Early structural changes to Müller glial cells in glaucoma

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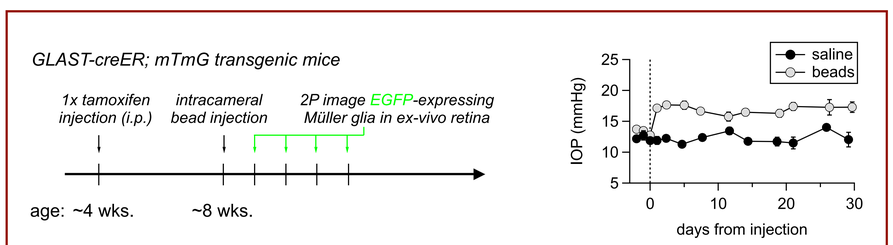
Background

Elevated intraocular pressure (IOP) is the most common risk factor for glaucoma, but the mechanisms linking high IOP and eventual vision loss are not well understood. Most work to date has focused on understanding how pathological events occurring at the optic nerve head contribute to disease progression. However, recent evidence indicates some of the earliest cellular changes in glaucoma may occur in the inner plexiform layer (IPL). In glaucoma, retinal glia undergo a variety of molecular and morphological changes referred to as reactive gliosis. Despite evidence that reactive gliosis contributes importantly to disease progression, little is known regarding how Müller cells, the primary glial cell type of the retina, respond to ocular hypertension early in disease or how gliosis contributes to vision loss. **We hypothesize that ocular hypertension-induced structural remodeling of Müller glia early in disease onset contributes to eventual degeneration of retinal ganglion cells (RGCs) in glaucoma.**

Methods

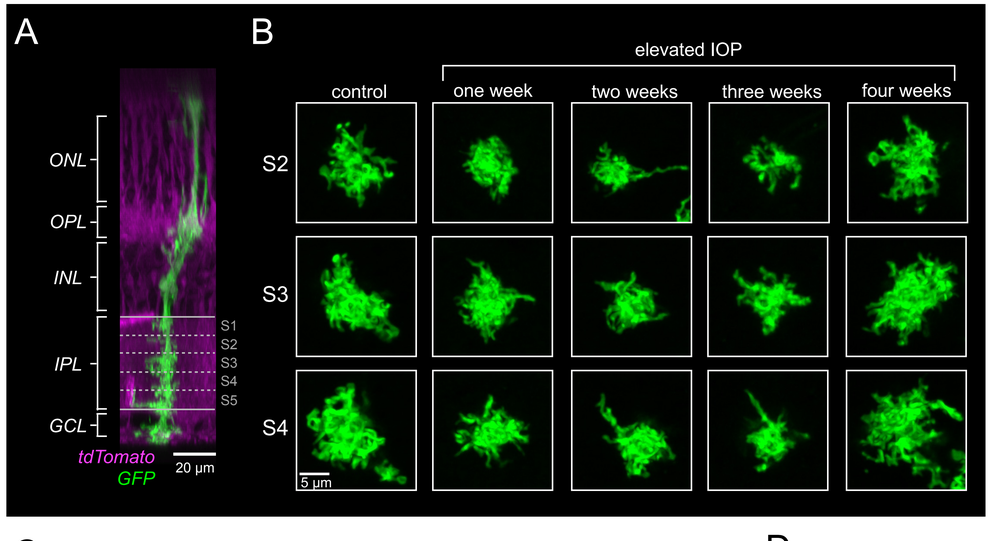


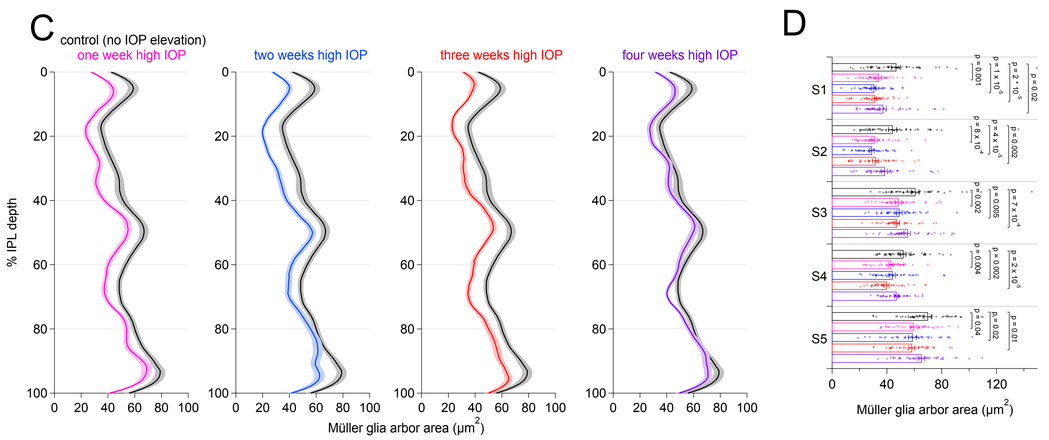
Microbead occlusion model: To experimentally elevate IOP, we injected polystyrene microbeads (10 µm diam.) into the anterior chamber of the right eyes of isoflurane-anesthetized mice. Left eyes were left un-injected or were injected with bead-free saline solution for use as controls. IOP was measured before and after bead/saline injections in awake mice.

Live imaging of fluorescently-labeled Müller glia: GFP expression was induced in a sparse subset of Müller cells via a single dose of tamoxifen in *GLAST-creER; mTmG* transgenic mice (Jackson labs strains 012586, 007576). All imaging was performed at 1-4 weeks after microbead injection using a flat-mounted *ex vivo* eyecup preparation in which a ~3x3 mm portion of the eye with lens and cornea removed was secured, vitreal side up, into a perfusion chamber on the stage of a two-photon fluorescence microscope. Eyecups were constantly perfused with a warmed (~32ºC) and oxygenated ACSF solution to maintain tissue health. Image stacks of individual Müller cells were acquired through the entire depth of the inner plexiform layer. We performed all imaging in live tissue to avoid fixation-induced morphological artifacts that we and others have noted in Müller cells.

Results

Müller cell peri-synaptic processes in the IPL transiently withdraw soon after IOP elevation





(A) Side projection of Müller cell with membrane-EGFP expression in *GLAST-creER; mTmG* mouse live retina. Approximate locations of IPL sublaminae denoted by dotted lines.

(B) Example *en face* view max-intensity z-projections from image stacks within different IPL sublamina in saline (control) or bead-injected eyes. For each timepoint, image stacks at different IPL depths are from the same Müller cells.

(C) Quantification of Müller cell process arbor size at different IPL depths for control (black) or hypertensive (colors) eyes. Thick lines with shaded areas show mean ± SEM of arbor area measurements from Müller cell image stacks. tdTomato fluorescence was used to define IPL borders (0 and 100% depth).

(D) Summary of Müller cell process arbor size for each of the five IPL sublamina. Bars with error bars show mean ± SEM. Circles show measurements from individual Müller cells. Note significant differences from control across all IPL sublamina at 1-3 weeks after bead injection.

Conclusions and Future Directions

- The territory of individual Müller cells shrinks in the IPL within one week of IOP elevation. Withdrawal of glial processes could lead to excitotoxicity via pathological overstimulation of glutamate receptors expressed on RGC dendrites.
  - *We are currently investigating how ocular hypertension-induced shrinkage affects how Müller cell processes interact with excitatory synapses onto RGC dendrites.*
  - *To examine whether retraction of Müller cell processes disrupts regulation of extracellular glutamate we are measuring light-evoked extracellular glutamate signals and NMDA receptor function in RGC dendrites after IOP elevation.*

- Unexpectedly, we found that after four weeks of elevated IOP, Müller cell processes were similar to control.
  - *We are additionally examining Müller cell morphology at later timepoints after bead injection to examine whether this apparent recovery is because there is a switch from withdrawal to expansion of Müller cell processes at ~4 weeks..*

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