COVID-19

https://doi.org/10.1038/s41556-023-01099-8

Linking SARS-CoV-2 to the circadian clock

Soumita Das

A study using a multi-organoid platform and state-of-the-art transcriptional profiling identifies potential therapeutic targets against SARS-CoV-2. The authors find that *CIART*, a gene involved in circadian regulation, promotes SARS-CoV-2 infection by regulating the retinoid X receptor pathway and fatty acid synthesis.

The lung is a highly complex organ comprising several cell types, including goblet, club and alveolar type I and type II cells. Recent advances in stem cell research have facilitated the development of lung organoids that mimic airway epithelial function and serve as models for respiratory infection[1–5]. For instance, studies have shown that after SARS-CoV-2 infection, the stem cell-based alveolosphere-derived type 2 cells are similar to those of individuals with COVID-19[3]. Single-cell transcriptomics of SARS-CoV-2-infected distal lung organoids has identified club cells as a primary viral target[4]. In addition, a complete lung organoid model developed by Tindle et al.[5] has shown that the proximal airway cells provide sustained viral infection, whereas the distal alveolar cells are responsible for the fatal host immune response after SARS-CoV-2 infection. In this issue of *Nature Cell Biology*, Tang et al.[6] model SARS-CoV-2 infection in lung airway organoids and lung alveolar organoids, along with cardiomyocytes generated from human pluripotent stem cells. The authors used these three models to transcriptionally profile SARS-CoV-2 infection at various multiplicities of infections (Fig. 1).

Tang et al.[6] started by profiling the multi-organoid platform, consisting of lung models and cardiomyocytes, with sequencing methods that included CUT&RUN, ATAC-seq and RNA-seq, and they identified several genes that influence SARS-CoV-2 infection, including *CIART* (which encodes circadian-associated repressor of transcription). The CIART (also known as CHRONO) protein functions as a transcriptional repressor, modulating the activity of the circadian master proteins BMAL1 and CLOCK[7]. Circadian rhythms have an important role in physiology and in the regulation of immune functions. For example, circadian oscillations of immune regulators allow the host to tackle microbial infections more efficiently at certain times of the day[8]. Moreover, circadian clocks modulate viral replication and the severity of infections[9]. Downregulation of BMAL1 increases the expression of interferon-stimulated genes in lung epithelial cells, thereby linking the circadian clock to inhibition of SARS-CoV-2 infection[9], and other work suggests that circadian rhythm can affect disease outcome in COVID-19[10]. Circadian rhythms also regulate the pharmacokinetics and efficacy of several therapeutics[11]. Consequently, better understanding of the effects of the circadian clock on SARS-CoV-2 infection may improve the clinical management of COVID-19. This heralds a potential new direction for circadian medicine in ameliorating the next pandemic.

Tang et al.[6] used CUT&RUN, ATAC-seq and RNA-seq analyses to show that CIART controls SARS-CoV-2 infection, at least in part, through regulation of the gene *NR4A1* (encoding nuclear receptor subfamily 4 group A member 1), which was identified from the multi-organoid analysis and was previously known to encode an important regulator of proliferation and apoptosis in tumour cells[12,13]. Transcriptional profiling and pharmacological inhibition further established that the retinoid X receptor (RXR) pathway regulates SARS-CoV-2 infection downstream of *CIART* and *NR4A1*[6]. The infection analyses of this multi-organoid platform, therefore, provide potential therapeutic targets for protection against COVID-19. Further research is required to confirm the potential of RXR as a drug target in the treatment of infection with SARS-CoV-2 and other viruses.

As Tang et al.[6] observed downregulation of RXR-pathway-associated genes in *CIART*-knockout (KO) organoids, and because previous studies have shown that the RXR signalling regulates fatty acid metabolism[14], the authors further analysed RNA-seq data from wild-type and *CIART*-KO airway organoids. Multiple genes encoding

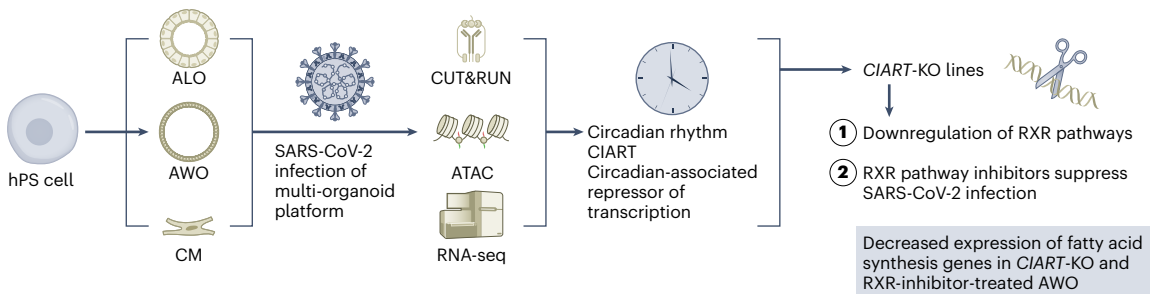

Fig. 1 | Identification of CIART in the multi-organoid platform after SARS-CoV-2 infection. Lung alveolar organoids (ALOs), lung airway organoids (AWOs) and cardiomyocytes (CMs) were derived from human pluripotent stem (hPS) cells. This multi-organoid platform was infected with SARS-CoV-2 at various multiplicities of infection, and the samples collected were used to study post-infection host transcriptional profiles. The authors identified CIART, a circadian rhythm regulator, as having altered expression after infection. CRISPR KO of *CIART* in the organoid platform resulted in downregulation of RXR pathways. Profiling *CIART*-KO lines revealed a link between CIART and fatty acid synthesis in the context of SARS-CoV-2 infection. Therefore, the CIART–RXR pathway could be useful for therapeutic screening in multi-organoid platforms.

molecules involved in fatty acid synthesis showed lower expression in *CIART*-KO organoids than in wild-type organoids, or in organoids treated with the RXR inhibitor HX531 than in control DMSO-treated organoids. The researchers next performed metabolic analyses of wild-type, *CIART*-KO and wild-type HX531-treated human pluripotent stem cell airway organoids and detected the downregulation of several fatty acids, which indicated that the circadian clock influences fatty acid synthesis[6]. Interestingly, cellular lipid synthesis has been shown to be required for SARS-CoV-2 replication[15] and offers an opportunity for pharmacological intervention. For example, the anti-obesity drug orlistat (which has been approved by the US Food and Drug Administration) inhibits the replication of SARS-CoV-2 variants, including the Delta variants, in vitro[15]. RXR modulators may offer a strategy for the development of antiviral drugs.

In contrast to animal models, organoid platforms lack possible inter-organ communication that is present in the whole body. Also, the absence of immune or endothelial cells in the organoid platform necessitates further attention from researchers to add these components individually to the cell model. Therefore, in future studies it would be useful to incorporate other cell types into the organoid platform, as well as vasculature, to further expand its physiological relevance. It will also be important to generate and study organoids from both healthy donors and individuals with COVID-19 to obtain reliable molecular assessments of viral susceptibility in individuals of different age, gender, ethnicity and basal metabolic index and to develop personalized treatment strategies for current and future pandemics.

Soumita Das[1,2] ✉
[1]Department of Biomedical and Nutritional Science, University of Massachusetts–Lowell, Lowell, MA, USA. [2]Department of Pathology, University of California, San Diego, San Diego, CA, USA.
✉e-mail: soumita_das@uml.edu

Published online: 13 March 2023

References

1. Miller, A. J. et al. *Nat. Protoc.* **14**, 518–540 (2019).
2. Sachs, N. et al. *EMBO J.* **38**, e100300 (2019).
3. Katsura, H. et al. *Cell Stem Cell* **27**, 890–904 e898 (2020).
4. Salahudeen, A. A. et al. *Nature* **588**, 670–675 (2020).
5. Tindle, C. et al. *eLife* **10**, e66417 (2021).
6. Tang, X. et al. *Nat. Cell Biol.* https://doi.org/10.1038/s41556-023-01095-y (2023).
7. Goriki, A. et al. *PLoS Biol.* **12**, e1001839 (2014).
8. Scheiermann, C., Kunisaki, Y. & Frenette, P. S. *Nat. Rev. Immunol.* **13**, 190–198 (2013).
9. Zhuang, X. et al. *iScience* **24**, 103144 (2021).
10. Haspel, J. et al. *J. Biol. Rhythms* **36**, 55–70 (2021).
11. Yu, F. et al. *Biochem. Pharmacol.* **203**, 115185 (2022).
12. Mullican, S. E. et al. *Nat. Med.* **13**, 730–735 (2007).
13. Deutsch, A. J. et al. *Blood* **123**, 2367–2377 (2014).
14. Ciaraldi, T. P. et al. *Ann. N.Y. Acad. Sci.* **967**, 66–70 (2002).
15. Chu, J. et al. *Nat. Metab.* **3**, 1466–1475 (2021).

Acknowledgements

S.D. is supported by an award from the Leona M. and Harry B. Helmsley Charitable Trust and by US National Institutes of Health grants 1R01DK107585, 1R01 AI155696 and R56 AG069689.

Competing interests

The author declares no competing interests.