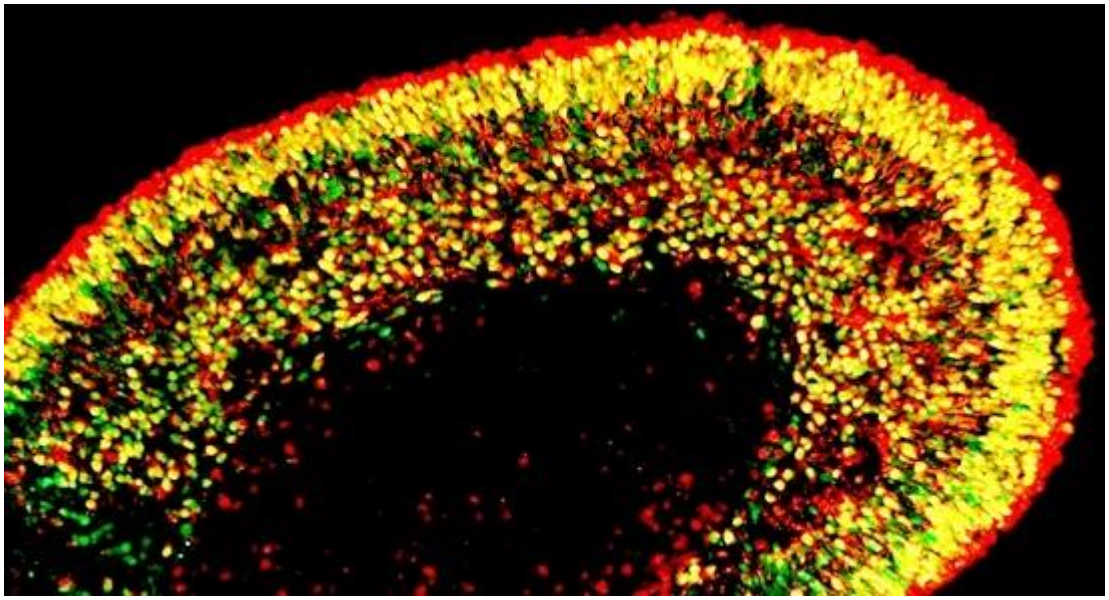
Neonates delivered to women infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been found to suffer from neurological complications, indicating that the fetal central nervous system (CNS) may be vulnerable to the virus. The retina is a component of the CNS, and SARS-CoV-2 mRNA has been found in the human retina. However, it is yet unknown how SARS-CoV-2 affects the fetal retina at various stages of development. In this work, the Chinese authors used retinal organoids (hEROs) derived from human pluripotent stem cells (hPSCs) to investigate how short-term exposure to the SARS-CoV-2 spike (S) protein affects different stages of retinal organoid development. Since retinal organoids express angiotensin-converting enzyme 2 (ACE2) and host transmembrane serine protease 2 (TMPRSS2), it was also investigated whether the S protein affects their expression.

Organoids derived from human pluripotent stem cells mimic the cell-type composition, structure, and function, which is important for studying basic developmental dynamics, disease models, and personalized therapeutic approaches. Previous studies have demonstrated that SARS-CoV-2 can infect neurons and neural progenitors. https://discovermednews.com/sars-cov-2-can-infect-and-replicate-human-motor-neurons/ In addition, Menuchin-Lasowski et al. demonstrated that SARS-CoV-2 was able to infect and replicate in photoreceptors and retinal ganglion cells of the retinal organoids *via* the ACE2 pathway (Menuchin-Lasowski Y, et al. SARS-CoV-2 infects and replicates in photoreceptor and retinal ganglion cells of human retinal organoids. Stem Cell Rep. 2022; 17 (4): 789–803).



About the study

The authors used human embryonic stem cell-derived retinal organoids (hEROs) as a model to investigate how the exposure to the SARS-CoV-2 S protein affects different stages of retinal development. The retina-specific markers, including the retinal progenitor marker CHX10, the retinal amacrine/ganglion cell marker HuC/D, the Müller cell marker vimentin, photoreceptor markers CRX and recoverin were characterized by immunostaining from day 60 to day 280. At early differentiation (from 60 to 90 days), the quantities of CHX10+ and HuC/D+ cells, located mainly in the inner retinal layer, were relatively high, but they declined over time. The formation of a pseudo-photoreceptor layer at day 90 indicated the early stage of photoreceptor layer development, while photoreceptor cells of the outer segment structures marked the late stage of photoreceptor layer development at day 280.

Subsequently, 90-day hEROs were treated with 1, 2, 5, and 10 µg/mL of S protein. Since the mRNA levels of toll-like receptor 4 (TLR4) and myeloid differentiation primary response 88 (MYD88) increased after exposure to 2 µg/ml of S protein, this concentration was selected for exposure to S protein on days 90 and 280. The impact of S protein on retinal development at both early and late stages was evaluated by immunofluorescence staining, RNA sequencing, and real-time polymerase chain reaction (RT-PCR). In human embryonic stem cells (hESCs) and hEROs, the expression of ACE2, a key receptor of SARS-CoV-2, and TMPRSS2, which facilitates viral entry into host cells *via* ACE2, was evaluated by RT-PCR.

The TUNEL assay determined cell apoptosis after 48 hours of S protein exposure.

Results

Expression of ACE2 mRNA increased while TMPRSS2 mRNA decreased in hEROs from day 60 to 280 compared to human embryonic stem cells (hESCs). ACE2+ cells were distributed throughout the neural retina, while the number of TMPRSS2+ cells was low. After exposure to S protein, there was no change in the mRNA levels of ACE2 and TMPRSS2 in hEROs at day 90 and day 280, suggesting that the upregulation of ACE2 was not required for the effects induced by S protein in hEROs.

RNA sequencing data demonstrated that S protein induced an inflammatory response in both the early and later stages of hEROs development. Expression of the pro-inflammatory MYD88 and TLR4 genes increased at day 90, while expression of the anti-inflammatory Transforming Growth Factor (TGF)-β and Inhibitor of DNA Binding 3 (ID3) genes decreased at day 280.

There was no difference in the number or morphology of three retinal cell types- photoreceptors, retinal ganglion cells, and progenitor cells- between hEROs treated with the S protein and non-treated hEROs at days 90 and 280. However, transcriptomic analysis revealed that the regulatory processes associated with lipid metabolism and the extracellular matrix disruption were involved in the impairments of retinal developmental induced by SARS-CoV-2. During early photoreceptor layer development, S protein primarily affected the nucleus and gene expression associated with lipid metabolism, while in the later stages of embryonic retinal development, exposure to S protein primarily affected gene expression associated with the extracellular matrix and cell membrane composition.

Conclusion

This work highlights different effects of SARS-CoV-2 S protein on both early and late stages of retinal development, and provides insights into the cellular and molecular mechanisms of these effects. The short-term exposure of hEROs to S protein primarily led to transcriptional alterations. Transcriptomic analysis showed an inflammatory response-related signature during both the early and advanced stages of retinal development. In addition, at early stages of embryonic retinal development, S protein primarily affected gene expression related to lipid metabolism. At later stages of embryonic retinal development, S protein

primarily affected gene expression associated with the extracellular matrix and cell membrane composition.

The authors suggested that further studies should investigate the impact of the SARS-CoV-2 on retinal development observed in retinal organoids derived from human pluripotent stem cells and the possible modulation of these pathways.

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