Complement pathway-mediated neurotoxicity of reactive astrocytes in a stem cell model of glaucoma

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INTRODUCTION

Retinal ganglion cells (RGCs) are the projection neurons of the retina that serve as the connection between the eye and the brain, allowing for visual perception, and damage to this pathway leads to vision loss or blindness. Astrocytes are found adjacent to RGCs within the optic nerve, where they maintain RGC health and proper function. Conversely, neuroinflammation occurs when astrocytes adopt a reactive state, leading to the degeneration of RGCs. As the highly localized nature of astrocyte reactivity in the optic nerve head is strongly correlated with the initial site of injury along RGC axons in glaucoma, the development of a human cellular model of these interactions would further our understanding of how astrocytes respond in neuroinflammatory conditions leading to the degeneration of RGCs.

Human pluripotent stem cells (hPSCs) serve as powerful *in vitro* models for retinogenesis as well as how cell types are adversely affected in disease states. Previous work from the Meyer lab has pioneered the derivation of RGCs from hPSCs, including cell lines derived from glaucoma patient cells. Previous work from the Meyer lab has also examined hPSC-derived astrocytes and RGCs in co-cultures, where astrocytes conferred a growth and maturation-promoting effect.

To establish a more relevant *in vitro* model of glaucoma, a critical need exists to develop a platform that takes the compartmentalization of RGCs into consideration. Microfluidic engineered platforms can isolate axonal compartments of neurons, providing a powerful approach for studies of how the RGC axonal compartment is specifically affected in glaucoma. Thus, the current study leverages a robust and reproducible in vitro model to recreate the spatial interactions of astrocytes upon human RGC axons.

Aim #1: to study the interactions between RGC axons and astrocytes, with a focus upon how reactive astrocytes modulate the RGC axonal compartment leading to neurodegeneration

Aim #2: to explore the role of the complement cascade in reactive astrocyte-mediated neuroinflammation.

DESIGN & METHODS

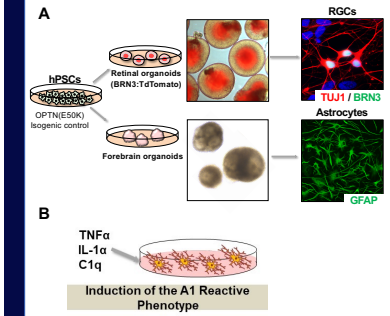

Figure 1. (**A**) hPSCs were grown as 3D retinal organoids or forebrain organoids for the differentiation of retinal ganglion cells (RGCs) and astrocytes, respectively. (**B**) Induction of reactivity in hPSC-derived astrocytes through incubation with TNFα, IL-1α and C1q.

RESULTS

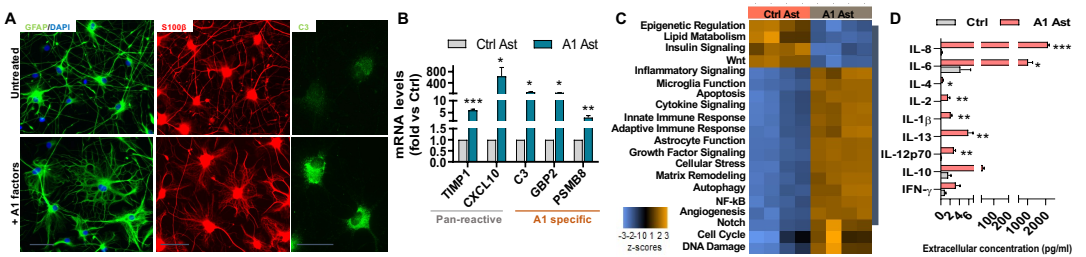

Figure 2. Induction of the A1 phenotype in astrocytes. hPSC-derived astrocytes were treated with A1 factors. (**A**) Treated astrocytes show morphological changes, as well as increased C3 expression, a specific characteristic of A1 astrocytes. (**B**) Upregulation of pan-reactive and A1-specific genes in astrocytes after incubation with A1 factors. Scale: 50μm. (**C**) NanoString analysis shows upregulation of inflammatory pathways in A1 astrocytes. (**D**) Increased levels of several pro-inflammatory cytokines in A1 astrocyte-conditioned media.

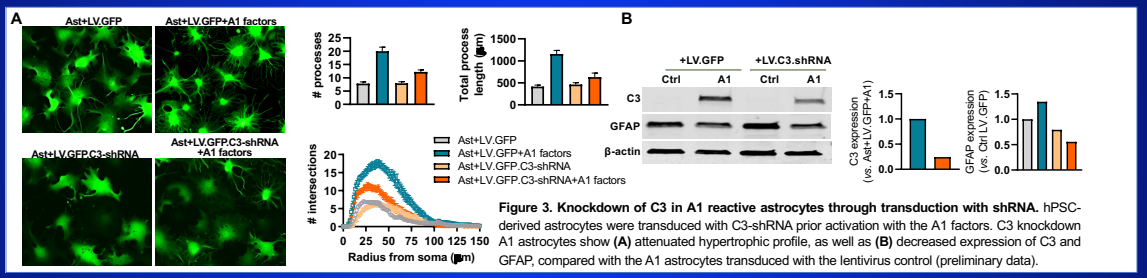

Figure 3. Knockdown of C3 in A1 reactive astrocytes through transduction with shRNA. hPSC-derived astrocytes were transduced with C3-shRNA prior activation with the A1 factors. C3 knockdown A1 astrocytes show **(A)** attenuated hypertrophic profile, as well as **(B)** decreased expression of C3 and GFAP, compared with the A1 astrocytes transduced with the lentivirus control (preliminary data).

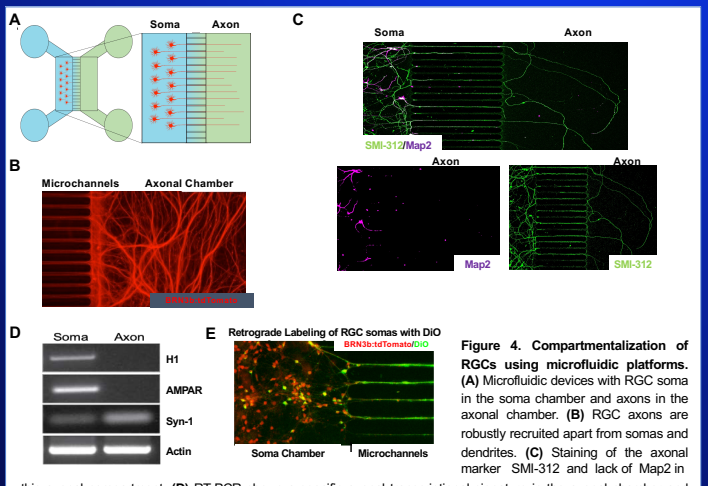

Figure 4. Compartmentalization of RGCs using microfluidic platforms. **(A)** Microfluidic devices with RGC soma in the soma chamber and axons in the axonal chamber. **(B)** RGC axons are robustly recruited apart from somas and dendrites. **(C)** Staining of the axonal marker SMI-312 and lack of Map2 in this axonal compartment. **(D)** RT-PCR shows a specific axonal transcriptional signature in the axonal chamber and lack of somatodendritic genes. **(E)** Retrograde labeling with DiO allows to identify somas corresponding to recruited axons apart from those whose axons had not been recruited into the axonal chamber.

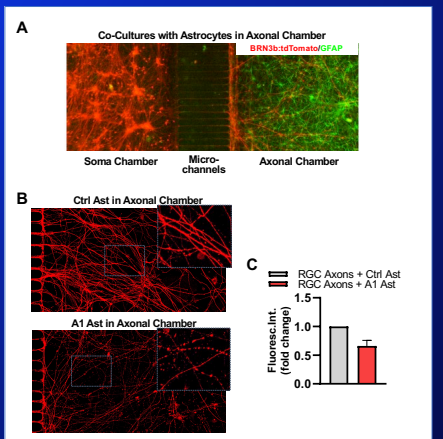

Figure 5. Neurotoxic effect of A1 astrocytes over RGC axons. (A) Astrocytes cultured in the axonal chamber mimicking the location of glia relative to RGCs in the optic nerve. **(B,C)** A1 reactive astrocytes induce neurotoxic effects and fragmentation of RGC axons (preliminary data).

CONCLUSIONS

- After incubation with TNFα, IL-1α and C1q, hPSC-derived astrocytes exhibit hypertrophic and pro-inflammatory profiles, as well as increased expression of A1-specific markers.
- Knockdown of complement C3 in reactive astrocytes seems to reduce their hypertrophic profile and features of reactivity.
- The use of microfluidic platforms allows to isolate RGC axons from the soma, which better mimics the compartmentalized characteristic of RGCs.
- Reactive astrocytes placed in the axonal compartment promote a toxic effect over RGC axons, including axonal fragmentation.

NEXT STEPS

- To study how the modulation of C3 in reactive astrocytes would impact on RGC axons.
- To explore axonal compartmentalization of RGCs differentiated from iPSCs derived from glaucoma patients, as well as the effect of reactive astrocytes over diseased RGCs.
- To expand upon these studies to determine how other neighboring glial cells may also contribute to the neurodegeneration of RGCs in a compartmentalized manner.

ACKNOWLEDGEMENTS

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