Critical Review

Functional Analysis of Optineurin and Some of Its Disease-associated Mutants

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Abstract

Optineurin is a multifunctional protein involved in a variety of cellular functions such as protein trafficking by vesicles, autophagy, and signal transduction. Certain mutations in optineurin (gene *OPTN*) are associated with neurodegenerative diseases like glaucoma and amyotrophic lateral sclerosis (ALS). Optineurin is also seen in pathological structures present in several other neurodegenerative diseases. In glaucoma, loss of vision occurs due to progressive degeneration of retinal ganglion cells, and perhaps loss of photoreceptor cone cells as well. Most of the glaucoma-associated mutations of optineurin are heterozygous missense mutations, whereas the ALS-associated mutations include deletion, truncation, and missense mutations. Optineurin mediates its functions by interacting with various proteins, often acting as an adaptor to provide a link between two or more proteins. Disease-causing mutations alter these interactions leading to functional defects in membrane vesicle trafficking, autophagy, signaling, aggregate formation, and other processes. Some of these functional defects, caused by glaucoma-associated mutants of optineurin, led to retinal cell death mediated by apoptosis and therefore may contribute to pathogenesis directly. Other mutations are likely to cause glaucoma by indirect mechanisms involving other cell types. Mechanisms of ALS pathogenesis by optineurin mutations are yet to be investigated in detail; however, some ALS-associated mutants cause defects in signaling, autophagy, and ubiquitin binding, which might contribute to pathogenesis. © 2015 IUBMB Life, 67(2):120–128, 2015

Keywords: *optineurin; glaucoma; amyotrophic lateral sclerosis; autophagy; neurodegenerative disease; protein function.*

Abbreviations: *ALS, amyotrophic lateral sclerosis; ATG, autophagy-related proteins; Bcl-2, B-cell lymphoma-2; BMDM, bone marrow-derived macrophages; CYLD, cylindromatosis (turban tumor syndrome) protein; C57BL6, "C57 black 6," "C57" or "black 6" (standard abbreviation: B6); GAP, GTPase-activating protein; HEK, human embryonic kidney cells; IRF-3, interferon regulatory factor-3; IFNβ, interferon-β; LC3-1, microtubule-associated protein 1 light chain 3; LPS, lipopolysaccharide; MYPT-1, myosin phosphatase targeting subunit-1; NEMO, NF-κB essential modulator; NF-κB, nuclear factor kappa B; PLK-1, Polo-like kinase-1; TNFα, tumor necrosis factor alpha; NSC-34, mouse motor neuron-like hybrid cell line; NTG, normal-tension glaucoma; OPTN, optineurin; POAG, primary open-angle glaucoma; Rab8, rat sarcoma (abbreviated as Ras)-related protein 8; RGC-5, retinal ganglion cell line-5; RIP, receptor-interacting protein; TBK1, TRAF family member-associated NF-κB activator kinase 1; TRAF, tumor necrosis factor (TNF) receptor-associated factor; TBC1D17, TBC 1 domain-related family protein 17 [TBC refers to the (Tre-2, Bub2p, and Cdc16p) domain]; TFR, transferrin receptor; TM, trabecular meshwork; UBD, ubiquitin-binding domain.*

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Introduction

Optineurin (gene *OPTN*) is an adaptor protein involved in several functions such as membrane vesicle trafficking, signal transduction, autophagy, cell survival, Golgi ribbon formation, and mitosis. Human optineurin is a 577-amino acid protein that contains multiple coiled-coil domains, a leucine zipper, a ubiquitin-binding domain (UBD), and a zinc finger at the C-terminus (1,2). These are schematically shown in Fig. 1. Optineurin is widely linked with various neurodegenerative diseases. Mutations in OPTN are associated with glaucoma and amyotrophic lateral sclerosis (ALS; refs. (3) and (4)). In addition, optineurin is present in pathological structures seen in several neurodegenerative diseases such as skein-like and round hyaline inclusions in ALS, senile plaques and neurofibrillary tangles in Alzheimer's disease, Lewy bodies in Parkinson's disease, and Pick bodies in Pick's disease (5). Optineurin is also linked with Paget's disease of the bone (6).

Optineurin is expressed in most of the tissues in humans and mice. In humans, it is expressed in ocular (retina, cornea, iris, etc.) as well as nonocular tissues such as heart, lung, brain, placenta, skeletal muscle, liver, and kidney (4,7). In the eye, it is present at maximum level in the pigmented epithelium and retinal ganglion cells. Its expression in various tissues and cells in significant amount indicates that optineurin has a common role to play in various types of cells. *OPTN* gene is evolutionarily conserved in mammals and is also seen in other vertebrates, such as chicken, frog, and zebra fish (2). However, its orthologs have not been identified in invertebrates, plants, and yeast.

Mutations in Optineurin Cause Disease

Mutations in the coding region of the gene optineurin are causatively associated with glaucoma, which is the second leading cause of bilateral blindness worldwide (8). Genetic as well as environmental factors contribute to glaucoma pathogenesis. It is a genetically heterogeneous group of optic neuropathies characterized by optic nerve head cupping and progressive loss of vision due to retinal ganglion cell death (9). Loss of other retinal cells, particularly photoreceptor cells, has been observed in human as well as experimental glaucoma (10,11). Increased intraocular pressure is one of the major risk factors in adult primary open-angle glaucoma (POAG). Mutations in optineurin are associated with normal-tension glaucoma (NTG) as well, which is a subtype of POAG with normal intraocular pressure (7). In a study of families affected with autosomal-dominant adult onset NTG, Rezaie et al. (4) identified mutations in OPTN as the cause of the disease in 16.7% of the families. Since then, several studies have identified the association of optineurin mutation with NTG (Fig. 1A; ref. [2]). These are mostly missense single-copy mutations, suggesting that these are dominant. Recently, mutations in optineurin were shown to cause familial as well as sporadic ALS (3). ALS-associated mutations of optineurin include deletions, missense, and nonsense mutations (Fig. 1B). ALS is a fatal disease, which involves degeneration of motor neurons in the spinal cord, brainstem, and primary cortex, resulting in paralysis of voluntary muscles (12). It has been suggested that loss of function as well as gain of function mechanisms are involved in ALS pathogenesis caused by mutations in optineurin. Mutations in optineurin that are associated with glaucoma are generally not associated with ALS. A review article by Maruyama and Kawakami (13) explains well the involvement of optineurin mutations in ALS pathogenesis.

Functions of Optineurin

Optineurin is involved in mediating several functions by interacting with many proteins directly or sometimes indirectly. In most of these functions, optineurin acts as an adaptor protein that links two different proteins (1,2). It does not have any enzymatic activity. The interactions of optineurin with various proteins are mediated by well-defined binding sites, as described in Fig. 2. Optineurin interacts with monoubiquitin with low affinity through its UBD. It binds to Lys 63-linked and linear (head-to-tail-linked) polyubiquitin chains with high affinity. It does not interact with Lys48-linked polyubiquitin chains (14). This selectivity of interaction with Lys63-linked and linear polyubiquitin chains is mediated by UBD and zinc finger domains, both of which bind to ubiquitin (15). A point mutation in UBD, D474N, abolishes its binding to ubiquitin (14). This ubiquitin-binding function of optineurin is implicated to be involved in mediating most of its function.

Vesicle Trafficking and Maintenance of Golgi Structure

Optineurin directly interacts with several proteins involved in membrane vesicle trafficking such as Rab8 [rat sarcoma (abbreviated as Ras)-related protein 8] GTPase, Huntingtin, myosin VI, and TBC 1 domain [TBC refers to the (Tre-2, Bub2p, and Cdc16p) domain]-related family protein 17 (TBC1D17; refs. 16–18). It is involved in regulating transferrin receptor (TFR) trafficking and recycling through its interaction with Rab8 (19,20). Optineurin is an effector of Rab8 because it binds preferentially with the activated form of Rab8 (Rab8-GTP) but not with its inactive form (Rab8-GDP; ref. 16). In addition to being an effector of Rab8, optineurin also functions as a negative regulator of Rab8-GTP by recruiting a GTPase-activating protein (GAP), TBC1D17, which inactivates Rab8 (21). TBC1D17 directly interacts with optineurin but not with Rab8. The interaction between Rab8 and TBC1D17 is mediated by optineurin. Activated Rab8 forms tubules emanating from endocytic recycling compartment that mediate recycling of TFR from recycling endosome to plasma membrane. Optineurin regulates TFR recycling by controlling the activity of Rab8 through TBC1D17 (21). Optineurin in conjunction with myosin VI (an actin-based motor protein) is also involved in regulating several other membrane vesicle trafficking pathways, which have been described in detail in recent reviews (1,2).

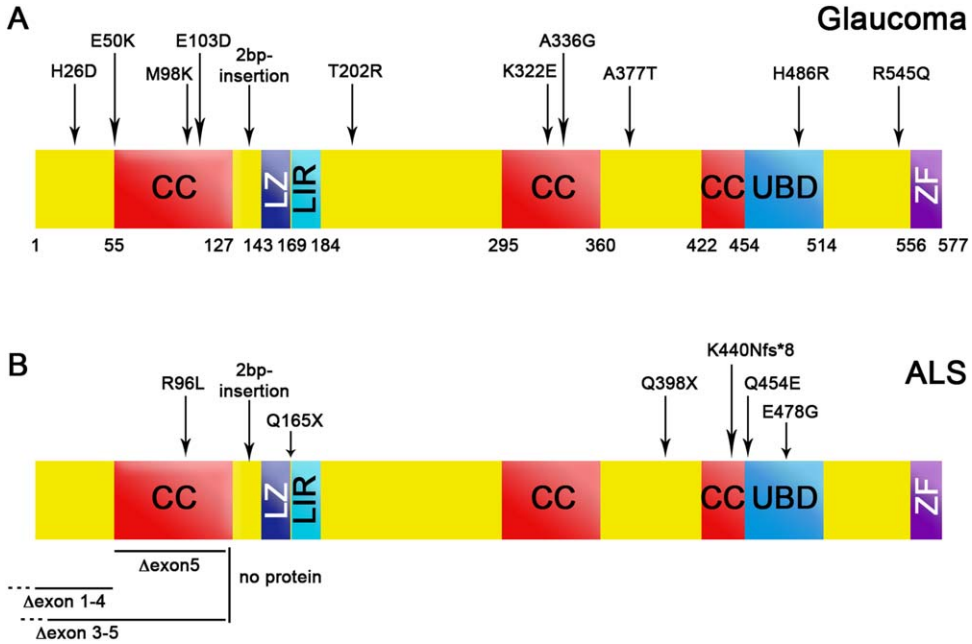

FIG 1 *Disease-associated mutations of optineurin. Schematic representation of human optineurin depicting its various domains; CC, coiled coil; LZ, leucine zipper; LIR, LC3-interacting region; UBD, ubiquitin-binding domain; ZF, zinc finger. Localization of disease-associated mutations in optineurin: (A) glaucoma and (B) ALS. M98K and R545Q are polymorphisms.*

Optineurin is also important for maintaining Golgi organization, with both its overexpression [in trabecular meshwork (TM), retinal pigment epithelium, and RGC-5 cells] and RNAi-mediated depletion causing fragmented or disconnected Golgi complex (18,22,23). Although the exact role of optineurin in Golgi maintenance is not known, its interaction with Rab8, Htt, and myosin VI have been implicated in this process (18).

Optineurin Functions as an Autophagy Receptor

Autophagy is one of the quality control mechanisms used by the cell to remove defective proteins and organelles by lysosomal

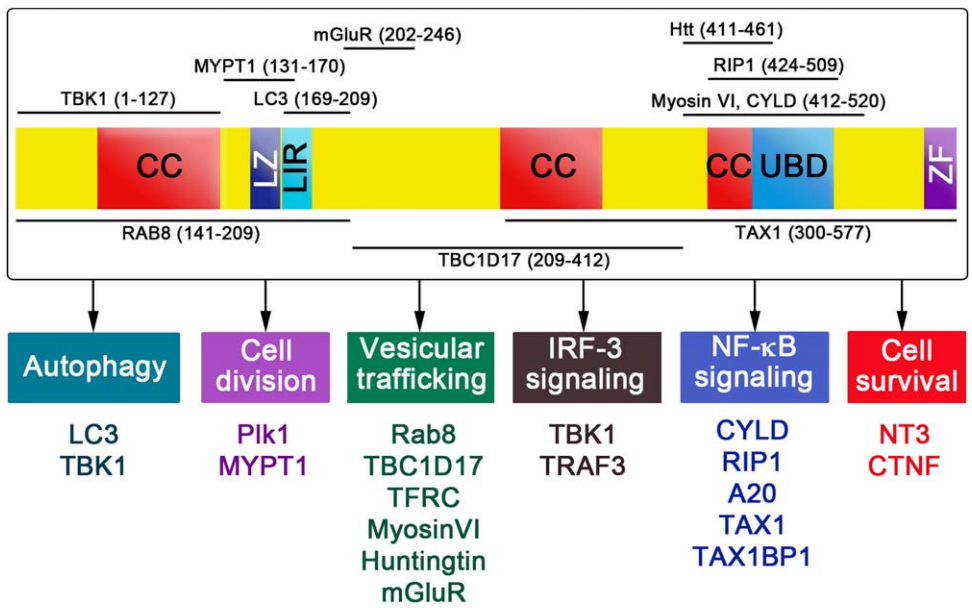

FIG 2 *Interactions and functions of optineurin. Optineurin is a multifunctional adapter protein, which performs its function by interacting with different proteins. Black horizontal bars depict regions of optineurin involved in interactions with indicated proteins with their delimitations (in amino acids) in brackets. Various functions performed by optineurin and proteins involved in those functions are depicted below the schematic of optineurin protein.*

degradation. When autophagy is induced, specialized membranous structures are formed which are known as autophagosomes (24). The formation of autophagosomes is a complex process that is initiated by the formation of an isolation membrane (also known as phagophore) near the endoplasmic reticulum. During maturation of the isolation membrane into autophagosomes, the cargo (cytoplasmic components and organelles) that need to be degraded are incorporated with the help of specialized proteins known as autophagy receptors. The autophagy receptors and associated cargos are recruited into autophagosomes by interaction with autophagosomal protein LC3 (microtubule-associated protein 1 light chain 3). During autophagy, LC3-1 is converted into LC3-II by conjugation to a lipid, phosphatidylethanolamine by coordinated action of several autophagy-related proteins (ATG proteins). Autophagy receptors bind with LC3 and also with ubiquitin, facilitating the recruitment of ubiquitinated cargo to autophagosomes (25). Optineurin was identified as an autophagy receptor involved in the clearance of cytosolic Salmonella by autophagy (26). Optineurin directly interacts with LC3 through a well-defined binding site, and phosphorylation of a Ser (Ser177 in human OPTN) in the LC3 interaction region enhances binding with LC3 resulting in efficient autophagy. UBD of optineurin is involved in binding to ubiquitinated cargo, although nonubiquitin-mediated autophagic function of optineurin has also been reported (27).

Role of Optineurin in Signaling

Optineurin was identified as a negative regulator of transcription factor NF-κB activation, because the knockdown of optineurin resulted in increased NF-κB activity (14,28). Overexpressed optineurin inhibits tumor necrosis factor alpha (TNFα)-induced NF-κB activation. It was proposed that optineurin inhibits TNFα-induced NF-κB activation by competing with NEMO for binding to polyubiquitinated receptor-interacting protein (RIP), an intermediate that is crucial in regulating IKK kinase activity and, thereby, NF-κB activity (14). This interaction of optineurin with polyubiquitinated RIP is mediated through the UBD of optineurin. Recently, it was shown that negative regulation of TNFα-induced NF-κB signaling by optineurin involves cylindromatosis (turban tumor syndrome) protein (CYLD), a deubiquitinase which deubiquitinates RIP (29). Optineurin directly interacts with CYLD to mediate deubiquitination of RIP by CYLD.

Optineurin is also involved in signaling to interferon regulatory factor-3 (IRF-3) phosphorylation and interferon-β (IFN-β) production in response to LPS and double-stranded RNA (30,31). UBD of optineurin is essential for this signaling (31). This signaling is involved in host immune defense in response to bacterial and viral infections. The protein kinase TRAF (TNF receptor-associated factor) family member-associated NF-κB activator kinase 1 (TBK1), which directly interacts with optineurin, is activated by LPS and double-stranded RNA, and optineurin is required for optimal activation of TBK1 to induce IFN-β (31,32).

Recently, two different mouse models have been generated to study the role of optineurin in NF-κB and IRF-3 signaling (31,33). In one model, a knock-in mouse has been created which expresses D474N mutation, which nearly completely abolishes the ubiquitin-binding function of optineurin. Bone marrow-derived macrophages (BMDM) derived from these mice are normal in LPS-induced NF-κB activation but compromised in LPS-induced TBK1-mediated IRF-3 activation and IFN-β production (31). In another model, C-terminal region of optineurin is deleted (Optn 470T), which completely eliminates ubiquitin binding (33). The Optn 470T mice, which express reduced levels of truncated OPTN (1–470 amino acids), showed embryonic lethality with incomplete penetrance, and this lethality was strain-specific, as seen in the 129 X C57BL/6 background but not when further backcrossed to C57BL/6 background. Those Optn 470T mice (of 129 X C57BL/6 background) that survived were normal. The BMDM and bone marrow-derived dendritic cells from Optn 470T mice are not defective in NF-κB regulation but are compromised in TBK1-mediated IRF-3 activation and IFN-β production. It is likely that in these mice, in the absence of optineurin, some other protein acts as an adaptor to mediate CYLD-dependent deubiquitination of RIP and NF-κB regulation. It is also likely that although expressed in most of the cells and tissues, optineurin has cell-specific functions because mutations in optineurin result in defects in certain specialized tissues/cells, whereas most of the other tissues/cells are unaffected. The D474N-OPTN in D474N mouse model is expressed at higher level than normal OPTN, and the D474N mutation does not completely abolish ubiquitin binding. This may explain the lack of lethality in these mice. In 470T mice, the role of optineurin in tissues and cells relevant for glaucoma and ALS is yet to be examined.

In contrast to these reports (31,33), Mankouri et al. (30) showed that optineurin negatively regulates IFN-β production in response to RNA virus infection in human embryonic kidney (HEK293) cells. The reason for this discrepancy is not clear; however, it could be due to the use of different cell types.

Regulation of Cell Cycle

Polo-like kinase-1 (PLK-1) is an important regulator of various mitotic events in eukaryotic cells governing the G_2M phase transition to cytokinesis. PLK-1 is activated at mitotic entry by phosphorylation at its Thr 210 residue. Myosin phosphatase complex (MYPT1 PP1β) inactivates PLK-1 by dephosphorylating this residue, and optineurin plays an important role in this process. PLK-1 phosphorylates optineurin at S177 and moves it to nucleus during mitosis (34). In the nucleus, optineurin promotes phosphorylation of MYPT1 by cyclin-dependent kinase-1, facilitating the interaction of MYPT1 with PLK-1, which leads to inactivation of PLK-1. Optineurin depletion causes failure in cell division (multinucleated cells) and defective chromosome separation. Thus, optineurin appears to act as a feedback regulator of PLK-1 activity.

Cytoprotective Functions of Optineurin

A cytoprotective function for optineurin in the retina was suggested by Rezaie et al. (4). They proposed that the loss of

cytoprotective function of optineurin on mutation leads to the death of retinal cells resulting in glaucoma. Later on, it was shown that knockdown of optineurin in the cell line RGC-5 results in apoptosis-like cell death due to reduced secretion of neurotrophins, particularly NT-3, and that addition of NT-3 to optineurin-deficient RGC-5 cells provided cytoprotection (35). Recently, it was shown that knockdown of optineurin in Neuro2a cells resulted in cell death via mitochondrial apoptotic pathway (36). This cell death induced by optineurin knockdown was due to activation of NF-κB because inhibition of NF-κB by withaferrin suppressed this cell death (36). Optineurin also has a cytoprotective role against oxidative stress-induced death of NIH3T3 cells (mouse embryonic fibroblast cells obtained from the National Institutes of Health, USA); however, this is yet to be shown in any cell type relevant for glaucoma or ALS (37).

Issue of the Identity of the RGC-5 Cell Line

In much of the research on the cell biology of glaucoma, researchers have found it difficult to work with purified primary RGCs, as one has limited access to small number of cells, which survive for only few passages. The introduction of the immortalized RGC cell line, termed RGC-5 by Krishnamoorthy et al. (38) in 2001, provided a very useful tool in this area of research. However, the publications by van Bergen et al. (39) in 2009 and Krishnamoorthy et al. (40) in 2013 showed that the RGC-5 cell line is not from rats at all, but is actually of murine origin and contains transformed photoreceptor cells, and is very similar to (or the same as) 661W photoreceptor cone cell line. In their latest analytical review, Sippl and Tamm (41) have summarized the available data on RGC-5 and offered some guidelines on the benefits and limitations of RGC-5 for research. We believe that RGC-5 is still a useful cell culture model to explore the mechanisms associated with glaucoma pathogenesis due to the following reasons: (a) it shows properties of neural precursor cells (39), and (b) in human glaucoma, outer retinal cells such as photoreceptor cone cells are also damaged in addition to RGCs (11). In several experimental animal models of glaucoma, the thickness of all the cell layers of retina is reduced due to cell death (10); and (c) the glaucoma-associated mutant of OPTN, E50K, induces death of RGC-5 cells but not of several other cells tested (42). This has been validated in transgenic mice expressing E50K-OPTN, which show reduced thickness of all the cell layers of retina (43). This also suggests that an authentic and immortalized human retinal ganglion cell line needs to be developed, characterized in great detail, and made available for researchers.

Functional Defects Caused by Mutants

Some of the mutants of optineurin cause a defect in its functions, which in some cases have been shown to be responsible for death of retinal cells. A few of these are listed below.

E50K Mutant Impairs Vesicle Trafficking and Autophagy

Loss of vision in glaucoma occurs due to the death of retinal ganglion cells, although other cells in the retina such as photoreceptor cone cells are also reduced (44). The possibility of functional defects in optineurin mutants, which are relevant for glaucoma, was examined by expressing various mutants of optineurin in the retinal cell line, RGC-5, and it was observed that two of these mutants, namely, E50K and M98K, induced significantly more cell death than wild-type OPTN (42,45). In transgenic mice expressing E50K-OPTN, the thickness of all retinal cell layers, including ganglion cells and photoreceptor cells, is seen to be reduced in the peripheral retina due to apoptotic cell death (43).

When E50K mutant is expressed in mammalian cells, it forms vesicle-like structures, often termed foci, which are larger than those formed by wild-type optineurin (19,22). This provided first indication that this mutant causes a defect in vesicle trafficking. Subsequent studies revealed that E50K mutant impairs endocytic trafficking and recycling of TFR, a process regulated by normal optineurin. This leads to the formation of larger foci positive for TFR (19,20). The mechanism of this defective trafficking caused by E50K mutant has been investigated, and it turns out that E50K mutant impairs Rab8-mediated TFR recycling. The E50K mutant enhances inactivation of Rab8 by recruiting the GAP protein, TBC1D17, more efficiently, resulting in reduced Rab8-mediated recycling of TFR (21,46). This impaired function of TFR is partly responsible for E50K-induced apoptotic death of retinal cells because coexpression of TFR inhibits E50K-induced cell death (46). The E50K-induced death of retinal cells is strongly inhibited by blocking the function of TBC1D17 either by knockdown or by a dominant negative mutant, suggesting that TBC1D17 plays a crucial role in E50K-induced cell death (46). In addition to its role in regulation of TFR recycling, TBC1D17, through its catalytic activity, is also involved in autophagy. The E50K mutant causes a block in autophagy, mediated by TBC1D17, leading to formation of larger autophagosomes. This block in autophagy is involved in mediating E50K-induced cell death because this cell death can be prevented by rapamycin, an inducer of autophagy (46). Thus, TBC1D17 mediates E50K-induced retinal cell death by two different mechanisms, impaired TFR recycling, and autophagy (Fig. 3). How TBC1D17 mediates E50K-induced block in autophagy is not clear. As TBC1D17 inhibits autophagy through its catalytic activity, it is likely to act on one of the Rabs involved in autophagy. However, the identity of the TBC1D17-regulated Rab GTPase involved in autophagy is not yet known.

The transgenic mice expressing E50K-OPTN show activation of glial cells, which might contribute to glaucoma phenotype (loss of retinal cells) of these mice (43). E50K-OPTN formed insoluble aggregates in HEK293 and also in neurons derived from induced pluripotent stem cells from E50K mutation-carrying patients with NTG (47). The E50K mutant shows enhanced interaction with a protein kinase, TBK1, and this enhanced interaction has been postulated to be involved

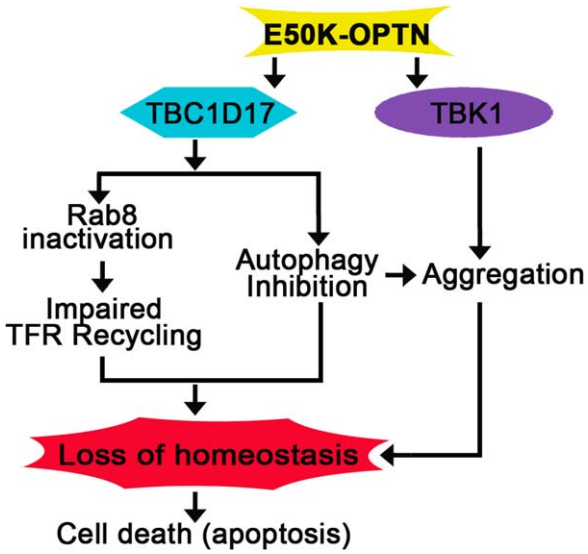

FIG 3 *Mechanism of E50K-induced disruption of homeostasis leading to death of retinal cells. Wild-type optineurin plays an important role in regulating TFR recycling and autophagy through its interaction with TBC1D17. E50K-OPTN shows enhanced recruitment of TBC1D17 causing defect in autophagy and repression of TFR recycling. This causes loss of cellular homeostasis and eventually cell death by apoptosis. E50K-OPTN also shows enhanced interaction with TBK1, which causes aggregate formation and may also contribute to cell death.*

in the formation of insoluble aggregates by E50K-OPTN and glaucoma pathogenesis (47). Impaired autophagy might also contribute to the formation of these aggregates (48). The *TBK1* gene is amplified in certain patients with NTG and TBK1 is known to phosphorylate optineurin to enhance its binding to LC3, which mediates autophagy (26,49,50). Whether enhanced interaction of E50K with TBK1 leads to altered phosphorylation of E50K-OPTN is yet to be examined.

Optineurin exists in the cells as hexamer, probably a dimer of trimer, and on treatment of cells with an oxidant, it forms nondisulfide-linked covalent trimer (23,51). The E50K mutant induces oxidative stress in the cell, leading to the death of retinal cells, which can be prevented by antioxidants. The oxidative stress generated by E50K-OPTN causes the formation of covalent trimers, and antioxidants prevent the formation of such covalent trimers of E50K-OPTN (51). This covalent trimer formation might contribute to pathogenesis; however, this needs to be tested experimentally.

M98K-OPTN Induces Autophagy

In the original study describing OPTN mutations as cause of NTG, it was shown that a polymorphism, M98K (where methionine at 98th position is changed to lysine), was found in 13.6% of glaucoma subjects, whereas only 2.1% control subjects showed this variation (4). Subsequently, it was shown that M98K polymorphism of OPTN is associated with glaucoma in certain ethnic groups such as the Japanese, Chinese, and Indian, but not in Caucasians (52–56). Recently, it was shown that M98K-OPTN, when overexpressed in retinal cells, induces autophagy leading to TFR degradation and cell death by apoptosis (45). Degradation of TFR plays a crucial role in this cell death because coexpression of TFR or inhibition of this degradation by lysosomal inhibitors provides protection against this cell death. Involvement of autophagy in M98K-induced TFR degradation and cell death was confirmed by knockdown of Atg5, a protein essential for autophagy, which prevented TFR degradation as well as cell death. Mutagenesis studies revealed that the function of UBD and LC3-binding sites are required for M98K-induced autophagy, caspase-3 activation, and cell death. Endogenous TFR is normally degraded by autophagy and M98K-OPTN enhances the delivery of TFR to autophagosomes for lysosomal degradation (45). These *in vitro* studies with M98K-OPTN using a retinal cell line have provided evidence to show that M98K mutation causes a functional defect in optineurin leading to cell death (Fig. 4). This might be relevant for glaucoma pathogenesis. However, this needs to be tested by using a transgenic animal model.

The E50K mutant impairs autophagy, whereas M98K mutant enhances autophagy, although both these mutants induce apoptotic death of retinal cells. These observations suggest that an optimum level of autophagy is crucial for the

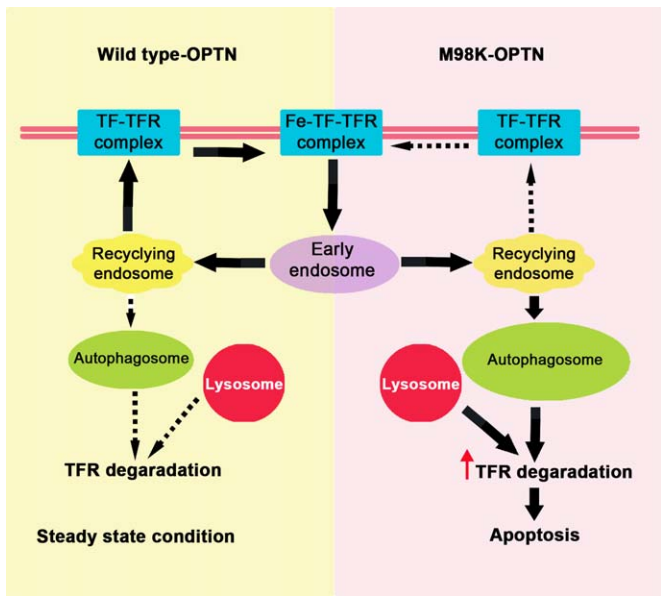

FIG 4 *A model showing steps involved in M98K-induced death of retinal cells. In normal cells, wild-type OPTN helps in regulating TFR levels by recycling most of it back to plasma membrane through recycling endosome. A proportion of TFR is degraded through autophagy. Optineurin is known to act as autophagy receptor. M98K-OPTN interacts with TFR more strongly and disrupts TFR turnover dynamics by enhancing its delivery to autophagosome rather than recycling endosomes. Consequently, increased lysosomal degradation of TFR leads to reduced cellular TFR levels causing autophagic cell death mediated by apoptosis.*

survival of retinal cells. However, the mechanisms involved in the induction of cell death by E50K and M98K mutants are different. The E50K-induced cell death involves generation of reactive oxygen species and is inhibited by antioxidants and also by the antiapoptotic protein B-cell lymphoma-2 (Bcl-2); however, M98K-induced cell death is not inhibited by antioxidants or Bcl2 (42,45). How such impaired or enhanced autophagy leads to caspase-3 activation and apoptosis is yet to be investigated. One common feature of E50K- and M98K-induced apoptosis of retinal cells is the impairment of TFR recycling and function. Although M98K mutant causes autophagic degradation of TFR leading to its reduced level and recycling, the E50K mutant causes impaired TFR recycling by inactivating Rab8. Thus, it appears that TFR function is critical for the survival of retinal cells.

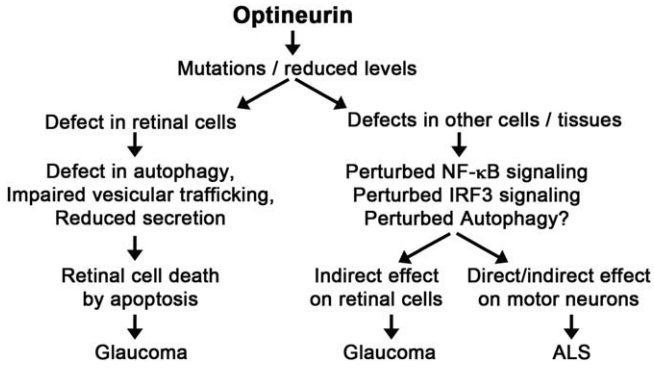

FIG 5 *Overview of defects caused by pathogenic optineurin mutations leading to diseases.*

H486R Mutant Is Defective in NF-κB Signaling

Optineurin inhibits basal as well as TNFα-induced NF-κB activation by mediating the interaction of the protein CYLD with its substrate, polyubiquitinated RIP (29). A glaucoma-associated mutant, H486R, is unable to inhibit such an activation (29,57). This H486R mutant is also defective in its interaction with CYLD, thus providing further evidence for the suggestion that the interaction between optineurin and CYLD is important for the negative regulation of NF-κB signaling by optineurin. In the presence of H486R-OPTN, overexpressed CYLD is unable to deubiquitinate RIP or inhibit NF-κB activation (29). The H486R mutation is located in UBD, and it affects binding to polyubiquitinated RIP to some extent. The loss of NF-κB inhibition by H486R-OPTN is largely due to impaired interaction with CYLD, although reduced binding to ubiquitinated RIP may also be a contributory factor.

Although this H486R mutant is defective in interaction with CYLD and in regulating TFNα-induced NF-κB activity, the relevance of this defect to pathogenesis of glaucoma is not clear. H486R-OPTN, when overexpressed, does not induce retinal cell death, and unlike E50K transgenic mice, the H486R-expressing transgenic mice do not show reduced thickness of retina, suggesting that unlike M98K and E50K mutants, the H486R mutant causes glaucoma by some indirect mechanism (44). Loss of vision in glaucoma can occur either due to direct effects on retinal cell survival or due to indirect effects (Fig. 5). Several indirect mechanisms have been proposed for RGC death in glaucoma pathogenesis such as glial cell activation to produce cytotoxic molecules such as TNFα, autoimmunity, and aberration in TM (58–60). Activation of NF-κB is seen in glaucomatous TM and also in autoimmune responses. Therefore, it is likely that defective NF-κB signaling by H486R mutant might contribute to glaucoma pathogenesis by indirect mechanisms. As transgenic mice expressing H486R mutant do not develop glaucoma phenotype, it is likely that the H486R mutation, which is very rare, may be cooperating with a mutation in another unidentified gene to cause glaucoma.

2bp-AG Insertion

The nonsense 2bp-AG insertion (691–692 in AG) was described as a glaucoma-associated mutation, which induces a premature stop codon in exon 6, leading to the formation of a truncated protein of 1–148 amino acids (4). Interestingly, this is one of the few mutations, which are also associated with ALS with rapid disease progression (57,61). The truncated protein produced by this mutant shows nuclear localization and induces cell death by apoptosis in retinal cells (62). The apoptosis induced by this mutant was much more than that induced by E50K, a severe glaucoma-causing mutant (62). Although the mechanism of induction of cell death is yet to be investigated, this mutant is likely to cause glaucoma by directly inducing death of retinal cells.

E478G, Associated with ALS, Is Seen to Be Defective in NF-κB Regulation, Ubiquitin Binding, and Autophagy

Another mutation, namely, E478G, is associated with familial as well as sporadic cases of ALS in heterozygous condition (3). So far, this mutation has not been found in any patient with glaucoma. This mutation lies in UBD, and binding studies show that it is indeed defective in ubiquitin binding. In a motor neuron cell line, NSC-34 (mouse motor neuron-like hybrid cell line), this mutant was unable to inhibit TNFα-induced NF-κB activation possibly due to loss of ubiquitin binding. This mutant is also defective in IRF3 signaling induced by melanoma differentiation-associated gene 5 or Toll-IL-1 receptor domain-containing adaptor-inducing IFN-β (63). As UBD is involved in several functions of optineurin such as vesicle trafficking, antiviral signaling, and autophagy, this mutant is likely to be defective in these functions of optineurin. However, this needs to be tested. How this mutant contributes to ALS pathogenesis is not clear; however, it is likely that defects in several functions of optineurin might contribute to ALS pathogenesis. Recently, Shen et al. (64) have shown that the E478G mutant is defective in autophagy-mediated degradation of protein aggregates formed by mutant huntingtin or truncated TDP43 (64). They suggest that this mutant, E478G, interferes with optineurin-mediated autophagy by a dominant negative mechanism.

Other Mutations

R96L has been reported to be an ALS-associated mutation (61), which shows enhanced interaction with TFR and Rab8,

but induces some cell death in retinal cells, although less efficiently than the E50K mutant (62). However, the mechanism of induction of cell death is yet to be investigated. Q398X is a nonsense mutation associated with ALS, which produces a truncated OPTN protein lacking UBD and zinc finger (3). This mutant does not inhibit NF-κB activation and causes defective IRF-3 signaling and transferrin uptake (3,62,63). As this mutant does not have UBD, it is likely to be defective in those functions of optineurin that require UBD.

Concluding Remarks and Future Directions

The function of optineurin in (i) membrane vesicle trafficking, (ii) signaling to transcription factor NF-κB and IRF3, and (iii) autophagy have been established. However, progress toward understanding the functional defects caused by disease-associated mutations has been explored only in a few mutations. The best studied mutation, E50K, causes death of retinal cells due to a block in autophagy as well as defective recycling of TFR (19,46). The M98K variant induces autophagy in retinal cells that leads to apoptotic death of retinal cells (45). Both these mutants require functional UBD to induce death in retinal cells; however, the role of ubiquitination, ligases, and ubiquitinated proteins involved are not yet identified. The role of phosphorylation in E50K- and M98K-induced cell death needs to be explored because OPTN directly interacts with the protein kinase TBK1, which is known to phosphorylate optineurin. How does impaired autophagy caused by M98K or E50K mutant lead to caspase activation and apoptosis? How does increased or decreased autophagy lead to caspase activation resulting in cell death by apoptosis? These are questions of much wider significance, which need to be addressed. How other glaucoma or ALS-associated mutants of optineurin affect its function is yet to be understood.

Optineurin is widely expressed in many tissues, but glaucoma-causing mutations do not appear to cause any disorder in any other tissue. How this cell type specificity is achieved by the mutants is yet to be understood. Loss or mutation of optineurin may affect other survival mechanisms such as secretion of neurotrophins. As autophagy is involved in the secretion of certain proteins, a possible link between autophagy and secretion of neurotrophins by retinal cells needs to be explored. Transgenic and knockout animal models are needed in addition to cell culture models to explore the role of optineurin in normal cellular functions and to understand the mechanisms of pathogenesis of glaucoma and ALS caused by optineurin mutants.

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