Chapter 3

E50K Mutant of Optineurin Selectively Induces Death of Retinal Ganglion Cells
3.1 Introduction

Glaucoma is the second major cause of bilateral blindness in the world. The etiology of the disease is quite varied and the major risk factor is raise of intraocular pressure (IOP). Whatever may be the etiology, the ultimate cause of the disease is the death of retinal ganglion cells (RGC). Though there are many theories on how the RGC death occurs, there is no clear picture of why it occurs.

One of the genes associated not only with normal tension glaucoma (NTG) but also primary open angle glaucoma (POAG) is $OPTN$, which codes for the protein optineurin. $OPTN$ is the only candidate gene reported for NTG till date. This subset of glaucoma does not show raise in IOP but yet, death of retinal ganglion cells occurs. Since there is no IOP increase, it becomes difficult to create animal models to understand this form of glaucoma. Certain missense mutations in the coding region of optineurin are associated with adult onset and juvenile onset open angle glaucoma. The mutation E50K (where glutamic acid at position 50 is mutated to lysine) is the most common disease-causing mutation of optineurin reported till date (Rezaie et al., 2002; Aung et al., 2005). However, little is known about the molecular mechanisms responsible for the pathogenesis of glaucoma caused by such mutations. Several functions for optineurin have been proposed as described earlier. However, none of these experiments explore the role of wild type optineurin or the pathogenicity of mutants in the eye, particularly retinal ganglion cells. All experiments have been done in non-ocular cell lines which do not hold patho-physiological relevance. It has been speculated that optineurin has a neuroprotective role in the eye and optic nerve (Rezaie et al., 2002), but it has not been demonstrated experimentally. Hence, we have conducted experiments using the retinal ganglion cell line (RGC-5), which is of
prime importance in glaucoma. Though the cell line is a rat cell line, it still holds the significance of what might be happening in the human counterpart. The retinal ganglion cell line (RGC-5) chosen for our study has all the appropriate cell characteristics, viz., Thy-1, Brn-3C, neuritin, NMDA receptor, GABA-B receptor and synaptophysin expression, and negative for GFAP, HPC-1 and 8A-1 (Krishnamoorthy et al., 2000).

We explored the possibility that the disease associated mutants of optineurin may inhibit the functions of, or even induce the death of retinal ganglion cells (RGCs), a cell type relevant for glaucoma. One of the mutants of optineurin, E50K, was able to induce cell death in RGCs but not in other cell lines tested. An attempt was made to characterize the cell death pathway because understanding the mechanism would help us to design treatment strategies to prevent this cell death.

3.2 Results

3.2.1 E50K mutant of optineurin induces death selectively in RGC-5 cells.

Because the primary defect in glaucoma is the death of retinal ganglion cells, we used the rat RGC cell line (RGC-5), which is a useful model to study physiology of retinal ganglion cells, to analyze the effect of optineurin mutant expression on the survival of RGCs. RGC-5 cells grown on coverslips were transfected with the plasmids expressing HA-tagged wild type optineurin (WT) or its mutants, as described in chapter 2. Transfected cells were stained with anti-HA antibody and examined by fluorescence microscopy. Expression of the mutant E50K resulted in the death of 22.6% ± 3.3% cells, as revealed by the loss of refractility, condensation of chromatin and decrease in cell size caused by the
shrinkage of cytoplasm (Figure 3.1A, 3.2A). These morphological features of E50K-induced cell death are similar to those of apoptosis. Interestingly, cells expressing other mutants, namely R545Q, H26D and H486R, which too have been linked to POAG, did not display any more cell death than the basal value shown by wild type optineurin (Figure 3.1A & 3.3). The induction of cell death by E50K was not caused by its higher level of expression as compared to WT optineurin which was confirmed by western blot analysis (Figure 3.1B). A mutant protein can induce stress in the endoplasmic reticulum because of improper folding, but the effect of E50K did not seem to be caused by ER stress because the level of calnexin (a chaperone protein induced by ER stress) did not increase upon expression of E50K (Figure 3.1B).

To understand whether E50K induced cell death is cell type specific or a common phenomenon in all cell types, experiments were done in non-ocular cell lines and one more ocular cell line. The ability of the E50K mutant to induce cell death appears to be selective to RGCs because neither this mutant nor WT optineurin was able to induce cell death in IMR32 (a neuronal cell line), HeLa or Cos-1 (Figure 3.1C, 3.2 B). The level of E50K expression in these cell lines was compared by western blot analysis. The differences in the level of E50K expression were less and were not likely to explain the selectivity of E50K to induce cell death in RGC-5 cells (Figure 3.1D). The level of endogenous protein was higher in RGC-5 cells than in other cell lines (Figure 3.1E). Moreover, in RGC-5 cells the optineurin band showed faster mobility. The effect of E50K overexpression was also checked in another ocular cell line, namely the human retinal pigment epithelial cell line or D407. Neither the wild type optineurin nor the E50K mutant was able to induce cell death in this cell line (Figure 3.1C,
Figure 3.1: E50K mutant of optineurin selectively induces the death of RGCs.

(A) Effect of expression of various mutants of optineurin on the induction of cell death in RGC-5 cells. Cells grown on coverslips were transfected with 150 ng plasmids expressing normal optineurin (WT) or its mutants. After 32 hours of transfection, cells were fixed and stained for optineurin (HA-tag antibody) to determine cell death. Data represents cell death in expressing cells (mean ± SD of at least 4 experiments) after subtracting the background cell death observed in nonexpressing cells, which was generally 1% to 3%. GFP was used as an additional control. *P<0.01 compared with WT optineurin-expressing cells (Student’s t test).

(B) Western blot showing expression of various mutants of optineurin and the level of expression of calnexin. Cdk2 was used as a loading control.

(C) E50K mutant does not induce cell death in D407, Cos-1, HeLa or IMR-32 cells. Cells grown on coverslips were transfected with 300 ng indicated plasmids and processed as described in (A).

(D) Western blot showing expression of E50K mutant in indicated cell lines. Cdk2 was used as loading control. Transfection conditions of E50K in various cell lines, grown in 24-well plates, were the same as those used for cell death assays.

(E) Western blot showing expression of endogenous optineurin using optineurin antibody.
**Figure 3.2: E50K mutant induces cell death only in RGC-5 and not in other cell lines (Cos-1, HeLa, IMR-32 & D407).**

(A) Images of RGC-5 cells expressing WT or E50K mutant of optineurin, showing the induction of cell death by this mutant. Arrowheads: E50K-expressing dead cells.

(B) Images of Cos-1, HeLa, IMR-32 and D407 cells expressing WT or E50K mutant of optineurin.
Figure 3.3: Localization of optineurin mutants in RGC-5 cells. RGC-5 cells were transfected with the mutant constructs, viz., R545Q, H26D and H486R and 32 hours after overexpression stained for optineurin with HA antibody. Images showing expression and localization of optineurin mutants, R545Q, H26D and H486R in RGC-5 cells.
3.2B). These results show that E50K mutant of optineurin selectively induces death of RGC-5 cells but not of other cell lines tested.

3.2.2 E50K induced cell death is inhibited by Bcl2 and requires caspases

Since it was observed that E50K induced cell death has features of apoptosis, the involvement of a few caspases was studied. Mutant caspase-1 (Gupta et al., 2002) and caspase-9s (Seol & Billiar, 1999) are dominant negatives of the respective caspases and Bcl2 is an anti-apoptotic protein present in the mitochondria. Cell death induced by E50K in RGC-5 cells was inhibited by the antiapoptotic protein Bcl2 (p<0.05). Expression of caspase-9s (an inactive variant of caspase-9 that inhibits caspase-9 function) and mutant caspase-1 significantly reduced the effect of E50K on cell death (p<0.05) (Figure 3.4A). The inhibitory effect of Bcl2, mutant caspase-1 and caspase-9s on E50K induced cell death was not caused by their effect on the expression of E50K protein as determined by western blotting (Figure 3.4B). These results suggest that caspases are required for E50K-induced cell death in RGC-5 cells. TUNEL assay of DNA fragmentation did not reveal any significant labeling of E50K-expressing cells over the control. Moreover, little activation of caspase-3 was observed on E50K mutant expression (Figure 3.5A, B).

3.2.3 Optineurin and E50K mutant potentiate TNF-α induced cell death in retinal ganglion cells

TNF-α is a cytokine that induces many signaling pathways and induces cell death in many types of cells. The expression of TNF-α and TNF-α receptor-1 is upregulated in the retina and optic nerve head in persons with glaucoma (Yan et al., 2000; Tezel et al., 2001; Yuan et al., 2000). Optineurin gene expression is
Figure 3.4: Effect of caspase inhibitors and Bcl2 on E50K-induced death of RGC-5 cells.

(A) Cells grown on coverslips were cotransfected with E50K expression plasmid (150 ng) along with plasmids expressing mutant caspase (mCasp)-1, caspase (Casp)-9s, or Bcl2 (150 ng each). After 32 hours of transfection, cells were fixed and stained with HA-tag antibody, and cell death was determined. Expression of mutant caspase-1, caspase-9s, and Bcl2 resulted in significant inhibition (P<0.05) of cell death.

(B) Cells grown in 24-well plates were transfected as described in (A), and, after 32 hours, cell lysates were made for Western blotting. E50K protein level was determined using HA antibody.
Figure 3.5: E50K expression leads to caspase-3 activation in RGC-5 cells.

(A) RGC-5 cells were transfected with 1μg of wild type optineurin or E50K mutant and allowed to express for 32 hours. Western blot showing a faint active caspase-3 band in E50K mutant transfected lane. RGC-5 cells treated with 1μM staurosporine for 7 hours was used as positive control for caspase-3 activation. Cdk2 was used as loading control.

(B) Images of RGC-5 cells showing active caspase-3 staining in E50K expressing cells undergoing cell death. Cells grown on coverslips were transfected with 150 ng plasmids expressing WT optineurin or its E50K mutant. After 32 hours of transfection, cells were fixed and stained for optineurin (HA-tag antibody, in green) and active caspase-3 (in red) to determine active caspase-3 in E50K transfected cells.
induced by TNF-α in many cells (Li et al., 2000; Schwamborn et al., 1998). An association between polymorphisms in the optineurin and the TNF-α genes has been suggested to increase the risk for glaucoma (Funayama et al., 2004; Lin et al., 2003). Therefore, we examined the effect of the expression of optineurin and E50K mutant on TNF-α induced cell death. Cells were transfected with WT optineurin or E50K and after 24 hours these cells were treated with TNF-α for 24 hours. TNF-α induced cell death was seen to be potentiated by both WT and E50K mutant; E50K expressing cells showed significantly more cell death than those expressing normal optineurin (Figure 3.6A, B). Surprisingly even wild type optineurin increased TNF-α induced death of RGC-5 cells (P<0.05).

3.2.4 E50K- induced cell death is inhibited by antioxidants

To understand the mechanism by which the E50K mutant induces cell death in RGC-5 cells, we investigated the possibility of E50K causing oxidative stress, which is known to lead to pathologic cell death. The ability of antioxidants to inhibit E50K-induced cell death was tested. In our experiments, three different antioxidants were used: N-acetylcysteine (NAC), Trolox (a water soluble homolog of vitamin E) and MnSOD (manganese superoxide dismutase which is a mitochondria specific enzyme). NAC is the precursor of glutathione, which is a major antioxidant in mammalian cells. Treatment with NAC resulted in an inhibition of E50K induced cell death (Figure 3.7A, B). Another antioxidant, Trolox was also able to reduce this cell death. Cotransfection of a plasmid-expressing MnSOD resulted in greater than 75% inhibition of E50K-induced cell death (Figure 3.7A, B). Interestingly, we note that the protection against cell death induced by the three different antioxidants, NAC, Trolox and MnSOD was
Figure 3.6: Effect of optineurin (WT & E50K) overexpression on TNF-α induced cell death in RGC-5 cells.

(A) RGC-5 cells grown on coverslips were transfected with 300ng of wild type optineurin or E50K mutant. 24 hours after transfection, cells were treated with murine TNF-α (10ng/ml) for 24 hours and later fixed and stained with HA antibody. Bar diagram showing the effect of optineurin overexpression on TNF-α induced cell death. *P< 0.05 compared with WT optineurin expressing TNF-α treated cells (Student's t test).

(B) Images of RGC-5 cells showing effect of WT optineurin and E50K mutant on the TNF-α induced death of RGC-5 cells.
significant (P<0.05). That this inhibitory effect was not caused by the reduced expression of E50K protein was shown by western blotting (Figure 3.7C). Cell death induced by E50K in the presence of TNF-α was also inhibited significantly by NAC and Trolox (P<0.05) (Figure 3.7D). These results suggest that oxidative stress induced by E50K plays an important role in cell death.

### 3.2.5 E50K overexpression increases ROS levels in RGC-5 cells

To investigate further the involvement of ROS (reactive oxygen species) in E50K-induced cell death, the levels of ROS in RGC-5 cells transfected with E50K mutant or WT optineurin were determined by using an ROS-sensitive probe, CM-H₂DCFDA. It is a non-fluorescent probe which upon entering the cell is cleaved by intracellular esterases that remove its acetate groups and shows green fluorescence on binding to ROS. Expression of E50K mutant resulted in an increase in ROS production, as shown by increased DCF fluorescence (Figure 3.8A). Treatment of E50K transfected cells with NAC and Trolox, resulted in reduced ROS production (Figure 3.8B). Cotransfection of a plasmid expressing MnSOD with E50K resulted in nearly complete loss of ROS (Figure 3.8B). These results suggest that E50K expression induces ROS production which is partially inhibited by NAC and Trolox and completely by MnSOD.

### 3.2.6 Sub-cellular localization of endogenous optineurin in mammalian cell lines

Altered subcellular localization of a mutant protein can affect its function. Therefore, the subcellular localization of exogenous optineurin and of E50K was examined. In addition, subcellular localization of endogenous optineurin was also analyzed. Initial studies on optineurin show that the endogenous protein localizes
Figure 3.7: Effect of antioxidants on E50K-induced cell death of RGC-5 cells.

(A, B) NAC or Trolox was added to the medium after 6 hours of cell transfection with E50K expression plasmid. After 22 hours of transfection, medium was replaced with fresh medium containing antioxidants. Cells were fixed and stained after 32 hours of transfection.

(C) Western blot showing the level of E50K mutant in the presence of antioxidants. Cells grown in 24-well plates were transfected with E50K plasmid alone or with MnSOD. Cells were then treated with NAC or Trolox after 6 hours of transfection as in (A, B). Cell lysates were prepared after 32 hours of transfection for Western blotting.

(D) Effect of antioxidants on E50K-induced cell death in presence of TNF-α. This experiment was performed as in (A) except that after 24 hours of transfection, murine TNF-α was added.
Figure 3.8: E50K mutant induces ROS production.
(A) RGC-5 cells were transfected with WT optineurin or E50K mutant plasmids. After 30 hours of transfection, cells were washed and incubated with CM-H$_2$DCFDA. After 1 hour, cells were washed and visualized by an inverted microscope. Representative fields showing DCF fluorescence and corresponding phase-contrast images are shown. H$_2$O$_2$-treated cells were used as positive control. UT, untreated cells. Expression of E50K and WT optineurin was confirmed in each experiment by fixing these cells and staining with HA antibody. Representative fields from the same coverslips are shown in the bottom panels.

(B) RGC-5 cells were transfected with E50K expression plasmid alone or with MnSOD. E50K-transfected cells were treated with 5 mM NAC or 0.2 mM Trolox and were subjected to ROS detection assay. Representative fields showing DCF fluorescence are shown. MnSOD-transfected cells showed no DCF fluorescence.
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to the Golgi apparatus (Schwamborn et al., 2000; Rezaie et al., 2002). But a later report provides evidence contradicting the Golgi localization of endogenous optineurin (Park et al., 2006). Hence, experiment was done to determine the exact intracellular localization of endogenous optineurin in RGC-5 cells. RGC-5 cells were transfected with GFP- p23 (plasmid codes for a mature form of rat p23 tagged with GFP at the N-terminus), which has been shown to localize to the Golgi network and intermediate compartment (Blum et al., 1999). The cells were then fixed and stained for endogenous optineurin. Majority of the endogenous optineurin showed diffuse distribution in the cytoplasm and a detectable amount of the protein colocalizes with GFP- p23 (Figure 3.9A). We also tested this using another ocular cell line, D407, by double staining the cells with optineurin and Giantin (another Golgi marker) antibodies. Unlike in RGC-5, we found here that apart from uniform distribution of the protein in the cytoplasm, a prominent localization in the Golgi (Figure 3.9B) was also seen as observed by colocalization with Giantin. Optineurin localization was checked by staining with optineurin antibody in the non-ocular cell lines Cos-1, HeLa and IMR-32. In Cos-1, besides a detectable amount of the protein in the cytoplasm, substantial amount of the protein was seen localizing in the Golgi region, which is in the perinuclear position whereas in HeLa and IMR-32, optineurin shows a similar distribution pattern like in RGC-5 (Figure 3.10). The above results indicate that optineurin localizes and distributes in a cell type-specific manner; in a few cell lines it shows diffuse distribution in the cytoplasm along with Golgi while in some others it shows prominent localization in the Golgi apart from cytoplasmic distribution.
Figure 3.9: Sub-cellular localization of endogenous optineurin in ocular cell lines.

(A) RGC-5 cells transfected with GFP-p23 (a known Golgi marker) were stained with optineurin antibody. Confocal sections show diffused distribution of optineurin in the cytoplasm. Colocalization of optineurin with the Golgi marker was also observed as yellow colour in merged images. Nuclear staining is shown in blue in the images.

(B) D407 cells were co-stained with Giantin (a Golgi marker) and optineurin antibodies. Optineurin showed distribution in the cytoplasm and a prominent staining in the Golgi as shown by yellow colour in merged images. One coverslip was stained with only optineurin to show that the staining pattern remains unaltered when stained with Giantin and there is no cross reactivity among the secondary antibodies.
Figure 3.10: Sub-cellular localization of endogenous optineurin in non-ocular cell lines.

Cos-1, HeLa and IMR-32 cell lines were plated on coverslips and after 24 hours stained with optineurin antibody. Immunofluorescence pictures showed that the protein has cytoplasmic distribution and in Cos-1 there is a prominent staining at the perinuclear position likely to be Golgi. Images also show negative controls for Cos-1, HeLa and IMR-32 where only secondary antibody staining was performed.
3.2.7 Expression and localization of overexpressed optineurin and its mutants

Since we found that endogenous optineurin shows cytoplasmic distribution along with Golgi localization, our next experiment was to see whether overexpressed optineurin and its mutants also behaved the same way. WT optineurin cloned in the pcDNA 3.1-HA vector was transfected in RGC-5 and stained with anti-HA antibody. Overexpressed optineurin showed diffused distribution in the cytoplasm (Figure 3.11A). The mutants of optineurin, viz., E50K, R545Q, H26D and H486R behaved like wild type optineurin in their expression and localization (Figure 3.3), except that the E50K mutant showed large vesicular structures distributed in the cytoplasm. However, the large vesicular structures seen in E50K transfectants were generally concentrated in the vicinity of Golgi region (Figure 3.11A).

To check whether the HA-tag is interfering with the localization pattern of optineurin, vectors were made which expressed optineurin and E50K mutant without any tag and the transfected cells were stained with optineurin antibody. Optineurin and E50K showed the same localization as in the HA-tagged vector (Figure 3.11B). It can thus be concluded that the HA-tag does not affect the localization pattern of optineurin and E50K.

3.3 Discussion

It has been suggested that optineurin has a cytoprotective function that is disrupted by mutations leading to glaucoma (Rezaie et al., 2002). Although this function of optineurin in RGCs is yet to be established, our results suggest that the E50K mutant has acquired the ability to induce cell death selectively in RGCs...
Figure 3.11: Subcellular localization of overexpressed optineurin and its mutant E50K in RGC-5 cells.
(A) RGC-5 cells were transfected with pcDNA3.1-HA-optineurin or E50K mutant and stained with HA and Giantin antibodies. Confocal sections show very little colocalization of overexpressed optineurin (WT) or E50K (green) with Golgi marker giantin (red) as seen by lack of yellow colour in the merged images. Optineurin shows diffused distribution in the cytoplasm. Nuclear DNA staining with DAPI is shown in blue in merged images. E50K forms large vesicular structures, which are usually concentrated in Golgi region.
(B) RGC-5 cells were transfected with pcDNA3.0-OPTN/E50K and stained with optineurin antibody. The staining pattern is similar to the tagged vectors of optineurin.
but not in other cells. This selectivity has relevance to the disease condition because even though optineurin shows ubiquitous expression in various tissues of the body, patients with a mutation in optineurin gene suffer from glaucoma alone, and are not reported to have any other disease history. The ability of E50K to potentiate TNF-α induced death of RGCs points to the role of environmental, as well as genetic factors in causing glaucoma. The finding that E50K mutant-induced cell death is mediated by oxidative stress indicates the potential of antioxidants in preventing or delaying glaucoma.

Interestingly, only the E50K mutant of optineurin was able to induce the death of RGC-5 cells; three other mutants were unable to do so. E50K is a unique mutation that has been found only in British patients or in patients of British descent. It has been suggested that a founder effect accounts for the E50K mutation frequency in British patients (Alward et al., 2003). It appears that E50K mutation alone is able to induce cell death when expressed at elevated levels. Glaucomas are generally multifactorial and are perhaps affected by multiple interacting loci (Libby et al., 2005). It is likely, therefore, that other mutants (H26D, H486R) of optineurin may require interaction with genetic modifiers for inducing cell death in RGCs.

Oxidative stress in a cell may occur because of an imbalance between the production and removal of ROS, and it has been implicated in nerve cell death in the eye (Maher & Henneken, 2005a, b). Cell death induced by oxidative stress can be prevented or reduced by blocking specific steps in the cell death cascade. Cell death induced by E50K in RGC-5 cells was inhibited by two structurally unrelated antioxidants (NAC and Trolox), which act by different mechanisms, suggesting that this cell death was mediated by oxidative stress. This suggestion
is further supported by the observations that E50K expression results in increased production of ROS, which is reduced by NAC and Trolox. MnSOD completely abolished ROS production by E50K and inhibited cell death. These results suggest that the inhibition of E50K-induced cell death by NAC and Trolox is mediated, at least in part, by their ability to reduce ROS levels. Cell death induced by oxidative glutamate toxicity in RGC-5 cells has features of classic apoptosis and oxytosis (Maher & Henneken, 2005b). This type of cell death has morphologic features of apoptosis (rounding of cells, shrinkage of cytoplasm) but does not involve DNA fragmentation or caspase-3 activation (Maher & Henneken, 2005b). E50K-induced cell death is morphologically similar to apoptosis but does not show any significant increase in DNA fragmentation. Only a small increase in caspase-3 activation was seen. Taken together, these results suggest that the E50K mutant induces a type of cell death in RGC-5 cells that has characteristics of classic apoptosis and oxytosis.

How does the E50K mutant induce cell death in RGC-5 cells? Optineurin interacts with many proteins and the Rab8 interaction domain (amino acids 58-209) (Hattula & Peranen, 2000) is present in the vicinity of the E50K mutation site. It is thus likely that a conformational change induced by E50K mutation alters its interaction with Rab8. It has been reported that in response to a high level of oxidative stress (25 mM H$_2$O$_2$), optineurin translocates to the nucleus in a Rab8-dependent manner, whereas the E50K mutant is unable to do so (De Marco et al., 2006). Whether the loss of interaction with Rab8 or a gain of interaction with some other protein is responsible for the ability of E50K mutant to induce cell death in RGC-5 cells will require further investigation. A cytoprotective role of optineurin has been suggested in the retina, though this remains to be
demonstrated. In NIH 3T3 cells, optineurin (but not the E50K mutant) protects against cell death induced by a high level of oxidative stress (De Marco et al., 2006). Thus, it is likely that under certain conditions of stress, optineurin may have a cytoprotective function. However, our results suggest that a high level of WT optineurin can increase the TNF-α-induced death of RGC-5 cells, indicating that optineurin might not have a generalized cytoprotective function in these cells.

The mechanism of selectivity of E50K-induced cell death in RGC-5 cells (compared with other cells tested) is unclear. This selectivity is not the result of a higher level of E50K expression in RGC-5 cells. Earlier studies with neonatal rat retina have shown that, compared with other retinal cells, RGCs were more resistant to oxidative stress-induced cell death (Kortuem et al., 2000). In another report (Maher & Henneken, 2005b), it was observed that RGC-5 cells were less sensitive to oxidative stress-induced cell death than the hippocampal cell line HT22. Therefore, differential sensitivity of RGC-5 cells to oxidative stress is not likely to be the reason for the selectivity of E50K-induced cell death, which would require further investigation.

Earlier evidence regarding endogenous optineurin distribution were quite contradictory; some reports show that endogenous optineurin has Golgi localization (Schwamborn et al., 2000; Rezaie et al., 2002), whereas another reports argues that optineurin is not present in Golgi (Park et al., 2006). A recent report also shows localization of endogenous optineurin in Golgi (De Marco et al., 2006). We have shown that endogenous optineurin shows diffused cytoplasmic distribution which includes Golgi too, as shown by using two different Golgi markers, i.e., GFP- p23 and Giantin in two different cell lines- RGC-5 and D407.
Moreover, staining for optineurin in Cos-1, HeLa and IMR-32 also showed similar distribution pattern like in RGC-5 and D407 cell lines. On the other hand, overexpressed optineurin showed uniform cytoplasmic distribution and did not colocalize with Giantin. Other mutants, viz., H26D, H486R and R545Q also behaved similar to wild type optineurin. The most common mutation E50K showed large vesicular structures in the cytoplasm and most of them lie in the vicinity of Golgi. Overall the results suggest that although some amount of endogenous optineurin is present in Golgi, this is dependent on cell type.

3.4 Conclusion

The results described in this chapter show that

- **E50K mutant of optineurin has acquired the ability to induce cell death selectively in retinal ganglion cells.**
- **E50K induced cell death is inhibited by antioxidants, suggesting the potential of antioxidants to prevent or delay some forms of glaucoma, particularly NTG and**
- **optineurin affects TNF-\(\alpha\) induced cell death, suggesting that it may be a component of TNF-\(\alpha\) signaling pathway.**