Glaucomatous Optineurin (E50K) Mutation Disrupts Mitochondrial Homeostasis in Human Stem Cell Derived RGCs

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Background:

Retinal ganglion cells (RGCs) are highly energy dependent due to their continuous action potential firing requirements and long unmyelinated axons hence highly susceptible to mitochondrial dysfunctions, observed in glaucoma. Dr. Das's lab recently had identified Tank-binding kinase 1 (TBK1) inhibition by BX795 drug activates mitochondrial biogenesis and promotes RGC protection with glaucomatous Optineurin (OPTN-E50K) mutation. OPTN is a critical player for mitophagy. It is still not clear if activation of mito-biogenesis improved mitochondrial homeostasis which I investigated in this project.

Methods

To investigate mitochondrial homeostasis in human RGCs, I have used a robust well-characterized human stem cell differentiated RGC (hRGC) model with wild-type (WT) and E50K mutation background which Dr. Das's lab routinely uses. To investigate mitochondrial homeostasis, I used JC1 live cell mitochondrial dye which fluoresces red when bound to healthy mitochondria and green when bound to damaged mitochondria. I used this assay on hRGCs treated with BX795 and mitochondrial stressor CCCP and measured red to green mitochondria ratio on confocal z-stacks using ImageJ.

Results

Under basal level, we found $hRGCs^{WT}$ had a significantly increased healthy (red:green) mitochondria compared to $hRGCs^{E50K}$. This suggests E50K mutation disrupts mitochondrial homeostasis. To gain mitochondrial homeostasis, it is possible that $hRGCs^{E50K}$ will produce more mitochondria over time than the WT. Indeed, we observed significant increase in healthy mitochondria for $hRGCs^{E50K}$ at 3h and 24h of DMSO and BX795 treatment, but not for $hRGCs^{WT}$. We also observed under CCCP damage for 3h, $hRGCs^{E50K}$ had significantly higher amount of damaged mitochondria (p=0.051) while $hRGCs^{WT}$ maintained homeostasis.

Conclusion and Potential Impact

My study suggests glaucomatous OPTN-E50K mutation disrupts mitochondrial homeostasis and activation of mito-biogenesis by BX enriches healthy mitochondria leading to $hRGC^{E50K}$ protection. This study has high impact as further avenues for promoting mito-biogenesis could lead to glaucoma neuroprotection therapy.