The Early Transcriptomic Landscape of Pressure-Induced Optic Nerve Heads in ex-vivo Organotypic Human Eyes

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Introduction: Whole-globe human eyes were received from the Eyebank of Canada within 24h of enucleation according to an Ethics Board approved protocol. Physiological fluid flow was restored in the eyes by infusion of synthetic aqueous humor into the posterior chamber over 6 hours. For each pair, one eye was maintained at normal IOP of ~15 mmHg, and the contralateral eye maintained an elevated IOP of ~40 mmHg. Following perfusion the ONH was rapidly isolated from four pairs of eyes and prepared for total mRNA sequencing. Results were analyzed by paired differential expression analysis and pathways/geneset analyses. In parallel, four pairs of fixed perfused eyes were used for spatial transcriptomics of the ONH region using the Visium platform (10x Genomics). Data processing and visualization were performed on SpaceRanger and Loupe Browser. Differential analyses of ONH and LC identified by barcodes were performed within and between samples.

Methods: All eyes maintained physiological or elevated IOP for 6 hours. Differential expression, Gene Ontology and Gene Set Enrichment analyses revealed significantly altered genes and pathways associated with astrocyte and microglial reactivity, and mechanosensing. K-mean clustering of spatial profiling results identified a distinct ONH expression profile. A panel of astrocyte and cell-adhesion genes highlights the LC region relative to the rest of ONH. Preliminary differential analysis revealed altered genes related to cellular stress responses and oxidative phosphorylation in the LC.

Results: Total mRNA and spatial transcriptomic profiling of the perfused human ONH reveals significant neuroinflammatory changes in response to elevated IOP. These changes provide insight into molecular events relevant to early human ocular hypertension, and are consistent with patterns found early in animal models.

Conclusions: N/A