Co-Expression of Mitochondrial Genes and ACE2 in Cornea Involved in COVID-19

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A novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) associated with severe human infected disease coronavirus disease 2019 (COVID-19) outbreak starting from December 2019, in China, and the disease is quickly spreading worldwide. Despite being primarily a respiratory virus, COVID-19 can also present with nonrespiratory signs, including ocular symptoms as conjunctival hyperemia, chemosis, epiphora, increased secretions, ocular pain, photophobia, and dry eye.2 The presence of virus in tears, conjunctival swab specimens, and animal models of infectious increasing clinical and scientific evidence that eyes may serve as a potential site of virus replication. Moreover, immunohistochemical studies and single-cell RNA-sequencing datasets revealed both extra- and intra-ocular localization of SARS-CoV-2 entry factors, ACE receptor, and TMPRSS2 protease in human eyes.6 Together, these results suggest that ocular surface cells are susceptible to infection by SARS-CoV-2. Moreover, a recent genomewide association study (GWAS) study identified 3p21.31 as a most significant genetic locus being associated with COVID-19 induced respiratory failure.7 This locus covers a cluster of six genes consisting of SLC6A20, LZFHL1, CCR9, FCRC1, CXCR6, and XCR1, with the identified risk allele being associated with increased SLC6A20 and LZTFL1 expression. Of note, SLC6A20, LZTFL1, and FCRC1 are known to associate with eye development, electroretinography abnormal, and anterior eye segment morphology. However, whether these key factors for cellular susceptibility to viral infection have correlation in ocular surface cell remains unclear. Herein, we constructed co-expression and interactome networks and mapped genes implicated by COVID-19 GWAS onto corneal co-expression network to infer the function of susceptibility gene.

METHODS

Dataset Summary and Quality Control

An overview of our strategy to inform corneal mitochondrial susceptibility module of SARS-CoV-2 infection in cornea is
shown in Figure 1A. We began by collecting the several RNA-Seq datasets of the normal tissue of the cornea (n = 19 samples), retina (n = 310 samples), retinal pigment epithelium (RPE; n = 207 samples), induced pluripotent stem cell (iPSC)-derived retinal (n = 5 samples), and lungs (n = 546 samples) from the National Center for Biotechnol-
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Differential Expression Analysis

The transcriptome profiling of human keratoconus corneas was also downloaded from the GEO database (GSE77938). Only genes with Transcripts Per Million (TPM) > 1 were preserved in the down-stream analysis. The DESeq2 package was used to normalize expression levels and detect differentially expressed genes (q value cutoff is 0.05). Statistical analysis was done using the R project for statistical computing (http://www.r-project.org).

RESULTS

To understand the expression patterns of ACE2, TMPRSS2, and susceptibility genes in the cornea, we first compared the expression level of ACE2 in the cornea, retina, RPE, and lung tissues based on bulk RNA sequencing. As expected from prior literature, the cornea showed a higher ACE2 expression than the lungs both in terms of their TPM values (see Fig. 1A). ACE2 exhibits the highest co-expression correlation with TMPRSS2, SLC6A20C, and LZTFL1 in the cornea compared to the lungs and RPE (Fig. 1B). To gain more insight into the biological network of genes associated with SARS-CoV-2 entry factors and susceptibility gene, we performed k-means clustering algorithm to identify genes associated with ACE2 on cornea datasets (Fig. 1C). We identified 26 co-expression modules ranging in size from 111 to 1798 genes. One cluster contained ACE2, LZTFL1, and FYCO1 (cluster 7; 1434 genes). The strongest correlation with the eigengene (the principal component) of this ACE2 cluster was found for hub genes STK16 (r = 0.95, P = 1.07 × 10^-22), a member of NAK family that activate the AP-2 scaffolding protein vital to viral entry and propagation. TMPRSS2 belonged to a separate cluster 5 (603 genes) with hub gene SLC25A1 (r = 0.91, P = 4.45 × 10^-9), which involved in TNF-α and IFN-α triggered inflammation. Together these data suggest that the cornea may provide a susceptibility and entry portal for the SARS-CoV-2 entry.

DISCUSSION

To control and mitigate the impact of the COVID-19 pandemic, it is vital to gain greater understanding of the
routes and modes of transmission, including the role of the ocular surface. Using a cornea-relevant co-expression network to inform virus entry factors and GWAS interpretation, we were able to identify putative susceptibility genes for highly correlated with ACE2. Interestingly, we found that the ACE2 co-expression cluster and SARS-CoV-2 interactome were both enriched for mitochondrial functions. As previously described, the ACE2 not only serves as a critical determinant of CoV-2 transmissibility but also regulates mitochondrial functions.23 ACE2 overexpression regulates mitochondria-localized nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4, which is known to produce reactive oxygen species (ROS) in the mitochondria.24 Oxidative stress caused by ROS excessiveness was indicated as a major player in COVID-19 pathogenesis and severity.25 Additionally, several lines of evidence have established a link between inflammation and oxidative stress.26–28 For example, TNF-α induces calcium-dependent increase in mitochondrial ROS.29 Once SARS-CoV-2 enters the host cell, its RNAs, such as ORF-9b, also can directly manipulate mitochondrial function to release mitochondrial DNA (mtDNA) in the cytoplasm and activate mtDNA-induced inflammasome and suppress innate and adaptive immunity.30,31 Together, the proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-10, and CXCL-8, affect diverse physiological processes by driving cellular oxidative stress ROS generation. In turn, increased ROS production stimulates proinflammatory mediator release that contributes to mitochondrial dysfunction. As we know, the corneal cell, especially corneal endothelial cells, is a mitochondria-rich cell. Given the highly exposed position, the cornea receives a significant amount of high-tension atmospheric oxygen and the ultraviolet range, which result in the generation of ROS and subsequent oxidative stress. Moreover, ROS are a by-product of oxidative phosphorylation in mitochondria, which can subsequently result in further mitochondrial damage and a further increase in
ROS. Overall, a vicious oxidation / inflammatory cycle is more likely to have a potential impact of SARS-CoV-2 infection and immune responses in corneal cells.

In our study, we predicted that the mitochondrial related CMSM gene set was vital susceptibility genes of COVID-19, including five genes involved in respiratory electron transport which are being targeted by metformin. A favorable effect of metformin in patients with COVID-19 has been hypothesized as the drug might prevent virus entry into target cells via adenosine monophosphate-activated protein kinase activation and the phosphatidylinositol-3-kinase-protein kinase B-mammalian target of rapamycin signaling pathway. Because metformin is found to have the properties of anti-inflammation and anti-oxidation, it has also been used in the treatment of eye diseases, including age-related macular degeneration, glaucoma, and diabetic retinopathy. These may give insight into metformin that may lower the COVID-19 risk in eye infection. Notably, ECSIT, one of the CMSM genes, is a cytosolic adaptor protein involved in inflammatory responses and plays a regulatory role as part of the TAK1-ECSIT-TRAF6 complex that is involved in the activation of NF-κB by the TLR4 signal. In the previous study, treatment with drugs that inhibited NF-κB activation led to a reduction in inflammation and significantly increased mouse survival after SARS-CoV infection. Additionally, ECSIT is also essential for the association of RIG-I-like receptors (RIG-I or MDA5) to VISA in innate antiviral responses. Therefore, ECSIT may be used as a new drug target to protect against the development of severe forms of COVID-19 infection. Based on our results, we believe that significant insight into COVID-19 in the cornea can be gained using co-expression and interaction networks.

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**References**


