



# Distribution and localization of abnormally expressed OPTN proteins in RGC5 retinal ganglion cells and their effects on subcellular morphology

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**ABSTRACT.** The objectives of this study were to investigate the distributions of abnormally expressed optineurin (OPTN) proteins in retinal ganglion cells (RGC5s) of transgenic rats and their effects on subcellular morphological structures. Green fluorescent protein labeled EGFP wild-type (OPTN<sub>WT</sub>), E50K mutant type (OPTN<sub>E50K</sub>), and OPTN siRNA (si-OPTN) eukaryotic expression plasmids were constructed and transfected into RGC5s. Intracellular structures were labeled with organelle specific fluorescent dyes. Construct localization and cell morphologies were visualized by confocal fluorescence microscopy. OPTN<sub>WT</sub> was observed to be distributed as fine punctate fluorescent particles in the cytoplasm around the nucleus, along with exhibiting nuclear expression. OPTN<sub>E50K</sub> exhibited similar distribution but with non-uniform fluorescence particle size. si-OPTN distribution was similar to that of EGFP: uniform across the cytoplasm and nucleus. Compared with the negative control group, OPTN<sub>WT</sub> and OPTN<sub>E50K</sub> and to a lesser degree pEGFP-transfected cells

exhibited fracture and loss of myofilament proteins and mitochondrial swelling and cytoplasmic accumulation, along with abnormal lysosomal distribution and increased volume, and Golgi fragmentation. However, si-OPTN transfected cells exhibited no significant damage. Therefore, we demonstrated that the E50K mutation disrupts the uniformity of OPTN protein distribution upon exogenous overexpression. Furthermore, these results suggested that si-OPTN transfection, and thus potentially OPTN knockdown, did not impact subcellular morphology of RGC5 cells, whereas transfection, especially when combined with wild-type or mutant OPTN expression, led to substantial abnormalities in subcellular morphological structures. These findings lay a foundation for further research into the function of the OPTN protein.

**Key words:** OPTN; RGC5; Cell distribution; Subcellular morphology