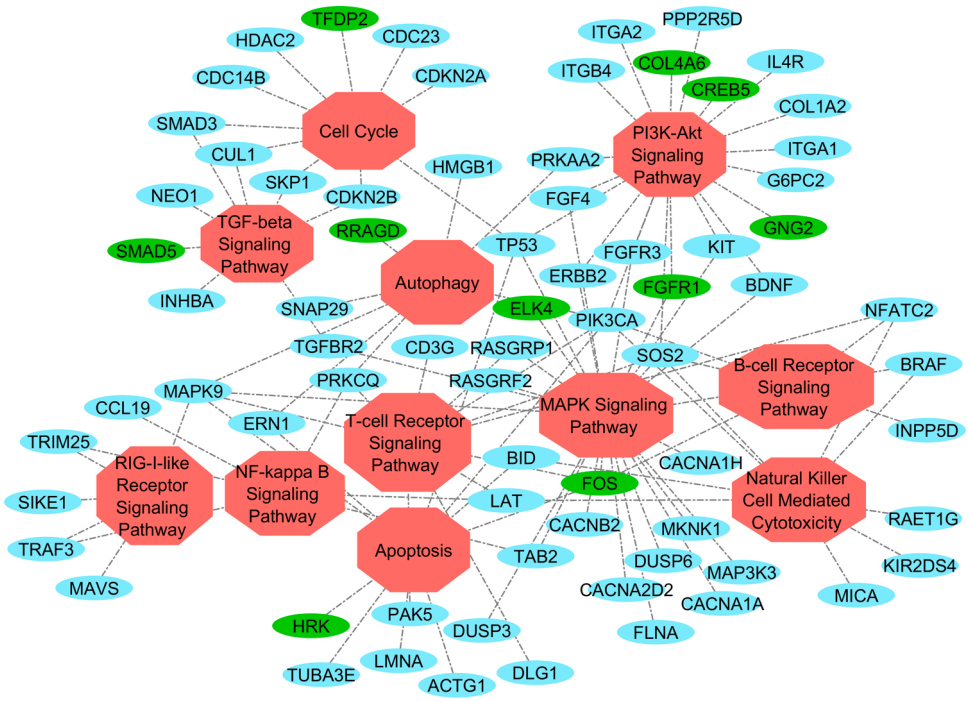

Fig. 3. Gene-pathway network showing the functionally enriched pathways and associated target genes of the predicted CHIKV miRNAs. Oval-shaped nodes indicate the target genes while the octagon-shaped nodes denote the enriched pathways. Green colored nodes indicate the target genes which were observed downregulated in the CHIKV infection model.

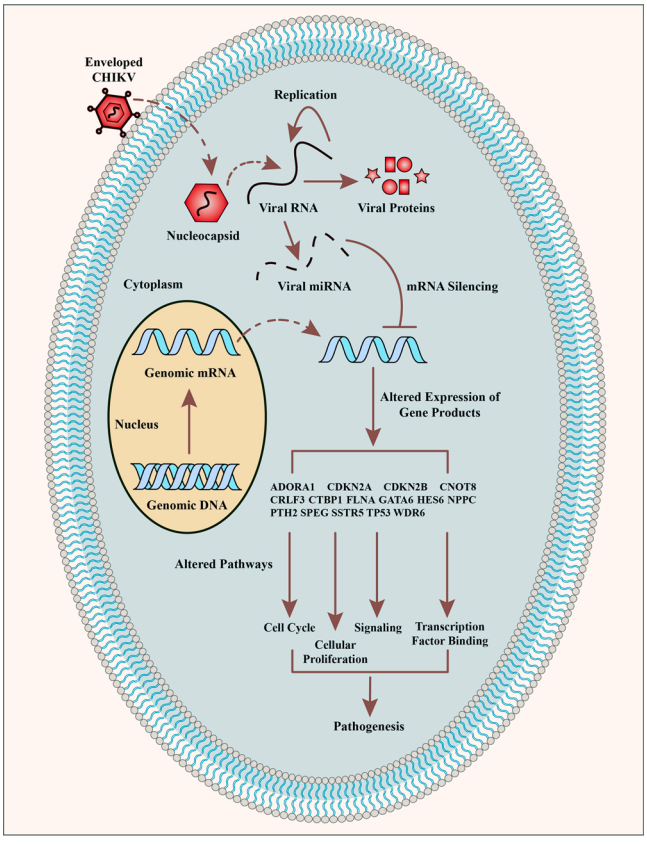

Fig. 4. Schematic diagram illustrating the mechanism of CHIKV pathogenesis. After entering into the host cell CHIKV releases its genomic RNA, which is translated into necessary proteins. Some of the RNAs are converted to miRNAs that target specific host mRNAs, thereby alter the expression level of the genes as well as the pathways associated with them. This results in viral pathogenesis.

that this virus may employ. Further experimental analysis of these miRNAs would enhance our understanding to better combat this virus in the future through novel therapeutic interventions.

Data availability

All data generated or analyzed during this study are available in online public repositories, within the article, and in the supplementary files.

Author contributions

MSI conceived the project. MSI and MAAKK designed the workflow. MSI and MAAKK performed the analyses. MSI and MAAKK wrote the manuscript. All authors reviewed the manuscript. MSI and MAAKK contributed equally to this work.

Funding

This study was supported by the research grant provided by the University of Barishal.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

The authors acknowledge the Department of Biochemistry & Biotechnology, University of Barishal for providing the research work supports. They also appreciate the contribution of **Shoaib Saikat**, a student of the Department of Biochemistry & Biotechnology, University of Barishal, who helped with providing background information for this research. The authors would also like to acknowledge **Dr. Abul BMMK Islam**, Professor, Dept. of Genetic Engineering and Biotechnology, University of Dhaka for sharing his computational resources for the

study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.compbiolchem.2021.107462.

References

Abere, B., et al., 2012. Proteomic analysis of chikungunya virus infected microglial cells. PLoS One 7. https://doi.org/10.1371/journal.pone.0034800 e34800.

Abraham, R., Mudaliar, P., Padmanabhan, A., Sreekumar, E., 2013. Induction of cytopathogenicity in human glioblastoma cells by chikungunya virus. PLoS One 8. https://doi.org/10.1371/journal.pone.0075854 e75854.

Ahmad, L., Mostowy, S., Sancho-Shimizu, V., 2018. Autophagy-virus interplay: from cell biology to human disease. Front. Cell Dev. Biol. 6 https://doi.org/10.3389/fcell.2018.00155, 155-155.

Barber, G.N., 2001. Host defense, viruses and apoptosis. Cell Death Differ. 8, 113–126. https://doi.org/10.1038/sj.cdd.4400823.

Barrett, T., et al., 2013. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res. 41, D991–995. https://doi.org/10.1093/nar/gks1193.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc.: Ser. B (Methodol.) 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Brandstadter, J.D., Yang, Y., 2011. Natural killer cell responses to viral infection. J. Innate Immun. 3, 274–279. https://doi.org/10.1159/000324176.

Chan, Y.K., Gack, M.U., 2015. RIG-I-like receptor regulation in virus infection and immunity. Curr. Opin. Virol. 12, 7–14. https://doi.org/10.1016/j.coviro.2015.01.004.

Chen, J., Wang, X., Liu, B., 2016. iMiRNA-SSF: improving the Identification of MicroRNA precursors by combining negative sets with different distributions. Sci. Rep. 6, 19062. https://doi.org/10.1038/srep19062.

Cho, W.C.S., 2007. OncomiRs: the discovery and progress of microRNAs in cancers. Mol. Cancer 6. https://doi.org/10.1186/1476-4598-6-60, 60-60.

Couderc, T., et al., 2008. A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathog. 4, e29. https://doi.org/10.1371/journal.ppat.0040029.

Dai, X., Zhuang, Z., Zhao, P., 2018. X. psRNATarget: a plant small RNA target analysis server (2017 release). Nucleic Acids Res. 46, W49–W54. https://doi.org/10.1093/nar/gky316.

Dhanwani, R., et al., 2012. Characterization of Chikungunya virus infection in human neuroblastoma SH-SY5Y cells: role of apoptosis in neuronal cell death. Virus Res. 163, 563–572. https://doi.org/10.1016/j.virusres.2011.12.009.

Ding, S.-W., Voinnet, O., 2007. Antiviral immunity directed by small RNAs. Cell 130, 413–426. https://doi.org/10.1016/j.cell.2007.07.039.

Gene Ontology Consortium: going forward. Nucleic Acids Res. 43, 2015, D1049–1056. https://doi.org/10.1093/nar/gku1179.

Ghosh, Z., Mallick, B., Chakrabarti, J., 2009. Cellular versus viral microRNAs in host–virus interaction. Nucleic Acids Res. 37, 1035–1048. https://doi.org/10.1093/nar/gkn1004.

Gkirtzou, K., Tsamardinos, I., Tsakalides, P., Poirazi, P., 2010. MatureBayes: a probabilistic algorithm for identifying the mature miRNA within novel precursors. PLoS One 5. https://doi.org/10.1371/journal.pone.0011843 e11843.

Grundhoff, A., Sullivan, C.S., 2011. Virus-encoded microRNAs. Virology 411, 325–343. https://doi.org/10.1016/j.virol.2011.01.002.

Hariharan, M., Scaria, V., Pillai, B., Brahmachari, S.K., 2005. Targets for human encoded microRNAs in HIV genes. Biochem. Biophys. Res. Commun. 337, 1214–1218. https://doi.org/10.1016/j.bbrc.2005.09.183.

Hoarau, J.J., et al., 2010. Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. J. Immunol. (Baltimore, Md. : 1950) 184, 5914–5927. https://doi.org/10.4049/jimmunol.0900255.

Hussain, M., Asgari, S., 2014. MicroRNA-like viral small RNA from dengue virus 2 autoregulates its replication in mosquito cells. Proc. Natl. Acad. Sci. 111, 2746–2751. https://doi.org/10.1073/pnas.1320123111.

Islam, M.S., Khan, M.A.A.K., Murad, M.W., Karim, M., Islam, A.B.M.M.K., 2019. 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. 91, 1584–1594.

Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., Tanabe, M., 2016. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 44, D457–462. https://doi.org/10.1093/nar/gkv1070.

Kincaid, R.P., Sullivan, C.S., 2012. Virus-encoded microRNAs: an overview and a look to the future. PLoS Pathog. 8 https://doi.org/10.1371/journal.ppat.1003018 e1003018.

Kinsella, R.J., et al., 2011. Ensembl BioMarts: a hub for data retrieval across taxonomic space. Database 2011. https://doi.org/10.1093/database/bar030 bar030.

Kohl, M., Wiese, S., Warscheid, B., 2011. Cytoscape: software for visualization and analysis of biological networks. Methods Mol. Biol. (Clifton, N.J.) 696, 291–303. https://doi.org/10.1007/978-1-60761-987-1_18.

Kruger, J., Rehmsmeier, M., 2006. RNAhybrid: microRNA target prediction easy, fast and flexible. Nucleic Acids Res. 34, W451–454. https://doi.org/10.1093/nar/gkl243.

Lester, S.N., Li, K., 2014. Toll-liK.e receptors in antiviral innate immunity. J. Mol. Biol. 426, 1246–1264.

Li, X., Zou, X., 2019. An overview of RNA virus-encoded microRNAs. ExRNA 1, 37. https://doi.org/10.1186/s41544-019-0037-6.

Lim, L.P., Glasner, M.E., Yekta, S., Burge, C.B., Bartel, D.P., 2003. Vertebrate microRNA genes. Science 299. https://doi.org/10.1126/science.1080372.

Lu, L.-F., Liston, A., 2009. MicroRNA in the immune system, microRNA as an immune system. Immunology 127, 291–298. https://doi.org/10.1111/j.1365-2567.2009.03092.x.

Mann, M., Wright, P.R., Backofen, R., 2017. IntaRNA 2.0: enhanced and customizable prediction of RNA-RNA interactions. Nucleic Acids Res. 45, W435–W439. https://doi.org/10.1093/nar/gkx279.

Manni, I., et al., 2009. The microRNA miR-92 increases proliferation of myeloid cells and by targeting p63 modulates the abundance of its isoforms. FASEB J. 23, 3957–3966. https://doi.org/10.1096/fj.09-131847.

Matusali, G., et al., 2019. Tropism of the Chikungunya virus. Viruses 11. https://doi.org/10.3390/v11020175.

Mishra, R., Kumar, A., Ingle, H., Kumar, H., 2020. The interplay between viral-derived miRNAs and host immunity during infection. Front. Immunol. 10 https://doi.org/10.3389/fimmu.2019.03079, 3079-3079.

Mogensen, T.H., Paludan, S.R., 2001. Molecular pathways in virus-induced cytokine production. Microbiol. Mol. Biol. Rev. 65, 131–150. https://doi.org/10.1128/MMBR.65.1.131-150.2001.

Mohanta, T.K., Sharma, N., Arina, P., Defilippi, P., 2020. Molecular insights into the MAPK cascade during viral infection: potential crosstalk between HCQ and HCQ analogues. Biomed Res. Int., 8827752 https://doi.org/10.1155/2020/8827752 (2020).

Nukui, M., Mori, Y., Murphy, E.A., 2014. A human herpesvirus 6A encoded miRNA: a role in viral lytic replication. J. Virol. https://doi.org/10.1128/jvi.02007-14.

Perez-Llamas, C., Lopez-Bigas, N., 2011. Gitools: analysis and visualisation of genomic data using interactive heat-maps. PLoS One 6. https://doi.org/10.1371/journal.pone.0019541 e19541.

Pruitt, K.D., Maglott, D.R., 2001. RefSeq and LocusLink: NCBI gene-centered resources. Nucleic Acids Res. 29 https://doi.org/10.1093/nar/29.1.137.

Ritchie, M.E., et al., 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43, e47. https://doi.org/10.1093/nar/gkv007.

Ross, R.W., 1956. The Newala epidemic: III. The virus: isolation, pathogenic properties and relationship to the epidemic. J. Hyg. 54, 177–191. https://doi.org/10.1017/S0022172400044442.

Rougeron, V., et al., 2015. Chikungunya, a paradigm of neglected tropical disease that emerged to be a new health global risk. J. Clin. Virol. 64, 144–152. https://doi.org/10.1016/j.jcv.2014.08.032.

Saxena, T., et al., 2013. Combined miRNA and mRNA signature identifies key molecular players and pathways involved in chikungunya virus infection in human cells. PLoS One 8. https://doi.org/10.1371/journal.pone.0079886 e79886.

Shen, W., Chen, M., Wei, G., Li, Y., 2012. MicroRNA prediction using a fixed-order markov model based on the secondary structure pattern. PLoS One 7. https://doi.org/10.1371/journal.pone.0048236 e48236.

Skalsky, R.L., Cullen, B.R., 2010. Viruses, microRNAs, and host interactions. Annu. Rev. Microbiol. 64, 123–141. https://doi.org/10.1146/annurev.micro.112408.134243.

Stern-Ginossar, N., et al., 2007. Host immune system gene targeting by a viral miRNA. Science (New York, N.Y.) 317, 376–381. https://doi.org/10.1126/science.1140956.

Tav, C., Tempel, S., Poligny, L., Tahi, F., 2016. miRNAFold: a web server for fast miRNA precursor prediction in genomes. Nucleic Acids Res. 44, W181–184. https://doi.org/10.1093/nar/gkw459.

Tempel, S., Tahi, F., 2012. A fast ab-initio method for predicting miRNA precursors in genomes. Nucleic Acids Res. 40 https://doi.org/10.1093/nar/gks146 e80-e80.

Thiberville, S.-D., et al., 2013. Chikungunya fever: epidemiology, clinical syndrome, pathogenesis and therapy. Antiviral Res. 99, 345–370. https://doi.org/10.1016/j.antiviral.2013.06.009.

Wang, Y., Lee, C.G.L., 2009. MicroRNA and cancer – focus on apoptosis. J. Cell. Mol. Med. 13, 12–23. https://doi.org/10.1111/j.1582-4934.2008.00510.x.

Wen-Tsai, J., Hung, J.L., 2008. PI3K-Akt signaling and viral infection. Recent Pat. Biotechnol. 2, 218–226. https://doi.org/10.2174/187220808786241042.

Wienholds, E., Koudijs, M.J., van Eeden, F.J.M., Cuppen, E., Plasterk, R.H.A., 2003. The microRNA-producing enzyme Dicer1 is essential for zebrafish development. Nat. Genet. 35, 217–218 doi:http://www.nature.com/ng/journal/v35/n3/suppinfo/ng1251_S1.html.

Xue, C., et al., 2005. Classification of real and pseudo microRNA precursors using local structure-sequence features and support vector machine. BMC Bioinformatics 6, 310. https://doi.org/10.1186/1471-2105-6-310.

Zhang, X., et al., 2018. Differences in genome characters and cell tropisms between two chikungunya isolates of Asian lineage and Indian Ocean lineage. Virol. J. 15, 130. https://doi.org/10.1186/s12985-018-1024-5.