Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration

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Keywords: AMD, autophagy, heterophagy, inflammasome, lysosome, oxidative stress, phagocytosis, proteasome, RPE

Abbreviations: 3-MA, 3-methyladenine; AMD, age-related macular degeneration; AMPK, 5’ adenosine monophosphate-activated protein kinase; ATG, autophagy-related; CNV, choroidal neovascularization; CQ, chloroquine; D, drusen; MERTK, c-mer proto-oncogene tyrosine kinase; MFGE8, milk fat globule-EGF factor 8 protein; MTOR, mechanistic target of rapamycin; MTORC1, MTOR complex 1; NLR family, pyrin domain containing 3 (NLRP3); POS, photoreceptor outer segment; ROS, reactive oxygen species; RPE, retinal pigment epithelium; SQSTM1, sequestosome 1; VEGF, vascular endothelial growth factor

Age-related macular degeneration (AMD) is a complex, degenerative and progressive eye disease that usually does not lead to complete blindness, but can result in severe loss of central vision. Risk factors for AMD include age, genetics, diet, smoking, oxidative stress and many cardiovascular-associated risk factors. Autophagy is a cellular housekeeping process that removes damaged organelles and protein aggregates, whereas heterophagy, in the case of the retinal pigment epithelium (RPE), is the phagocytosis of exogenous photoreceptor outer segments. Numerous studies have demonstrated that both autophagy and heterophagy are highly active in the RPE. To date, there is increasing evidence that constant oxidative stress impairs autophagy and heterophagy, as well as increases protein aggregation and causes inflammasome activation leading to the pathological phenotype of AMD. This review ties together these crucial pathological topics and reflects upon autophagy as a potential therapeutic target in AMD.

Introduction

AMD is a neurodegenerative disease, which is characterized by loss of central vision as a result of cellular dysfunction and cell loss at the macula. The macula is a highly specialized region of the central retina unique to humans and other primates that is normally responsible for achieving high acuity and color vision.1 Macular pathology, as occurs in AMD, results in difficulty in seeing letters on a page, reduced ability to distinguish contrasts, distortion of straight lines, central visual field defects, scotomas and color vision impairment (Fig. 1). AMD is the most common cause of visual impairment in Western countries.2 Worldwide, approximately 50 million elderly people suffer from AMD, and the number of cases is expected to rise 3-fold over the next 20 years, suggesting that AMD is becoming a major public health issue.3,4 Approximately 10 million Americans are affected by AMD, and the costs of treatment are in excess of $340 billion US.5,6 The loss of vision results primarily from the progressive degeneration and death of RPE cells, which secondarily impairs the function of rods and cones. Phenotypically, AMD can be divided into two main forms: dry (atrophic) and wet (exudative) type and further subdivided into early and late stage disease. The early stage of dry AMD is asymptomatic, although pigment mottling, accumulation of intracellular lysosomal lipofuscin, and extracellular drusen deposits can be detected.7,8 The late stage of dry AMD, also known as geographic atrophy, is characterized by discrete areas of RPE loss and impairment of the overlying retinal photoreceptor cells. In wet AMD, aberrant blood vessels sprout from the choroidal capillaries and penetrate through the Bruch’s membrane leading to subretinal membranes, hemorrhage, retinal edema and damage to retinal cells. If left untreated, late stage fibrosis and permanent visual loss may occur. Interestingly, AMD shares several risk factors with other neurodegenerative and aggregation diseases.1 These risk factors include: aging, genetic
The RPE layer consists of hexanocuboidal epithelial cells which have two types of apical microvilli directed toward the interphotoreceptor matrix and the outer segment tips of rods and cones (Fig. 3). The short microvilli facilitate transepithelial transport. The RPE cells are a central regulator of vision, playing an essential role in maintaining the functionality and survival of photoreceptor cells. The RPE can, for example, metabolize and recycle retinoids, secrete growth factors for photoreceptors and choriocapillaries, and control the transport of nutrients into, and waste products out of, the retina. Moreover, RPE cells are phagocytically the most active cells in the whole body, whereby up to 10% of the POS length can become phagocytosed by RPE cells on a daily basis in a process called heterophagy. Since each human RPE cell is responsible for the upkeep of 30–40 photoreceptors, this is an enormous metabolic challenge for the endolysosomal system of the RPE, which is needed for the degradation of the ingested POS. The loss of the normally nondividing RPE cells during aging and AMD increases the metabolic burden on neighboring cells. In the long run, autofluorescent, lipid-protein aggregates called lipofuscin accumulate in the lysosomes of RPE cells.

The process of photoreceptor disc shedding and renewal and the role of RPE cells in the process were first revealed in the 1960s by Young and colleagues. The renewal of the POS with the help of RPE is essential for the survival of rods and cones, and the diminished phagocytic capacity of RPE cells has been associated with degenerative diseases of the retina. Heterophagy of the POS occurs at the apical side of the RPE cells that is intimately associated with the photoreceptor layer. Once the discs have been internalized, the phagosome moves from the apical to the basal surface where the contents of the phagosome become degraded. Although the process is not completely understood, the phagocytosis of POS can be divided into four distinct stages: recognition and attachment of the POS discs, their ingestion, formation of the phagosome and its fusion with a lysosome, and degradation.

A number of molecules essential for the recognition, binding and internalization of POS have been identified. The integrin ITGAV-ITGB5 (αVβ5) is, for example, required for the binding of outer segments, while MERTK (c-mer proto-oncogene tyrosine kinase) is essential for triggering their ingestion. Protein tyrosine kinase 2 becomes activated through the binding process, being phosphorylated by MERTK, thus linking the signaling between integrin ITGAV-ITGB5 and MERTK. MFGE8 (milk fat globule-EGF factor 8 protein), a ligand of integrin ITGAV-ITGB5, is needed for the regulation of the circadian rhythm of phagocytosis. A defect in the initial stage of phagocytosis results in photoreceptor death, as demonstrated in the Royal College of Surgeons rat which carries a mutation in the Merk gene. In addition, age-related decrease in the level of integrin ITGAV-ITGB5 leads to the accumulation of lipofuscin within the lysosomes in RPE cells and decreased retinal adhesion, both of which secondarily evoke vision loss.

Although some of the molecular mechanisms involved in the initial stages of phagocytosis or heterophagy are already known (see above), the processes of phagosome maturation and the final degradation of POS still remain obscure. Proteins involved in the maturation of the phagosome have been identified in macrophages—cells in which phagocytosis has been studied much more extensively than other cell types. We have also reported that some of the phagosome maturation proteins are expressed in RPE cells of rats, indicating that similar signaling pathways may be involved in the RPE-related phagosome maturation. Cytoskeletal elements, particularly actin filaments and microtubule-dependent motor proteins, play a critical role in the internalization of phagosomes by RPE cells. Abnormal cytoskeletal

**RPE cells in AMD Pathology**

The degeneration of RPE cells is widely considered to take place first during AMD development. The substantial oxygen consumption, lipid peroxidation products from the ingested photoreceptor outer segments (POS) and almost constant exposure to light, predispose the metabolically active RPE cells to chronic oxidative stress. Oxidatively damaged molecules, such as carboxyethylpyrrole, malondialdehyde, 4-hydroxynonenal, and advanced glycation end products, accumulate in the macular area and serve as a source for chronic oxidative stress. Age-related increase in oxidative stress is concurrent with increased accumulation of auto-oxidative lipofuscin in the lysosomes of RPE cells, as well as drusen formation in the extracellular space between the RPE and the Bruch’s membrane (Figs. 3 and 4). In addition to oxidative stress and protein aggregation, immunological events are involved in the pathogenesis of AMD. These include the production of several types of inflammation-related molecules, the recruitment of leukocytes, such as macrophages and dendritic cells, as well as activation of the complement pathway, alternate complement pathway, inflammasomes and microglia cells.

![Figure 1. Symptoms of AMD include distortion of straight lines, central visual field defects, central dark spots, contrast sensitivity alterations and color-vision impairment.](image-url)
Oxidized low-density lipoproteins and lipid peroxidation end products reduce the degradation of phagocytosed POS material, and increase cellular stress in the RPE cell. Our recent findings implicate βA3/A1-crystallin as a novel lysosomal component in the RPE that could regulate both phagocytosis and autophagy. Since βA3/A1-crystallin has been found in human drusen material, and our recent studies also show that Nuc1 rats with a spontaneous mutation in the Cryba1 gene develop deposits between the basal side of the RPE and Bruch’s membrane during aging (Fig. 5), it is tempting to speculate that perturbation of normal phagocytosis and/or autophagy could lead to some manifestations of AMD. Also, an experimental disruption of lysosomal function has provided support for the possible role of lysosomes in the development of AMD.

Impairment of Lysosomal Function in RPE

Lysosomal clearance may be disturbed by various mechanisms during the degeneration of RPE cells and development of AMD. Lysosomes possess a multifunctional capacity to cope with different cleaning processes when proteins destined for degradation arrive via endocytosis, phagocytosis (heterophagy) or autophagy. Cathepsins are proteases with a biological task to degrade proteins; so far, CTSA, B, D, E and S have been characterized in RPE cells. The main responsibility of CTSD is the degradation of POS and rhodopsin into glycopeptides within the RPE lysosomes; CTSD-deficient mice develop retinal degeneration. CSTs (cystatins), which are inhibitors of lysosomal cysteine proteases, are highly expressed in RPE cells. A CST3 (encoding cystatin C) gene variant is associated with an increased risk of developing advanced AMD. Due to this cystatin variant, important cellular functions, such as cellular transport and regulation of proteolytic balance in the extracellular space become affected, which are thought to be involved in the RPE degeneration and AMD development. This observation is supported by the earlier documentation indicating a relationship between the increased level of serum CST3 and the incidence of AMD.

Figure 2. AMD risk factors associated with different phenotypes of macular degeneration. Phenotypically, AMD can be divided into early and late stages of dry and wet AMD. (A) Pigment mottling and extracellular drusen deposition can be detected in the early stage of dry AMD (arrow). (B) The late stage of dry AMD develops a large geographic atrophy lesion in the macula (arrow). (C) Hemorrhages are usually observed in the early neovascularization process in wet AMD (arrow). (D) Fibrotic disciform lesion develops in wet AMD if not treated by anti-VEGF agents.

Oxidized low-density lipoproteins and lipid peroxidation end products reduce the degradation of phagocytosed POS material, and increase cellular stress in the RPE cell. The intra- and lysosomal storage of these metabolites is considered to represent the initial stage of lipofuscinogenesis. The A2-E (N-retinylidene-N-retinylethanol-amine) fluorophore is a harmful component of lipofuscin, the latter being a photosensitizer and auto-oxidant that can increase the mitochondrial stress and irreversibly inhibit lysosomal cathepsin activity upon light exposure, leading to increased damage of the RPE. It is thought that, once formed, lipofuscin cannot be degraded by proteasomal or lysosomal enzymes or become transported into the extracellular space via exocytosis. Furthermore, maintenance of an acidic lysosomal pH is critical. Elevated lysosomal pH is observed in RPE cells from ABCA4 knockout mice (a model for Stargardt’s disease) and in cultured human ARPE-19 cells exposed to A2E. Thus, POS clearance and lysosomal enzyme activity seem to be related to lysosomal pH. Clinical findings from AMD patients indicate that mitochondrial damage and excessive lipofuscin accumulation precede the atrophy of outer retinal layers and the subsequent loss of visual function. Oxidative stress and mitochondrial dysfunction in RPE cells. Oxidative stress can induce electron leakage from the mitochondrial electron transport chain, followed by formation of hydroxyl radicals by Fenton-type reactions, and production of superoxide, hydrogen peroxide and hypochlorite as a consequence of many enzymatic reactions. An imbalance between the generation and the suppression of reactive oxygen species (ROS) can
This could result in the formation of oxygen radicals and cause oxidative modification of proteins, lipids and nucleic acids. For example, many proteins isolated from RPE cell-derived lipofuscin have been modified and decorated by the markers of oxidative stress, such as malondialdehyde, hydroxynonenal, advanced glycation end products and advanced glycosylation end product-specific receptor. Interestingly, Höhn et al. observed that due to the iron, lipofuscin is able to sustain the production of cytotoxic oxidants independently of the mitochondria. Together with an increased accumulation of lipofuscin, impaired lysosomal function, continuous light exposure, and oxidants, this can induce defects in mitochondrial functions, which can further increase the oxidative stress. Due to the high metabolic activity, impaired mitochondrial function is assumed to lead to the accelerated degeneration of RPE cells and secondarily to photoreceptor cell death. Taken together, the Fenton reaction can occur in mitochondria leading to their damage, but also in lysosomes where it can directly contribute to lipofuscin formation. However, it must be noted that most of the RPE lipofuscin is derived from retinal POS.

The concurrent increase in the structural alterations of mitochondria also coincides with the pathology of AMD. Oxidative stress leads to mitochondrial DNA damage, increases ROS generation and reduces the metabolic capacity. Mitochondrial DNA is more susceptible to oxidative damage and light exposure than nuclear DNA. Recent findings support the idea that there is increased mitochondrial stress and dysfunction in the RPE cells of AMD patients. Therefore, selective removal of oxidatively damaged mitochondria by autophagy (called mitophagy) might be essential for cell survival.

**Impaired Proteasomal and Autophagosomal Proteolysis in RPE Cells and AMD**

Increased oxidative stress evokes misfolding of proteins via oxidation/glutathione conjugation. Primarily, molecular chaperones (heat shock proteins) repair the misfolding damage, but if this fails, soluble proteins become ubiquitinated and are targeted into proteasomes for degradation (Fig. 6). The proteasomal activity may also decline during aging, which leads to aggregation of oxidized and ubiquitinated proteins—a process that can lead to undesirable effects, for example, due to protein unfolding and damage, especially in age-related conditions.

The central retina is exposed to an exceptionally high burden of oxidative stress, which increases during aging. Epidemiologic, genetic, and molecular pathology studies support the role of oxidative stress in the pathogenesis of AMD. The retina and RPE cells provide an ideal environment for the generation of ROS. Oxidative stress is mainly caused by retinal irradiation, lipid peroxidation, photochemical damage of retinal chromophores, and the respiratory burst. These oxidative processes are thought to contribute to the clinical manifestation of pigment dispersion, accumulation of intracellular lysosomal lipofuscin, and extracellular drusen deposits.

Mitochondrial hydrogen peroxide and lysosomal iron react in the Fenton reaction, producing hydroxyl radicals. Some oxidation products polymerize to form undegradable lipofuscin, which accumulates in the lysosomes. It has been speculated that lipofuscin itself could be cytotoxic because of its ability to provide a redox-active surface by incorporating oxidatively labile iron. This could result in the formation of oxygen radicals and cause oxidative modification of proteins, lipids and nucleic acids.

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**Figure 3.** Cross-sectional illustration of the eye and retina. Light reflects via the cornea and lens to the retina. RPE cells have a central role in the pathogenesis of AMD. They absorb light, transport metabolites and nutrients between photoreceptors and the choriocapillaris, produce growth factors, control tissue ionic balance, phagocytose shed tips of photoreceptor outer segments, regulate vitamin A metabolism and visual cycle, and create the blood–retinal barrier. Abbreviations used: R, rods; C, cones; RPE, retinal pigment epithelium; POS, photoreceptor outer segments; BM, Bruch’s membrane; L, lipofuscin; D, drusen; ch, choriocapillaris. Red arrow indicates choroidal neovascularization in the wet AMD process.
an inflammatory response that further provokes long-term, low-grade inflammation in retinal cells, thus, speeding up the aging process (Fig. 6). The swelling, destabilization and dysfunction of lysosomes caused by the accumulation of fibrillar amyloid β (Aβ) in the lysosomes of microglia cells results in the activation of NLRP3 (NLR family, pyrin domain containing 3) inflammasomes. Additionally, the lysosomal rupture by silica crystals and aluminum salts leads to a similar result. Lysosomal destabilization using the lysosmotropic agent L-leucyl-l-leucine methyl ester also activates the NLRP3 inflammasomes in ARPE-19 cells. NLRP3 is an intracellular pattern-recognition receptor, which responds to a wide variety of danger signals by inducing the formation of a multiprotein complex called the inflammasome, and thereby initiating an inflammatory response. Although the exact mechanism of NLRP3 activation has not been elucidated yet, the contribution of CTSB released from the ruptured lysosomes has been strongly suggested. Mitochondrial dysfunction and subsequent oxidative stress are also well known activators of the NLRP3 inflammasome. We have recently shown that oxidative stress is capable of inducing activation of the NLRP3 inflammasomes in human ARPE-19 cells. Oxidative stress, hypoxia, the unfolded protein response or inflammation can activate autophagy, and are present in AMD pathology. Autophagy activity is strongly regulated by the signaling of mechanistic target of rapamycin (MTOR), the inhibition of which results in the partial dephosphorylation of ATG13, activation of the ULKs (unc-51-like kinases) and recruitment of RB1CC1 (RB1-inducible coiled-coil 1). The ULK1/2-ATG13-RB1CC1 complex plays a crucial role in the formation of double-membrane autophagic vacuoles, autophagosomes. Other important proteins in autophagosome formation include ATG14, BECN1, PIK3C3, PIK3R4 and UVRAG. Microtubule-associated protein 1 light chain 3 α is a ubiquitin-like protein that connects autophagy to the proteasomal clearance system via ubiquitin and SQSTM1 binding sites.

Failure of the RPE cells to use autophagy can result in accumulation of aggregation-prone proteins, cellular degeneration and finally cell death. In addition, a decline in the autophagy flux is usually accompanied by the accumulation of SQSTM1 in large perinuclear aggregates or inclusion bodies which are also positive for ubiquitin, as has been reported to happen in numerous neurodegenerative diseases, including Alzheimer, Parkinson and Huntington diseases.

**Autophagy decline and inflammasome activation.** To date, there is strong evidence showing that decreased autophagy flux is associated with RPE damage and AMD pathology. When autophagic capacity declines simultaneously with an increased lipofuscin accumulation, accelerated ROS production and elevated protein aggregation can occur, which may activate an inflammatory response that further provokes long-term, low-grade inflammation in retinal cells, thus, speeding up the aging process (Fig. 6). The swelling, destabilization and dysfunction of lysosomes caused by the accumulation of fibrillar amyloid β (Aβ) in the lysosomes of microglia cells results in the activation of NLRP3 (NLR family, pyrin domain containing 3) inflammasomes. Additionally, the lysosomal rupture by silica crystals and aluminum salts leads to a similar result. Lysosomal destabilization using the lysosmotropic agent L-leucyl-l-leucine methyl ester also activates the NLRP3 inflammasomes in ARPE-19 cells. NLRP3 is an intracellular pattern-recognition receptor, which responds to a wide variety of danger signals by inducing the formation of a multiprotein complex called the inflammasome, and thereby initiating an inflammatory response. Although the exact mechanism of NLRP3 activation has not been elucidated yet, the contribution of CTSB released from the ruptured lysosomes has been strongly suggested. Mitochondrial dysfunction and subsequent oxidative stress are also well known activators of the NLRP3 inflammasome.

We have recently shown that oxidative stress is capable of inducing activation of the NLRP3 inflammasomes in human ARPE-19 cells. Moreover, oxidized mitochondrial DNA leaking out from damaged mitochondria is a direct agonist of NLRP3. In normal circumstances, autophagy controls the activation of the NLRP3 inflammasome, for example by degrading the inflammasome components and effector molecules. Conversely,
when autophagy declines, inflammasomes become activated—most probably through the dysregulation of mitochondrial homeostasis.\textsuperscript{118,123}

It is well known that accumulated intracellular lipofuscin and extracellular drusen deposits increase the risk for progression of AMD.\textsuperscript{66,124,125} Among other effects, drusen may play a role in inflammasome activation. Drusen material isolated from donor AMD eyes can activate the NLRP3 inflammasome pathway in human mononuclear cells.\textsuperscript{126} Immunohistochemical analyses have revealed that drusen are composed of many intracellular-derived proteins which can regulate proteolytic processes.\textsuperscript{13,109,127}

Drusen formation may be associated with decreased autophagy, and increased transcytosis and exocytosis in RPE cells, which can further be involved in the development of AMD.\textsuperscript{89,109,128} Some exosome and autophagy markers have been detected in drusen.\textsuperscript{109} We have observed that SQSTM1 accumulates in the macular RPE cells rather than in peripheral retina, revealing an impaired autophagy flux in AMD.\textsuperscript{110} In addition, most of the drusen from AMD patients show strong ubiquitin positivity, while the SQSTM1 staining could be observed only intracellularly. This might imply that SQSTM1 is mostly degraded by the autophagic pathway and, unlike ubiquitin, SQSTM1 is not exocytosed to the extracellular space of the RPE cells. Recent reports have argued that ubiquitin and SQSTM1 strongly colocalize in perinuclear aggregates.\textsuperscript{129} However, we found that only ubiquitin was detectable in the extracellular drusens of AMD samples. As a consequence, we hypothesize that autophagosomes do not fuse with cell membranes and are not involved directly in exocytosis that contributes to AMD pathology.

**Complexity of autophagy as a pharmacological target in AMD therapy.** Regulation of the signaling pathways involved in autophagy is certainly a potential therapeutic target for AMD treatment. However, targeting autophagy may be more complex than that, since a) autophagy is a fundamental housekeeping process in all cells, and too little or too much autophagy can result in cellular dysfunction, b) autophagy pathways will differ depending on the stimulus signal(s), and c) AMD has both degenerative characteristics including protein deposits, and in certain cases proliferative characteristics as occurs in the wet AMD form. To date, there is no consensus as to whether autophagy inhibitors or activators would be beneficial in AMD therapy, and how they should be used in different phenotypes of AMD in prevention or in therapy. The induction of autophagy can be attained through inhibition of the mitogen-activated protein kinase/extracellular...
signal-regulated kinase, class I phosphoinositide 3-kinase-AKT and MTOR signaling pathways. Autophagy inhibitors can be broadly classified into early or late stage inhibitors depending on the autophagy pathway they act upon (Fig. 7).

Early stage inhibitors of autophagy, such as wortmannin, 3-methyladenine (3-MA) and LY294002, can act upon an upstream regulator of autophagy, the class III PtdIns3K. In retinal ganglion cells following optic nerve transection, the inhibition of autophagy by wortmannin and 3-MA, as well as the late stage inhibitor bafilomycin A₁, can decrease cell viability, suggesting a cell-protective role of autophagy in neurodegenerative diseases. Conversely, blocking autophagy with 3-MA in developing retinal neuroepithelium or after optic nerve axotomy results in abnormal retinal tissue formation and function or attenuation of axonal swelling and degeneration, respectively. Late stage inhibitors of autophagy including antimalarial drugs and broad-spectrum antibiotics such as fluoroquinolones, impose their action on the lysosomal part of the autophagosome-lysosomal pathway and prevent fusion of autophagosomes with lysosomes. Similarly to early stage inhibitors, the late ones have also been tested in cancer cell lines and cancer animal models. The antimalarial drug chloroquine (CQ) has been used in cancer therapy due to its inhibitory effect on autophagy, besides its additional, autophagy-independent toxic effect on cancer cells. CQ and other quinines have been associated with cases of retinal toxicity, particularly when provided at higher doses for longer times. In vitro, CQ can induce lipid accumulation and block phagocytosis in ARPE-19 cells in a dose- and time-dependent manner.

Neuronal transmitter blockers such as HTR1A (5-hydroxytryptamine (serotonin) receptor 1A, G protein-coupled) agonists offer a therapeutic option for retinal degenerations such as AMD, diabetic retinopathy or retinitis pigmentosa. In both, an in vitro system and an in vivo mice model of atrophic AMD, the HTR1A receptor agonist 8-hydroxy-2-(dipropylamino)tetralin (8-OH DPAT) can protect the retina from degeneration by reducing oxidative damage. This study showed that autophagy- and photo-oxidative stress-derived lipofuscin accumulation can be reduced by 8-OH DPAT in cultured ARPE-19 cells, possibly through stimulation of MTOR phosphorylation, which can lead to decreased autophagy induction (Fig. 7).

Recent findings have revealed that rapamycin-induced inhibition of MTOR complex 1 (MTORC1), and, therefore, activation of autophagy, can slow the aging and neurodegenerative processes in mice. Interestingly, RPE degeneration is associated with increased sensitivity and enhanced activity of MTORC1 in experimental AMD studies. Rapamycin prevents the development of harmful AMD-related aging signs in RPE cells. MTOR regulates the detrimental dedifferentiation and hypertrophy of RPE cells exposed to oxidative stress, whereas rapamycin treatment can prevent these effects and preserve photoreceptor functions. In addition, rapamycin inhibits choroidal neovascularisation (CNV) by interfering with the
changes without cellular proliferation, whereas CNV development is based on the choroidal endothelial cell proliferation in wet AMD. The inhibition of autophagy potentiates anti-angiogenic effects and might be used together with anti-VEGF therapy in wet AMD. However, the autophagy inducer rapamycin functions as a VEGF inhibitor and reduces CNV activity, as discussed above. Due to these opposite effects, further results and experimental models are required to determine whether autophagy activation or inhibition is a goal in AMD therapy. Recent clinical experiences provide the possibility to apply drugs through intravitreal injections. This could elegantly circumvent a number of side effects of putative autophagy-related therapies when applied systemically. Autophagy-targeted gene therapy for treatment of AMD is also an interesting future option. However, accumulating lysosomal lipofuscin may be a limiting factor in the regulation of autophagy flux. Overall, considering AMD as a degenerative age-related disease it may be tempting to state that functional autophagy may prevent RPE cell degeneration and AMD development, although documentation varies in the different models studied.

**Conclusions**

During the past several years, our understanding of the mechanisms leading to RPE degeneration and AMD development has greatly increased. Various genetic and environmental risk factors associated with lysosomal damage, including accumulation of lipofuscin and drusen, and induction of chronic inflammation all can lead to decreased autophagy flux in the RPE cells and AMD progression. Therefore, it should be appreciated that autophagy may represent an important therapeutic target in AMD. In particular, the autophagy-regulating kinases AMPK and MTOR can be potential therapeutic targets for preventing RPE cell degeneration and AMD progression. Autophagy-targeted gene therapy for treatment of AMD is also an interesting future option. However, accumulating lysosomal lipofuscin may be a limiting factor in the regulation of autophagy flux. Overall, considering AMD as a degenerative age-related disease it may be tempting to state that functional autophagy may prevent RPE cell degeneration and AMD development, although documentation varies in the different models studied.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

This work was supported by the EVO grants of Kuopio University Hospital (K.K.), the Finnish Cultural Foundation and its North Savo Fund (K.K.), the Finnish Eye Foundation (K.K.), the Finnish Funding Agency for Technology and Innovation (K.K.), Health Research Council of the Academy of Finland (K.K., A.K.), and the Päivikki and Sakari Sohlberg Foundation (A.K.), National Institutes of Health (USA) grants EY019688 and EY021626 (MEB), EY019037-S (D.S.) D.S. is a recipient of the Sybil B. Harrington Special Scholar award for Macular Degeneration from Research to Prevent Blindness, the Hungarian Scientific Research Fund (OTKA PD 101316) and the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 “VÉD-ELEM” project. The project is implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.
49. Koike M, Shiba M, Ohbasa Y, Nakashima H, Koga T, Kametaka S, et al. Involvement of two different cell death pathways in retinal atrophy of catarhepin D-deficient mice. Mol Cell Neurosci 2005; 22:146-61; PMID:15676526; http://dx.doi.org/10.1016/j.mcn.2004.07.005

50. Paraoan L, Grierson I, Maden BE. Analysis of expressed sequence tags of retinal pigment epithelium: cystatin C is an abundant transcript. Int J Biochem Cell Biol 2008; 40:763-71; PMID:18063475; http://dx.doi.org/10.1016/j.biocel.2007.11.004

51. Pang J, Liu J, Liu W, Reigada D, Nguyen J, Laties AM, Mitchell CH. Restoration of lysosomal pH in RPE cells from cultured human and AR-C57/C mice: pharmacological approaches and functional recovery. Invest Ophthalmol Vis Sci 2008; 49:772-80; PMID:18325027; http://dx.doi.org/10.1167/iovs.07-0675

52. Baltsar GC, Guha S, Lu W, Lim J, Boeze-Bataglia K, Laties AM, et al. Acidic nanoparticles are trafficked to lysosomes and restore an acidic lysosomal pH and degradative function to compromised ARPE-19 cells. PLoS One 2012; 7:e49635; PMID:23272048; http://dx.doi.org/10.1371/journal.pone.0049635

53. Kurz T, Karlsson M, Brunk UT, Nilsson SE, Frennesson C. ARPE-19 retinal pigment epithelial cells are highly resistant to oxidative stress and exercise strict control over their lysosomal redox-active iron. Autophagy 2009; 5:494-501; PMID:19223677; http://dx.doi.org/10.4161/auto.5.4.7961

54. Feher J, Kovacs I, Attico M, Cavallotti C, Papale A, Balacco Gabrieli C. Mitochondrial alterations of retinal pigment epithelium in age-related macular degeneration. Neurobiol Aging 2006; 27:983-93; PMID:15979212; http://dx.doi.org/10.1016/j.neurobiaging.2005.05.012

55. Holz FG, Bindewald-Wirtlich A, Fleckenstein M, Gao J, Vollmer-Snarr HR, et al. The age lipid A2E may contribute to lysosomes and restore an acidic lysosomal pH and degradative function to compromised ARPE-19 cells. J Biol Chem 2008; 283:24770-80; PMID:18621729; http://dx.doi.org/10.1016/j.jbc.2008.01.009

56. Hammer M, Richter S, Guehrs KH, Schweitzer D. Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis. FEBS J 2005; 272:1360-7; PMID:15704664; http://dx.doi.org/10.1111/j.1742-4658.2005.03523.x

57. Koike M, Shibata M, Ohsawa Y, Nakanishi H, Koga T. Mitochondria-derived reactive oxygen species: implications for mechanisms of aging. Frontiers in Aging Cell 2012; 1:47-54; PMID:23625902; http://dx.doi.org/10.3389/fncel.2012.00047

58. Baek A, Bowers A. The mitochondrial energy transduction system and the aging process. Am J Physiol Cell Physiol 2007; 292:C670-80; PMID:17020935; http://dx.doi.org/10.1152/ajpcell.00213.2006

59. King A, Gottlieb E, Brooks DG, Murphy MP, Duniaef JL. Mitochondria-derived reactive oxygen species mediate blue light-induced death of retinal pigment epithelial cells. Photochem Photobiol 2004; 79:470-5; PMID:15191057; http://dx.doi.org/10.1562/LE-03-17.1

60. Godley BE, Shamsi FA, Liang FQ, Jarrett SG, Davies S, Boulton M. Blue light induces mitochondrial DNA damage and free radical production in epithelial cells. J Biol Chem 2005; 280:21061-6; PMID:15797866; http://dx.doi.org/10.1074/jbc.M502194200

61. Nordgaard CL, Karunadharma PP, Feng X, Ohlen TW, Ferrington DA. Mitochondrial proteomics of the retinal pigment epithelium at progressive stages of age-related macular degeneration. Invest Ophthalmol Vis Sci 2008; 49:2848-55; PMID:18344451; http://dx.doi.org/10.1167/iovs.07-1352

62. Lin H, Xu H, Liang FQ, Liang H, Gupta P, Havey AN, et al. Mitochondrial DNA damage and repair in RPE associated with aging and age-related macular degeneration. Invest Ophthalmol Vis Sci 2011; 52:3521-9; PMID:21273542; http://dx.doi.org/10.1167/iovs.10-49563

63. Nakamura T, Lipton SA. Molecular mechanisms of nitrative stress-mediated protein misfolding in neurodegenerative diseases. Cell Mol Life Sci 2007; 64:1609-20; PMID:17451343; http://dx.doi.org/10.1007/s00018-006-6525-0

64. Grune T, Jung T, Merker K, Davies S. Decreased cell death pathways in retinal atrophy of cathepsin S-deficient mice. Invest Ophthalmol Vis Sci 2010; 51:3039-49; PMID:20457149; http://dx.doi.org/10.1167/iovs.09-3909

65. Kannan R, Sreekumar PG, Hinton DR. Novel roles for α-crystallins in retinal function and disease. Prog Retin Eye Res 2003; 22:146-61; PMID:12729554; http://dx.doi.org/10.1016/S1044-5750(03)00035-4

66. Holz FG, Bindewald-Wirtlich A, Fleckenstein M, Gao J, Vollmer-Snarr HR, et al. The age lipid A2E may contribute to lysosomes and restore an acidic lysosomal pH and degradative function to compromised ARPE-19 cells. J Biol Chem 2008; 283:24770-80; PMID:18621729; http://dx.doi.org/10.1016/j.jbc.2008.01.009

67. Koike M, Shibata M, Ohsawa Y, Nakanishi H, Koga T. Mitochondria-derived reactive oxygen species: implications for mechanisms of aging. Frontiers in Aging Cell 2012; 1:47-54; PMID:23625902; http://dx.doi.org/10.3389/fncel.2012.00047

68. Jarrett SG, Davies S, Boulton M. Blue light induces mitochondrial DNA damage and free radical production in epithelial cells. J Biol Chem 2005; 280:21061-6; PMID:15797866; http://dx.doi.org/10.1074/jbc.M502194200

69. Nordgaard CL, Karunadharma PP, Feng X, Ohlen TW, Ferrington DA. Mitochondrial proteomics of the retinal pigment epithelium at progressive stages of age-related macular degeneration. Invest Ophthalmol Vis Sci 2008; 49:2848-55; PMID:18344451; http://dx.doi.org/10.1167/iovs.07-1352

70. Lin H, Xu H, Liang FQ, Liang H, Gupta P, Havey AN, et al. Mitochondrial DNA damage and repair in RPE associated with aging and age-related macular degeneration. Invest Ophthalmol Vis Sci 2011; 52:3521-9; PMID:21273542; http://dx.doi.org/10.1167/iovs.10-49563

71. Nakamura T, Lipton SA. Molecular mechanisms of nitrative stress-mediated protein misfolding in neurodegenerative diseases. Cell Mol Life Sci 2007; 64:1609-20; PMID:17451343; http://dx.doi.org/10.1007/s00018-006-6525-0

72. Grune T, Jung T, Merker K, Davies S. Decreased cell death pathways in retinal atrophy of cathepsin S-deficient mice. Invest Ophthalmol Vis Sci 2010; 51:3039-49; PMID:20457149; http://dx.doi.org/10.1167/iovs.09-3909

73. Kannan R, Sreekumar PG, Hinton DR. Novel roles for α-crystallins in retinal function and disease. Prog Retin Eye Res 2003; 22:146-61; PMID:12729554; http://dx.doi.org/10.1016/j.preteyeres.2012.06.001
91. Fernandes AF, Zhou J, Zhang X, Bian Q, Sparrow J, Taylor A, et al. Oxidative inactivation of the proteasome in retinal pigment epithelial cells. A potential link between oxidative stress and up-regulation of interleukin-8. J Biol Chem 2008; 283:20745-50; PMID:18527488; http://dx.doi.org/10.1074/jbc.M800262000

92. Li Y, Wang YS, Shen XF, Hui YN, Han J, Zhao W, et al. Alterations of activity and intracellular distribution of the 20S proteasome in ageing retinal pigment epithelial cells. Exp Gerontol 2008; 43:1114-22; PMID:18817863; http://dx.doi.org/10.1016/j.exger.2008.08.052

93. Jung T, Cataldi B, Grune T. The proteasomal system. Mol Aspects Med 2009; 30:191-236; PMID:19737762; http://dx.doi.org/10.1016/j.mamach.2009.04.001

94. Kawaguchi Y, Kowaci JJ, McLaurin A, Vance JM, Ito A, Yao TP. The decaetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell 2005; 121:757-78; PMID:16475357; http://dx.doi.org/10.1016/j.cell.2005.09.035

95. Boyault C, Zhang Y, Carlon C, Gilquin B, Kwon SH, et al. HDAC6 controls major cell response to misfolded protein stress. Genes Dev 2007; 21:2172-81; PMID:17785525; http://dx.doi.org/10.1101/gad.156407

96. Ryhänen T, Viiri J, Hyttinen JM, Uusitalo H, Salminen A, Kaarniranta K. hnRNP F and HDAC inhibition and tubulin acetylation on perinuclear protein aggregation in human retinal pigment epithelial cells. Mol Vis 2010; 16:1399-414; PMID:20680098

97. Mirer SK, Rao HV, Qi X, Cai J, Sugre A, Dunn WA Jr., et al. Autophagy in the retina: a potential role in age-related macular degeneration. Adv Exp Med Biol 2012; 723:83-90; PMID:22188339; http://dx.doi.org/10.1007/978-1-4416-06310-1_12

98. Salminen A, Kaarniranta K, Kauppinen A. Autophagy-mediated proteolysis in human retinal pigment epithelial cells. J Biol Chem 2007; 282:24131-45; PMID:17588030; http://dx.doi.org/10.1074/jbc.M700282200

99. Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. Curr Opin Cell Biol 2010; 22:124-31; PMID:20304776; http://dx.doi.org/10.1016/j.cob.2010.09.014

100. Mizushima N, Komatsu M. Autophagy: renaissance of p62/sequestosome 1 in neurofibrillary tangles in Alzheimer’s disease. Biochim Biophys Acta 2002; 1580:57-66; PMID:11910758; http://dx.doi.org/10.1016/S0005-2728(01)00710-2

101. Kuusisto E, Salminen A, Alafuzoff I. p62 is a common component of cytoplasmic inclusions in protein aggregation diseases. Am J Pathol 2002; 160:255-63; PMID:12718649; http://dx.doi.org/10.1016/S0002-9440(10)64650-1

102. Baek H, Thal DR, Del Tredici K. Nerve cells immunoreactive for p62 in select hypothalamic and brainstem nuclei of controls and Parkinson’s disease cases. J Neural Transm 2011; 118:809-19; PMID:21502746; http://dx.doi.org/10.1007/s00702-010-0508-2

103. Haras J, Hartman M, Roche C, Zeng SG, O’Shea A, Sharp FA, et al. Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. J Biol Chem 2011; 286:5978-97; PMID:21228274; http://dx.doi.org/10.1074/jbc.M110.114493

104. Korolchuk VI, Mansilla A, Menzies FM, Rubinsztein DC. Autophagy in the retina: a potential role in age-related macular degeneration. Autophagy 2009; 5:653-64; PMID:19270489; http://dx.doi.org/10.4161/auto.5.4.8163

105. Zatloukal K, Stumptner C, Fuchsbichler A, Heid M, et al. Autophagy in the retina: a role for 5-fluorouracil in a colon cancer cell line via Chloroquine enhances the chemotherapeutic activ- ity of 5-fluorouracil in a colon cancer cell line via chloroquine enhances the chemotherapeutic activity of Chloroquine enhances the chemotherapeutic activity of Chloroquine enhances the chemotherapeutic activity of...
136. Maycotte P, Aryal S, Cummings CT, Thorburn J, Morgan MJ, Thorburn A. Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. Autophagy 2012; 8:200-12; PMID:22252008; http://dx.doi.org/10.4161/auto.8.2.18554

137. Michaelides M, Stover NB, Francis Pj, Welsberg RG. Retinal toxicity associated with hydroxychloroquine and chloroquine: risk factors, screening, and progression despite cessation of therapy. Arch Ophthalmol 2011; 129:30-9; PMID:21226266; http://dx.doi.org/10.1001/archophthalmol.2010.321

138. Hickley NM, Al-Maskari A, McKibbin M. Chloroquine and hydroxychloroquine toxicity. Arch Ophthalmol 2011; 129:1506-7; PMID:22084231; http://dx.doi.org/10.1001/archophthalmol.2011.321

139. Thampi P, Rao HV, Mitter SK, Cai J, Mao H, Li H, et al. The SSTR1a receptor agonist 8-Oh-DPAT induces protection from lipofuscin accumulation and oxidative stress in the retinal pigment epithelium. PLoS One 2012; 7:e34468; PMID:22509307; http://dx.doi.org/10.1371/journal.pone.0034468

140. Wilkinson JE, Burmeister L, Brooks SV, Chen CC, Friedline S, Harrison DE, et al. Rapamycin slows aging in mice. Aging Cell 2012; 11:675-82; PMID:22587563; http://dx.doi.org/10.1111/j.1474-9726.2012.00832.x

141. Rodríguez-María N, Germain F, María G, Firze PS, Boya P. Autophagy promotes survival of retinal ganglion cells after optic nerve axotomy in mice. Cell Death Differ 2012; 19:162-9; PMID:21701497; http://dx.doi.org/10.1038/cdd.2011.88

142. Zhao C, Yasumura D, Li X, Marthes M, Lloyd M, Nielsen G, et al. mTOR-mediated dedifferentiation of the retinal pigment epithelium initiates photoceptor degeneration in mice. J Clin Invest 2011; 121:369-83; PMID:21135502; http://dx.doi.org/10.1172/JCI44393

143. Li Y, Huang D, Xia X, Wang Z, Luo L, Wen R. CCR3 and choroidal neovascularization. PLoS One 2011; 6:e17106; PMID:21358803; http://dx.doi.org/10.1371/journal.pone.0017106

144. Deidentifier NS, Kureki AM, Fonse J, Tang W, Tolentino MJ, Bennett J. Systemic rapamycin inhibits retinal and choroidal neovascularization in mice. Mol Vis 2004; 10:964-72; PMID:15623986

145. Stahl A, Puschel L, Marin G, Gross NJ, Felgen N, Hansen LL, et al. Rapamycin reduces VEGF expression in retinal pigment epithelium (RPE) and inhibits RPE-induced spouting angiogenesis in vitro. FEBs Lett 2008; 582:3097-102; PMID:18703055; http://dx.doi.org/10.1016/j.febslet.2008.08.005

146. Qin S, De Vries GW. α2 But not α1 AMP-activated protein kinase mediates oxidative stress-induced inhibition of retinal pigment epithelium cell phagocytosis of photoreceptor outer segments. J Biol Chem 2008; 283:6744-51; PMID:18195011; http://dx.doi.org/10.1074/jbc.M708848200

147. Herrera-Martín G, Hoyer-Hansen M, García-García C, Fumarola C, Farkas T, López-Rivas A, et al. TAK1 activates AMPK-dependent cytoprotective autophagy in TRAIL-treated epithelial cells. EMBO J 2009; 28:677-85; PMID:19197243; http://dx.doi.org/10.1038/emboj.2009.8

148. Nishikawa T, Truono NH, Okaji Y, Sunami E, Shuno Y, Sasaki K, et al. The inhibition of autophagy potentiates anti-angiogenic effects of sulforaphane by inducing apoptosis. Angiogenesis 2010; 13:227-38; PMID:20694744; http://dx.doi.org/10.1007/s10456-010-9180-2

149. Chekhonin VP, Shein SA, Korchagina AA, Gurina OJ. Vegf in tumor progression and targeted therapy. Curr Cancer Drug Targets 2012; PMID:23167597

150. Juuti-Uusitalo K, Vaajasaari H, Ryhänen T, Narkilahti S, Suuronen R, Matinlinna J, et al. Efflux protein expression in human stem cell-derived retinal pigment epithelial cells. PLoS One 2012; 7:e30089; PMID:22272278; http://dx.doi.org/10.1371/journal.pone.0030089

151. Rolling F, Le Meur G, Stieger K, Smith AJ, Weber M, Deschamps JY, et al. Gene therapeutic prospects in early onset of severe retinal dystrophy: restoration of vision in RPE65 Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigment- ed epithelium. Bull Mem Acad R Med Belg 2006; 161:497-508, discussion 508-9; PMID:17503728

152. Pastore N, Blomenkamp K, Annunziata F, Piccolo P, Maria Sepe R, et al. Gene transfer of master autophagy regulator TFEB results in clearance of toxic protein and correction of hepatic disease in alpha-1-anti-trypsin deficiency. EMBO Mol Med 2013; 5:397-412; PMID:23381957; http://dx.doi.org/10.1002/emmm.201202046