Generation of CRISPR-Cas9 Engineered OPTNE478G Human Embryo Stem Cell Line for Investigation into Mitophagy Defects

Maverick Tebbe[1], Saajid Kathri[2], Michelle Surma[3], Arupratan Das[3]*
[1]Indiana University School of Medicine; [2]Purdue University; [3]Department of Ophthalmology, Eugene and Marilyn Glick Eye Institute

Background: Optineurin (OPTN) is a mitophagy adaptor protein linking damaged, ubiquitinated mitochondria to autophagosomes for lysosomal degradation. The E50K and E478G OPTN mutations are associated with normal tension glaucoma and ALS, respectively. Currently, it is unknown whether the E50K mutation selectively affects retinal ganglion cells and the E478G mutation selectively affects motor neurons. Given OPTN's role in mitophagy, investigating the mitophagy defects in human stem cell-differentiated (hRGCs) and induced motor neurons (iMNs) harboring these mutations provides an avenue to explore the cellular mechanisms of these diseases.

Methods: This project generated a OPTN-E478G human embryonic stem cell (hESC) line through CRISPR-Cas9 gene editing. gRNA oligomers were annealed and cloned via transformation of DH5α cells. Following sequencing, the gRNA-Cas9-GFP plasmid and donor plasmid with E478G insert were transfected into H7-WT-hESCs. The CRISPR-Cas9 system cut and repaired the double stranded break by homology-directed repair introducing the E478G mutation. GFP-positive colonies were isolated, expanded, and screened by PCR and restriction enzyme digestion. Western blot analysis of E50K and E478G mutants for hRGCs and iMNs were performed following introduction of the mitochondrial uncoupler CCCP, inducing mitophagy.

Results: Insertion of the gRNA into the Cas9-GFP plasmid was confirmed by sequencing. The hESCs were successfully transfected with the E478G plasmid as confirmed by restriction enzyme digestion of SapI and BspHI. Isolation of this stem cell population is ongoing and will be screened with restriction enzymes prior to sequencing. Western blots of NBR1 showed increased levels in WT-hRGCs and iMNs (E50K and E50K corrected), but not in E50K-hRGCs, indicating impaired mitophagy.

Conclusions: Our study reveals distinct mitophagy defects seen in the OPTN mutations E50K and E478G for the RGCs and iMNs. This project lays the groundwork for further studies, including live cell imaging, OPTN activation, and LC3b lipidation, to further define the implications of these mutations on mitophagy.