Commentary
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Optineurin, a multifunctional protein involved in glaucoma, amyotrophic lateral sclerosis and antiviral signalling

Glaucomas are a heterogeneous group of neurodegenerative eye diseases that cause blindness and are one of the leading causes of blindness worldwide. Loss of vision in glaucoma occurs due to death of retinal ganglion cells (RGCs) in the optic nerve head (Quigley 1999). Some of the genes known to be involved in causing glaucoma in adults are myocilin, optineurin and WDR36 (Stone *et al.* 1997; Rezaie *et al.* 2002; Monemi *et al.* 2005; Ray and Mookherjee 2009). Mutations in the coding region of the gene *OPTN*, which codes for the protein optineurin, are associated with certain types of glaucoma. In the original study, families affected with normal tension glaucoma (a sub-type of adult onset primary open angle glaucoma) were analysed for mutations in optineurin (Rezaie *et al.* 2002). Subsequent studies have shown that in sporadic cases of glaucoma mutations in optineurin are rare, accounting for only about 1% of the cases. Almost all the glaucoma-associated mutations in *OPTN* are single copy alterations. Most of these mutations are missense mutations. One of the glaucoma-associated mutants (E50K) causes death of retinal ganglion cells *in vitro* as well as in transgenic mice providing support to the suggestion that mutations in optineurin cause glaucoma (Chalasani *et al.* 2007; Chi *et al.* 2010).

A recent report described that certain mutations in optineurin are the cause of familial amyotrophic lateral sclerosis (ALS) (Maruyama *et al.* 2010). Like some forms of glaucomas, ALS is an adult onset progressive neurodegenerative disorder whose hallmark is the selective death of motor neurons of primary motor cortex, brainstem, and spinal cord. Mutations in optineurin were found in familial as well as sporadic cases of ALS. Three types of mutations were observed, two of these being homozygous. One of these homozygous mutations was deletion of exon 5, observed in familial ALS, and the other was Q398X nonsense mutation found in both familial as well sporadic cases. A heterozygous missense mutation E478G was observed in familial ALS (Maruyama *et al.* 2010). What are the functional defects caused by mutations in optineurin? To address this question we need to understand the function of normal optineurin.

Optineurin interacts with several proteins which are involved in various functions (table 1, figure 1). On the basis of interactions and other experiments various functions have been proposed for optineurin such as regulation of exocytosis and vesicle traffic from the Golgi to the plasma membrane, organization of the Golgi stacks, regulation of signalling to transcription factor NF-κB, antiviral signalling, metabotropic glutamate receptor signalling and regulation of gene expression (Hattula and Peranen 2000; Anborgh *et al.* 2005; Weisschuh *et al.* 2007; Zhu *et al.* 2007; Chalasani *et al.* 2009; del Toro *et al.* 2009; Mankouri *et al.* 2010). Optineurin is phosphorylated on serine and tyrosine residues and exists as homo-hexamers (Ying *et al.* 2010). Several optineurin-interacting proteins such as Rab8, Huntingtin, myosin VI and TBC1D17 are involved in regulating vesicular membrane traffic in various cells. Knockdown of optineurin affects structure of the Golgi and reduces transport from the Golgi to the plasma membrane (Sahlender *et al.* 2005). However, there is no report of any disruption of this transport (from the Golgi to the plasma membrane) or exocytosis by any disease-associated mutant although overexpression of the E50K mutant and to a lesser extent of wild-type optineurin causes breakdown of the Golgi (Park *et al.* 2006). Recently we and others have shown that knockdown of optineurin reduces endocytic trafficking of transferrin and its receptor (transferrin receptor, TfR) to the recycling endosomes (Nagabhushana *et al.* 2010; Park *et al.* 2010). A glaucoma-causing mutant of optineurin (E50K) impairs

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trafficking of TfR possibly due to altered interactions with Rab8 and transferrin receptor. This impaired trafficking results in accumulation of TfR in large vesicular structures formed by E50K leading to lower level of TfR at the cell surface and hence reduced uptake of transferrin by the E50K expressing cells. It was suggested that impaired trafficking caused by the E50K mutant might be the cause of cell death induced by this mutant in RGCs (Nagabhushana *et al.* 2010; Park *et al.* 2010). Transport of neurotrophins in the axons is crucial for the survival of neuronal cells. Blockade of axonal transport has been reported in glaucoma in humans and in experimental animal models (Pease *et al.* 2000). However, the molecules whose defective trafficking by E50K optineurin causes RGC death in glaucoma need to be identified. Is the defective trafficking of neurotrophins, transferrin or their receptors (or some other associated molecules) responsible for RGC death?

Another function of optineurin is regulation of signalling to the transcription factor NF-κB. NF-κB plays a key role in the expression of many genes involved in regulating immune response, apoptosis, cell cycle and its deregulation is involved in the pathogenesis of many diseases including some neurodegenerative disorders. Upon treatment of cells with TNFα, trimerization of TNF receptor results in the assembly of a signalling complex at the cytoplasmic side of the plasma membrane. In this complex RIP is ubiquitinated (by the addition of Lys63-linked ubiquitin chains) which then recruits NF-κB essential modulator (NEMO), the regulatory sub-unit of a kinase complex, IKK. This leads to activation of the catalytic subunits of the IKK complex, IKKα and β, that phosphorylate inhibitor of κB (IκB) resulting in its degradation. This enables nuclear translocation of NF-κB to activate transcription of target genes (Hayden and Ghosh 2008). Knockdown of optineurin increases basal as well as TNFα-induced NF-κB activity whereas overexpressed optineurin inhibits it. This negative regulation of NF-κB activity is believed to be the result of competition of optineurin with NEMO for binding to polyubiquitinated RIP (Zhu *et al.* 2007). C-terminal half of optineurin shows considerable homology with NEMO. However the regulation of NF-κB activity by optineurin is likely to be far more complex because optineurin interacts with two other negative regulators of NF-κB- CYLD, a deubiquitinase and A20, a ubiquitin editing enzyme (Chalasani *et al.* 2009). In human T-lymphotropic virus type 1 (HTLV-1) infected cells optineurin interacts with TAX1BP1 and a viral protein TAX1 resulting in sustained activation of NF-κB and ubiquitination of TAX1 (Journo *et al.* 2009). Thus role of optineurin in the regulation of NF-κB is cell type and stimulus dependent.

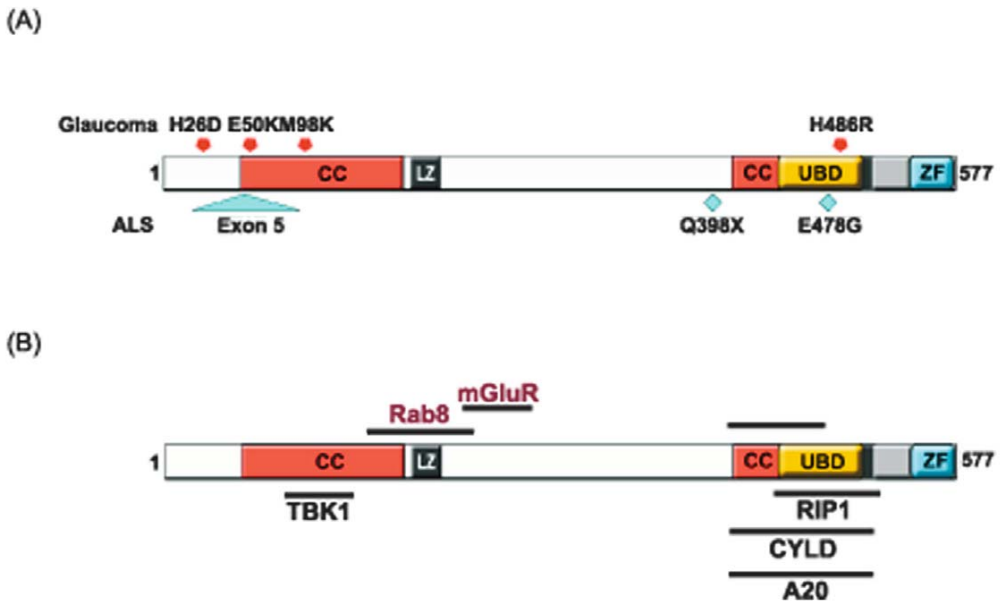

Figure 1. Schematic representation of optineurin showing domain organization, key mutations and binding sites of interacting proteins. (**A**) A schematic showing various domains of optineurin and the locations of glaucoma (red ⬟) and ALS associated (blue ◇) mutations. M98K is a polymorphism. (**B**) Interacting proteins of optineurin and their binding sites on optineurin. CC, coiled coil; LZ, leucine zipper; UBD, ubiquitin-binding domain; ZF, zinc finger.

Table 1. Optineurin interacting proteins and their functions

Interacting protein	Function	References
Rab8	Vesicular trafficking, cellular morphogenesis	Hattula and Peranen 2000
Myosin VI	Exocytosis, Golgi morphogenesis, polarized EGFR trafficking and migration	Sahalender *et al.* 2005 Chibalina *et al.* 2010
Huntingtin	Vesicular trafficking, post Golgi trafficking to lysosome	Del Toro *et al.* 2009
Transferrin receptor	Endocytic recycling	Nagabhushana *et al.* 2010, Park *et al.* 2010
RIP1	NF-κB signalling	Zhu *et al.* 2007
TRAF3	IFN-β induction and antiviral responses	Mankouri *et al.* 2010
TBK1	IFN-β induction and antiviral responses	Mankouri *et al.* 2010 Morton *et al.* 2008
TAX1BP1	NF-κB signalling	Journo *et al.* 2009
TAX1 and TAX2	HTLV- mediated NF-κB activation	Journo *et al.* 2009
mGluR1, mGluR5	Metabotropic glutamate signalling	Anborgh *et al.* 2005
TFIIIA	Transcription?	Moreland *et al.* 2000
CYLD	NF-κB signalling?	Chalasani *et al.* 2009
A20	NF-κB signalling?	Chalasani *et al.* 2009
TBC1D17	Vesicular trafficking?	Chalasani *et al.* 2009
UXT	NF-κB signalling?	Chalasani *et al.* 2009
ZBTB33	Cell growth and development	Chalasani *et al.* 2009
BAT4	Immunity	Chalasani *et al.* 2009

Do the glaucoma- and ALS-associated mutations affect distinct aspects of cellular functions of optineurin (membrane trafficking, NF-κB signalling)? All the three ALS associated mutants of optineurin showed no inhibition of basal as well as TNFα-induced NF-κB activity although wild-type optineurin showed good inhibition. These experiments were done in mouse NSC-34 cells, a neuroblastoma and spinal-cord hybrid cell line relevant for ALS (Maruyama *et al.* 2010). The authors suggest that defective NF-κB regulation by mutants of optineurin may be contributing to ALS. In contrast, the glaucoma-associated E50K mutant attenuates TNFα induced NF-κB activation more strongly than wild-type optineurin (Sudhakar *et al.* 2009). Both hyperactivation and severe inhibition of NF-κB have been proposed to be responsible for neurodegeneration (Mattson and Meffert 2006). The effect of ALS-associated mutations of optineurin on vesicle trafficking is yet to be analysed. Since ubiquitin-binding domain (UBD) is required for both endocytic trafficking as well as NF-κB regulation, the Q398X (which lacks UBD) and E478G (which is likely to have lost the function of UBD) mutants may be defective in vesicle trafficking. The E478G mutant shows altered sub-cellular distribution which appears to be similar to the localization pattern of UBD-defective D474N mutant (Maruyama *et al.* 2010; Nagabhushana *et al.* 2010).

What is the mechanism by which mutations in optineurin cause ALS? The dominant and recessive mutations are likely to cause ALS by different mechanisms. In case of recessive mutations the level of optineurin protein will decrease and loss of functions of optineurin is likely to cause the disease. The dominant heterozygous mutation E478G showed increased optineurin protein in the cell body and neurites, and also showed different sub-cellular distribution (Maruyama *et al.* 2010). The authors suggested that increased NF-κB activity caused by mutation in optineurin and/or impaired intracellular trafficking may be contributing to the disease pathogenesis. Increased NF-κB activity has been reported in sporadic ALS motor neurons which may increase optineurin protein level because NF-κB is involved in inducing optineurin gene expression by activating the promoter (Sudhakar *et al.* 2009).

Neuropathology of ALS, like some other neurodegenerative diseases, is characterized by large intracytoplasmic aggregates or inclusions of largely ubiquitinated proteins. Motor neurons from a subject with E478G mutation (familial ALS) showed intracytoplasmic inclusions which were optineurin

positive. These inclusions were also positive for ubiquitin, a constituent of inclusions seen in many neurodegenerative diseases. Interestingly these optineurin positive inclusions were also seen in some cases of sporadic ALS in which optineurin was not mutated but other genes (SOD1, TDP-43) were mutated (Maruyama *et al.* 2010). Therefore optineurin may have a much broader role in ALS. The vesicular structures or foci formed by the glaucoma-causing mutant E50K and to a lesser extent wild-type optineurin are positive for ubiquitin. The E50K mutant increases ubiquitination of transferrin receptor (Nagabhushana *et al.* 2010). Thus one of the functions of mutated optineurin in ALS and glaucoma may be to increase ubiquitination. Overexpression of mutant huntingtin disrupts interaction of optineurin and Rab8 and their localization to the Golgi disrupting trafficking from TGN to lysosomes thereby inducing formation of autophagosomes (del Toro *et al.* 2009). The role of optineurin in autophagosome formation and its relevance to glaucoma or ALS, if any, needs further investigation.

In response to viral infection our innate immune system produces type I interferons (IFNα/IFNβ) which activate transcription of many genes to produce antiviral state in the host cells. However, a tight regulation of cytokine signalling is vital to limit unwarranted inflammatory tissue damage. Loss of IFNβ production can lead to immunodeficiency leading to reduced ability of the host to combat viral infection. Optineurin negatively regulates the production of IFNβ in response to RNA virus infection and this signalling involves interaction of optineurin with antiviral protein kinase TBK1 and ubiquitin ligase TRAF3 (Mankouri *et al.* 2010). These authors suggest that optineurin may be a general negative regulator of inflammation and a potential target for antiviral therapy. In accordance with this, a recent genome wide association study has found that optineurin is associated with Paget disease, an inflammatory disease of the bone (Albagha *et al.* 2010).

Thus the two major functions regulated by optineurin are membrane trafficking and signalling to transcription factor NF-κB. Optineurin interacts with diverse array of proteins and, like huntingtin, acts as a platform to assemble multimolecular complexes to mediate various cellular and molecular functions. Altered interactions of optineurin mutants with cellular proteins may be the main cause for defective functions leading to disease phenotype. Since the level of optineurin is regulated, altered level of optineurin due to mutation or other factors may also contribute to disease pathogenesis. Ubiquitin-binding function of optineurin and regulation of ubiquitination of cellular proteins by optineurin are likely to be crucial for normal cell physiology and disease pathogenesis caused by mutations in optineurin. Differential effects of mutations on various functions of optineurin may be responsible for the selectivity of disease phenotype.

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