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RAPID COMMUNICATION

Epigenetic regulation of SARS-Cov-2 spike protein modulates cellular viability and nascent RNA transcription in neuronal cells



SARS-CoV-2 has been demonstrated to be highly susceptible to neuron- and glial-like cells,¹ but there is no consensus regarding how the virus affects neurons following neuroinvasion. It is therefore unknown if and how SARS-CoV-2 maintains the homeostasis of neuronal cells. Herein, we revealed that the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein acted as an extracellular remodeling component to undermine epigenetic regulation, perturb nascent RNA (nsRNA) transcription, promote mitochondrial biogenesis, and increase cellular viability in SH-SY5Y cells. Through boosting the expression of KDM6B, RBD proteins decreased H3K27me3. Down-regulation of KDM6B was able to decrease molecular and cellular abnormalities previously mediated by all RBD mutants (K417N/L452R/T478K: KLT; L452R/E484Q: LE; N501Y), including NRF2 expression levels, ROS scavenging capacity, mitochondrial activity, and cell proliferation capacity. Further, we also investigated whether RBD proteins affected mitochondrial biogenesis epigenetically. After treatment with the LE mutant, ChIP-gPCR revealed that H3K27me3 was repressed in the NRF2 promoter regions, resulting in NRF2 activating. This study, therefore, uncovered a novel epigenetic mechanism by which SARS-CoV-2 RBD proteins regulate mitochondrial biogenesis via an extracellular signal transduction channel and remodel neuronal cell proliferation.

COVID-19's ability to evade the immune system and its fast dissemination are both tightly linked to mutations in RBD proteins.² To determine whether RBD proteins may enhance cellular viability, SH-SY5Y cells were grown with RBD proteins (Fig. 1A). Finally, we identified that RBD proteins increased cellular viability in a dose-dependent manner (Fig. 1B). The LE mutant exhibited the greatest improvement in cellular viability, from 107% to 116%

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(Fig. 1C). However, the results were not observed in respiratory epithelial HBE cells, suggesting that this effect may, at least in part, be specific to neuronal cells (Fig. 1D–F). Particularly, all RBD proteins could improve the proliferative capability of cells (Fig. 1G). Hence, our research suggests that RBD proteins could affect neural cell proliferation.

There is a close relationship between mitochondrial function and cellular proliferation. RBD proteins dramatically boosted the fluorescence intensity of mito-trackerlabeled SH-SY5Y cells (Fig. S1A). In contrast to cellular proliferation, the N501Y mutant displayed the greatest increase in mitochondrial bioactivity (Fig. S1B). Subsequent examination with a transmission electron microscope (TEM) found that RBD proteins significantly increased mitochondrial counts by 1.68-1.86 times (Fig. S1C, D). Mitochondrial biogenesis, a cellular physiological response to external stress, is necessary for cellular homeostasis.³ All RBD proteins significantly improved the expression of mitochondrial biogenesis-essential DRP1 and MFF proteins (Fig. S1E-G). Importantly, the N501Y mutant considerably increased MFF (Fig. S1F), which was consistent with the highest rise in mitochondrial bioactivity (Fig. S1B, D), indicating that MFF was the driving force behind the N501Y mutant's increase in mitochondrial bioactivity in neuronal cells.

Numerous investigations have demonstrated that ROS elimination and mitochondrial biogenesis are tightly connected. RBD mutants dramatically decreased total intracellular ROS (cytoROS); nevertheless, no statistically significant difference was observed in the RBD wild-type group (Fig. S2A, B). It is widely accepted that mitochondrial ROS (mtROS) plays a critical role in controlling cellular stress responses. While all RBD mutants were able to eliminate mtROS, the LE mutant was the most effective. Interestingly, both RBD proteins were effective for ROS scavenging, although RBD WT was only weakly effective relative to its mutant (Fig. S2B, C). NRF2 has been linked to

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the regulation of mitochondrial biogenesis and ROS homeostasis.⁴ After receiving RBD treatment for 48 h, NRF2 expression was greatly elevated (Fig. S3A). NQO1 and HO-1, the downstream target genes of NRF2, are involved in the supervision process of oxidative stress. Moreover, the LE mutant significantly activated the transcription of HO-1 and NQO-1, but not KLT (Fig. S2B, C). This comparative experiment demonstrates that the E484Q mutation site is unquestionably a key site for the transcriptional activation of HO-1 and NQO-1, whereas none of the mutation sites in KLT, including L452R, have transcriptional activation.

NRF2 is transcriptionally regulated by epigenetic factors, such as KDM6B and EZH2. Because 48-h RBD treatment did not significantly affect the levels of KDM6B and H3K27me3 (Fig. S4), we decided to focus on shorter RBD treatments. EZH2 and H3K27me3 reduced considerably after 24-h RBD treatment, but KDM6B was modestly raised (Fig. S3D-H). ChIP-qPCR assay revealed that H3K27me3 was substantially suppressed at the *NRF2* promoter regions following the LE mutant treatment (Fig. S3I). Thus, we hypothesized that the RBD protein epigenetically activated *NRF2* in neuronal cells via the extracellular signal transduction pathway, thereby regulating mitochondrial biogenesis and ROS homeostasis.

The down-regulation of *NRF2* and the enhancement of H3K27me3 indicate that *NRF2* was favorably controlled by KDM6B (Fig. S5A–C). Further study revealed that post-transcriptional suppression of KDM6B could partially restore H3K27me3 (Fig. S5D, E), NRF2 expression (Fig. S5F, G), and cytoROS (Fig. S5H) to control levels. Nonetheless, mito-chondrial bioactivity appeared to be preserved, as in LE and KLT mutants (Fig. S5I), but cell proliferation potential did not differ from that of controls. Thus, we successfully demonstrated that KDM6B directly regulates mitochondrial biogenesis and cellular proliferation in neuronal cells by epigenetically controlling *NRF2* expression following RBD protein treatment.

RT-qPCR, microarrays, and RNA-seq can only detect the quantity of steady-state RNAs. Here, we examined the nascent RNAs (nsRNAs) by capturing nsRNA transcripts with an EU-labeling approach, followed by RNA-seq (nsRNA-seq; Fig. S6A). We identified a total of 76 up-regulated and 96 down-regulated genes after 12-h RBD treatment by nsRNA-seq (Fig. S6B). The fate of transcripts is determined by the post-transcriptional degradation mechanism of nsRNA;

overexpression of nsRNA indicates completed transcriptional activation, and vice versa.⁵ The tight expression interaction network generated by the up-regulation of nsRNAs, which mainly affected genes associated with ribosomes, was observed (Fig. S6C, D). In contrast, the interactions between the down-regulated nsRNAs were minimal, and only HSPA5, an intervention target of the coronavirus, exhibited a strong molecular interaction. The down-regulated nsRNAs have stronger connections to MARK and Rap 1 signaling pathways (Fig. S6D). Overall, although additional research is required, the transcriptional profile of nsRNAs provides a glimpse into the effects of SARS-Cov-2 on neuronal cells and demonstrates a cascade of gene regulation intimately tied to its life cycle.

We could still demonstrate that RBD proteins functioned in a non-classical and neuron-specific manner, activating intracellular epigenetic regulatory mechanisms, and regulating *NRF2* expression to affect mitochondrial biogenesis and cellular proliferation in neuronal cells despite the study's limitations (Fig. S7, 8). It is noteworthy that such a novel method could reconstruct the cellular epigenetic regulatory system and control cell growth state via extracellular signal transduction. This could provide fresh insight into how SARS-CoV-2 might affect neuronal cells through epigenetic mechanisms and contribute to understanding the neuropathogenesis of SARS-CoV-2 infection.

Author contributions

Y.J. and S.G.Y. conceived the project. S.G.Y. wrote the manuscript. X.K., P.L., and C.H.K. performed the experiments. J.Y.Z. performed the nsRNA-seq. Z.B. completed the data analysis. J.H.P., B.Q.X., and N.M. made constructive comments on the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interests.

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Figure 1 Receptor-binding domain (RBD) proteins promote cellular viability and proliferation in SH-SY5Y cells (A) Sequence alignment of RBD proteins; Red, mutated amino acids (B) SH-SY5Y cells were treated with different concentrations of RBD for 48 h and the cellular viability was detected (C, D) CCK8 was used to detect the cell viability of SH-SY5Y (C) and HBE (D) treated with RBD proteins. The absorbance of the control wells was taken as 100%, and cell viability was expressed as a percentage of the control. All assays were performed independently three times (E) Representative fluorescence images of EdU staining of SH-SY5Y and HBE cells. Red, proliferating cells positively stained with EdU; blue, cell nuclei stained with DAPI. Scale bar = 20 μ m (F, G) Cellular proliferation curve of HBE (F) and SH-SY5Y (G) cells after treatment with RBD and its mutants. Five randomly selected views per sample were used to calculate the EdU-positive rate. Data were represented as mean \pm standard deviation. *P* values were calculated using one-way ANOVA followed by Dunnett's multiple comparisons tests. ns, no significant difference; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 versus control group. The experiments depicted in this figure have been reproduced three times.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.001.

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