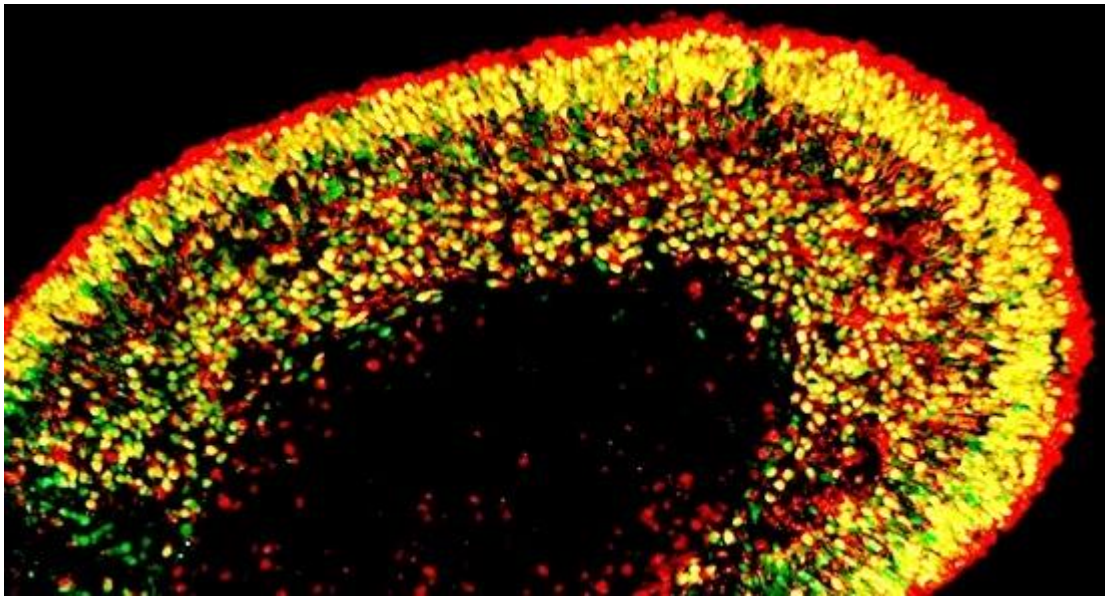
Neonates delivered to women infected with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been found to suffer from neurological complications, indicating that the fetal central nervous system (CNS) may be vulnerable to the virus. The retina is a component of the CNS, and SARS-CoV-2 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), the authors 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 can 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 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, and photoreceptor markers CRX and recoverin, were characterized by immunostaining from day 60 to 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. The late stage of photoreceptor layer development at day 280 was marked by the presence of photoreceptor cells in the outer segment structures.

Subsequently, 90-day hEROs were treated with 1, 2, 5, and 10 µg/mL of the SARS-CoV-2 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 the SARS-CoV-2 S protein, this concentration of the S protein was selected for exposure 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) tests. The expression of ACE2, a key receptor of SARS-CoV-2, and TMPRSS2,

which facilitates viral entry into host cells *via* ACE2, in human embryonic stem cells (hESCs) and hEROs was evaluated by RT-PCR. After 48 hours of the S protein exposure, cell apoptosis was determined by the TUNEL assay.

Results

In hEROs from day 60 to 280, expression of mRNA for ACE2 increased while mRNA for TMPRSS2 decreased compared to human embryonic stem cells (hESCs). ACE2+ cells were distributed throughout the neural retina, while the number of TMPRSS2+ cells was low. The exposure to the S protein did not change the mRNA levels of ACE2 and TMPRSS2 in hEROs at day 90 and day 280, suggesting that the effects induced by the S protein in hEROs do not require the ACE2 upregulation.

In both early and later stages of hEROs development, exposure to the S protein induced an inflammatory response. Expression of genes encoding pro-inflammatory MYD88 and TLR4 increased at day 90. The expression of genes encoding anti-inflammatory transforming growth factor (TGF)-β and inhibitor of DNA binding 3 (ID3) decreased at day 280.

The number or morphology of three retinal cell types: photoreceptors, retinal ganglion cells, and progenitor cells did not differ between hEROs treated with the S protein and non-treated hEROs at days 90 and 280. However, retinal development was impaired by the SARS-CoV-2 S protein, involving regulatory processes associated with lipid metabolism and extracellular matrix. During early development of the photoreceptor layer, the S protein primarily affected gene expression associated with lipid metabolism, while in the later stages of embryonic retinal development, exposure to the 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 the 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.

At the early stages of embryonic retinal development, the S protein primarily affected gene

expression related to lipid metabolism. At later stages of embryonic retinal development, the 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 SARS-CoV-2 on retinal development observed in retinal organoids and the possible modulation of these pathways.

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