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Functional Defects Caused by Glaucoma – Associated Mutations in Optineurin

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1. Introduction

Glaucomas are a heterogeneous group of optic neuropathies characterized by progressive loss of retinal ganglion cells (RGCs) leading to visual field defects. The distinctive pattern of optic nerve degeneration results in glaucomatous cupping. The atrophy of optic nerve cells initially leads to loss of peripheral vision and visual field loss increases with increased damage to optic nerve. Worldwide glaucoma is the second leading cause of blindness affecting more than 70 million people [1, 2]. Traditionally elevated intraocular pressure (IOP) is considered as a major risk factor for glaucomatous neuropathy. In addition to increased IOP, other risk factors include age, genetic and environmental factors, myopia, primary vascular dysregulation and hypertension [3, 4].

Glaucoma has been classified into different types based on various criteria. One of the widely used classifications depends on the nature of iridio-corneal angle [5]. Primary open angle glaucomas (POAGs) are the most common and clinically well defined subsets of glaucomas among Caucasians [6]. As its name suggests, in POAG there is no anatomical hindrance to the flow of aqueous humor as the angle structures remain 'open'. However, the drainage of humor is still inefficient resulting in an increase in IOP. Based on the age of onset, POAG can be juvenile (5-35 years) or adult onset (onset after 45 years) [6]. POAGs are usually chronic and largely asymptomatic, with gradual elevation of IOP and consequent visual field loss. In a significant fraction of POAG, glaucoma occurs even in the absence of elevation of IOP. These are recognized as normal tension glaucomas (NTG) [7].



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Angle closure glaucomas (ACGs) are relatively rare among Caucasians and usually are acute. It is the most common form of glaucoma in Asian population [8, 9]. In ACGs, the iridiocorneal angle is closed, blocking the drainage of aqueous humor and resulting in elevation of IOP. People with shallower anterior chamber, with hypermetropia and hence narrower angles, are more susceptible to ACGs. Unlike POAG, ACG can be associated with symptoms like eye pain, blurred vision, headache, nausea, and hence is usually detected earlier [10].

In developmental or congenital glaucoma, developmental anomalies in tissues like trabecular meshwork and Schlemm's canal cause optic neuropathies [5].

2. Genetic basis of glaucoma

Glaucomas are genetically heterogeneous. Very few cases of glaucoma exhibit typical Mendelian inheritance, though familial history increases the risk factor [11, 12]. Majority of glaucoma cases appear to be multifactorial that are affected by multiple genetic and (or) environmental factors. In certain cases, mutations in some genes may cause glaucoma only when present in a susceptible genetic background. These and other complexities confound genotype-phenotype associations, making it difficult to identify genes that actually cause the disease. As a result, only a small fraction of glaucomas are associated with mutations in specific genes. Genetic studies have led to the identification of over 20 chromosomal loci that have been linked to glaucoma: GLC1A-1N, GLC3A-3C [5]. However, only five genes have so far been linked to glaucoma. While four genes - Myocilin/TIGR (trabecular meshwork inducible glucocorticoid response), Optineurin, NTF4 (neurotrophin 4) and WDR36 (WD repeat 36), have been shown to be associated with POAGs, CYP1B1 (cytochrome p450-1B1) has been linked to congenital glaucoma [5, 6, 11, 13, 14]. But mutations in CYP1B1 have been shown to be associated with POAG also [15, 16]. A better understanding of the genetic basis of the disease, with the genes involved, is critical for early detection of the disease and development of therapeutic agents that can target specific pathways.

Mutations in the gene *OPTN*, which encodes the protein optineurin (optic neuropathy inducing), cause NTG and amyotrophic lateral sclerosis (ALS) [17, 18]. Both of these are neurodegenerative diseases. Like glaucoma, ALS is also a progressive disease, which involves degeneration of motor neurons in the primary cortex, brainstem and spinal cord [19]. Optineurin is also seen in pathological structures present in some other neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [20]. Despite its association with glaucoma almost a decade ago, the cellular functions of optineurin, and how its mutations alter these functions, are beginning to be understood only now. This review focuses on the recent advances in cellular functions of optineurin and defective molecular events because of optineurin mutations.



Figure 1. Disease associated mutations in optineurin. Schematic of optineurin, showing its various domains. CC-coiled coil, UBD- ubiquitin binding domain, LZ- leucine zipper, LIR- LC3 interacting region, ZF- zinc finger. A. Various glaucoma causing mutations identified in the optineurin. Of these, R545Q and M98K are polymorphisms. B. Amyotro-phic Lateral Sclerosis (ALS) associated mutations in optineurin. Deletion of some of the exons have been found in ALS patients but not in glaucoma patients. C. Schematic shows regions of optineurin interacting with various proteins. Htt-Huntingtin, a protein found to be mutated in Huntington's disease; mGluR- metabotropic glutamate receptor; MYPT1- myosin phosphatase targeting subunit 1; TBK1- TANK binding kinase 1; RIP1- receptor interacting protein 1.

3. Glaucoma-associated mutations in optineurin

Rezaie et al. (2002) showed that certain mutations in the coding region of the gene *OPTN* are associated with 16.7% of the families with NTG, the only gene to be implicated in this sub-type of POAG. One of the mutations, in which glutamic acid at 50th position is replaced by lysine (E50K), segregates with the disease in a large family affected with NTG [18]. This provided strong evidence for the conclusion that this mutation in optineurin causes glaucoma. Such strong evidence is not available for other mutations of optineurin but some of the mutations

have not been found in normal population. The E50K mutation was found in 13.5% of affected families [18]. Subsequent studies have identified several other mutations in optineurin that are associated with adult onset NTG and in rare cases of juvenile onset glaucoma. However, the frequency of optineurin mutation in sporadic cases is low, generally less than 1%. A polymorphism in optineurin, M98K, is associated with glaucoma in some South Asian populations but not in Caucasians [21, 22]. Most of these optineurin mutations are missense mutations (mutation which leads to replacement of the pre-existing amino acid with another). One of the rare mutations is an insertion in exon5, which would lead to production of a truncated protein due to frameshift [18] (Figure 1A). Certain point mutations that do not cause a change in amino acid sequence, for example, V148V, have also been reported [23]. Recently, certain mutations in optineurin have been shown to cause ALS [17, 24-26]. These mutations are mostly different from those that cause glaucoma (Figure. 1B). Almost all the glaucoma-associated mutations of optineurin are single copy alterations, indicating therefore, that these are likely to be dominant. An alternate possibility is that these point mutations cause a loss of function and the resulting haploinsufficiency may cause the disease.

4. Interaction of optineurin with cellular proteins

Optineurin is predominantly a coiled coil protein of 577 amino acids [27] (Figure 1). It has a well defined ubiquitin-binding UBAN domain (UBD) [28], and a zinc finger domain, which is also believed to bind to ubiquitin [29]. Optineurin interacts with a diverse array of cellular proteins through multiple interaction domains [28, 30-45] (Figure 1C). Over 20 proteins are known to interact with optineurin but functional significance of only some of these interactions is known. Emerging evidences suggest that optineurin is an adaptor protein with no known enzymatic or catalytic activity. Therefore, its functions are likely to be mediated by interaction with other proteins [46, 47].

5. Functions of optineurin

Optineurin is a multifunctional protein involved in regulating various cellular functions such as signal transduction, membrane vesicle trafficking, autophagy, NF- κ B signalling, and cell survival [46, 47] (Figure 2). These functions are mediated through interaction with a wide variety of proteins.

5.1. Role of optineurin in vesicular trafficking

Vesicular trafficking is one of the most fundamental processes of eukaryotic cells. As the name suggests, it is the process of movement of cargo packaged in the vesicles or cell organelles across the cytosol inside the cell. It ensures supply of nutrients and signals to various compartments of the cell, crosstalk between the various organelles inside the cell, secretion and exocytosis [48, 49]. In a typical vesicular trafficking event, four basic steps are involved -

selection of cargo and budding of a vesiculo-tubular transport intermediate, movement of this vesicle on a cytoskeletal track, tethering or docking with an appropriate target compartment and finally fusion of the vesicle with the target membrane [50]. Several proteins like small GTPases, motor proteins, SNAREs (Soluble *N*-ethylmaleimide sensitive-factor-Attachment Protein Receptors), tethers, etc. mediate different steps of vesicular trafficking. One family of proteins, which mediates virtually all these steps in vesicular trafficking, is a class of Ras superfamily of small GTPases, the Rab GTPases (Ras-like GTPases in brain) [51, 52]. Rab GTPases confer identity to certain vesicular intermediates and organelles inside the cell, e.g. Rab5 associates with early endosome or sorting endosome and acts as a marker for it. Apart from imparting vesicle identity to some organelles, these Rab GTPases act as master regulators of trafficking events controlling vesicle budding, vesicle fusion, signal transduction and motility [53]. Rab GTPases function as molecular switches in the cell as they exist in two different forms, a GTP-bound active form that is membrane associated, and a GDP-bound inactive form that is cytoplasmic.



Figure 2. Functions of optineurin. Optineurin is involved in several cellular pathways. Schematic shows various functions performed by optineurin inside the cell. Proteins shown in the boxes are the ones involved in these pathways. Most of these proteins are involved in direct interaction with optineurin.

Rab GTPases mediate their functions mainly through effector proteins. By definition, effectors are the proteins, which preferentially bind to the membrane associated activated form of Rabs [54]. Given the importance of trafficking in normal cellular functions, it is not surprising that defects in trafficking have been implicated in many diseases, including glaucoma [3, 55-57].

Since optineurin interacts with multiple proteins like Rab8, huntingtin, myosinVI, transferrin receptor (TfR), TBC1D17 etc. that are involved in various intra-cellular trafficking pathways, its role in vesicular trafficking is evident [30, 34, 36, 43, 45]. But the exact mechanisms by which optineurin performs its functions in trafficking are being uncovered only recently. Rab8 is a GTPase involved in exocytosis, trafficking at recycling endosome, insulin dependent GLUT4 trafficking at plasma membrane, transferrin receptor recycling etc [30, 58-63]. Optineurin preferentially interacts with activated (GTP-bound) form of Rab8; therefore, it is an effector of some of the functions of Rab8 [34]. MyosinVI is an actin based motor protein involved in various trafficking pathways [64]. Optineurin, in conjunction with myosinVI, is required for maintenance of Golgi ribbon structure [30], polarized delivery of EGF receptor to the plasma membrane [65], sorting of AP-1Bdependent cargo to the basolateral domain in polarized cells [66] and secretory vesicle fusion at the plasma membrane [67]. Most of these processes are mediated by Rab8, also an optineurin-interacting protein. Optineurin was earlier identified as Huntingtin-interacting protein [68]. Later study showed that optineurin interacts with Rab8 through its N-terminus and recruits huntingtin to Rab8-positive vesicles [34]. Rab8 recruits optineurin to link huntingtin and myosinVI to coordinate the movement of vesicles on microtubule and actin tracks [30]. This has been reviewed in detail recently [46].

Studies from our laboratory and others have shown that optineurin interacts with TfR and mediates its trafficking [31, 32]. However, the mechanism by which optineurin regulates this, is not very clear. Recently we have shown that optineurin mediates TfR recycling by regulating the function of Rab8 through interaction with TBC1D17, a GTPase activating protein (GAP) [45] (Figure 3A). Optineurin directly interacts with TBC1D17 and also with Rab8 through adjacent but distinct binding sites. TBC1D17 does not bind directly with Rab8 and requires optineurin for this interaction. Optineurin essentially functions as an adaptor protein to recruit TBC1D17, a Rab GAP to its target Rab, Rab8, leading to inactivation of Rab8 [45]. This is a novel mechanism of regulation of Rab8 GTPase by its effector through a complex negative feedback mechanism.

5.2. Regulation of NF-κB by optineurin

Nuclear factor κ B (NF- κ B) is a family of inducible transcription factors, which is involved in regulating expression of genes involved in cell survival, immunity, inflammation, cell cycle, apoptosis etc. [69, 70] (Figure 4). Deregulation of NF- κ B is associated with several human disorders including chronic inflammation, cancer, glaucoma and neurodegeneration [71].



Figure 3. A model showing regulation of Rab8 by optineurin and its defective regulation by the E50K mutant. A. GTP-bound active Rab8 performs its various functions by its interaction with effector proteins. Optineurin, an effector of Rab8, binds to the activated form of Rab8. Upon binding to activated Rab8, optineurin recruits a GAP, TBC1D17, in close proximity to Rab8. This leads to inactivation of Rab8 and thus maintenance of homeostasis. B. E50K-optineurin causes enhanced inactivation of Rab8 by recruiting TBC1D17 more efficiently.



Figure 4. Schematic showing functions of transcription factor NF-\kappaB. NF- κ B is an inducible transcription factor. After its activation, it can activate transcription of various genes (shown in the boxes) and hence regulate various pathways.

It is generally kept in an inactive state in the cytoplasm through interaction with IkB (inhibitor of kappa B) inhibitory proteins. Activation of NF-kB can occur either via canonical (classical) or noncanonical (alternate) pathway. In classical pathway, upon stimulation of cells with a cytokine such as TNF α (tumor necrosis factor α), the inhibitory proteins IkB α and IkB β are phosphorylated. This phosphorylation and consequent ubiquitination marks them for degradation by ubiquitin proteasome system. This allows NF-kB (p50-p65 complex) to move to the nucleus, where it acts as a transcriptional activator. Upon binding of TNF α to its cell surface receptor, TNFR1 (TNF α receptor 1), a signalling complex is formed in the cytoplasm, which consists of several proteins including TRADD (TNFR1-associated death domain protein), TRAF2 (TNF receptor associated factor 2) and RIP (receptor interacting protein). This leads to activation of IkB kinase (IKK), which consists of the catalytic sub-units IKK α and β , and the regulatory sub-unit NEMO / IKK- γ . Activation of IKK involves addition of polyubiquitin chains to RIP, which then binds to NEMO that leads to activation of catalytic sub-units of IKK phosphorylates IkB proteins leading to their degradation by ubiquitin-proteasome system (Figure 5A).

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Figure 5. Schematic showing the regulation of TNF α -induced NF- κ B signalling by optineurin and defective regulation caused by its H486R mutant. A. Binding of TNF α to its receptor leads to receptor trimerization, which promotes assembly of a multimolecular complex on TNF receptor in which ubiquitination of RIP takes place. Then NEMO is recruited to ubiquitinated RIP, which leads to activation of IKK. Active IKK phosphorylates IkB, which acts as a trigger for ubiquitination and degradation of IkB. This leads to the release of p50/p65 complex of NF- κ B and movement to the nucleus leading to transcription activation. B. Optineurin regulates this process by acting as a competitive inhibitor of NEMO

and binds to ubiquitinated RIP by displacing NEMO. Optineurin then recruits CYLD (a deubiquitinase) to the molecular complex thus facilitating deubiquitination of polyubiquitinated RIP by CYLD leading to downregulation of downstream pathway. C. In the case of H486R mutation in optineurin, CYLD is not recruited to ubiquitinated RIP resulting in accumulation of ubiquitinated RIP. This leads to constitutive activation of NF-kB.

Role of optineurin in TNF α and NF- κ B signalling was long suspected, when it was observed that it shares 53% similarity to NEMO, which led to its earlier nomenclature, NRP (NEMO related protein) [27]. It is induced by TNF α [42]. The role of optineurin in NF- κ B signalling was shown by Zhu et al. [28]. Their work showed that optineurin acts as a negative regulator of TNF α -induced NF- κ B signalling by binding to polyubiquitinated RIP [28]. Later, optineurin was shown to interact with CYLD, product of a tumor suppressor gene *CYLD* involved in cylindromatosis or turban tumor syndrome [36]. CYLD is a deubiquitinated RIP [36, 73-75]. By interacting with CYLD and also with polyubiquitinated RIP, optineurin facilitates deubiquitination of polyubiquitinated RIP by CYLD [76]. In the absence of optineurin, CYLD is unable to deubiquitinate RIP, leading to accumulation of polyubiquitinated RIP, resulting in enhanced basal NF- κ B activity. Thus, in NF- κ B signalling optineurin acts as an adaptor protein that brings together an enzyme (CYLD) and its substrate (polyubiquitinated RIP) together [76] (Figure 5B).

Optineurin gene expression is induced by cytokines such as TNF α and interferons [27, 77]. Human optineurin promoter has been cloned and characterized [77] and harbours, among others, NF- κ B sites. TNF α induces optineurin gene expression in various cells [42, 77]. This induction is mediated by NF- κ B, which binds to a site in optineurin promoter [77]. The NF- κ B-binding site in optineurin promoter is located very close to the transcription start site, and is essential for TNF α mediatied induction. The activation of NF- κ B is tightly regulated by complex feedback loops. Like many of its regulators, expression of optineurin, a negative regulator of NF- κ B, is governed by NF- κ B. Thus, there is a feedback loop in which TNF α induced NF- κ B enhances expression of optineurin, which itself negatively regulates NF- κ B activation [77].

The NF- κ B activity is elevated in the cells of trabecular meshwork obtained from the eyes of glaucoma patients of diverse etiology [78]. Trabecular meshwork controls aqueous outflow that regulates intraocular pressure. Elevated NF- κ B activity, due to increased interleukin-1 level, protects glaucomatous trabecular meshwork cells from oxidative stress induced apoptotic cell death [78]. NF- κ B p50-deficient mice show glaucoma-like pathological features such as age induced death of RGCs, hypertrophy of astrocytes with an enlargement of axons, decreased number of axons in optic nerve leading to excavation of the optic nerve head and production of autoantibodies against RGCs [79]. Therefore, it appears that NF- κ B plays a cytoprotective role in various tissues of the eye. Overexpressed optineurin is known to protect NIH3T3 fibroblasts from oxidative stress-induced cell death [80]. Whether increased level of NF- κ B in glaucomatous trabecular meshwork cells leads to enhanced optineurin level or optineurin-mediated cytoprotection, is yet to be investigated.

Optineurin interacts with UXT (ubiquitously expressed transcript) [36], a protein involved in the regulation of NF-κB signalling [81]. UXT is localized predominantly in the nucleus and

interacts specifically with NF- κ B. UXT forms a complex with NF- κ B and is recruited to the NF- κ B enhanceosome upon stimulation by TNF α [81]. Enhanceosome is a protein complex that binds to the "enhancer" region of a gene, which can be upstream or downstream of the promoter, or within a gene. It accelerates the gene's transcription [82, 83]. However, functional significance of optineurin-UXT interaction has not been investigated.

5.3. Role of optineurin in autophagy

Autophagy is one of the intracellular quality control mechanisms for removing and degrading defective proteins and organelles in the lysosomes [84]. During induction of autophagy, specialized membranous structures known as autophagosomes are formed, which engulf the cargo (cytoplasmic components and organelles) and deliver it to the lysosomes [85]. LC3 (microtubule-associated protein 1 light chain 3) is present in autophagosomal membranes. Overexpressed GFP conjugated LC3 or endogenous LC3 upon immunostaining is seen predominantly in autophagosomes; therefore, LC3 serves as a very useful marker for autophagosomes [86]. LC3 on autophagosomes interacts with autophagy receptors, which help in recruiting ubiquitinated proteins and organelles to autophagosomes. Autophagy receptors are believed to play a crucial role in the selection and recruitment of cargo to autophagosomes by simultaneously binding to LC3 and ubiquitinated cargo [85, 87, 88]. Optineurin was identified as an autophagy receptor due to its ability to bind LC3 and ubiquitin directly and simultaneously through well defined binding sites [37]. Optineurin is involved in clearance of cytosolic Salmonella in macrophages [37]. However, so far no specific protein of Salmonella has been identified that binds to optineurin and is targeted to autophagosomes for degradation. Overexpressed normal optineurin and its E50K mutant induce formation of autophagosomes in retinal ganglion cells in culture and also in transgenic mice expressing E50K-optineurin [89].

5.4. Role of optineurin in cell survival and cell death

One of the glaucoma-associated optineurin mutations (2 bp insertion in exon 5) leads to frameshift resulting in truncation of a major part of the protein. This mutant protein is unlikely to be functional; therefore it was speculated that optineurin has a cytoprotective role in the retina that is lost by mutations [18]. Some support for this hypothesis was provided by experiments in which overexpressed optineurin protected NIH3T3 cells from oxidative stressinduced cell death whereas a glaucoma-causing mutant, E50K, did not [80]. However, this protective effect of optineurin against oxidative stress is yet to be tested in cells relevant for glaucoma or ALS. Recently, using a mouse retinal ganglion cell line, RGC-5, it was shown that knockdown of endogenous optineurin results in induction of apoptotic cell death due to reduced secretion of neurotrophin 3 (NT-3) and ciliary neurotrophic factor (CNTF) [90]. Addition of NT3 to the medium was able to suppress this cell death. The level of NT-3 or CNTF mRNA was not affected significantly upon knockdown of optineurin. Knockdown of optineurin resulted in breakdown of the Golgi structure [30, 90] and accumulation of NT-3 positive vesicles due to a block in vesicle trafficking in the secretory pathway [90]. Overexpression of optineurin sensitizes RGC-5 cells to TNF α -induced cell death but interestingly, in Hela cells, overexpressed optineurin does not increase TNF α -induced cell death. In fact, in Hela cells optineurin inhibits $\text{TNF}\alpha$ -induced cell death [91]. This is consistent with the observation that an interplay between polymorphism in $\text{TNF}\alpha$ and optineurin gene increases the risk of glaucoma [92]. Thus it appears that maintenance of optimum level of optineurin is important for survival of RGCs. The mechanism by which optineurin causes different effects in RGCs and in Hela cells is not known.

5.5. Regulation of mitosis by optineurin

Polo-like kinase (Plk1) is an important regulator of various events in cell division cycle such as G2/M (Gap2 of interphase to mitosis) transition, centrosome maturation, chromosome segregation and cytokinesis. The precise control of these events depends on the kinase activity of Plk1 [93-95]. During mitosis optineurin is phosphorylated by Plk1 at Ser177 that leads to its relocalization to the nucleus from the Golgi. In the nucleus optineurin enhances phosphorylation of MYPT1 (myosin phosphatase target subunit 1) by Cdk1 (cyclin dependent kinase 1) that leads to binding of MYPT1 with Plk1 and inactivation of Plk1. Knockdown of optineurin leads to defects in chromosome separation and formation of multinucleate cells [39]. Formation of multinucleate cells upon optineurin knockdown has been observed in RGC-5 cells also [90]. Thus optineurin is involved in a feedback mechanism by which Plk1 modulates localization of optineurin that in turn regulates Plk1 activity and mitosis progression [39].

5.6. Role of optineurin in antiviral signalling

Our body responds to viral infection through innate immune response and produces type I interferons (IFN α / IFN β). These induce signalling to activate transcription of many genes to produce an antiviral state in the cells [96]. A tight regulation of this antiviral signalling is necessary to prevent unwanted tissue damage due to inflammatory response. Optineurin has emerged as one such negative regulator limiting IFN β production in response to RNA virus infection [40]. This negative regulation of IFN β production is mediated by interaction of optineurin with TBK1 (TANK binding kinase 1), a protein kinase involved in the activation of IRF3/7 (interferon regulatory factor 3/7) transcription factors [97]. Optineurin inhibits TBK1-mediated phosphorylation of IRF3 induced by Sendai virus or extracellular poly (I:C) [98]. But another group has suggested that optineurin is an activator of TBK1 and mediates IFN β production in response to lipopolysaccharide or poly (I:C) [99]. UBD of optineurin plays an essential role in this process. However, a negative regulatory role for optineurin in innate immune response is supported by the observation that optineurin inhibits IRF3 activation in response to MDA5 (melanoma differentiation associated gene 5) or TRIF (TIR-domain-containing adapter-inducing interferon- β) overexpression [98].

6. Functional defects caused by optineurin mutants

Considering the importance of diverse cellular functions optineurin assists in, defects caused by its mutants are imperative. Recent work has revealed some of the normal cellular functions of optineurin. However, our understanding of functional defects due to mutations in optineurin, is only beginning to emerge. So far, functional defects caused by only two disease associated mutants are known. Here we are providing some insight into how optineurin mutants might be leading to defective cellular functions.

6.1. Defective NF-κB regulation

Aberrant NF- κ B signalling has been implicated in many neurodegenerative diseases like Alzheimer's, Parkinson's and Huntington's diseases, and glaucoma [100, 101]. Recently it has been shown that a glaucoma-associated mutant of optineurin, H486R, is defective in inhibiting TNF α -induced NF- κ B activation [76]. The H486R mutant is associated with JOAG and POAG patients, and this mutant has not been found in any normal individual [23, 102]. This mutation lies in the ubiquitin-binding domain (Figure 1A). The H486R mutant shows drastically reduced interaction with CYLD and also shows somewhat reduced interaction with polyubiquitinated RIP [76]. The inability of H486R mutant to inhibit TNF α -induced NF- κ B activation is primarily due to defective interaction with CYLD although reduced interaction with RIP may also contribute to a small extent. This conclusion is supported by the finding that overexpressed CYLD was unable to deubiquitinate RIP and inhibit TNF α -induced NF- κ B activity in presence of the H486R mutant [76] (Figure 5C). Thus it is clear that the interaction of optineurin with CYLD plays a crucial role in the regulation of TNF α -induced NF- κ B activation [76].

What is the mechanism of pathogenesis of glaucoma caused by the H486R mutant? In glaucoma, loss of vision occurs due to the death of retinal ganglion cells in the optic nerve head. Several mechanisms have been implicated as cause of RGC death in glaucoma such as direct effect on RGCs, activation of glial cells to secrete cytotoxic proteins like TNF α , changes in trabecular meshwork, and autoimmunity [3, 103]. However, unlike E50K mutant, the H486R mutant does not cause RGC death in cell culture or in transgenic mice [91, 104]. Therefore, it is likely that indirect effects through other cells might contribute to H486R-induced glaucoma. Increased NF-kB activity is associated with autoimmune response and also with glaucomatous trabecular meshwork [78, 79, 105]. Deregulation of NF-κB by H486R mutant provides a basis for exploring its indirect mechanisms of neurodegeneration associated with glaucoma. Since CYLD knockout mice show autoimmune defects [106], it is possible that the H486R mutant, by blocking the function of CYLD, might also cause autoimmune defects relevant for glaucoma. Whether increased NF-kB activity associated with glaucomatous trabecular meshwork [78] is a cause or an effect of elevated IOP is not known. The relevance of NF-κB deregulation by H486R-optineurin to elevated IOP is not known but an interesting possibility is that increased NF-kB activity in trabecular meshwork might cause increased IOP by altering growth or other properties of trabecular meshwork cells.

The ALS-associated mutant E478G is unable to inhibit TNF α -induced NF- κ B activation but the molecular mechanism of this defect is not known [17]. This mutant is predicted to be defective in binding to ubiquitin but this is yet to be tested. It would be of interest to know whether this mutant is defective in binding to CYLD or not. Relevance of defective NF- κ B regulation by E478G mutant to disease pathogenesis is not clear.

6.2. Defective cell survival and membrane vesicle trafficking

The E50K is a dominant mutation [18], which upon overexpression induces death of RGC-5 cells in culture but not of other cell lines tested. None of the other glaucoma-associated mutants tested (H26D, H486R, R545Q) induced RGC death [91]. This suggests that the E50K mutant causes glaucoma by directly inducing death of RGCs. Transgenic mice expressing E50K mutant showed apoptotic death of RGCs suggesting, therefore, that RGC-5 cell line is a useful cell culture model to study molecular mechanisms of pathogenesis of glaucoma [104]. The E50K transgenic mice showed degeneration of entire retina resulting in reduced thickness of retina [104]. The E50K-induced death of RGCs is mediated by oxidative stress although the mechanism of induction of oxidative stress by E50K is not known. The oxidative stress is due to formation of reactive oxygen species probably produced by mitochondria because E50Kinduced RGC death and production of reactive oxygen species were abolished by coexpression of mitochondrial superoxide dismutase [91]. The E50K mutant inhibits endocytic trafficking and recycling of transferrin receptor leading to accumulation of transferrin receptor in large foci/vesicular structures (recycling endosomes, autophagosomes). This defective Rab8 mediated TfR trafficking by E50K mutant is due to altered interaction of this mutant with Rab8 and transferrin receptor [31, 32]. Optineurin functions as an adaptor protein to mediate negative regulation of Rab8 by the GTPase activating protein, TBC1D17. The E50K mutant recruits TBC1D17 more efficiently to the multimolecular complex leading to enhanced inactivation of Rab8 by TBC1D17. This leads to inhibition of Rab8-mediated TfR trafficking and recycling. This hypothesis is supported by the observation showing that E50K-optineurin dependent inhibition of transferrin receptor trafficking can be prevented by knockdown of TBC1D17 or by expressing a catalytically inactive mutant of TBC1D17. A constitutively active mutant of Rab8, Q67L also reverses E50K-optineurin induced inhibition of transferrin receptor trafficking [45]. Whether E50K-induced TBC1D17-mediated Rab8 inactivation, or defective TfR trafficking, play a role in RGC death, is yet to be investigated. A blockade in axonal vesicular trafficking of brain-derived neurotrophic factor and its receptor, that are vital for RGC survival, has been considered as one of the causes for glaucomatous cell death [107, 108].

It appears that the molecular mechanism of defective TfR trafficking by the E50K mutant is somewhat complex. Optineurin forms a multimolecular complex containing Rab8 and TfR as seen by co-immunoprecipitation [31, 32]. Co-immunoprecipitation identifies protein-protein interactions, which may be direct or indirect (mediated by another protein) [109]. The E50K mutant forms a stronger complex with transferrin receptor and Rab8. Stronger colocalization of E50K mutant with Rab8 and transferrin receptor in the same structures/foci provides support for this suggestion [31]. But, direct interaction between E50K mutant and Rab8 is lost as shown in mammalian cells and also by yeast two-hybrid assay [45, 104]. Based on these observations it appears that in the multimolecular complex, direct interaction between E50K mutant and Rab8 is lost but indirect interaction (through other proteins) is increased. Therefore, it is likely that the functional positioning of these proteins in the multimolecular complex is altered in such a way that the inactivation of Rab8 by TBC1D17 is increased in E50K-expressing cells [45]. This is depicted schematically in Figure 3.

Optineurin plays a role in maintaining the structure of the Golgi complex and expression of E50K mutant results in breakdown of the Golgi [110]. However, the molecular mechanism of this effect of E50K mutant and its relevance to RGC death are not known. Whether Golgi breakdown is a contributory factor for E50K-induced defective trafficking and hence RGC death is not clear. The relationship between Rab8 inactivation and Golgi breakdown by E50K is yet to be investigated.

6.3. Defective autophagy

Formation of aggregates is one of the hallmarks of many neurodegenerative diseases like Alzheimer's, Parkinson's, Huntington's diseases and prion deseases. Accumulation of aggregates is indicative of either an inability to degrade mutant protein or an overall inhibition of the cellular trafficking and degradative machinery [111-113].

Overexpression of optineurin results in the formation of vesicular structures or foci. Some of these foci are autophagosomes and overexpression of E50K mutant results in the formation of larger autophagosomes [89]. This formation of larger autophagosomes by E50K mutant is perhaps due to a block in autophagy, which partly contributes to E50K-induced death of RGCs. This conclusion is supported by the observation that rapamycin, an inducer of autophagy, reduces E50K-induced death of RGC-5 cells [89]. However, the mechanism of increased formation of larger autophagosomes in E50K expressing cells is not known. Interaction of E50K with ubiquitinated proteins is perhaps required for autophagosome formation because inactivation of UBD by point mutation in E50K causes nearly complete loss of foci formation [31].

6.4. Other defects of optineurin mutants

RNA virus infection is sensed by components of innate immune response, including RIG-1 (retinoic acid inducible gene 1), MDA5 (melanoma differentiation associated gene 5) and Toll like receptors [114-116]. This sensing of receptors leads to activation of TBK1 and IRF3 [117]. Optineurin is a negative regulator of IRF3 activation, which is involved in IFN β production [40]. ALS-associated mutants of optineurin, E478G and Q398X, are defective in this negative regulation [98]. Whether any of the glaucoma-associated mutants show this defect is yet to be examined.

Optineurin interacts with proteins involved in immunity, IK-cytokine and BAT4 [36]. But the functional significance of these interactions is not known.

7. Conclusions and future directions

Optineurin functions as an adaptor protein and thereby plays a crucial role in several functions including vesicle trafficking in the secretory and recycling pathways, NF- κ B signalling, control of mitosis, Golgi organization, autophagy and antiviral signalling. The relationship between

these different functions of optineurin is not clear. Since optineurin is an adaptor protein, mutations in it can lead to altered interactions with other proteins impairing its normal cellular functions. Identifying the functions that are affected by disease-associated mutations of optineurin is a major challenge towards understanding the molecular mechanisms of etiopathogenesis of neurodegenerative disease like glaucoma. Presently, our understanding of the molecular mechanisms of functional defects caused by E50K mutation, the best studied mutant, is far from complete. Several questions remain to be answered. How does E50K mutation cause a block in autophagy? Does E50K mutant cause inhibition of secretion of neutrophins/survival factors? Is Rab8 involved in this process? Does impaired transferrin receptor trafficking or function contribute to E50K-induced RGC death? How does H486R mutant cause glaucoma? Does it cause autoimmune defects by impairing the function of CYLD? How do other mutants of optineurin alter its function? Why some mutations cause ALS and others cause glaucoma? Are mutations of optineurin also prevalent in other neurodegenerative diseases? Is interaction of optineurin or its mutants altered with huntingtin or its mutants? If so, what is its relevance for Huntington's disease and glaucoma? Role of various mutants of optineurin in affecting known functions of optineurin needs to be examined. This would help in understanding the molecular mechanisms of pathogenesis of glaucoma and other neurodegenerative diseases. Most of the optineurin mutants do not directly induce death of RGC-5 cells upon overexpression, indicating, therefore, that these optineurin mutations might cause glaucoma by indirect mechanisms involving defects in other cells/tissues (Figure 6]. Survival of RGCs is influenced by other accessory cells like glial cells. Role of optineurin mutants in autoimmunity and glial cell activation needs to be explored.



Figure 6. Overview of role of optineurin mutations in causing Glaucoma.

Functional defects caused by mutations in optineurin in cells other than RGC, especially glial cells could also be relevant for glaucoma pathogenesis. However, molecular mechanism of such effects and relevance to glaucoma needs to be established. Transgenic and knockout animal models are needed to understand the complex and diverse mechanisms involved in the pathogenesis of glaucoma and ALS caused by mutations in optineurin.

Abbreviations

RGC, Retinal ganglion cells; IOP, Intraocular pressure; POAG, Primary open angle glaucoma; JOAG, Juvenile open angle glaucoma; NTG, Normal tension glaucoma; ACG, Angle closure glaucoma; TIGR, trabecular meshwork inducible glucocorticoid response; WDR36, WD repeat 36; CYP1B1, cytochrome p4501B1; NTF3/4, neurotrophin3/4; ALS, amyotrophic lateral sclerosis; UBD, ubiquitin-binding domain; GLUT4, glucose transporter member 4; EGF, epidermal growth factor; GAP, GTPase activating protein; NF- κ B, Nuclear factor κ B; I κ B, inhibitor of kB; TNF α , tumor necrosis factor α ; TNFR1, tumor necrosis factor Receptor1; TRADD- TNFR1-associated DEATH domain protein; TRAF2, TNF receptor associated factor 2; RIP, receptor interacting protein; IKK, I κ B kinase; NEMO, NF- κ B essential modifier; UXT, ubiquitously-expressed transcript; LC3- microtubule-associated protein 1 light chain 3; CTNF, ciliary neurotrophic factor; PIk1, Polo-like kinase; MYPT1, myosin phosphatase target subunit 1; Cdk1, cyclin dependent kinase 1; IRF3, Interferon regulatory factor 3; TBK1, TANK binding kinase; MDA5, melanoma differentiation associated gene 5; TRIF, TIR-domain-containing adapter-inducing interferon- β ; ROS, reactive oxygen species; RIG1, retinoic acid inducible gene 1.

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