Investigating the Role of ANGPTL2 in Glaucomatous Trabecular Meshwork

Aileen Huang[1], Chenna Sugali[1], Jiannong Dai[1], Kamesh Dhamodaran[1], Weiming Mao[1]

[1]Indiana University School of Medicine, Department of Ophthalmology

Background and Hypothesis:

Glaucoma is a progressive optic neuropathy and the leading cause of irreversible blindness worldwide, primarily driven by elevated intraocular pressure (IOP). IOP elevation often results from impaired aqueous humor outflow through the trabecular meshwork (TM), where extracellular matrix (ECM) remodeling and cytoskeletal changes increase tissue stiffness and cause dysfunction. Our RNA sequencing study identified angiopoietin-like 2 (ANGPTL2), a TGFβ2-inducible gene, in the TM. Since ANGPTL2 is a secreted protein involved in ECM remodeling in other tissues and TGFβ2 is a well-known glaucomatous insult, we hypothesize that ANGPTL2 contributes to glaucomatous changes in the TM.

Experimental Design:

GTM3 cells were transfected with either ANGPTL2 siRNA (following dose optimization via western blot) or an ANGPTL2 overexpression plasmid (generated by subcloning ANGPTL2 cDNA into a GFP vector and confirmed by Sanger sequencing). The cells were treated with or without 5ng/ml TGF-β2 to simulate profibrotic conditions. Protein expression of ANGPTL2, fibronectin, and collagen I was assessed by western blotting. Immunocytochemistry with phalloidin and ECM markers was used to evaluate cytoskeletal organization and ECM remodeling.

Results:

ANGPTL2 knockdown reduced fibronectin and collagen I levels and decreased phalloidin-labeled actin stress fibers, in the presence or absence of TGFβ2. Overexpression of ANGPTL2 increased actin stress fibers.

Conclusions and Potential Impact:

Our preliminary data indicate that ANGPTL2 likely contributes to TM cytoskeletal remodeling and ECM accumulation in the TM. Further studies are required to validate these findings. Also, ex vivo perfusion culture and in vivo mouse studies are needed to determine the role of ANGPTL2 in IOP regulation.