Identification of oligosaccharyltransferase as a host target for inhibition of SARS-CoV-2 and its variants

Yi-Jiao Huang1, Hui Zhao1, Xun Huang1, Yong-Qiang Deng1, Xiao-Feng Li1, Qing Ye1, Rui-Ting Li1, Yan-Peng Xu1, Tian-Shu Cao1 and Cheng-Feng Qin1,2,253

Dear Editor,

According to the report of WHO (https://covid19.who.int/), COVID-19, caused by the pandemic pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to over 250 million confirmed cases and at least 5 million deaths, as of November 2021. Vaccine administration is currently the most effective way to control the COVID-19 pandemic, while novel variants of SARS-CoV-2 with concerning mutations have thrived throughout the world. Many of these variants have been evidenced to enhance viral transmissibility, fitness, infectivity, and even evade protection from vaccines1. Thus, there is an urgent need for developing effective antiviral drugs against SARS-CoV-2. So far, a large panel of antiviral agents showed promising efficiency against SARS-CoV-2 in either preclinical studies or clinical trials2–4.

Targeting host proteins associated with SARS-CoV-2 represents an alternative strategy to antagonize the emerging variants. Therapies that target the host–virus interface, which is relatively conserved between reported SARS-CoV-2 strains, could present broad-spectrum antiviral potentials5,6. Some studies have mapped the SARS-CoV-2–host interactome5,7, however, it remains a challenge to systematically explore the critical host proteins that have been already or potentially targeted by drugs.

The genome of SARS-CoV-2, which is about 29.8 kb in size, comprises two flanking untranslated regions and 14 open reading frames (ORFs) that encode 27 proteins, and shares ~80% nucleotide sequence identity with SARS-CoV8. SARS-CoV-2 contains four structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins8. The spike, located on viral surface, is constituted by the homotrimers of S protein, and responsible for the recognition of host cell receptor(s)9. M protein, containing three transmembrane domains, can shape the virions and promote membrane curvature to facilitate the binding with N proteins, which bind virus RNA genome through different mechanisms9. E protein executes an important role in the assembly and release of virus, and is implicated in modulating viral pathogenesis9. Since these structural proteins play critical roles in the virion assembly and infection of SARS-CoV-2, we screened the host factors that interact with these proteins. We found that the oligosaccharyltransferase (OST) complex is closely associated with E, M and S proteins, and blockade of OST by NGI-1 can significantly inhibit the infection of both SARS-CoV-2 and its variants.

To identify the potential host factors that interact with SARS-CoV-2 structural proteins, we first synthesized codon-optimized virus cDNAs of E, M, S and N proteins, and cloned these ORFs into mammalian expression vectors. The expressed structural proteins contain an N-terminal 3× Flag tag and a C-terminal Twin-Strep-tag. We next expressed these structural proteins in HEK293T, and screened the interacting host proteins by affinity-purification-mass spectrometry (AP-MS). We found that E, M and S proteins can associate with many N-Glycosylation-related proteins, especially the OST complex, which can catalyze the N-linked glycosylation of newly synthesized proteins10 (Fig. 1a). In addition to E, M, S and N proteins, we also detected the interacting proteins of another SARS-CoV-2-derived protein, 3a, which has been reported as a potential structural glycoprotein in SARS-CoV11, similar to SARS-CoV-2 M protein. Several N-Glycosylation-related proteins can interact with 3a

Correspondence: Cheng-Feng Qin (qincf@bmi.ac.cn)
1State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, China
2Research Unit of Discovery and Tracing of Natural Focus Diseases, Chinese Academy of Medical Sciences, Beijing, China
These authors contributed equally: Yi-Jiao Huang, Hui Zhao, Xun Huang.
**Fig. 1** (See legend on next page.)
Supplementary Fig. S1b). Blockade of either STT3A or N-Glycosylation, proteins E, M and S proteins, showed between the structural proteins and STT3A/STT3B by Huang et al. PNGase F, an amidase that can remove N-linked oligosaccharides, which is consistent with other lower than 180 kDa (Fig.1b), indicating that spike is heavily N-Glycosylated, and OST complex may play an role. There was no obvious variation observed for 3a and S proteins after PNGase F treatment (Supplementary Fig. S1a). These results indicated that E, M and S proteins may be N-Glycosylated in host cells. To examine this hypothesis, we treated the pull-down samples with PNGase F, an amidase that can remove N-linked oligosaccharides from glycoproteins. As shown in Fig. 1b, after PNGase F treatment, the major spike band shifted to lower than 180 kDa (Fig. 1b), indicating that spike is heavily N-Glycosylated, which is consistent with other studies. Different from S protein, both E and M proteins displayed broad bands and main bands. After PNGase F treatment, the broad bands were decreased, while the main bands of E and M (~20 kDa and ~30 kDa, respectively) were increased (Fig. 1b), suggesting that both E and M proteins may be glycosylated, while other post-translational modification or oligomerization may also occur. There was no obvious variation observed for 3a and N proteins after PNGase F treatment (Supplementary Fig. S1a). These results indicated that E, M and S proteins were N-Glycosylated, and OST complex may play an important role in mediating this post-translational modification.

There are two OST protein isoforms in mammalian cells. The two isoforms are composed of a catalytic subunit (encoded by the paralogs STT3A or STT3B) and accessory subunits. We then examined the interaction between the structural proteins and STT3A/STT3B by pull-down assay. Among all tested proteins, three N-Glycosylated proteins, E, M and S proteins, showed strong interaction with both STT3A and STT3B (Fig. 1c; Supplementary Fig. S1b). Blockade of either STT3A or STT3B by siRNA or CRISPR-Cas9 sgRNA suppressed SARS-CoV-2 infection (Fig. 1d, e). These results indicated that both OST isoforms could modulate functions of E, M and S proteins, and thus are critical for SARS-CoV-2 infection.

We next sought to confirm whether OST can serve as a potential target for treating SARS-CoV-2 infection. NGI-1, a small molecule that directly targets STT3A and STT3B, is an inhibitor of N-linked glycosylation with pan-flaviviral activity. We first detected whether NGI-1 affected the N-glycosylation of SARS-CoV-2 E, M and S proteins. When treated with NGI-1, S protein, which was overexpressed in HEK293T, was detected at lower molecular weight, indicating that NGI-1 decreased the N-glycosylation of spike (Fig. 1f), while E and M proteins showed no obvious decrease (Supplementary Fig. S2a). We next analyzed the modification changes of S protein with quantitative mass spectrometry. We detected two N-glycosylated sites of high abundance of spike, N1074 and N1194, and the proportion of N-glycosylated peptides of spike at N1074 site decreased from 87.39% to 65.92% (Fig. 1g). These results suggested that NGI-1 could decrease the N-Glycosylation of SARS-CoV-2.

Then, we investigated the antiviral effect of NGI-1 in human colorectal adenocarcinoma Caco2 cells. After 3-day infection, we examined virus replication in both the culture medium and Caco2 cells, and found a strong inhibition effect of NGI-1 on SARS-CoV-2 (Fig. 1h, i; Supplementary Fig. S2b). The half inhibitory dose-response curve was fitted by GraghPad Prism 8.
therapies that target the host as a broad-spectrum anti-coronavirus drug. Results indicated that the OST inhibitor NGI-1, can serve 229E (Supplementary Fig. S3a, b). Taken together, these findings suggest that the OST inhibitor NGI-1, can serve as a potential target for treating COVID-19. Indeed, we found that blockade of the catalytic subunit, STT3A or STT3B, of OST, suppressed SARS-CoV-2 infection.

In the present study, we focused on the potential therapies that target the host–virus interface, especially the interactions involving structural proteins of SARS-CoV-2. By AP-MS, we found that E, M and S proteins were all N-glycosylated, and were closely related to OST-variants. A recent study showed that SARS-CoV-2 variants are supposed to be sensitive to NGI-1. In addition, by comparing several concerned variants with SARS-CoV-2, we found that mutations in structural proteins, especially spike, have no effect on the existing or potential N-linked glycosylation sites (sequons, (NXT/S/C; X≠p)10). Interestingly, the SARS-CoV-2 variant 501Y.V3 contained the T20N mutation in S protein, which may generate an additional N-glycosylation site that can be catalyzed by OST (from TRT to NRT). The biological impact of T20N mutation remains to be determined. Moreover, the appropriate SI of NGI-1 in various cell lines supports further validation in animal models.

Acknowledgements
This work was supported by grants from the National Key R&D Program of China (2020YFC0841000, 2020YFA0707801, and 2021YFC0863300) and the National Natural Science Foundation of China (NSFC, 32000663, 82073621). C.-F.Q. was supported by the National Science Fund for Distinguished Young Scholar (81925025), and the Innovative Research Group (81621005) from the NSF, and the Innovation Fund for Medical Sciences (2019I2M-S-049) from the Chinese Academy of Medical Sciences.

Author contributions
C.-F.Q. conceived and supervised the project. Y.-J.H. designed the experiments and performed the affinity purification, drug treatment and cell experiments with the help from Y.-Q.D., X.-F.L., and Q.Y.; Y.-J.H. and R.-T.L. performed mass spectrometry analysis; Y.-J.H. and H.Z. analyzed the data; Y.-J.H. and C.-F.Q. wrote the manuscript.

Conflict of interest
The authors have filed a patent related to the results reported in this work.

Publisher’s note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s41421-021-00354-2.

Received: 10 May 2021 Accepted: 11 November 2021
Published online: 30 November 2021

References
1. Plante, J. A. et al. The variant gambit: COVID-19’s next move. Cell Host Microbe 29, 506–515 (2021).
2. Wang, M. et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 30, 269–271 (2020).
3. Wang, G. et al. Dalbavancin binds ACE2 to block its interaction with SARS-CoV-2 spike protein and is effective in inhibiting SARS-CoV-2 infection in animal models. Cell Res. 31, 17–24 (2021).
4. Zu, S. et al. 25-Hydroxycholesterol is a potent SARS-CoV-2 inhibitor. Cell Res. 30, 1043–1045 (2020).
5. Gordon, D. E. et al. SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature 583, 459–468 (2020).
6. Prussia, A., Thepchati, P., Snyder, J. P. & Plempner, R. K. Systematic approaches towards the development of host-directed antiviral therapeutics. Int. J. Mol. Sci. 12, 4027–4052 (2011).
7. Cava, C., Bentoli, G. & Castiglioni, I. A protein interaction map identifies existing drugs targeting SARS-CoV-2. BMC Pharmacol. Toxicol. 21, 65 (2020).
8. Wu, A. et al. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 27, 325–328 (2020).
9. Bhat, E. A. et al. SARS-CoV-2. Insight in genome structure, pathogenesis and viral receptor binding analysis — an updated review. Int. Immunopharmacol. 95, 107465 (2021).
10. Shimal, S. & Gilmore, R. Oligosaccharyltransferase structures provide novel insight into the mechanism of asparagine-linked glycosylation in prokaryotic and eukaryotic cells. Glycobiology 29, 288–297 (2019).
11. Oostra, M., de Haan, C. A., de Groot, R. J. & Rottier, P. J. Glycosylation of the severe acute respiratory syndrome coronavirus triple-spanning membrane proteins 3a and M. J. Virol. 80, 2326–2336 (2006).
12. Tian, W. et al. O-glycosylation pattern of the SARS-CoV-2 spike protein reveals an “O-Follow-N” rule. Cell Res. 31, 1123–1125 (2021).
13. Yao, H. et al. Molecular architecture of the SARS-CoV-2 virus. Cell 183, 730–738 e713 (2020).
14. Puschnik, A. S. et al. A small-molecule oligosaccharyltransferase inhibitor with pan-flaviviral activity. Cell Rep. 21, 3052–3059 (2017).
15. Gasas-Sanchez, A. et al. Protein N-glycosylation is essential for SARS-CoV-2 infection. bioRxiv. https://doi.org/10.1101/2021.02.05.429940 (2021).