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Review

Defects in autophagy caused by glaucoma-associated mutations in optineurin

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ABSTRACT

Certain mutations in optineurin (gene *OPTN*) are associated with primary open angle glaucoma. Optineurin is ubiquitously expressed but it shows high level of expression in certain cells and tissues including retinal ganglion cells. It interacts with many proteins, often acting as an adaptor to link two or more proteins. These interactions play a crucial role in mediating various functions of optineurin such as membrane vesicle trafficking, autophagy, signal transduction etc. Autophagy is basically a quality control mechanism to remove damaged proteins and organelles through lysosomal degradation. Optineurin was identified as an autophagy receptor that directly interacts with autophagosomal protein, LC3, and ubiquitin. These interactions are important for autophagy receptor function. Autophagy receptors recruit their cargo and take it to autophagosomes which fuse with lysosomes to form autolysosomes where degradation of proteins takes place. Optineurin interacts with a motor protein, myosinVI, and this interaction is involved in mediating fusion of autophagosomes with lysosomes. A glaucoma-associated mutant of optineurin, E50K, impairs autophagy as well as vesicle trafficking, leading to death of retinal cells by apoptosis. E50K-OPTN-induced block in autophagy is dependent on a GTPase activating protein, TBC1D17. The E50K mutant also causes other changes in the cells such as altered interaction with TBK1 protein kinase, aggregate formation, generation of reactive oxygen species and inhibition of proteasome, which may contribute to pathogenesis. A polymorphism of optineurin, M98K, associated with glaucoma, causes enhanced autophagy leading to transferrin receptor degradation and apoptotic death of retinal cells. M98K-OPTN-induced autophagic cell death is dependent on Rab12 GTPase. Thus, an optimum level of optineurin-mediated autophagy is crucial for survival of retinal cells, and impaired autophagy is likely to contribute to glaucoma pathogenesis. How impaired autophagy caused by optineurin mutants leads to apoptosis and cell death, is yet to be explored.

Contents

Abbreviations: ALS, Amyotrophic Lateral Sclerosis; ATG, Autophagy-related proteins; CDK1, Cyclin dependent kinase-1; CMA, Chaperon-mediated autophagy; CYLD, cylindromatosis (turban tumor syndrome) protein; GAP, GTPase activating protein; LC3-1, Microtubule-associated protein 1 light chain 3; NEMO, NF-κB essential modulator; NF-kB, Nuclear factor kappa B; NTG, Normal tension glaucoma; OPTN, Optineurin; POAG, Primary open-angle glaucoma; Rab8, Rat sarcoma (abbreviated as Ras)-related protein 8; RGC, Retinal ganglion cells; ROS, Reactive oxygen species; RIP, Receptor interacting protein; TBK1, TRAF family member-associated NF-kappa-B activator kinase 1 (TRAF: Tumor necrosis factor (TNF) receptor-associated factor); TBC1D17, TBC1 domain family member 17; TFRC, Transferrin receptor-1; UBD, Ubiquitin-binding domain.

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1. Introduction

Mutations in the gene *OPTN* that codes for the protein optineurin are associated with glaucoma and ALS (amyotrophic lateral sclerosis) (Maruyama et al., 2010; Rezaie et al., 2002). Both of these are neurodegenerative diseases. There are several types of glaucomas. In adults, POAG (primary open angle glaucoma) and angle closure glaucoma are major types of glaucoma. In POAG the increase in intraocular pressure (IOP) is the major risk factor. However, in NTG (normal tension glaucoma), a sub-type of POAG, the intraocular pressure remains in the normal range and it accounts for about 30% of POAG cases (Fingert, 2011). POAG causes permanent bilateral blindness due to progressive loss of retinal ganglion cells in the optic nerve head. Other cells in retina such as photoreceptor cone cells are also lost in POAG (Agudo-Barriuso et al., 2013; Choi et al., 2011; Harada et al., 2007). Rezaie et al., in 2002 identified mutations in the coding region of *OPTN* that were associated with 16.7% of families affected with autosomal dominant adult onset NTG (Rezaie et al., 2002). One of the mutations, E50K, segregated with the disease phenotype in a very large family, providing strong evidence to support the hypothesis that mutations in *OPTN* cause NTG (Rezaie et al., 2002). Subsequently, several studies reported association of *OPTN* mutations with NTG in familial as well as sporadic cases of NTG and occasionally with POAG (Alward et al., 2003; Ayala-Lugo et al., 2007). Most of the *OPTN* mutations are missense single copy mutations suggesting, therefore, that these are likely to be dominant. A 2-bp insertion that causes frameshift and truncation was also associated with NTG (Rezaie et al., 2002). In 2010, Maruyama et al., showed that certain mutations in *OPTN* cause ALS, a fatal disease that results from loss of motor neurons in the brainstem, primary cortex and spinal cord leading to paralysis of voluntary muscles (Maruyama et al., 2010). ALS causing mutations in *OPTN* include deletions, truncations and missense mutations (Fig. 1). Most of the mutations associated with ALS are not associated with glaucoma (Fig. 1). On the basis of the nature of mutations, it was suggested that loss of function as well as gain of function mechanisms are involved in the pathogenesis of glaucoma and ALS. Optineurin is also seen in pathological structures found in several neurodegenerative diseases including Alzheimer's disease and Parkinson's disease (Osawa et al., 2011).

2. Structure of optineurin

Optineurin is mostly a coiled coil protein which does not have any enzymatic activity. Human optineurin is a 577 amino acid protein which migrates as a 74 kDa polypeptide on SDS-PAGE. In the cell optineurin forms hexamers (Ying et al., 2010). Upon induction of oxidative stress in the cell it forms covalent trimers which are not linked by disulfide bonds (Gao et al., 2014). Optineurin has a well defined ubiquitin-binding domain (UBD), zinc finger domain and LC3-interacting region (LIR) (Fig. 1). It interacts directly with several proteins in the cell through well defined binding sites. C-terminal half of optineurin shows homology with NF-κB essential modulator (NEMO or IKKγ), regulatory sub-unit of IKK complex, which is involved in signaling to transcription factor NF-κB (Schwamborn et al., 2000).

3. Sub-cellular localization of optineurin

Optineurin is predominantly a cytosolic protein. However, a small fraction of optineurin is present in the Golgi, endocytic vesicles and autophagosomes (Nagabhushana et al., 2010; Park et al., 2010; Rezaie et al., 2002; Wild et al., 2011; Ying and Yue, 2012). Overexpressed optineurin forms vesicles some of which are autophagosomes and some are TFRC (transferrin receptor)-positive endosomes (Sirohi et al., 2013). Upon induction of acute oxidative stress, it migrates to nucleus (De Marco et al., 2006). However, *OPTN* does not have a defined nuclear localization signal.

4. Interactions and functions of optineurin

Optineurin interacting proteins have been identified using several methods such as yeast two-hybrid screening, immunoprecipitation and GST pull-down assays (Chalasani et al., 2009; Kachaner et al., 2012b). Nature and function of these interacting proteins provided initial clues about the cellular functions of optineurin. Most of the functions of optineurin are mediated by its ability to link two or more proteins (Fig. 1). UBD of optineurin is involved in most of its functions such as signaling to NF-κB, autophagy and vesicle trafficking. It binds to polyubiquitinated proteins with high affinity and shows selectivity towards Lys63-linked and linear polyubiquitin chains (Laplantine et al., 2009). It does not interact with Lys48-linked polyubiquitin chains (Zhu et al., 2007). This selectivity is mediated by UBD and zinc finger domain, which also binds to ubiquitin. Mutation of a conserved amino acid Asp474 to Asn (D474N) in the UBD abolishes binding of optineurin to ubiquitin.

5. Optineurin and autophagy

Ubiquitin proteasome system and autophagy are two major pathways for degradation of proteins in the cell. Macro-autophagy (hereafter referred to as autophagy) is a catabolic mechanism involved in the maintenance of cellular homeostasis by degrading long lived proteins, damaged and aggregated proteins and organelles through lysosomal pathway (Levine and Klionsky, 2004). The