CORRESPONDENCE

Downregulated *miR-451a* as a feature of the plasma cfRNA landscape reveals regulatory networks of IL-6/IL-6R-associated cytokine storms in COVID-19 patients

Penghui Yang[1], Yingze Zhao[2], Jie Li[3,4], Chuanyu Liu[3,8], Linnan Zhu[3,8], Jie Zhang[2], Yeya Yu[3,5], Wen-Jing Wang[3], Guanglin Lei[1], Jin Yan[1], Fang Sun[1], Chengrong Bian[1], Fanping Meng[1], Zhe Xu[1], Changqing Bai[1], Beiwei Ye[2], Yuanyuan Guo[2,6], Liumei Shu[2], Xiaoju Yuan[2], Ning Zhang[7], Yuhai Bi[7], Yi Shi[7], Guizhen Wu[2], Shaogeng Zhang[1], George F. Gao[2,6], Longqi Liu[3,8], William J. Liu[2] and Hai-Xi Sun[3]

Cellular & Molecular Immunology (2021) 18:1064–1066; https://doi.org/10.1038/s41423-021-00652-5

Rapidly spreading coronavirus disease 2019 (COVID-19) is currently affecting the world. Specifically, cytokine storms are a key feature in a substantial number of COVID-19 patients,[1] and studies from our group and others suggest that the IL-6/IL-6R cascade plays a dominant role in symptom-correlated cytokine storms.[2,3] Cell-free circulating RNAs (cfRNAs) in plasma carry information from pathologic sites, and they have been reported to play important roles in disease development;[4] however, their involvement in COVID-19 has not yet been clarified. Here, we report the characteristics of plasma cfRNA profiles of COVID-19 patients, and we found that no SARS-CoV-2 RNA is present in the plasma of COVID-19 patients. Compared with healthy donors, significantly higher mRNA expression of *IL-6R* was observed; *miR-451a*, a known negative regulator of *IL-6R* translation, was downregulated, which may promote IL-6R expression at the protein level. In addition, three upregulated long noncoding RNAs (lncRNAs) carrying *miR-451a* binding sites might function as miRNA sponges to compete with *IL-6R* for *miR-451a* in COVID-19 patients. Taken together, we provide the cfRNA landscape of COVID-19 patient plasma and describe the possible mechanisms underlying elevated cytokine storms in COVID-19 patients. These findings will contribute to the identification of drug targets for this new disease.

We analyzed the cfRNAs from three healthy donors and five COVID-19 patients (Fig. 1a), and no SARS-CoV-2 RNA was detected (Fig. 1b, c). We detected 33,562 human genes, including 57% protein-coding genes, 4% miRNA genes, 15% lncRNA genes, and 24% other noncoding genes (Fig. 1d and Fig. S1a). Compared with healthy donors, we identified 2583 upregulated and 192 downregulated cfRNA genes in all COVID-19 patients (Fig. 1e). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses revealed that the functions of the upregulated genes are significantly enriched in antivirus-related pathways such as "type I interferon signaling pathway" and "innate immune response and response to virus" (Fig. 1f). In particular, many interferon-stimulated genes (ISGs) were upregulated in COVID-19 patients, including *ISG15*, *IFI6*, *IFI16*, and *IFI27L* (Fig. 1f), which is consistent with a previous study reporting that IFNα expression is increased in COVID-19 patients.[5] Among these ISGs, *ISG15* may be related to prolonged viral latency of SARS-CoV-2.[6] A previous study found that the IL-6 concentration in COVID-19 patient plasma was higher than the normal range (0–7 pg/ml).[5] We also detected high expression of *IL-6R* in COVID-19 patients compared with healthy donors (Fig. 1f). this high level of *IL-6R* transcription might be a result of increased type I interferon signaling.[7] Moreover, regarding stages of disease progression, expression levels of interferon-stimulated genes, and *IL-6R* showed a downward trend from stage 1 to stage 4 (Fig. S1b and Supplementary Table S2).

In addition to increased RNA expression of *IL-6R*, we also found *miR-451a*, a reported translational repressor of IL-6R,[8] to be one of the top five downregulated microRNA (miRNA) genes in COVID-19 patients (Fig. 1g, Fig. S1c, d, Supplementary Table S1 and Supplementary Table S2). Moreover, expression levels of *miR-451a* showed an upward trend from stage 1 to stage 4 (Fig. S1b and Supplementary Table S3), suggesting that decreased *miR-451a* may promote expression of IL-6R in COVID-19 patients at the protein level. LncRNAs can act as miRNA sponges to inhibit miRNA function.[9] We identified in COVID-19 patients three upregulated lncRNAs, *LOC105371414*, *LOC105374981*, and *LOC107987081*, carrying *miR-451a* binding sites (Fig. 1g, h and Fig. S1d), which may compete with *IL-6R* for *miR-451a* binding. We also found that *miR-374a*, the target of which is *CCL2*,[10] was downregulated in COVID-19 patients (Fig. 1g). Derepression of CCL2 may confer acute respiratory distress syndrome (ARDS) and cause cytokine storms in COVID-19 patients.[11] We also detected 16 upregulated lncRNAs carrying *miR-374a* binding sites (Fig. 1g and Fig. S2b). In healthy donors, *miR-451a/miR-374a* can maintain the normal level of IL-6R/CCL2 by targeting *IL-6R/CCL2* mRNAs. However, in COVID-19 patients, decreased expression of *miR-451a/miR-374a* and its

[1]Fifth Medical Center of Chinese PLA General Hospital, National Clinical Research Center for Infectious Diseases, Beijing, China; [2]NHC Key Laboratory of Biosafety, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China; [3]BGI-Shenzhen, Shenzhen, China; [4]BGI Education Center, University of Chinese Academic of Sciences, Shenzhen, China; [5]BGI College, Zhengzhou University, Zhengzhou, China; [6]School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing, China; [7]CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China and [8]Shenzhen Bay Laboratory, Shenzhen, China
Correspondence: Shaogeng Zhang (zhangsg302@hotmail.com) or George F. Gao (gaofu@chinacdc.cn) or Longqi Liu (liulongqi@genomics.cn) or William J. Liu (liujun@ivdc.chinacdc.cn) or Hai-Xi Sun (sunhaixi@genomics.cn)
These authors contributed equally: Penghui Yang, Yingze Zhao, Jie Li, Chuanyu Liu, Linnan Zhu.

Received: 28 January 2021 Accepted: 3 February 2021
Published online: 26 February 2021

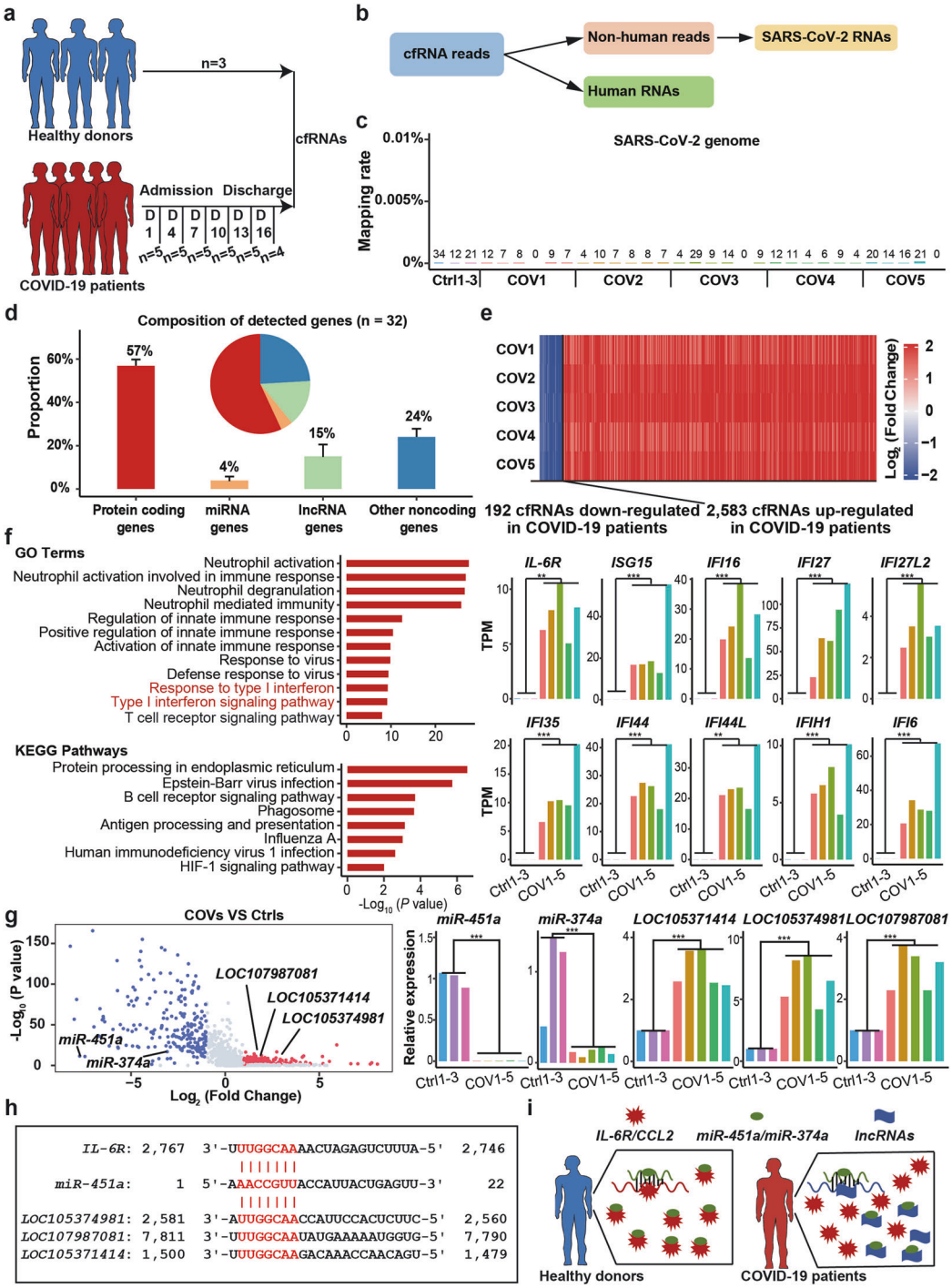

Fig. 1 The landscape characteristics of cell-free circulating RNAs (cfRNAs) in the plasma of COVID-19 patients; no SARS-CoV-2 RNA was detected in COVID-19 patient plasma. **a** Schematic diagram showing the sample preparation procedure in this study. cfRNAs were collected from 3 healthy donors and 5 COVID-19 patients. **b** The computational pipeline to detect human RNAs and SARS-CoV-2 RNAs. **c** Bar plot showing the mapping rate and number of mapped reads against the SARS-CoV-2 genome ($n = 32$). **d** Bar plot and pie plot showing the composition of detected genes in all samples. Data are shown as the mean ± SD ($n = 32$). **e** Heatmap showing fold changes (relative to healthy donors) of 2,583 upregulated cfRNA genes and 192 downregulated cfRNA genes in COVID-19 patients. Blue and red represent $\log_2$-transformed fold changes < 0 and > 0, respectively. **f** GO and KEGG enrichment analyses of 2,583 upregulated cfRNA genes in COVID-19 patients (left). Expression levels (TPMs) of *IL-6R* and representative interferon-stimulated genes (ISGs) are shown on the right. **g** Volcano plot showing up- and downregulated microRNA (miRNA) and long noncoding RNA (lncRNA) genes in COVID-19 patients relative to healthy donors (left). Relative expression of *miR-451a*, *LOC105371414*, *LOC105374981* and *LOC107987081* is shown on the right. **h** Base-pairing interaction between *miR-451a* and *IL-6R* (top) and the three upregulated lncRNAs (bottom). *miR-451a* target sites (seed sequences) are highlighted in red. **i** A proposed model for the regulatory network of *miR-451a*, *IL-6R* and lncRNAs in healthy donors and COVID-19 patients. Asterisks indicate statistically significant differences: $^{**}P < 0.01$; $^{***}P < 0.001$

binding to lncRNAs may promote expression of IL-6R/CCL2 at the protein level (Fig. 1i). These results suggest that decreased *miR-451a/miR-374a* and enhanced lncRNA levels may exacerbate IL-6-induced cytokine storms by promoting *IL-6R/CCL2* translation in COVID-19 patients.

The cytokine storm in COVID-19 patients is characterized by increased IL-6[2]. However, the mechanisms of cytokine storm-correlated symptoms from the perspective of cfRNAs remain unclear. Our study identified obvious differences in cfRNA molecules between COVID-19 patients and healthy donors. We found activation of type I interferon-responsive genes and low expression of *miR-451a*, which may lead to uncontrolled expression of *IL-6R* at both mRNA and protein levels, enhancing cytokine storms in COVID-19 patients. Furthermore, the three lncRNAs identified as upregulated in COVID-19 patients may compete with *IL-6R* for *miR-451*a to reverse overexpression of IL-6R at the protein level. Collectively, our work provides the cfRNA landscape of plasma in COVID-19 patients, offers insight into the potential mechanism to understand the elevated cytokine storms caused by *IL-6* in COVID-19 patients and may shed light on drug development for this new disease.

ACKNOWLEDGEMENTS

The study was supported by CAMS Research Units of Adaptive Evolution and Control of Emerging Viruses (2018RU009) and Beijing New-star Plan of Science and Technology (Z181100006218080). WJL is supported by the Excellent Young Scientist Program of the National Natural Science Foundation of China (81822040) and the National Youth Talent Support Program. L.L. is funded by the National Natural Science Foundation of China (31900466). C.L. is funded by the China Postdoctoral Science Foundation (2020T130080ZX).

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41423-021-00652-5.

Competing interests: The authors declare no competing interests.

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