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Deficiency of HTR4 and ADRB1 caused by SARS-CoV-2 spike may partially explain multiple COVID-19 related syndromes including depression, cognitive impairment, loss of appetite, heart failure, and hypertension

Dear Editor,

Previously, in this journal, we reported that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) blocked sorting nexin 27 (SNX27)-mediated endocytic recycling of angiotensin-converting enzyme 2 (ACE2) and glucose transporter type 1 (GLUT1). By its postsynaptic density protein 95/Disc large protein/Zonula occludens 1 (PDZ) domain, SNX27 recognizes PDZ domain binding motif (PBM) in multiple endocytic recycling membrane proteins, which are functional in cell surface. For instance, cell surface levels of serotonin (5-hydroxytryptamine [5-HT]) receptor 4 (HTR4) and beta-1 adrenergic receptor (ADRB1) are dependent on endocytic recycling from endosomes to the plasma membrane mediated by SNX27. Inhibition of HTR4 is known to cause major depressive disorder (MDD), anxiety, cognitive impairment, and loss of appetite. Inhibition of ADRB1 could induce heart failure and hypertension. Depression, anxiety, cognitive impairment, loss of appetite, heart failure, and hypertension are also observed in acute Coronavirus Disease 19 (COVID-19) and long COVID patients. However, the detailed mechanism how these symptoms occur is not conclusive. To address this question, we hypothesize that the plasma membrane localization of HTR4 and ADRB1 is reduced by SARS-CoV-2 S.

First, we validated the interaction between HTR4 and SNX27 by GST pulldown experiments. As shown in Fig.1A, bacterial expressed GST-tagged PDZ domain of SNX27 (GST-SNX27 PDZ), and GST-SNX27, but not GST could pull down Flag-tagged HTR4 (Flag-HTR4). In order to recycle cargoes back to plasma membrane, SNX27 associates with Vps26, a subunit of retromer composed of Vps35, Vps29 and Vps26. Previously, we uncovered that SARS-CoV-2 S abrogated SNX27-Vps26A interaction. However, S-T1238A, a mutant losing binding affinity to SNX27, did not affect the interaction between SNX27 and Vps26A. To examine whether SARS-CoV-2 S affects the plasma membrane localization of HTR4, we performed the confocal immunofluorescent analysis experiments. Compared with GFP, GFP-S but not GST-S-T1238A mutant reduced the surface level of Flag-HTR4 in both HEK293T cells (Fig. 1B and C) and HeLa cells (Fig. 1D and E). We concluded that SARS-CoV-2 S could suppress the endocytic recycling of HTR4 mediated by SNX27, resulting in the reduction of surface level of HTR4.

Next, we confirmed the interaction between ADRB1 and SNX27 by GST pulldown experiments. GST-SNX27 PDZ but not GST could pull down HA-tagged ADRB1 (HA-ADRB1) (Fig. 2A). To examine whether SARS-CoV-2 S affects the plasma membrane localization of ADRB1, we carried out the confocal immunofluorescent analysis experiments. Compared with GFP, GPP-S but not GSP-S-T1238A mutant reduced the surface level of HA-ADRB1 in both HEK293T cells (Fig. 2B and C) and HeLa cells (Fig. 2D and E). We concluded that SARS-CoV-2 S could inhibit the endocytic recycling of ADRB1 mediated by SNX27, leading to the reduction of surface level of ADRB1.

Depression, anxiety, cognitive impairment, loss of appetite, heart failure, and hypertension are symptoms of COVID-19 patients. Our current finding supports a model that depressive symptoms, anxiety cognitive impairment, loss of appetite, heart failure, and hypertension are partially due to the deficiency of HTR4 and ADRB1 by SARS-CoV-2 S. To fulfill their functions, HTR4 and ADRB1 are delivered from endosome to plasma membrane by SNX27 and retromer (Fig. 2F). However, upon SARS-CoV-2 infection, SARS-CoV-2 S suppressed endocytic recycling of HTR4 and ADRB1 by inhibiting the interaction between SNX27 and Vps26A. Subsequently, surface levels of HTR4 and ADRB1 were reduced, leading to depression, anxiety, cognitive impairment, loss of appetite, heart failure, and hypertension (Fig. 2F).

Previously, we discussed that adverse events of S-based mRNA vaccines against SARS-CoV-2, such as myocarditis, pericarditis, cervical dystonia and ataxia, may be due to the deficiency of ACE2 and GLUT1 by S. In current study, we uncovered the role for SARS-CoV-2 S in suppressing endocytic recycling of HTR4 and ADRB1 by targeting SNX27. ADRB1 positively regulates heart rate, heart contraction and systemic arterial blood pressure, while HTR4 is important for procognitive effects, antidepressant effects and appetite. Therefore, by targeting SNX27, SARS-CoV-2 S may increase the incidence of depressive symptoms, anxiety, heart failure, hypertension, and loss of appetite. Because of less inhibition of SNX27- mediated endocytic recycling, T1238A mutant of SARS-CoV-2 S would be a better backbone for mRNA vaccine against SARS-CoV-2.

In conclusion, we reveal that SARS-CoV-2 S reduces the surface level of HTR4 and ADRB1. SARS-CoV-2 S suppresses endocytic recycling of HTR4 and ADRB1 by abrogating SNX27-Vps26A interaction, reducing the surface levels of HTR4 and ADRB1. Our results provide the link between SARS-CoV-2 and HTR4/ADRB1 trafficking, which advances our understanding of HTR4/ADRB1 deficiency by SARS-CoV-2 and will explain the multiple COVID-19 related diseases caused by the decrease of HTR4/ADRB1, such as depression, cognitive impairment, loss of appetite, heart failure, and hypertension. Also, our study could predict some side effects of S-based mRNA vaccine against SARS-CoV-2.

https://doi.org/10.1016/j.jinf.2022.11.021
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Fig. 1. SARS-CoV-2 S reduces surface level of HTR4. (A) HTR4 interacts with SNX27 and its PDZ domain in GST pulldown experiments. HEK293T cells were transfected with constructs expressing Flag-HTR4 and cell lysates were used in a pulldown assay with purified GST, GST-SNX27, or GST-SNX27 PDZ. Input represents 5% of total cell lysates. (B) SARS-CoV-2 S reduces surface level of HTR4 in HEK293T cells. HEK293T cells transfected with the constructs expressing Flag-HTR4 and GFP-tagged SARS-CoV-2 S, T1238A mutant of SARS-CoV-2 S, or GFP were fixed with methanol and stained with antibodies against Flag (red) and GFP (green). Scale bar: 10 μM. (C) Relative HTR4 intensity was normalized by quantifying at least 20 HEK293T cells through Image J. ****, p value < 0.0001. (D) SARS-CoV-2 S reduces surface level of HTR4 in HeLa cells. HeLa cells transfected with Flag-HTR4 and GFP-S, GFP-S T1238A mutant, or GFP were fixed with 4% paraformaldehyde and stained with antibodies against Flag (red) and GFP (green). Scale bar: 10 μM. (E) Relative HTR4 intensity was normalized by quantifying at least 20 HeLa cells through Image J. ****, p value < 0.0001.
Fig. 2. SARS-CoV-2 S reduces surface level of ADRB1. (A) ADRB1 associates with PDZ domain of SNX27. Lysates from HEK293T cells transfected with constructs expressing HA-ADRBI were pulled down by GST or GST-SNX27 PDZ. Input represents 1% of total cell lysates. (B) SARS-CoV-2 S reduces surface level of ADRB1 in HEK293T cells. HEK293T cell transfected with the constructs expressing HA-ADRBI and GFP-tagged SARS-CoV-2 S, T1238A mutant of SARS-CoV-2 S, or GFP were fixed methanol and stained with HA (red) and GFP (green) antibodies. Scale bar: 10 μM. (C) Relative ADRB1 intensity was normalized by quantifying at least 20 HEK293T cells through Image J. ****, p value < 0.0001. (D) SARS-CoV-2 S reduces surface level of ADRB1 in HeLa cells. HeLa cells transfected with the constructs expressing HA-ADRBI and GFP-tagged SARS-CoV-2 S, T1238A mutant of SARS-CoV-2 S, or GFP were fixed methanol and stained with HA (red) and GFP (green) antibodies. Scale bar: 10 μM. (E) Relative ADRB1 intensity was normalized by quantifying at least 20 HeLa cells through Image J. ****, p value < 0.0001. (F) Schematic model of how SARS-CoV-2 S inhibits endocytic recycling of HTR4 and ADRB1 mediated by SNX27. Endocytic recycling of HTR4 and ADRB1 from endosome to plasma membrane is mediated by SNX27 and retromer. SARS-CoV-2 S could inhibit plasma membrane targeting of HTR4 and ADRB1 by competing with Vps26A for its association of SNX27, resulting in the reduction of HTR4 and ADRB1 on the cell surface.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (82272306 and 82072270), Taishan Scholars Program, and the Academic Promotion Program of Shandong First Medical University (2019J001).

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L. Lv, A. Li, L. Jiang et al.

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