Published in final edited form as:

Exp Eye Res. 2009 April ; 88(4): 724–728. doi:10.1016/j.exer.2008.11.002.

THE ROLE OF PROTEOLYTIC CELLULAR SYSTEMS IN TRABECULAR MESHWORK HOMEOSTASIS

Paloma B. Liton, Pedro Gonzalez, and David L. Epstein

Duke University, Department of Ophthalmology, Erwin Road, Box 3802, Durham, NC 27710

Abstract

The molecular mechanisms contributing to the progressive malfunction of the trabecular meshwork (TM)/Schlemm's canal (SC) conventional outflow pathway during aging and in Primary Open Angle Glaucoma (POAG) are still poorly understood. Progressive accumulation of damaged and cross-linked proteins is a hallmark of aging tissues and has been proposed to play a major role in the tissue abnormalities associated with organismal aging and many age-related diseases. Such progressive accumulation of damaged proteins with age is believed to result from both, increased oxidative stress that results in faster rates of protein damage, as well as from a functional decline in the cellular proteolytic machinery that eliminates misfolded and damaged proteins. Here, we review the reported data that supports the occurrence of oxidative damage and the alterations in the intracellular proteolytic systems in the TM in aging and POAG. Finally, we discuss how the functional decline of the cellular proteolytic machinery in the TM might lead to the observed physiologic alterations of the outflow pathway in glaucoma.

INTRODUCTION

Malfunction of the trabecular meshwork (TM)/Schlemm's canal (SC) conventional outflow pathway has been long associated with elevated intraocular pressure (IOP) and therefore, increased risk of developing primary open angle glaucoma (POAG), a blinding disease affecting more than 70 million people worldwide (Leske et al., 2008). Despite the efforts of our laboratory and several others, the fundamental abnormality occurring in the TM/SC tissue with age and disease that leads to a decrease in aqueous humor (AH) outflow still remains obscure. Here, we will propose and evaluate a potential role of impaired clearance of oxidatively damaged macromolecules in the pathogenesis of POAG.

THE GARBAGE CATASTROPHE THEORY OF AGING AND DISEASE

Aging is a complex phenomenon associated with a progressive accumulation of deleterious changes that results in a gradual decline in cellular and physiological function, diminished capacity to respond to stress, and increased probability of degenerative diseases (Beckman and Ames, 1998). In 1954, Denham Harman proposed for the first time the "free-radical theory of aging", emphasizing the connection between an oxidative environment and the aging process (Harman, 1956). According to his theory, free radical reactions resulting from normal aerobic metabolism are responsible for the age-associated damage at the cellular and tissue levels. Later

CORRESPONDING AUTHOR: Paloma B. Liton: E-mail: paloma.liton@duke.edu, Duke University Eye center, AERI 4004, Erwin Road, Box 3802, Durham, NC 27710, USA, Phone: (919) 681 4085, Fax: (919) 684 8983.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

on, in 1972, he expanded his theory and postulated the “mitochondrial theory of aging”, which proposes that mitochondria are the source of the majority of reactive oxygen species (ROS) produced by the cell, and that damage to mitochondrial DNA causes a concomitant decrease in mitochondrial function. Such decrease in mitochondrial function further increases the production of damaging free-radicals, leading to a “vicious cycle” of escalating ROS production and mitochondrial damage. (Harman, 1972). The validity of the free radical theory of aging has been extensively supported by numerous *in vivo* and *in vitro* studies showing that age-related changes accelerate under the influence of oxidative stress, while various antioxidants slow aging (Barja, 2004).

Although most oxidatively damaged biomolecules and organelles are successfully removed by cellular proteolytic systems, the recycling machinery is inherently imperfect and is affected by the aging process. Thus, many types of cells and tissues accumulate oxidatively damaged material during aging and age-related disorders, which are believed to impede normal cellular function and tissue homeostasis. This hypothesis, first proposed by Sheldrake back in 1974 in his “waste product theory of aging” (Sheldrake, 1974), and recently revived by Terman as “garbage catastrophe theory of aging” (Terman, 2001), suggests that cellular aging is caused by the accumulation of intracellular waste products that cannot be destroyed or eliminated. It is further proposed that age-related “garbage” accumulation primarily occurs in long-lived postmitotic or terminally differentiated cells, like TM cells, in which waste material cannot be diluted by cell division. (Grune et al., 2005).

Accumulation of biological waste material, represented mainly by lipofuscin, defective mitochondria and cytoplasmic protein aggregates, within neurons, retinal pigmented epithelial cells, cardiac myocytes, and other long-lived postmitotic cells has been associated with a number of age-related diseases, including age-related macular degeneration, Alzheimer’s disease, Parkinson’s disease, cardiomyopathies, and atherosclerosis (Keller et al., 2004; Kiffin et al., 2006; Terman et al., 2007).

OXIDATIVE STRESS, AGING, AND PATHOPHYSIOLOGY OF THE OUTFLOW PATHWAY

Cells in the outflow pathway are subjected to chronic oxidative stress through ROS that are both present in the AH and generated by the normal metabolism of the cells (Spector et al., 1998). Exposure to ROS has been suspected to contribute to the morphological and physiological alterations of the aqueous outflow pathway in aging and POAG (reviewed in (Kumar and Agarwal, 2007; Sacca et al., 2007; Aslan et al., 2008). Indeed, decreased antioxidant potential, increased expression of oxidative stress markers, and increased oxidative DNA damage, peroxidized lipids, as well as the upregulation of inducible nitric oxide synthase have been described in the TM of glaucoma patients (Babizhayev and Bunin, 1989; Izzotti et al., 2003; Ziangirova and Antonova, 2003; Ferreira et al., 2004; Sacca et al., 2005; Fernandez-Durango et al., 2008). Moreover, a recent paper by He et al. has demonstrated an increase in mitochondrial ROS production in TM primary cultures from glaucoma donors (He et al., 2008). Interestingly, exposure of TM cells to chronic oxidative stress *in vitro* leads to elevated mitochondrial ROS production that mediates the upregulation of endothelial leukocyte adhesion molecule-1 (Li et al., 2007), a previously reported glaucoma marker (Wang et al., 2001; Liton et al., 2006).

PROTEOLYTIC SYSTEMS IN AGING AND DISEASE

Cells are not static structures but rather continuously rebuild themselves by degrading organelles as well as intracytoplasmic and phagocytosed proteins into their low-molecular-weight constituents via the cellular degradative pathways (Ciechanover and Schwartz, 1994).

In addition to this continuous turnover, abnormally synthesized proteins or proteins incorrectly modified, are also eliminated by the proteolytic cellular machinery. Four major proteolytic systems have been identified in eukaryotic cells: The ubiquitin-proteasome system, the calpains, the Lon proteases, and the lysosomal compartment. Age-related alterations of the components of the cellular proteolytic machinery have been implicated in the accumulation of waste material with age and in the increased risk of degenerative diseases (Cuervo and Dice, 1998).

The Ubiquitin-Proteasome System

The proteasome is a autocatalytic complex ubiquitously present in eukaryotic cells that plays an important role in intracellular proteolysis and in cellular functions that require protein turnover, stress responses, and elimination of misfolded and damaged proteins. Proteasomes exists in different oligomeric assemblies including: the 20S proteasome, the 26S proteasome; and the immunoproteasome (Hanna and Finley, 2007). The intracellular distribution of proteasomes varies depending on a particular cell type and its differentiation stage (Jung et al., 2007). 20S and 26S proteasomes are abundant in both the nucleus and the cytoplasm, and immunoproteasomes specifically concentrate at the endoplasmic reticulum (ER). In the majority of the cell types, the highest proteasome to protein ratio is generally located near the cell membrane, co-localizing with a high ratio of oxidized proteins to total proteins. However, specific studies regarding the localization of the proteasome in TM cells have not been conducted.

The proteasomal system is characterized by a high degree of specificity towards its substrates. While ubiquitinylation targets proteins for degradation by the 26S proteasomes, 20S proteasome units have been shown to degrade selectively oxidized proteins. The first recognition site of oxidized and misfolded proteins by the proteasome is the exposure of hydrophobic amino acid residues to the surface. Since oxidized protein can be ubiquitinated, both 20S and 26S proteasome units appear to play an important role in preventing the accumulation of oxidized proteins in the cell (Breusing and Grune, 2008).

It is now well accepted that proteasome activity declines with age and that this decline may contribute to the aging process as well as to different age-related pathologies (Cuervo and Dice, 1998; Dahlmann, 2007). The age-related proteasomal dysfunction could result from the formation of cross-linked protein aggregates, which inhibit proteasome activity and thus amplify their accumulation. Additionally, the age-related loss of antioxidant capacity affects the proteasomal system, particularly the 26S proteasome, which has been shown to be sensitive to oxidative inactivation (Breusing and Grune, 2008).

Similar to what has been reported in other tissues, the cells of the TM may experience a decline of proteasomal activity with age. A comparative analysis conducted in primary cultures of TM cells from young and old donors showed that TM cells from old donors (ages 66–73) showed a 3-fold increase in oxidized proteins and a 7.5-fold decrease of proteasome activity, compared to those from young donors (ages 9–25) (Caballero et al., 2004). The observed loss of proteasome function was not associated with decreased proteasome content, consistent with the notion that inhibition of proteasome activity with aging in the TM cells could be associated with “clogging” of the proteasome with protein aggregates and/or oxidation of the proteasome components. In addition, we also reported that chronic oxidative stress leads to the accumulation of oxidized proteins and a concomitant decrease in proteasome activity in primary cultures of human TM cells (Caballero et al., 2003). More recently results reported by Govindarajan et al suggest that impaired proteasome activity in the cells of the TM may be associated with POAG (Govindarajan et al., 2008).

Proteasome dysfunction can also result from other types of cellular stress, such as the presence of some genetic mutations. It is now well-established and demonstrated by several groups that disease-causing myocilin (MYOC) mutants are not secreted into the extracellular media but are intracellularly sequestered in form of misfolded large aggregates within the ER (Caballero and Borras, 2001; Jacobson et al., 2001; Joe et al., 2003; Gobeil et al., 2004; Liu and Vollrath, 2004; Yam et al., 2007). Liu and Vollrath further showed that a portion of misfolded mutant MYOC is retrotransported out of the ER, conjugated with ubiquitin, and degraded by proteasome (Liu and Vollrath, 2004). Therefore, any alteration in proteasome function due to oxidative stress or aging would also be expected to increase the rate of accumulation of misfolded mutant MYOC in the ER and contribute to the pathogenic effect of these mutant proteins in the TM.

The Lysosomal Proteolytic Pathway

Initially described by De Duve as membrane-bound organelles containing a variety of acid hydrolases (De Duve and Wattiaux, 1966), lysosomes represent the cell's "garbage disposal" system and are defined as the terminal organelle responsible for the degradation of all worn-out organelle and long-lived proteins, including extracellular proteins and membrane-bound receptors. Material to be degraded is delivered to the lysosomal system by two different pathways: (i) the endocytic pathway, by which extracellular molecules are internalized by pinocytosis, phagocytosis, or receptor-mediated endocytosis; and (ii) the autophagic pathway, responsible for the destruction of most long-lived endogenous proteins and damaged or obsolete organelles (Luzio et al., 2007).

The lysosomal degradative pathway is the major proteolytic system affected during the aging process (Cuervo and Dice, 1998). Lysosomes of long-lived postmitotic cells become more abundant and larger with age. They exhibit increased levels of lipofuscin or "age pigment", a brown-yellow polymeric complex composed primarily of cross-linked proteins and lipid residues (Terman et al., 1999; Sitte et al., 2000). Although lipofuscin has long been considered an innocent hallmark of aging, there is accumulating evidence of its harmful properties. One the most important consequences of intralysosomal lipofuscin accumulation is its interference with the lysosomal degradative capacity. First, cells respond to the accumulation of waste material by upregulating the synthesis of lysosomal enzymes, which are preferentially delivered to the lipofuscin-loaded lysosomes and are thereby misplaced and prevented from performing their normal role within late endosomes, autophagic vacuoles, and phagosomes (Grune et al., 2005; Terman and Brunk, 2006). Second, by mechanisms that are still unknown, the intralysosomal accumulation of waste material affects the activity of the lysosomal enzymes in several cell types. Such decline in the lysosomal degradative capacity further affects the cellular recycling processes. For example, the reported decreased proteasome activity in lipofuscin-loaded fibroblasts is believed to result from defective proteasome renewal due to lysosomal failure (Terman et al., 1999) (Cuervo et al., 1995). This might well explain the observed proteasome dysfunction in TM cells under chronic oxidative stress and with age (Caballero et al., 2003; Caballero et al., 2004).

Supporting this hypothesis, it has been recently reported that exposure of confluent cultures of porcine TM cells to a hyperoxic environment results in the accumulation of lipofuscin-loaded lysosomes and autophagic vacuoles content. The expression of several lysosomal cathepsins was found to be upregulated at both mRNA and proteins levels in the oxidatively stressed cultures. However, despite the increase in lysosomal mass and lysosomal enzyme content, the stressed cultures did not display increased lysosomal proteolytic activity. These data suggest that intralysosomal accumulation of oxidized material might impair lysosomal function in TM cells (Liton et al., 2008).

In 2005, our laboratory reported a significant increase in the number of cells positively stained for senescence-associated β -galactosidase (SA- β -Gal) activity in the glaucomatous outflow pathway (Liton et al., 2005). SA- β -Gal, which is defined as β -galactosidase activity detectable at pH 6 in senescence cells, was first described by Dimri et al. (Dimri et al., 1995) and soon became a widely used biomarker for cellular senescence. Although the nature of such activity remains unknown, SA- β -Gal has been proposed to be a manifestation of residual lysosomal β -galactosidase activity at a suboptimal pH that becomes detectable due to the increased lysosomal content in aged cells (Kurz et al., 2000). Accordingly, oxidatively stressed TM cells demonstrated increased levels of SA- β -Gal activity associated with the increase in lysosomal mass *in vitro* (Liton et al., 2008). Whether the observed increase in SA- β -Gal activity in the glaucomatous TM tissue is due to the accumulation of lipofuscin-loaded lysosomes still needs to be determined.

Impaired lysosomal function in the aging TM can potentially cause very detrimental effects in overall tissue physiology. Lysosomal enzyme activities have been detected in the human AH and in the TM tissue, and are thought to play a crucial role in tissue remodeling (Yue et al., 1987; Weinreb et al., 1991). In addition, TM cells are known to be actively phagocytic, capable of ingesting endogenous and exogenous material, thus keeping the trabecular outflow channels free of potentially obstructive debris. A number of reports suggest a potential negative effect of oxidative stress on phagocytosis. For instances, diminished phagocytic capacity, believed to be due to lipofuscin accumulation, has been described in cultured retinal pigment epithelial cells (Sundelin et al., 1998; Kaemmerer et al., 2007). Likewise, oxidative stress and cytoplasmic saturation with indigestible material has been reported to impair phagocytosis of apoptotic cells in atherosclerotic plaques (Schrijvers et al., 2005).

Compromised lysosomal function has been associated with an array of age-related disorders (Bahr and Bendiske, 2002; Terman et al., 2007). Although still not studied in detail, several evidences support a potential lysosomal dysfunction in glaucoma, including the observation of increased hydrolase activities and lipid peroxidation in the TM of glaucomatous eyes (Babizhayev and Bunin, 1989; Coupland et al., 1993). Consistent with this are observed ultrastructural changes in the TM, such as accumulation of pigment granules in the cytoplasm, lipid droplets, autophagic vacuoles, and the presence of extracellular lysosomes (Rohen, 1982; Sihota et al., 2001; Cracknell et al., 2006). Moreover, membrane-limited vesicles filled with granular material have been described in several types of glaucoma, including POAG, normal-tension glaucoma, and corticosteroid-induced glaucoma (Luetjen-Drecoll and Rohen, 1996). It is also noteworthy that the development of glaucoma has been associated with several mucopolysaccharidoses, a group of congenital lysosomal storage diseases, characterized by extracellular deposition of glycosaminoglycans and the presence of fibrillogranular and multimembranous membrane-bound inclusions distributed primarily in the outflow pathway and in the cornea (Nowaczyk et al., 1988; Cantor et al., 1989; Cahane et al., 1990; Mullaney et al., 1996). Even though glaucoma induced by lysosomal storage diseases belongs to the so-called secondary open angle glaucomas, this type of glaucoma highlights the potential critical role of the lysosomal system in proper outflow pathway function.

The Calpain Proteolytic System

The calpain system constitutes the third major proteolytic cellular system. Calpains comprise a family of sixteen intracellular non-lysosomal Ca^{2+} -regulated cysteine proteases located in the cytosol, ER, and Golgi apparatus in most mammalian tissues, which have been suggested to be involved in a number of processes during differentiation, life and death of the cell. One of their principal roles is regulating cell adhesion, cell spreading or migration, as well as the transduction of extracellular signals (Suzuki et al., 2004).

Beyond these physiological roles, calpains contribute to the pathogenesis of major human diseases, including Parkinson's disease, Alzheimer's disease, and Type 2 diabetes (Nixon, 2003). More importantly, the contribution of calpains in disease pathogenesis seems to increase with aging, suggesting that either age-related factors influence the regulation of calpains activation and/or that calpains themselves contribute to the aging process (Nixon, 2003). A well-established example, supported by several *in vitro* and *in vivo* models, is the mechanism of cataract formation. Overactivation of calpains in the aging lens, triggered by an increase in intracellular Ca^{2+} levels, results in deregulated proteolysis of crystallins, the water-soluble proteins involved in maintaining lens transparency. Such improperly processed crystallins precipitate in form of aggregates, thus contributing to lens opacity associated with cataracts. (Biswas et al., 2004).

Consistent with the upregulation of calpains in other damaged neuronal tissue, proteomics and Western-blot analysis of optic nerve tissue demonstrated an increased presence of calpain-1 in glaucomatous versus normal optic nerve (Bhattacharya et al., 2006). A different group found that inhibition of calpains significantly protected cells in the ganglion cell layer from cell death, suggesting that overactivation of the calpain system might be somehow implicated in the death of retinal ganglion cell that occurs in glaucoma (McKernan et al., 2007).

Elevated levels of calpain-1 protein have been also recently reported in the TM from glaucoma donors (Govindarajan et al., 2008). However, in contrast to what has been described in other tissues, the glaucomatous TM showed decreased calpain protease activity compared with control TM. By a series of elegant experiments, Govindarajan et al. demonstrated that such decreased enzymatic activity resulted from the inactivation of calpain-1 by isolevuglandins (IsoLGs) in the glaucomatous TM. IsoLGs are products of lipid peroxidation that damage proteins by covalent adduction and cross-linking thereby interfering with their normal function. The higher occurrence of IsoLGs in the glaucomatous outflow pathway is supported by the work of Babizhayev and Bunin reporting increased levels of lipid peroxidation in the TM in POAG (Babizhayev and Bunin, 1989).

The physiological implications of diminished calpain activity in the glaucomatous outflow pathway need to be studied in more detail. One potential consequence is the interference with the proteasomal degradative pathway. Proteins and peptides modified by IsoLGs can significantly reduce the activity of the proteasome 20S. *In vitro* studies showed that calpain-1 modified by IsoLGs undergoes ubiquitination, and it is more prone to form large aggregates and impair the cellular proteasome activity (Govindarajan et al., 2008).

Another potential outcome of the presence of lower levels of active calpains in glaucomatous TM cells might be the improper proteolytic processing of myocilin. Calpain-2 has recently been reported to be involved in the intracellular endoproteolytic cleavage of myocilin, yielding a C-terminal domain that is secreted together with the full-length protein, and an N-terminal domain that remains retained in the ER (Sanchez-Sanchez et al., 2007). Two different regions of myocilin are involved in its processing by calpains: the olfactomedin-like domain, which acts as a calpain binding site, and the cleavage site, located in the linker domain. Adequate conformation of the olfactomedin domain is required for myocilin proteolysis by calpain. Interestingly, glaucoma-associated myocilin mutations, which have been shown to reduce proteolytic cleavage, are located in the olfactomedin domain. The functional meaning of the proteolytic processing of myocilin is still unknown. It has been suggested that it might contribute to regulate its interaction with other proteins, decreasing its ability to form high molecular weight aggregates by cross-linking proteins (Aroca-Aguilar et al., 2005); however, this needs to be confirmed. Also, it would be very interesting to investigate whether the decreased calpain activity in the glaucomatous TM correlates with decreased proteolytic cleavage of wild-type myocilin.

The Mitochondrial Lon Proteases

The mitochondrial respiratory chain is one of the main sources of endogenous ROS and related oxidants, the production of which increases with age. In order to limit the extent of the oxidative damage, mammalian mitochondria contain both antioxidant systems to reduce and remove ROS, as well as a proteolytic system by which oxidatively modified proteins can be eliminated. The mitochondrial proteolytic system, ubiquitously expressed in all cell types, comprises three major ATP-dependent oligomeric proteases: Lon, Clp-like, and AAA proteases. These proteases contribute to the degradation of short-lived, misfolded or damaged proteins and to the maintenance of mitochondrial genome stability (Bulteau et al., 2006). In particular, Lon protease is believed to play a crucial role in the degradation of oxidized proteins within the mitochondria and for the preservation of mitochondrial structural and functional integrity (Ngo and Davies, 2007). Downregulation of the human Lon protease results in disruption of mitochondrial structure, loss of function and cell death (Bota et al., 2005).

Similar to the proteasomal and lysosomal proteolytic systems, alterations in the Lon protease system with age have been reported in several studies (Bulteau et al., 2006). Although aging seems to affect the Lon expression levels in a tissue-specific manner, results from all studies are consistent with a decline of the efficiency of the enzyme with age. Moreover, the decline in Lon protease activity was found to be associated with a decrease in the activity of aconitase, an essential Krebs cycle enzyme, known to be inactivated by oxidation in the mitochondrial matrix (Bota et al., 2002).

Mitochondrial dysfunction has been implicated in the aging process, as well as a number of age-associated diseases such as Parkinson's disease and Alzheimer's disease, associated with increased levels of mitochondrial-derived free radicals and oxidative damage (Ngo and Davies, 2007). Abu-Amero et al. reported a spectrum of mitochondrial abnormalities in patients with POAG, including a decrease in mitochondrial respiratory activity and mtDNA sequence alterations (Abu-Amero et al., 2006). A more recent study has shown that TM cells from glaucoma donors have higher levels of endogenous ROS, lower ATP levels, and decreased mitochondrial membrane potential compared to TM cells from control donors (He et al., 2008). Also, our laboratory has shown that exposure of TM primary cultures to chronic oxidative stress induces mitochondrial ROS production and the expression of inflammatory mediators previously observed in glaucoma donors (Li et al., 2007). Despite all this evidence supporting a potential role of mitochondrial disruption and oxidative stress in the pathogenesis of glaucoma, no one, to our knowledge, has studied, how the mitochondrial proteolytic system changes in the TM with age and in glaucoma.

CONCLUDING REMARKS

Cells within the outflow pathway present a low basal proliferative rate and are continuously subjected to different types of stress, including oxidative and phagocytic stress, which might predispose them over time to accumulate substantial amounts of nondegradable intracellular oxidized material. Similar to what has been observed in other aging postmitotic tissues, this deposition of biological garbage might impair TM cellular and tissue function homeostasis and, hence, might explain the morphological and physiological alterations observed in the TM/SC tissue with age and in POAG.

BILBIOGRAPHY

Abu-Amero KK, Morales J, Bosley TM. Mitochondrial abnormalities in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2006;47:2533–41. [PubMed: 16723467]

- Aroca-Aguilar JD, Sanchez-Sanchez F, Ghosh S, Coca-Prados M, Escibano J. Myocilin mutations causing glaucoma inhibit the intracellular endoproteolytic cleavage of myocilin between amino acids Arg226 and Ile227. *J Biol Chem* 2005;280:21043–51. [PubMed: 15795224]
- Aslan M, Cort A, Yucel I. Oxidative and nitrative stress markers in glaucoma. *Free Radic Biol Med* 2008;45:367–76. [PubMed: 18489911]
- Babizhayev MA, Bunin A. Lipid peroxidation in open-angle glaucoma. *Acta Ophthalmol (Copenh)* 1989;67:371–7. [PubMed: 2801038]
- Bahr BA, Bendiske J. The neuropathogenic contributions of lysosomal dysfunction. *J Neurochem* 2002;83:481–9. [PubMed: 12390510]
- Barja G. Free radicals and aging. *Trends Neurosci* 2004;27:595–600. [PubMed: 15374670]
- Baudry M, DuBrin R, Beasley L, Leon M, Lynch G. Low levels of calpain activity in Chiroptera brain: implications for mechanisms of aging. *Neurobiol Aging* 1986;7:255–8. [PubMed: 3018604]
- Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev* 1998;78:547–81. [PubMed: 9562038]
- Bhattacharya SK, Crabb JS, Bonilha VL, Gu X, Takahara H, Crabb JW. Proteomics implicates peptidyl arginine deiminase 2 and optic nerve citrullination in glaucoma pathogenesis. *Invest Ophthalmol Vis Sci* 2006;47:2508–14. [PubMed: 16723463]
- Biswas S, Harris F, Dennison S, Singh J, Phoenix DA. Calpains: targets of cataract prevention? *Trends Mol Med* 2004;10:78–84. [PubMed: 15102361]
- Bota DA, Ngo JK, Davies KJ. Downregulation of the human Lon protease impairs mitochondrial structure and function and causes cell death. *Free Radic Biol Med* 2005;38:665–77. [PubMed: 15683722]
- Bota DA, Van Remmen H, Davies KJ. Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. *FEBS Lett* 2002;532:103–6. [PubMed: 12459471]
- Boveris A, Fraga CG. Oxidative stress in aging and disease. *Mol Aspects Med* 2004;25:1–4. [PubMed: 15051311]
- Breusing N, Grune T. Regulation of proteasome-mediated protein degradation during oxidative stress and aging. *Biol Chem* 2008;389:203–9. [PubMed: 18208355]
- Bulteau AL, Szweda LI, Friguet B. Mitochondrial protein oxidation and degradation in response to oxidative stress and aging. *Exp Gerontol* 2006;41:653–7. [PubMed: 16677792]
- Caballero M, Borras T. Inefficient processing of an olfactomedin-deficient myocilin mutant: potential physiological relevance to glaucoma. *Biochem Biophys Res Commun* 2001;282:662–70. [PubMed: 11401512]
- Caballero M, Liton PB, Challa P, Epstein DL, Gonzalez P. Effects of donor age on proteasome activity and senescence in trabecular meshwork cells. *Biochem Biophys Res Commun* 2004;323:1048–54. [PubMed: 15381105]
- Caballero M, Liton PB, Epstein DL, Gonzalez P. Proteasome inhibition by chronic oxidative stress in human trabecular meshwork cells. *Biochem Biophys Res Commun* 2003;308:346–52. [PubMed: 12901875]
- Cahane M, Treister G, Abraham FA, Melamed S. Glaucoma in siblings with Morquio syndrome. *Br J Ophthalmol* 1990;74:382–3. [PubMed: 2116163]
- Cantor LB, Disseler JA, Wilson FM 2nd. Glaucoma in the Maroteaux-Lamy syndrome. *Am J Ophthalmol* 1989;108:426–30. [PubMed: 2508477]
- Ciechanover, AJ.; Schwartz, AL., editors. Cellular proteolytic systems. New York: Wiley-Liss; 1994.
- Coupland SE, Heimann H, Hoffmann F, Penfold PL, Billson FA. Increased hydrolase activities in the human trabecular meshwork of glaucomatous eyes. *Ger J Ophthalmol* 1993;2:107–12. [PubMed: 8097935]
- Cracknell KP, Grierson I, Hogg P, Majekodunmi AA, Watson P, Marmion V. Melanin in the trabecular meshwork is associated with age, POAG but not Latanoprost treatment. A masked morphometric study. *Exp Eye Res* 2006;82:986–93. [PubMed: 16297910]
- Cuervo AM, Dice JF. How do intracellular proteolytic systems change with age? *Front Biosci* 1998;3:D25–43. [PubMed: 9407152]
- Cuervo AM, Palmer A, Rivett AJ, Knecht E. Degradation of proteasomes by lysosomes in rat liver. *Eur J Biochem* 1995;227:792–800. [PubMed: 7867640]

- Dahlmann B. Role of proteasomes in disease. *BMC Biochem* 8 Suppl 2007;1:S3.
- De Duve C, Wattiaux R. Functions of lysosomes. *Annu Rev Physiol* 1966;28:435–92. [PubMed: 5322983]
- Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 1995;92:9363–7. [PubMed: 7568133]
- Fernandez-Durango R, Fernandez-Martinez A, Garcia-Feijoo J, Castillo A, de la Casa JM, Garcia-Bueno B, Perez-Nievas BG, Fernandez-Cruz A, Leza JC. Expression of nitrotyrosine and oxidative consequences in the trabecular meshwork of patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2008;49:2506–11. [PubMed: 18296660]
- Ferreira SM, Lerner SF, Brunzini R, Evelson PA, Llesuy SF. Oxidative stress markers in aqueous humor of glaucoma patients. *Am J Ophthalmol* 2004;137:62–9. [PubMed: 14700645]
- Gobeil S, Rodrigue MA, Moisan S, Nguyen TD, Polansky JR, Morissette J, Raymond V. Intracellular sequestration of hetero-oligomers formed by wild-type and glaucoma-causing myocilin mutants. *Invest Ophthalmol Vis Sci* 2004;45:3560–7. [PubMed: 15452063]
- Govindarajan B, Laird J, Salomon RG, Bhattacharya SK. Isolevuglandin-modified proteins, including elevated levels of inactive calpain-1, accumulate in glaucomatous trabecular meshwork. *Biochemistry* 2008;47:817–25. [PubMed: 18085799]
- Grune T, Merker K, Jung T, Sitte N, Davies KJ. Protein oxidation and degradation during postmitotic senescence. *Free Radic Biol Med* 2005;39:1208–15. [PubMed: 16214036]
- Hanna J, Finley D. A proteasome for all occasions. *FEBS Lett* 2007;581:2854–61. [PubMed: 17418826]
- Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956;11:298–300. [PubMed: 13332224]
- Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc* 1972;20:145–7. [PubMed: 5016631]
- He Y, Leung KW, Zhang YH, Duan S, Zhong XF, Jiang RZ, Peng Z, Tombran-Tink J, Ge J. Mitochondrial complex I defect induces ROS release and degeneration in trabecular meshwork cells of POAG patients: protection by antioxidants. *Invest Ophthalmol Vis Sci* 2008;49:1447–58. [PubMed: 18385062]
- Izzotti A, Sacca SC, Cartiglia C, De Flora S. Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. *Am J Med* 2003;114:638–46. [PubMed: 12798451]
- Jacobson N, Andrews M, Shepard AR, Nishimura D, Searby C, Fingert JH, Hageman G, Mullins R, Davidson BL, Kwon YH, Alward WL, Stone EM, Clark AF, Sheffield VC. Non-secretion of mutant proteins of the glaucoma gene myocilin in cultured trabecular meshwork cells and in aqueous humor. *Hum Mol Genet* 2001;10:117–25. [PubMed: 11152659]
- Joe MK, Sohn S, Hur W, Moon Y, Choi YR, Kee C. Accumulation of mutant myocilins in ER leads to ER stress and potential cytotoxicity in human trabecular meshwork cells. *Biochem Biophys Res Commun* 2003;312:592–600. [PubMed: 14680806]
- Jung T, Bader N, Grune T. Oxidized proteins: intracellular distribution and recognition by the proteasome. *Arch Biochem Biophys* 2007;462:231–7. [PubMed: 17362872]
- Kaemmerer E, Schutt F, Krohne TU, Holz FG, Kopitz J. Effects of lipid peroxidation-related protein modifications on RPE lysosomal functions and POS phagocytosis. *Invest Ophthalmol Vis Sci* 2007;48:1342–7. [PubMed: 17325182]
- Keller JN, Dimayuga E, Chen Q, Thorpe J, Gee J, Ding Q. Autophagy, proteasomes, lipofuscin, and oxidative stress in the aging brain. *Int J Biochem Cell Biol* 2004;36:2376–91. [PubMed: 15325579]
- Kiffin R, Bandyopadhyay U, Cuervo AM. Oxidative stress and autophagy. *Antioxid Redox Signal* 2006;8:152–62. [PubMed: 16487049]
- Kumar DM, Agarwal N. Oxidative stress in glaucoma: a burden of evidence. *J Glaucoma* 2007;16:334–43. [PubMed: 17438430]
- Kurz DJ, Decary S, Hong Y, Erusalimsky JD. Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *J Cell Sci* 2000;113 (Pt 20):3613–22. [PubMed: 11017877]
- Leske MC, Wu SY, Hennis A, Honkanen R, Nemesure B. Risk factors for incident open-angle glaucoma: the Barbados Eye Studies. *Ophthalmology* 2008;115:85–93. [PubMed: 17629563]

- Li G, Luna C, Liton PB, Navarro I, Epstein DL, Gonzalez P. Sustained stress response after oxidative stress in trabecular meshwork cells. *Mol Vis* 2007;13:2282–8. [PubMed: 18199969]
- Liton PB, Challa P, Stinnett S, Luna C, Epstein DL, Gonzalez P. Cellular senescence in the glaucomatous outflow pathway. *Exp Gerontol* 2005;40:745–8. [PubMed: 16051457]
- Liton PB, Lin Y, Luna C, Li G, Gonzalez P, Epstein DL. Cultured Porcine Trabecular Meshwork Cells Display Altered Lysosomal Function when Subjected to Chronic Oxidative Stress. *Invest Ophthalmol Vis Sci* 2008;49:3961–9. [PubMed: 18469195]
- Liton PB, Luna C, Challa P, Epstein DL, Gonzalez P. Genome-wide expression profile of human trabecular meshwork cultured cells, nonglaucomatous and primary open angle glaucoma tissue. *Mol Vis* 2006;12:774–90. [PubMed: 16862071]
- Liu Y, Vollrath D. Reversal of mutant myocilin non-secretion and cell killing: implications for glaucoma. *Hum Mol Genet* 2004;13:1193–204. [PubMed: 15069026]
- Luetjen-Drecoll, E.; Rohen, JW. Morphology of Aqueous Outflow Pathways in Normal and Glaucomatous Eyes. In: Ritch, R.; Shields, MB.; Krupin, T., editors. *The Glaucomas*. St. Louis; Mosby: 1996. p. 89-123.
- Luzio JP, Pryor PR, Bright NA. Lysosomes: fusion and function. *Nat Rev Mol Cell Biol* 2007;8:622–32. [PubMed: 17637737]
- McKernan DP, Guerin MB, O'Brien CJ, Cotter TG. A key role for calpains in retinal ganglion cell death. *Invest Ophthalmol Vis Sci* 2007;48:5420–30. [PubMed: 18055788]
- Mullaney P, Awad AH, Millar L. Glaucoma in mucopolysaccharidosis 1-H/S. *J Pediatr Ophthalmol Strabismus* 1996;33:127–31. [PubMed: 8965238]
- Ngo JK, Davies KJ. Importance of the lon protease in mitochondrial maintenance and the significance of declining lon in aging. *Ann N Y Acad Sci* 2007;1119:78–87. [PubMed: 18056957]
- Nixon RA. The calpains in aging and aging-related diseases. *Ageing Res Rev* 2003;2:407–18. [PubMed: 14522243]
- Nowaczyk MJ, Clarke JT, Morin JD. Glaucoma as an early complication of Hurler's disease. *Arch Dis Child* 1988;63:1091–3. [PubMed: 3140740]
- Rohen JW. Presence of matrix vesicles in the trabecular meshwork of glaucomatous eyes. *Graefes Arch Clin Exp Ophthalmol* 1982;218:171–6. [PubMed: 7084692]
- Sacca SC, Izzotti A, Rossi P, Traverso C. Glaucomatous outflow pathway and oxidative stress. *Exp Eye Res* 2007;84:389–99. [PubMed: 17196589]
- Sacca SC, Pascotto A, Camicione P, Capris P, Izzotti A. Oxidative DNA damage in the human trabecular meshwork: clinical correlation in patients with primary open-angle glaucoma. *Arch Ophthalmol* 2005;123:458–63. [PubMed: 15824217]
- Sanchez-Sanchez F, Martinez-Redondo F, Aroca-Aguilar JD, Coca-Prados M, Escribano J. Characterization of the intracellular proteolytic cleavage of myocilin and identification of calpain II as a myocilin-processing protease. *J Biol Chem* 2007;282:27810–24. [PubMed: 17650508]
- Schrijvers DM, De Meyer GR, Kockx MM, Herman AG, Martinet W. Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2005;25:1256–61. [PubMed: 15831805]
- Sheldrake AR. The ageing, growth and death of cells. *Nature* 1974;250:381–385.
- Sihota R, Lakshmaiah NC, Walia KB, Sharma S, Pailoor J, Agarwal HC. The trabecular meshwork in acute and chronic angle closure glaucoma. *Indian J Ophthalmol* 2001;49:255–9. [PubMed: 12930118]
- Sitte N, Merker K, Von Zglinicki T, Davies KJ, Grune T. Protein oxidation and degradation during cellular senescence of human BJ fibroblasts: part II--aging of nondividing cells. *Faseb J* 2000;14:2503–10. [PubMed: 11099468]
- Spector A, Ma W, Wang RR. The aqueous humor is capable of generating and degrading H₂O₂. *Invest Ophthalmol Vis Sci* 1998;39:1188–97. [PubMed: 9620079]
- Sundelin S, Wihlmark U, Nilsson SE, Brunk UT. Lipofuscin accumulation in cultured retinal pigment epithelial cells reduces their phagocytic capacity. *Curr Eye Res* 1998;17:851–7. [PubMed: 9724002]
- Suzuki K, Hata S, Kawabata Y, Sorimachi H. Structure, activation, and biology of calpain. *Diabetes* 2004;53(Suppl 1):S12–8. [PubMed: 14749260]

- Terman A. Garbage catastrophe theory of aging: imperfect removal of oxidative damage? *Redox Rep* 2001;6:15–26. [PubMed: 11333111]
- Terman A, Abrahamsson N, Brunk UT. Ceroid/lipofuscin-loaded human fibroblasts show increased susceptibility to oxidative stress. *Exp Gerontol* 1999;34:755–70. [PubMed: 10579636]
- Terman A, Brunk UT. Oxidative stress, accumulation of biological 'garbage', and aging. *Antioxid Redox Signal* 2006;8:197–204. [PubMed: 16487053]
- Terman A, Gustafsson B, Brunk UT. Autophagy, organelles and ageing. *J Pathol* 2007;211:134–43. [PubMed: 17200947]
- Wang N, Chintala SK, Fini ME, Schuman JS. Activation of a tissue-specific stress response in the aqueous outflow pathway of the eye defines the glaucoma disease phenotype. *Nat Med* 2001;7:304–9. [PubMed: 11231628]
- Weinreb RN, Jeng S, Miller AL. Lysosomal enzyme activity in human aqueous humor. *Clin Chim Acta* 1991;199:1–5. [PubMed: 1834372]
- Yam GH, Gaplovska-Kysela K, Zuber C, Roth J. Aggregated myocilin induces russell bodies and causes apoptosis: implications for the pathogenesis of myocilin-caused primary open-angle glaucoma. *Am J Pathol* 2007;170:100–9. [PubMed: 17200186]
- Yue BY, Elner VM, Elner SG, Davis HR. Lysosomal enzyme activities in cultured trabecular-meshwork cells. *Exp Eye Res* 1987;44:891–7. [PubMed: 3653279]
- Ziangirova GG, Antonova OV. [Lipid peroxidation in the pathogenesis of primary open-angle glaucoma]. *Vestn Oftalmol* 2003;119:54–5. [PubMed: 12934509]