Glaucomatous E50K Mutation in the Optineurin Gene Causes Mitophagy Defects for Retinal Ganglion Cells but Not for Motor Neurons

Authors: Harshavardhan Sanaka[1,3], Michelle Surma[1], Leah LaCross[1,4], Kavitha Anbarasu[1,2], and Arupratan Das[1,2,4,*]

[1] Department of Ophthalmology, Eugene and Marilyn Glick Eye Institute, Indiana University, Indianapolis, IN 46202, USA; [2] Department of Medical and Molecular Genetics, Indiana University, Indianapolis, IN 46202, USA; [3] Indiana University School of Medicine, Indianapolis, IN 46202, USA; [4]Stark Neurosciences Research Institute, Indiana University, Indianapolis, IN 46202, USA

*Correspondence: arupdas@iu.edu

Background: Clearance of damaged mitochondria by lysosomes, known as mitophagy, is critical for maintaining mitochondrial homeostasis and cellular energy balance. Optineurin (Optn) is the central player for mitophagy found to be mutated among normal tension glaucoma ($OPTN^{E50K}$) and in some familial forms of amyotrophic lateral sclerosis ($OPTN^{E478G}$) patients. It is critical to understand how mitophagy mechanisms are altered for these inherited mutations in stem cells and differentiated neurons to gain insight into the disease's developmental aspect.

Methods: We utilized human embryonic stem cell-derived retinal ganglion cells (hRGCs) and induced motor neurons (iMNs) with and without the $OPTN^{E50K}$ mutation. The cells were treated with DMSO (vehicle control) or with 10 μM CCCP, an uncoupler that induces mitochondrial damage. We then analyzed the activation status of critical mitophagy players including Optn, Parkin, Pink and Lc3b. We further analyzed if $Optn^{E50K}$ mutant forms aggregate in iMNs as observed for hRGCs.

Results: We found that $OPTN^{E50K}$ mutation causes attenuated activation for Optn, Parkin, and LC3b in hRGCs under mitochondrial damage, while iMNs and hPSCs maintained healthy mitophagy. Additionally, mutant iMNs do not display distinct Optineurin aggregates, unlike in mutant hRGCs.

Conclusion: Our results suggest that the $Optn^{E50K}$ mutation may disrupt mitophagy in hRGCs but not in iMNs and stem cells. In addition, the lack of Optn aggregates in the $OPTN^{E50K}$ mutant iMNs suggests an alternative pathway that inhibits the aggregate formation, presumably for maintaining healthy mitophagy for longer survival.

Impact: The results from this project lay the groundwork for further investigation of the mechanisms behind mitophagy and its relation to glaucoma and ALS. Understanding what causes certain cell types to degrade while others remain healthy is key to understanding the genotype-phenotype specificity for inherited gene disorders.