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Letter to the Editor

Inhibition of endocytic recycling of ACE2 by SARS-CoV-2 S protein partially explains multiple COVID-19 related diseases caused by ACE2 reduction

Dear editor

In this Journal, Li and colleagues described the comparative biology of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor ACE2 and provided some explanation of the host range.[1] Reduction of ACE2 protein level leads to a variety of diseases, such as lung injury, hypertension and abnormal coagulation.[2] It has been reported that the protein level of ACE2 decreases during SARS-CoV-2 infection.[3] To explain this phenomenon, we hypothesize that SARS-CoV-2 disrupts the homeostasis of ACE2 by interfering its trafficking to plasma membrane.

Sorting nexin 27 (SNX27) mediates endocytic recycling of cargo proteins containing C-terminal PSD95/Dlg1/ZO-1 (PDZ)-binding sequences from endosomes to the plasma membrane, preventing their lysosomal degradation.[4] Human ACE2 (hACE2) contains a SNX27-PDZ domain binding motif DVQTSF in its C terminus (Fig. 1A), indicating that ACE2 may interact with SNX27. Indeed, GST pulldown experiments demonstrated that hACE2 but not hACE2T803A/F805A associated with both PDZ domain of SNX27 and full length SNX27 (Fig. 1B). Immunofluorescence staining

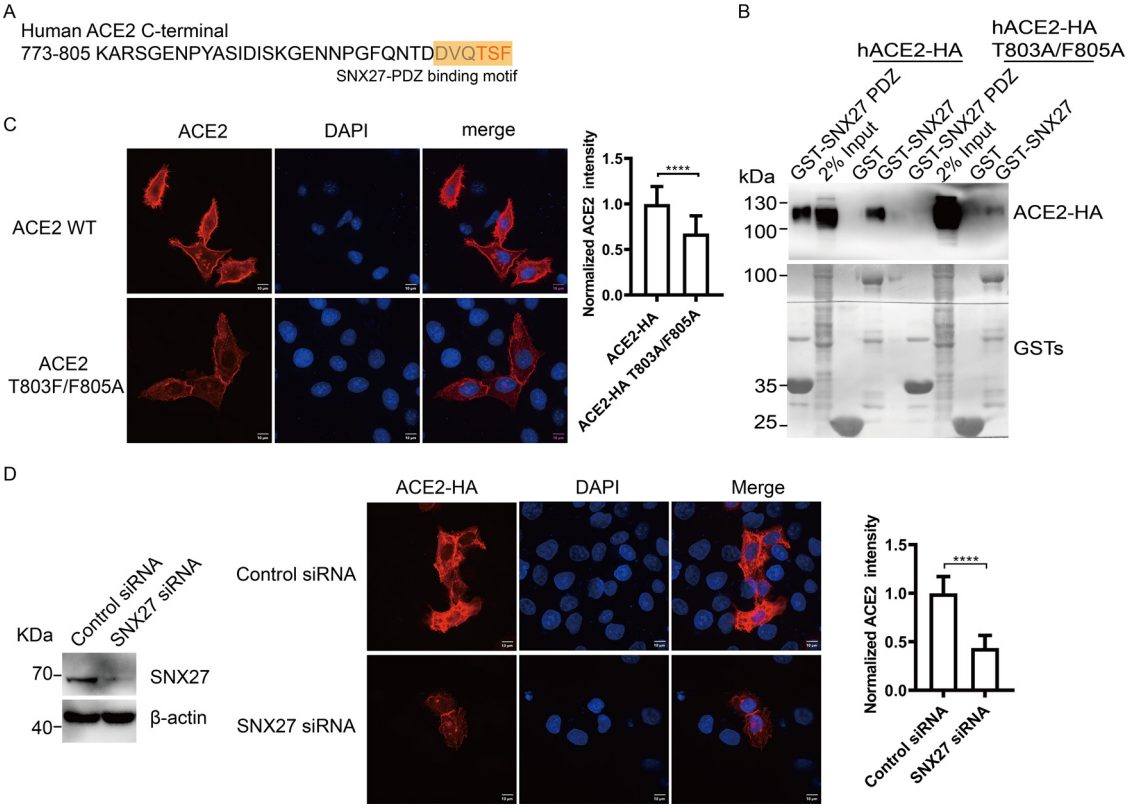

Fig. 1. Endocytic recycling of ACE2 is mediated by SNX27. (A) Human ACE2 contains a SNX27 PDZ binding motif in the C-terminus. (B) Both SNX27 and its PDZ domain associate with ACE2 but not T803A/F805A mutant of ACE2 in GST pulldown experiments. Lysates from 293T cells transfected with the constructs expressing HA-tagged wild type hACE2 and T803A/F805A mutant of hACE2 were pulled down by GST–SNX27, GST-SNX27 PDZ or GST. Input represents 2% of total cell lysates. (C) Surface level of T803A/F805A mutant of ACE2 is weaker than that of wild type ACE2. HeLa cells transfected with the constructs expressing HA-tagged wild type hACE2 and T803A/F805A mutant of hACE2 were stained with HA antibody. ACE2-HA and T803A/F805A mutant of ACE2-HA are in red. Nucleus stained with DAPI is in Blue. Scale bar: 10 μM. Relative ACE2 intensity was normalized by quantifying at least 20 cells through Image J. ****, p value $<$ 0.0001. (D) Silencing SNX27 by siRNA reduces surface level of ACE2. HeLa cells treated with siRNA against SNX27 for 3 days and then transfected with the construct expressing HA-tagged wild type hACE2 were stained with HA antibody. ACE2-HA is in red. Nucleus stained with DAPI is in Blue. Scale bar: 10 μM. Relative ACE2 intensity was normalized by quantifying at least 20 cells through Image J. ****, p value $<$ 0.0001. HeLa cells were treated with siRNA against SNX27 for 3 days and then blotted with antibodies against SNX27 or β-actin.

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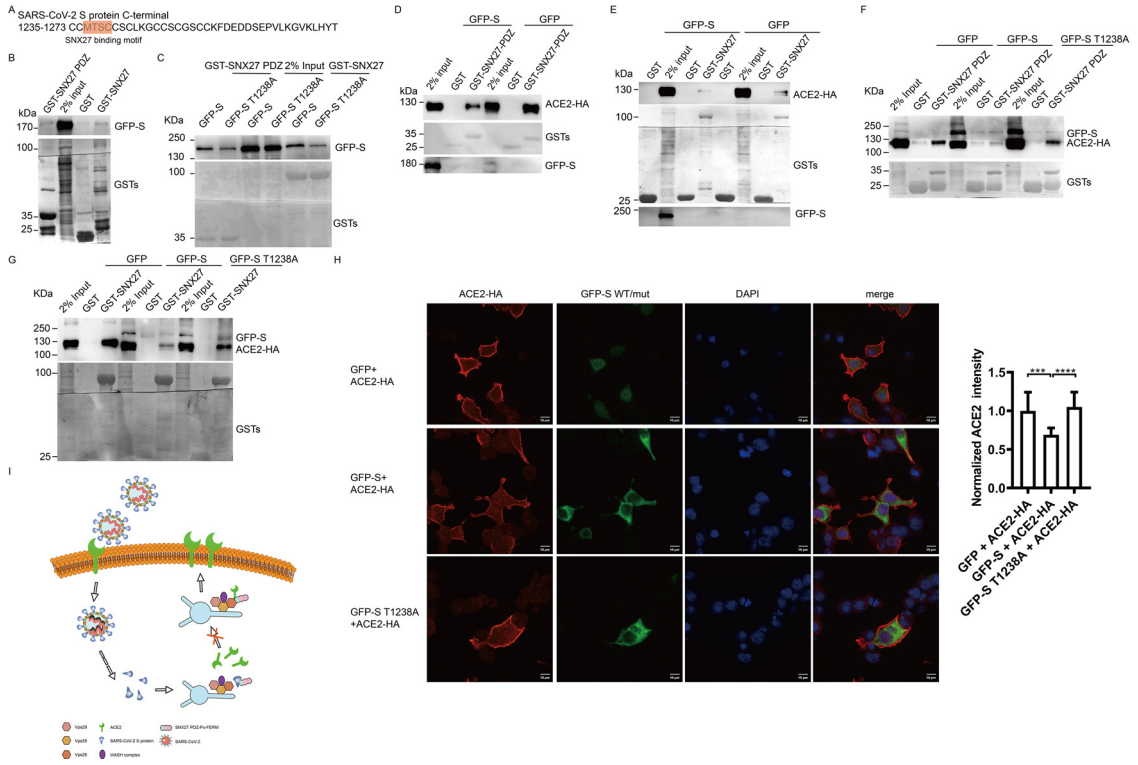

Fig. 2. SARS-CoV-2 S protein reduces the surface level of ACE2 by associating with SNX27. (A) SARS-CoV-2 S protein contains a SNX27 binding motif in the C-terminus. (B) Both SNX27 and its PDZ domain associate with SARS-CoV-2 S. Lysates from 293T cells transfected with the constructs expressing GFP-tagged SARS-CoV-2 S were pulled down by GST–SNX27, GST-SNX27 PDZ or GST. Input represents 2% of total cell lysates. (C) Both SNX27 and its PDZ domain associate with SARS-CoV-2 S but not T1238A mutant of SARS-CoV-2 S in GST pulldown experiments. Lysates from 293T cells transfected with the constructs expressing GFP-tagged SARS-CoV-2 S or T1238A mutant of SARS-CoV-2 S were pulled down by GST–SNX27 or GST-SNX27 PDZ. Input represents 2% of total cell lysates. (D) SARS-CoV-2 S inhibits the interaction between ACE2 and SNX27 PDZ domain in GST pulldown experiments. Lysates from 293T cells transfected with the constructs expressing HA-tagged ACE2 with GFP-tagged SARS-CoV-2 S or GFP were pulled down by GST–SNX27 PDZ or GST. Input represents 2% of total cell lysates. (E) SARS-CoV-2 S suppresses the interaction between ACE2 and SNX27 in GST pulldown experiments. Lysates from 293T cells transfected with the constructs expressing HA-tagged ACE2 with GFP-tagged SARS-CoV-2 S or GFP were pulled down by GST–SNX27 or GST. Input represents 2% of total cell lysates. (F) SARS-CoV-2 S but not T1238A mutant of SARS-CoV-2 S inhibits the interaction between ACE2 and SNX27 PDZ domain in GST pulldown experiments. Lysates from 293T cells transfected with the constructs expressing HA-tagged ACE2 with GFP-tagged SARS-CoV-2 S, T1238A mutant of SARS-CoV-2 S, or GFP were pulled down by GST–SNX27 PDZ or GST. Input represents 2% of total cell lysates. (G) SARS-CoV-2 S but not T1238A mutant of SARS-CoV-2 S suppresses the interaction between ACE2 and SNX27 in GST pulldown experiments. Lysates from 293T cells transfected with the constructs expressing HA-tagged ACE2 with GFP-tagged SARS-CoV-2 S, T1238A mutant of SARS-CoV-2 S, or GFP were pulled down by GST–SNX27 or GST. Input represents 2% of total cell lysates. (H) Compared with GFP, GFP-S but not GSP-S-T1238A mutant reduces the surface level of ACE2. HeLa cells transfected with the constructs expressing HA-tagged ACE2 with GFP-tagged SARS-CoV-2 S, T1238A mutant of SARS-CoV-2 S, or GFP were stained with HA antibody. ACE2-HA is in red. SARS-CoV-2 S, T1238A mutant of SARS-CoV-2 S, and GFP are in green. Nucleus stained with DAPI is in Blue. Scale bar: 10 μM. Relative ACE2 intensity was normalized by quantifying at least 20 cells through Image J. ***, p value < 0.001; ****, p value < 0.0001. (I) Model of how SARS-CoV-2 S blocks ACE2 transport mediated by SNX27. Endocytic recycling of ACE2 is mediated by SNX27. SARS-CoV-2 S could inhibit plasma membrane targeting of ACE2 by suppressing the association of SNX27 and ACE2, leading to the reduction of ACE2.

showed that the surface signal of ACE2T803A/F805A was weaker than that of wild type ACE2, suggesting that binding to SNX27 was crucial for the plasma membrane targeting of ACE2 (Fig. 1C). Moreover, silencing SNX27 by siRNA reduced the surface level of ACE2, confirming that ACE2 was delivered to the cell surface through SNX27 (Fig. 1D). Taken together, those results demonstrated that endocytic recycling of ACE2 was mediated by SNX27.

SARS-CoV-2 spike (S) protein also contains a potential SNX27 binding motif MTSC in the cytoplasmic tail (Fig. 2A), indicating that S may associate with SNX27. GST pulldown experiments confirmed that S interacted with both PDZ domain of SNX27 and full length SNX27 (Fig. 2B). Further GST pulldown experiments showed that S but not T1238A mutant of S associated with both PDZ domain of SNX27 and full length SNX27, suggesting T1238 in S was critical for its SNX27 association (Fig. 2C). Since both S and ACE2 interacted with PDZ domain of SNX27, we hypothesized that SARS-CoV-2 S competed with ACE2 for associating with SNX27. To test this hypothesis, we performed GST pulldown experiments and found that overexpression of GFP-S but not GFP reduced the binding ability of ACE2 to PDZ domain of SNX27 or full length SNX27 (Fig. 2D and E). However, when overexpressing SARS-CoV-2 S-T1238A in which the SNX27 binding affinity was abolished, the association of ACE2 with SNX27 PDZ or SNX27 was recovered, suggesting that S blocked SNX27-ACE2 interaction through its association with SNX27 (Fig. 2F and G). Compared with GFP, GFP-S but not GSP-S-T1238A mutant reduced the surface level of ACE2, which suggested that S suppressed the endocytic recycling of ACE2 through SNX27 (Fig. 2H). Taken together, SARS-CoV-2 S inhibited the endocytic recycling of ACE2 mediated by SNX27.

Consistent with our study, recent studies found that ACE2 surface localization was mediated by SNX27 and SARS-CoV-2 S associated with SNX27.[5–8] However, those studies did not explore the role for SARS-CoV-2 S in the endocytic recycling of ACE2. Our finding advanced our understanding of many phenomena caused by ACE2-deficiency. In the last two years, long COVID concept has been gradually accepted.[9] Upon SARS-CoV-2 infection, endocytic recycling of ACE2 mediated by SNX27 could be suppressed by SARS-CoV-2 S and surface level of ACE2 would be decreased (Fig. 2I), which could lead to many long COVID diseases due to the deficiency of ACE2. Meanwhile, some side effects of S-based SARS-CoV-2 vaccine may due to the reduction of ACE2 recycling by S. For instance, myocarditis and pericarditis appeared after administra-

tion of BNT162b2 from BioNTech and mRNA-1273 from Moderna.[10] It is possible that ACE2-dificiency by SARS-CoV-2 S causes those side effects. Because T1238A mutant of SARS-CoV-2 S no longer suppresses endocytic recycling of ACE2, this mutant could be a better design for mRNA vaccine against SARS-CoV-2.

In this study, we revealed the mechanism how SARS-CoV-2 S protein lowered the surface level of ACE2. SARS-CoV-2 S decreased the surface level of ACE2 by inhibiting endocytic recycling of ACE2 mediated by SNX27. ACE2 reduction by SARS-CoV-2 is considered as a critical driver for COVID-19 pathology, which could cause multiple diseases, such as lung injury, hypertension and abnormal coagulation. Our study provided new ideas for understanding some symptoms of SARS-CoV-2 infection, which could help the treatment of various diseases caused by SARS-CoV-2.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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