

Analysis of the Binding Partners of Clusterin in their Role in Increased Intraocular Pressure in Glaucoma

Clay Hepp¹, Anoop Magesh¹, Avinash Soundararajan², Padmanabhan Pattabiraman²

¹Indiana University School of Medicine, ²Indiana University School of Medicine, Department of Ophthalmology

Background and Objective:

Elevated intraocular pressure (IOP) is a risk factor for primary open-angle glaucoma (POAG). Clusterin (CLU) is a secretory chaperone protein found in trabecular meshwork tissue that is implicated with POAG risk. In this study, we aimed at understanding the role of CLU and its binding partners in IOP homeostasis and POAG pathology.

Methods:

Normal trabecular meshwork (NTM) cell lines were used. Half of the NTM cell lines were transfected with adenovirus empty (AdMT) while the other half of the NTM cell lines were transfected with adenovirus clusterin with histidine tag (AdCLUHIS). AdCLUHIS allows for the overexpression of CLU HIS in the NTM cells. After 72 hours of transfection, the media and cell lysate were collected. As CLU is a secretory chaperone protein, the media was analyzed. Immunoprecipitation (IP) was conducted to isolate CLU HIS and all the proteins bound to it. Western blot analysis was conducted to confirm IP worked successfully. Once it was confirmed that CLU HIS with all its binding partners was isolated successfully using IP, the media samples were sent to proteomics to determine all the specific proteins that are bound directly to CLU.

Results:

Western blot analysis confirmed that the overexpression of CLU HIS was successfully accomplished through adenovirus transfection. In addition, Western blot analysis confirmed that IP worked successfully. At the current moment, results of proteomics are still being developed, so the specific binding partners of CLU are still unknown at the time.

Conclusions and Potential Impact:

Our preliminary study suggests that CLU can be overexpressed via adenovirus and analyzed via IP. Understanding this allows for the purification of the protein and its attached binding partners. Identifying these binding partners can be novel targets for improving aqueous humor outflow through trabecular meshwork to decrease IOP and decrease one's risk for POAG.