Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Genes & Diseases xxx (xxxx) xxx

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://ees.elsevier.com/gendis/default.asp

RAPID COMMUNICATION

Coronaviruses RNA interacts with host miR-500a-5p and miR-501-5p to regulate multiple pathways

To the Editor,

The Coronavirus disease is the contagious disease. Clinical manifests vary from no symptoms, upper and lower respiratory tract infections, and even severe acute respiratory distress syndrome and multiple organs failure. Recently, three of new beta-coronaviruses have emerged to cause serious and widespread illness and death, such as the Severe Acute Respiratory Syndrome Coronaviruses (SARS-CoV), Middle East Respiratory Syndrome Coronaviruses (MERS-CoV), and 2019-nCoV (the causative pathogen of Coronavirus Disease 2019 (COVID-19). The COVID-19 is continuing to spread globally. However, the detailed molecular mechanisms have not yet been fully clarified. Since the similarity of the pathological features among 2019-nCoV, SARS-CoV and MERS-CoV, we could speculate the biological features of current COVID-19 by researching those of MERS-CoV and SARS-CoV. Therefore, we analyzed expression levels of miRNA and mRNA profiles of MERS-CoV, SARS-CoV and 2019-nCoV from five public GEO datasets (Table S1). As a result, we found 16 miRNAs potentially target a group of hub genes clustering together to tune the immune response. Notably, viral RNAs have the ability to sponge miR-500a-5p and miR-501-5p, indicating viral RNAs sequestering these two miRNAs to trigger genes expression and contribute to pathogenesis. We hope this study will pave the way to better understand 2019-nCoV pathogenesis and develop the innovative therapeutics.

In this study, a group of differentially expressed miRNAs in GSE139516 was validated in the other data set of GSE81852 (Fig. S1A and Table S2). In order to determine the differentially expressed genes (DEGs) after 2019-nCoV, SARS-CoV and MERS-CoV infection, a meta-analysis was performed in the four data sets of GSE139516, GSE147507, GSE30589 and GSE17400 (Fig. S1B). As a result, 82 up-regulation and 155 down-regulation of coexpressed genes were shown in Figure 1A and Table S3. We then conducted KEGG pathways (Table S4) and GO functional annotations analysis (Table S5, S6) among these DEGs. Additionally, the hub genes of DEGs-encoded proteins were constructed into a network using the online tool STRING. Based on an inverse relationship between the significantly different expression of miRNAs and their targeted genes, we constructed the core miRNA-mRNA regulatory network *in silico* analysis. Consequently, we found 10 out of 11 downregulated miRNAs cooperatively targeting the hub genes in Figure 1B. For example, downregulated expression of miR-501-5p/miR-500a-5p is associated with elevated expression of *NR3C1*, *KPNA1*, *EIF5* and *CSF3* after viral infection. Notably, *CSF3* is a cytokine coding gene and *NR3C* is an inflammation repression gene. These results strongly imply that miR-501-5p/miR-500a-5p may act as the switch to tune the inflammatory response via the transcriptional activation of *EIF5* to coordinate the expressions of *NR3C1* and *CSF3*. As shown in Figure 1B, we found out these upregulated genes involved in both direct viral effects (viral life cycle and viral process) and indirect effects produced by immunopathogenetic factors (inflammatory response and cytokine-mediated signaling pathways). For example, tumor necrosis factor alpha-induced protein 3 (TNFAIP3) is a key regulator of NF-κB activity, which is involved in the cytokine-mediated immune and inflammatory responses in Epstein—Barr virus (EBV) positive tumor cells of classical Hodgkin lymphoma. More importantly, TNFAIP3 acts as a deubiquitinating enzyme in inflammation response of gastric cancer cells.[1] Besides, itchy E3 ubiquitin protein ligase (ITCH) has the ubiquitinating function in the regulation of immune responses. With respect to the downregulated DEGs, there were six overexpressed miRNAs linking to the targeted genes involving the function of the cell cycle (Fig. 1C). Thus, one can image that when viruses infect human lung

Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2022.07.008

Please cite this article as: , Coronaviruses RNA interacts with host miR-500a-5p and miR-501-5p to regulate multiple pathways, Genes & Diseases, https://doi.org/10.1016/j.gendis.2022.07.008

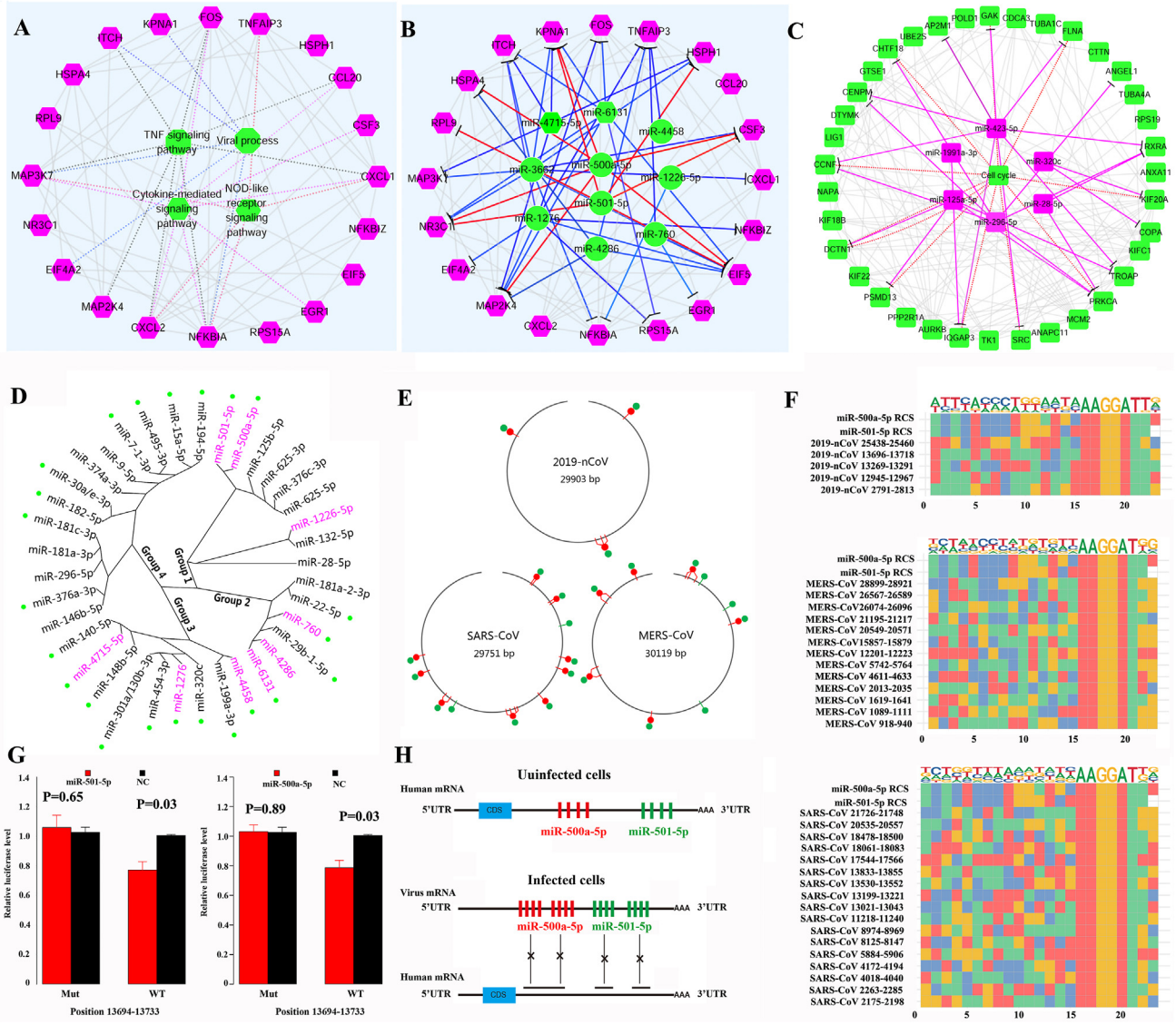

Figure 1 Viral RNA regulates host miRNA-mRNAs regulatory network. Downregulated miRNAs dysregulated hub genes involved in the numeric pathways**(A, B)**, and the upregulated miRNAs together with targeting genes involved in cell cycles **(C)**. Gray lines indicate the interactive networks of hub genes. Purple hexagons and quadrilaterals represent increased expression of genes (A, B) and miRNAs (C), while green colors indicate decreased expression of miRNAs and genes after coronaviruses infections. Dysregulated genes enriched in multiple pathways (A) by miR-500a-5p/miR-501-5p (red lines) along with other miRNAs (blue lines) (B). Purple lines (C) highlight the relationship of downregulated genes by miRNAs, and red dash lines indicate some genes clustering in the cell cycle pathway. The binding sites are classified into 4 groups **(D)** based on the similarity of nucleotides. Downregulated miRNAs are colored in purple while upregulation miRNA are colored in black. The green dots beside miRNAs refer to binding sites in the 5′ part of miRNA. The physical locations **(E)** and the binding sequences **(F)** of specific binding sites were illustrated in the viral RNA genomes. Green dots (E) represent miR-501-5p, while red dots represent miR-500a-5p. The bases (F) completely pare at positions 2—7 from the 5′ part of miR-501-5p, implying miR-501-5p obtains the most strongly sponging ability. "2019-nCoV 13,696—13,718" indicates 2019-nCoV RNA sequence at the position ranging from 13,696 to 13,718. Evidence of the interaction between 2019-nCoV and miR-501-5p and miR-500a-5p was seen in Figure G **(G)**. The relative luciferase levels were estimated as the ratio between each value versus the basic vector. Reduced firefly luciferase expression indicates miRNAs bind to the cloned 2019-nCoV sequence. miR-500a-5p and miR-501-5p were able to reduce the firefly luciferase when cotransfected with 2019-nCoV wide-type (WT) sequence. Wild-type and mutated (Mut) Cov-2019 sequences in miRNAs binding sites were respectively coexpressed with mimic-miRNAs (miR-500a-5p and miR-501-5p) and mimic-miR-control (NC). The experiments were repeated double times. Schematic model illustrating coronaviruses regulated gene expressions via sponging host's miR-501-5p and miR-500a-5p **(H)**. RCS, reverse complement sequence.

Please cite this article as: , Coronaviruses RNA interacts with host miR-500a-5p and miR-501-5p to regulate multiple pathways, Genes & Diseases, https://doi.org/10.1016/j.gendis.2022.07.008

cells, they would trigger the inflammation and immune response via the miRNA-mRNAs regulatory network.

Recently, it was reported a viral RNA could sequester host's miRNA in infected cells.[2–4] In order to understand whether coronavirus could act on host's miRNAs, firstly, we conducted pair-wise alignment among the genomic RNAs of 2019-nCoV, SARS-CoV and MERS-CoV. We secondly performed the predictive analysis of the targeting miRNAs using the common nucleotide sequences, and found more than 935 targeting miRNAs manually by searching the database of miRDB and RNAhybrid (Fig. S2 and Table S7). Finally, we matched the differentially expressed miRNAs to these targeting miRNAs, and found 9 out of 11 (82%) downregulated miRNAs and 30 of 43 (70%) upregulated miRNAs have at least one predicted binding site in viral nucleotide sequences (Table S8). In the viral genomic sequences, one binding site can predict to sponge several miRNAs, for example, miR-301a and miR-103b-3p share the same binding sites. This result implied that different miRNAs might compete with each other when combined to some binding sites, and each viral RNA had the relative specific binding ability based on the similarity of the nucleotide sequences. Therefore, we classified these binding sites into four groups. As shown in Figure 1D, miR-500a-5p and miR-501-5p together with 4 miRNAs are grouped into one cluster as Group 1. It is well known that binding sites in the 5′ part of miRNAs obtain the most stringent binding ability.[5] Therefore, the other 4 miRNAs would loss the potential binding ability due to the binding sites in the non-5′ part of miRNAs. The sponging ability to low expressed miRNAs should be submerged by the over-expressed miRNAs in Group 2 and 3. Therefore, miR-760, miR-4286, miR-4715 and miR-1276 might have less chance to bind viral RNAs due to the high expression levels of miR-199a-3p, miR-320c, miR-454-3p, miR-140-5p, miR-148-5p and miR-103b-3p and miR-301a, which occupy the common binding sites (Fig. 1D). While miRNAs in Group 4 will compete with each other to occupy the very similar binding sites due to all co-overexpression levels. Because viral RNAs sponging could lead to downregulation of miRNAs, we refined the lowly expressed miRNAs into two groups (Fig. S3). One has the stringent binding sites like miR-500a-5p and miR-501-5p, the other has no or limited specific binding ability. Notably, there are 5, 17, 13 and 5, 15, 10 specific stringent binding sites of miR-501-5p and miR-500a-5p in 2019-nCoV, SARS-CoV and MERS-CoV RNA, respectively (Fig. 1E). In fact, the binding sites of miRNA-500a-5p and miR-501-5p are very similar in both physical locations and bases (Fig. 1E, F). The bases completely pare at positions 2—7 from the 5′ part of miR-501-5p, implying miR-501-5p obtains the most strongly sponging ability. There are totally 14 binding sites with perfect paring at position 2—7 of miR-500a-5p and 16 moderate stringent binding sites with G:U parings. Thus, the binding sites in viral RNAs will sponge more miR-501-5p than miR-500a-5p. We further test the interaction of miR-500a-5p/miR-501-5p and the potential binding sites of 2019-nCoV (Table S9 and Fig. S4). The luciferase activity decreased significantly in the HEK293 cells cotransfected with the viral RNA sequence at the position ranging from 13,694 to 13,733 and miR-500a-5p/miR-501-5p, comparing to the negative control-transfected cells (Fig. 1 G). The other four binding sites were not validated for 2019-nCoV (Fig. S5). Taken together, we can image that when these coronaviruses infect human cells, they will replicate and deposit its nucleic acid into cytoplasm of infected cells. In turn, viral RNAs utilize the multiple binding sites to sequester the host miRNAs like miR-500a-5p and miR-501-5p, leading to the overexpression of the endogenous target genes involved in the inflammatory response.

In summary, we present a coronavirus RNA regulating miRNA-mRNA pathway, which elicits a strong viral effects and host immune responses in infected cells.

Availability of data and materials

Gene expression data of GES30589, GSE81852, GSE139516, GSE17400 and GSE147507 were from the Gene Expression Omnibus (GEO) repository.

Patient consent for publication

This study does not involve human data and tissue.

Author contributions

JHW, QG and LHT designed the research and/or analyzed the data. JJP, FY, JSH and SFW carried out the validated studies. JHW, QG, LHT, FY and SFW wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interests

Authors declare no conflict of interests.

Acknowledgements

We would like to thank the authors who donated the GEO datasets.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.07.008.

References

1. Du B, Liu M, Li C, et al. The potential role of TNFAIP3 in malignant transformation of gastric carcinoma. *Pathol Res Pract*. 2019;215(8):152471.
2. Ebert MS, Sharp PA. Emerging roles for natural microRNA sponges. *Curr Biol*. 2010;20(19):R858—R861.
3. Nersisyan S, Engibaryan N, Gorbonos A, Kirdey K, Makhonin A, Tonevitsky A. Potential role of cellular miRNAs in coronavirus-host interplay. *PeerJ*. 2020;8:e9994.
4. Fulzele S, Sahay B, Yusufu I, et al. COVID-19 virulence in aged patients might Be impacted by the host cellular microRNAs abundance/profile. *Aging Dis*. 2020;11(3):509—522.
5. Zhang K, Zhang X, Cai Z, et al. A novel class of microRNA-recognition elements that function only within open reading frames. *Nat Struct Mol Biol*. 2018;25(11):1019—1027.

Qi Guo[a,1], Linghui Tan[b,1], Jiajia Pan[b,1], Songfang Wu[c,1], Jiansong Huang[a], Fang Yu[d], Jinghan Wang[e,*]

[a] *Department of Nephrology, The First Affiliated Hospital, School of Medicine Zhejiang University, Hangzhou, Zhejiang 310003, PR China*
[b] *Zhejiang University School of Medicine, Hangzhou, Zhejiang 310003, PR China*
[c] *Shanghai Xuhui Central Hospital/Zhongshan-Xuhui Hospital, Fudan University, Shanghai 200000, PR China*
[d] *Department of Pathology, The First Affiliated Hospital, School of Medicine Zhejiang University, Hangzhou, Zhejiang 310003, PR China*
[e] *Department of Hematology, The First Affiliated Hospital, School of Medicine Zhejiang University, Hangzhou, Zhejiang 310003, PR China*

*Corresponding author. The First Affiliated Hospital, School of Medicine Zhejiang University, 79# Qingchun Road, Hangzhou, Zhejiang 310003, PR China. Fax: +86 571 87236702.
E-mail address: 1513084@zju.edu.cn (J. Wang)

9 April 2022

[1] These authors contributed equally to this work.

Please cite this article as: , Coronaviruses RNA interacts with host miR-500a-5p and miR-501-5p to regulate multiple pathways, Genes & Diseases, https://doi.org/10.1016/j.gendis.2022.07.008