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Review

Targeting the ER-autophagy system in the trabecular meshwork to treat glaucoma

Andrew R. Stothert, Sarah N. Fontaine, Jonathan J. Sabbagh, Chad A. Dickey*

Department of Molecular Medicine, Byrd Alzheimer's Research Institute, University of South Florida, Tampa, FL 33613, USA

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ABSTRACT

A major drainage network involved in aqueous humor dynamics is the conventional outflow pathway, which is gated by the trabecular meshwork (TM). The TM acts as a molecular sieve, providing resistance to aqueous outflow, which is responsible for regulating intraocular pressure (IOP). If the TM is damaged, aqueous outflow is impaired, IOP increases and glaucoma can manifest. Mutations in the *MYOC* gene cause hereditary primary open-angle glaucoma (POAG) by promoting the abnormal amyloidosis of the myocilin protein in the endoplasmic reticulum (ER), leading to ER stress-induced TM cell death. Myocilin accumulation is observed in approximately 70–80% of all glaucoma cases suggesting that environmental or other genetic factors may also promote myocilin toxicity. For example, simply preventing myocilin glycosylation is sufficient to promote its abnormal accretion. These myocilin amyloids are unique as there are no other known pathogenic proteins that accumulate within the ER of TM cells and cause toxicity. Moreover, this pathogenic accumulation only kills TM cells, despite expression of this protein in other cell types, suggesting that another modifier exclusive to the TM participates in the proteotoxicity of myocilin. ER autophagy (reticulophagy) is one of the pathways essential for myocilin clearance that can be impacted dramatically by aging and other environmental factors such as nutrition. This review will discuss the link between myocilin and autophagy, evaluating the role of this degradation pathway in glaucoma as well as its potential as a therapeutic target.

Contents

1. Introduction

Aqueous humor is a watery, ionic fluid, much like plasma in the blood, produced by the ciliary body that fills the anterior chamber of the eye (Abu-Hassan et al., 2014). Unlike the vitreous humor in the vitreous chamber of the eye, aqueous humor is constantly produced; therefore, in order to maintain a homeostatic environment, it must be constantly recycled out of the eye. Regulatory aqueous outflow occurs by two pathways in the anterior chamber: the conventional pathway, comprised mainly of the TM, and the unconventional outflow pathway, comprised of drainage channels located at the angle between the ciliary muscle and the iris. The conventional pathway regulates upwards of 85% of the aqueous

* Corresponding author.
E-mail address: cdickey@health.usf.edu (C.A. Dickey).

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outflow that occurs in the anterior chamber (Tamm, 2009).

TM is the major component of the conventional outflow pathway, comprised of TM cells, juxtacanalicular connective tissue (JCT), the inner walls of Schlemm's canal, Schlemm's canal, the collector channel, and finally the episcleral vein, which carries aqueous humor back to systemic circulation (Lutjen-Drecoll, 1999; Swaminathan et al., 2014). The TM is located at the iridocorneal junction of the anterior chamber, the area where the iris forms a junction with the cornea. In addition to giving the eye its shape, aqueous humor is responsible for providing nutrients to the avascular structures of the anterior chamber, including the lens and cornea (Abu-Hassan et al., 2014; Tamm, 2009). However, one of the more important functions aqueous humor provides to the microenvironment of the eye is producing IOP (Abu-Hassan et al., 2014), which is largely regulated by resistance of outflow provided by the TM in the conventional outflow pathway. Studies have shown that resistance to outflow is increased with age and in ocular disorders, such as glaucoma, where IOP elevation is a pathological hallmark (Tamm, 2009).

Glaucoma is a neurodegenerative protein misfolding disorder, characterized by retinal ganglion cell (RGC) death and optic nerve (ON) axon damage leading to progressive irreversible vision loss (Gupta and Yucel, 2007; Llobet et al., 2003; Porter et al., 2015; Stothert et al., 2014; Wentz-Hunter et al., 2004). Glaucoma is the second leading cause of blindness worldwide, with over 60 million individuals suffering from the disease. In the United States alone, it is estimated that 2–3 million people have glaucoma, with over 120,000 individuals suffering blindness due to the disease (Fingert et al., 2002; Gupta and Yucel, 2007; Tamm, 2002). The most common forms of glaucoma include open-angle glaucoma, angle-closure glaucoma, normal-tension glaucoma, and congenital glaucoma. Although all forms of glaucoma are seen throughout the population, over 90% of cases involve a form of POAG (Bruttini et al., 2003; Shepard et al., 2007). In POAG aqueous drainage channels remain exposed, but the TM network is damaged preventing proper outflow (Bruttini et al., 2003). POAG arising from distinct mechanisms is often associated with accumulation of the glycoprotein myocilin, within the TM and Schlemm's canal (Burns et al., 2011; Jacobson et al., 2001; Konz et al., 2009; Lutjen-Drecoll et al., 1998; McDowell et al., 2012).

2. Normal intracellular myocilin processing

Understanding the normal features of myocilin protein could help to elucidate why this protein accumulates in the glaucomatous eye. Myocilin is a ubiquitous 504 amino acid (aa) secreted glycoprotein expressed in both ocular and non-ocular tissues. In the eye, myocilin is found in the TM, sclera, ciliary body, choroid, cornea, iris, retina, and ON (Karali et al., 2000). Outside the eye, myocilin is expressed in tissues of the peripheral nervous system (Kwon et al., 2013). Structurally, myocilin consists of an N-terminal region containing a signal peptide sequence (aa 1–32), a helix-turn-helix (aa 18–58) and a leucine zipper domain containing two coiled-coil domains (aa 74–110 and aa 118–186) responsible for coordinating protein–protein interactions (Resch and Fautsch, 2009; Tamm, 2002). Myocilin is a glycoprotein that normally undergoes asparagine-linked glycosylation at aa residues 57–59 (Asn-Glu-Ser) (Amaravadi et al., 2011; Jacobson et al., 2001). The C-terminal globular region contains the olfactomedin (OLF) domain (aa 230–504) (Donegan et al., 2012; Tamm, 2002), an evolutionarily conserved region of amino acids first discovered in the olfactory neuroepithelium. In humans, the OLF domain is found within a group of glycosylated proteins important in the organization of the nervous system during development (Anholt, 2014; Burns et al., 2011). Though myocilin is expressed throughout the body, its physiological function is yet unknown. Recent studies have suggested the importance of myocilin in oligodendrocyte differentiation and axonal myelination (Kwon et al., 2014), and myocilin may play a role in retinal cell programmed death during development (Koch et al., 2014).

The secretory pathway is responsible for synthesis, folding, and delivery of a wide range of cellular proteins, including myocilin (Barlowe and Miller, 2013; Farhan and Rabouille, 2011). This pathway is particularly important in correctly coordinating the localization of newly formed proteins to specific organelles and to the extracellular space (Farhan and Rabouille, 2011). The pathway is comprised of the rough endoplasmic reticulum (ER), ER-exit sites, ER-to-Golgi intermediate compartment (ERGIC), the Golgi complex, and post-Golgi carriers (Barlowe and Miller, 2013). During ribosome synthesis of a polypeptide, proteins that are to be secreted are targeted by a signal recognition particle (SRP), a molecule that targets hydrophobic peptide signal sequences on the N-terminal domain of a protein. SRPs direct the translocation of newly forming polypeptides into the ER membrane via translocons, or channels in the ER membrane, for continued translation and post-translational modifications required for secretion. Once in the ER membrane, post-translational modifications such as signal peptide cleavage, glycosylation, disulfide bond formation, and glycosidase trimming occur to ensure proper protein folding.

The cleavage of the N-terminal signal peptide sequence is a common modification for many proteins that enter the ER, including myocilin. Cleavage of this sequence occurs via signal peptidase complex (SPC), a 4-subunit polypeptide. Most of the SPC subunits are required for cell viability, except the Sec11 subunit, which contains the protease active site (Barlowe and Miller, 2013). The SPC cleaves off the N-terminal signal peptide sequence, exposing glycosylation sites on the protein (Barlowe and Miller, 2013; Farhan and Rabouille, 2011). Asparagine –linked (N-linked glycosylation) is a post-translational modification where oligosaccharides are attached to asparagine residues in the N-terminal domain of the protein (Schwarz and Aebi, 2011). Oligosaccharyl transferase enzyme (OST), an 8-subunit polypeptide, carries out the addition of oligosaccharides. Cells require the majority of the OST subunits for viability, though the Stt3 domain is dispensable and only required for catalytic activity. N-linked glycosylation of a protein is thought to be important in thermodynamic stability, solubility, and protein folding (Schwarz and Aebi, 2011). Glycosylated proteins in the ER membrane then form disulfide bonds at free sulfhydryl groups on cysteine residue side chains due to the oxidizing environment within the ER lumen. A specialized family of disulfide isomerases is required for the formation, reduction and isomerization of these disulfide bonds, ensuring correct protein folding (Farquhar et al., 1991). Once the signal sequence is cleaved, proteins are glycosylated and disulfide bonds form. Following this process, the final step required for proper protein folding is the trimming of glucose residues from the protein oligosaccharide core. Glucosidases (Gls1/Gls2) are enzymes that rapidly trim glucose residues, allowing for proper chaperone folding (Helenius and Aebi, 2004). Mns1 and Htm1, enzymes that cleave mannose residues, generate signals for protein degradation by recognizing proteins that are terminally misfolded (Jakob et al., 1998).

After post-translational modifications, proteins are exported to ER-exit sites, areas that have a high concentration of coat-protein complex II (COPII) (Farhan and Rabouille, 2011; Lee et al., 2004). COPII is a molecule that forms a coat around properly folded proteins, promoting vesicle formation for Golgi transport. COPII vesicle formation is activated by the ER GTPase Sar1, which interacts with Sec proteins responsible for protein recruitment to form Sec23–24 and Sec13–31 complexes. The Sec23–24 complex is vital for protein capture and the Sec13–31complex is necessary for COPII coat