SARS-CoV-2 Spike protein S2 subunit modulates γ-secretase and enhances amyloid-β production in COVID-19 neuropathy

Guanqin Ma1, Deng-Feng Zhang1,2,3, Qing-Cui Zou2, Xiaochun Xie1, Ling Xu1,2,3, Xiao-Li Feng2, Xiaohong Li1, Jian-Bao Han2, Dandan Yu1,2,3, Zhong-Hua Deng2, Wang Qu2, Junyi Long2, Ming-Hua Li2, Yong-Gang Yao1,2,3,4✉ and Jianxiong Zeng1,2,3,5✉

Dear Editor,

SARS-CoV-2-induced multi-lineage neural cell dysregulation has been documented1. SARS-CoV-2 infection elevates neuroinflammation2, alters brain structure3, leads to abnormal accumulation of neurodegenerative amyloid-β (Aβ) and phosphorylated tau4,5, and increases the risk of cognitive impairment6 in COVID-19 patients. However, the mechanism underlying neurological dysfunctions following SARS-CoV-2 infection remains largely unknown.

To evaluate long-term impact of SARS-CoV-2 infection to the brain, the hACE2 transgenic mice as described previously7 were intranasally infected with a single low dose (1 × 102 TCID50) of prototyped SARS-CoV-2 and maintained for up to 30 days post infection (dpi) (Fig. 1a). Presence of SARS-CoV-2 was found in cortex at 7 dpi but not at 30 dpi by the viral Spike protein immunostaining (Supplementary Fig. S1a). We found a remarkable activation of Iba1+ microglia and GFAP+ astrocytes in the hippocampus and cortex of infected mice at 30 dpi (Supplementary Fig. S1b–e), suggesting a persistent neuroinflammation. We looked for further brain changes by analyzing transcriptomics of the hippocampal tissues at 30 dpi (Supplementary Fig. S2a). A series of dysregulated genes or pathways were identified in response to SARS-CoV-2 infection (Supplementary Table S1). Gene ontology analysis revealed that the upregulated genes were mainly enriched in pathways related to antiviral immune response and aging, while the downregulated genes were enriched in neuronal function-related pathways such as synaptic vesicle clustering (Fig. 1b). Specifically, the neuroinflammatory genes Trem2, Ifitm3 and Gfap were significantly upregulated, whereas the neuronal genes Map2 and Synapsin II (Syn2) were downregulated. Unexpectedly, mRNA levels of amyloid precursor protein (APP) processing-related genes such as Bace1, Aph1, Presenilin 1 (Psen1), Nicastrin (Ncstn), and Psenen were unchanged (Fig. 1c). The upregulation of Trem2 and Gfap, the downregulation of Map2 and Syn2, and the un-alteration of Bace1 and Psen1 were validated by quantitative real-time PCR (Supplementary Fig. S2b). Such expression patterns were also observed in the brain transcriptomic dataset obtained from COVID-19 patients by single-nucleus RNA sequencing8 (Supplementary Fig. S3a–c). These results suggest that the presence of the neurodegenerative hallmarks in COVID-19 brain might not be regulated at the transcriptional level but through an unknown regulatory mechanism.

To explore potential mechanisms underlying COVID-19-related neuropathology, we tested whether SARS-CoV-2 membrane protein plays a role in this process. The γ-secretase complex, comprising PEN-2, APH-1, PS1 and NCT, is a critical membrane complex contributing to Aβ production in Alzheimer’s disease (AD) pathogenesis9. Initially, we conducted co-immunoprecipitation (co-IP) in...
Ma et al. Cell Discovery (2022) 8:99

Fig. 1 (See legend on next page.)
HEK293T cells and found that SARS-CoV-2 Spike S2 subunit (S-S2), but not S-S1 protein, interacted individually with PEN-2 (Fig. 1d), APH-1 (Fig. 1e), PS1 (Fig. 1f) and NCT (Fig. 1g), and even bound to all these four components (Fig. 1h). The inverse co-IP could validate the interactions between S-S2 and PS1 or NCT (Supplementary Fig. S4a, b). To determine whether C-terminal transmembrane domain in S-S2 constitutes the structural basis for its interaction with γ-secretase, we examined membrane (M) protein of SARS-CoV-2 but found no interaction with PEN-2 and PS1 (Supplementary Fig. S4c, d), suggesting a specific interaction between S-S2 and γ-secretase. We next performed glutathione s-transferase (GST) pull-down and found that S-S2 can directly bind to PS1 and NCT (Supplementary Fig. S4e, f). Immunocytochemistry assay showed the co-colocalization of S-S2 with γ-secretase components individually in Hela cells (Supplementary Fig. S4g–j) and in the brain sections of infected mice at 7 dpi (Supplementary Fig. S4k).

SARS-CoV-2 Omicron variant (BA.1) Spike S2 subunit possesses six mutations (N764K, D796Y, N856K, Q954H, N969K, and L981F) compared to the prototype. To see whether these mutations would interfere with its interaction with γ-secretase, co-IP assay in HEK293T cells showed that Omicron S-S2 not only interacted efficiently with PS1 and NCT (Supplementary Fig. S5a, b), but also had a comparable binding capacity to PS1 and NCT as prototyped S-S2 (Supplementary Fig. S5c, d), suggesting Omicron BA.1 S-S2 is capable of binding to γ-secretase.

An enzymatic cleavage of the APP by both β-secretase and γ-secretase, acting together, produces Aβ, which can cause widespread neuropathy within brain and is a pathological hallmark of AD. The cleavage site of γ-secretase is located on C-terminal APP, namely APP C-terminal 99 fragment (APP-C99) only contains the cleavage site of γ-secretase. As a result, APP intracellular domain (AICD) at C-terminal C99 domain is produced by γ-secretase cleavage. To examine whether the interaction between S-S2 and γ-secretase modulates the cleavage activity, we initially detected the production of AICD. Immunoblot showed that prototyped S-S2 promoted the production of flag-tagged AICD, whereas the expression of APP and NCT was largely unchanged (Fig. 1i). This was validated by the observation of the increased production of flag-tagged AICD in U251-C99 cells while the expression of APP and NCT was largely unaltered (Fig. 1j). Similarly, Omicron S-S2 also increased the production of flag-tagged AICD, while the expression of APP and PS1 was unchanged (Fig. 1k). These results demonstrate that the increased production of AICD from the APP cleavage was caused by S-S2 modulation of γ-secretase.

HEK-APP69 cells transfected with prototyped S-S2, but not the M, produced higher level of Aβ40 than non-transfected cells via enzyme-linked immunosorbent assay (ELISA), while a similar increase of Aβ40 was also observed upon the transfection of IFITM3 as a positive control (Supplementary Fig. S6a). To further evaluate this effect, we used neuronal cells including U251 and mouse primary neurons, both endogenously expressing APP protein. Lentiviral transduction of prototyped S-S2 or IFITM3 invariably caused the increase of Aβ40 or Aβ42 production as compared to empty-vector lentivirus transduction in U251 cells (Supplementary Fig. S6b) and mouse primary neurons (Fig. 1l), whereas lentiviral transduction of the M did not have such an effect. As expected, mouse primary neurons transduced with lentiviral Omicron-S-S2 produced higher Aβ40 and Aβ42 levels (Supplementary Fig. S6c). These results demonstrate
that SARS-CoV-2 Spike S2 subunit can modulate γ-secretase to increase Aβ production.

To investigate whether S-S2 modulates γ-secretase in vivo, we examined hippocampal and cortical tissues of APPswe/PSEN1ΔE9 mice, which have mutated human APP (Swedish mutations K595N/M596L) and the human PSEN1/PS1 lacking exon 9 [14], 2 months after AAV delivery of S-S2. Immunohistochemistry showed a widespread overexpression of S-S2 in hippocampal tissues (Supplementary Fig. S7a). Measurement of soluble and insoluble Aβ levels using ELISA showed that soluble Aβ42 species, but not insoluble Aβ40 and Aβ42 and soluble Aβ40, were markedly increased in cortical tissues of APP/PS1ΔE9 mice with S-S2 overexpression relative to empty vector group (Supplementary Fig. S7b–e). Similarly, immunostaining showed a significant increase of Aβ burden in cortical and hippocampal tissues of APP/PS1ΔE9 mice after S-S2 delivery (Fig. 1m). The delivery of S-S2 increased the Aβ plaque-deposited area in cortical and hippocampal tissues of APP/PS1ΔE9 mice (Fig. 1n). Overall, overexpression of SARS-CoV-2 S-S2 in hippocampus exacerbated Aβ burden in APP/PS1ΔE9 mice.

Neuroinflammation, an important factor in AD pathogenesis, promotes Aβ pathology [15]. A significant increase of Iba1+ microglia and GFAP+ astrocytes (Supplementary Fig. S8a–c) was observed in hippocampal tissues of APP/PS1ΔE9 mice after delivery of S-S2. Staining of microglial marker TMEM119 validated the elevated neuroinflammation following S-S2 delivery (Fig. 1o, p). These results demonstrated that S-S2 overexpression increased Aβ deposition and caused neuroinflammation in Aβ pathology of APP/PS1ΔE9 mice. Both the area covered by NeuN-labeled neuronal cells and the thickness of NeuN-labeled CA1 subfield (Supplementary Fig. S8d–f) were not significantly altered in hippocampal tissues following S-S2 delivery, suggesting that S-S2 overexpression might not cause neuronal loss after AAV delivery for 2 months.

In summary, we have identified S-S2 subunit as a γ-secretase modulatory protein and revealed a previously unknown mechanistic insight into COVID-19-related neuropathological sequelae (Supplementary Fig. S9). A systematical examination of multiple Omicron subvariants (Supplementary Fig. S10) on potential brain dysfunction would be inspired in future studies. The Spike protein could function as an immune switch to increase γ-secretase activity and Aβ production and contribute to neurological changes in COVID-19 patients.
8. Sisodia, S. S. & St George-Hyslop, P. γ-Secretase, notch, Aβ and alzheimer’s disease: where do the presenilins fit in? Nat. Rev. Neurosci. 3, 281–290 (2002).
9. Mannar, D. et al. SARS-CoV-2 Omicron variant: Antibody evasion and cryo-EM structure of spike protein-ACE2 complex. Science 375, 760–764 (2022).
10. O’Brien, R. J. & Wong, P. C. Amyloid precursor protein processing and Alzheimer’s disease. Annu. Rev. Neurosci. 34, 185–204 (2011).
11. Knopman, D. S. et al. Alzheimer disease. Nat. Rev. Dis. Prim. 7, 33 (2021).
12. Hung, A. Y., Koo, E. H., Haass, C. & Selkoe, D. J. Increased expression of beta-amyloid precursor protein during neuronal differentiation is not accompanied by secretory cleavage. Proc. Natl. Acad. Sci. USA 89, 9439–9443 (1992).
13. Hur, J. Y. et al. The innate immunity protein IFITM3 modulates gamma-secretase in Alzheimer’s disease. Nature 586, 735–740 (2020).
14. Jankowsky, J. L. et al. Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase. Hum. Mol. Genet. 13, 159–170 (2004).
15. Pascoal, T. A. et al. Microglial activation and tau propagate jointly across Braak stages. Nat. Med. 27, 1592–1599 (2021).