Autophagic Regulation of Retinal Pigment Epithelium Homeostasis

Ya-Ju Chang1*, Ja-an Annie Ho1, Shih-lan Hsu2 and Li-Chen Wu3

1Department of Biochemical Science and Technology, National Taiwan University, Taipei, Taiwan
2Department of Medical Education and Research, Taichung Veterans General Hospital, Taichung, Taiwan
3Department of Applied Chemistry, National Chi Nan University, Puli, Taiwan

Abstract

Retinal pigment epithelium (RPE) is a single layer of hexagonal pigmented cells located in the outmost part of the neurosensory retina. RPE cells are vital for the health of retina. Dysfunction of RPE resulted from consistent exposures to oxidative stress has been reportedly to cause retinal degenerations, such as age-related macular degeneration (AMD). RPE cells exert different types autophagy to maintain retina hemostasis. This review summarizes molecular and cellular autophagic mechanisms that are revealed in RPE to respond to stress. The evidence in support of autophagy dysfunction or aging that result in diseases is also discussed.

Keywords: Retinal pigment epithelium; Autophagy; Phagocytosis; Mitochondrial dynamics

Introduction

Retinal pigment epithelium (RPE) is a single layer of hexagonal pigmented cells located in the outmost part of the neurosensory retina. Several activities have been identified of these cells including the supplement of nutrients and oxygen to retina, the sustainment of visual cycle through metabolizing vitamin A, the absorbing of scattered light to reduce photo-oxygenation via melanosomes, the performance of receptor-mediated phagocytosis of photoreceptor outer segment (POS) fragments for assuring viability and functionality of photoreceptors, the secretion of signaling modulators for communicating with adjacent cells, transportation of ions, and supporting of immune privilege [1]. These functions make RPE an important role in visual processing system. Dysfunction of RPE resulted from consistent exposures to oxidative stress has been reportedly to cause retinal degenerations, such as age-related macular degeneration (AMD).

Autophagy is a basic catabolic mechanism by which cells degrade intracellular unnecessary or dysfunctional components inside lysosomes (Figure 1). This process promotes cellular survival by saving cellular energy during extreme starvation through breakdown and recycle of cellular components. There are at least three types of autophagy addressed in mammalian cells, macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) [2]. Macroautophagy and CMA are prominent under stress conditions. Basal level of these two activities is detectable in most cell types. During macroautophagy, cytosolic components are enclosed within a double-membrane structure, autophagosome, which fuses with lysosomes to degrade the enclosed contents by hydrolases [3].

Mechanisms such as heat shock response [4] and unfolded protein response are effective regulatory machinery for proteostasis. Proteins can not only be degraded through lysosomal delivery approach such as macro/microautophagy, but also through chaperone-targeted approach to directly cross lysosomal membrane into lumen, known as chaperone-mediated autophagy [5]. The high removal selectivity of CMA makes this system an efficient degradation system, and assures CMA a regulatory role in cellular activity [6]. This review summarizes molecular and cellular autophagic mechanisms that are revealed in RPE to respond to stress (Table 1) [7-14]. The evidence in support of autophagy dysfunction or aging resulting in diseases is also discussed.

Autophagy and heterophagy in RPE

The heterophagy of POS by RPE is essential to the longevity of photoreceptors (Figure 1). The renewal of POS is regulated by circadian rhythms via the shedding of distal tips POS, which are degraded and engulfed by RPE, and are eventually digested by lysosomal enzymes. All-trans-retinol (ROL) in the degradation products are recycled and converted to 11-cis-retinal (11CR) by visual cycle to replenish chromophore for reproduction of photobleached pigments. Once converted by light, 11CR becomes all-trans-retinal (ATR), which is then flipped by ATP-binding cassette transporter 4 (Abca4) from lumen of the outer segment discs of photoreceptors to cytoplasm. The reduction of ATR is subsequently performed to generate ROL by retinol dehydrogenase 8 (Rdh8). As mentioned above, ROL is transferred to RPE to regenerate 11CR. It is indicated that delayed clearance of 11CR from photoreceptors in Abca4−/−Rdh8−/− mice causes severe dystrophy of photoreceptors and RPE cells [15]. Mice deficient in Abca4 and Rdh8 (Abca4−/−/Rdh8−/−) demonstrate increased levels of autophagy and mitophagy using microtubule-associated protein light chain 3II (LC3II) and Park2 as indicators, respectively [16]. Moreover, under strong photobleaching condition, ATR and 11CR form cytotoxic N-retinylidine-N-ethanolamine (A2E) and other bisretinoids, which are components of ocular lipofuscin and are accumulated in outer segments causing human dry and wet AMD [15]. It is not surprising that RPE cells gain lysosomal lipofuscin mostly from retinal POS [17].

Recently, the linkage of heterophagy (phagocytosis) of POS and the regeneration of retinal is suggested via a noncanonical form of autophagy, LC3-3-associated phagocytosis (LAP), in which molecules of autophagy pathway are recruited to the process of phagocytosis [18]. Single-membrane phagosomes with lipidated LC3-II demonstrate increased levels of autophagy and released microtubule-associated protein light chain 3II (LC3II) and Park2 as indicators, respectively [16]. Moreover, under photobleaching condition, ATR and 11CR form cytotoxic N-retinylidine-N-ethanolamine (A2E) and other bisretinoids, which are components of ocular lipofuscin and are accumulated in outer segments causing human dry and wet AMD [15]. It is not surprising that RPE cells gain lysosomal lipofuscin mostly from retinal POS [17].

*Corresponding author: Li-Chen Wu, Department of Applied Chemistry, National Chi Nan University, Puli, Taiwan, Tel: (049)2910960-4890; E-mail: lw25@ncnu.edu.tw

Received October 20, 2015; Accepted November 13, 2015; Published November 24, 2015

Citation: Chang YJ, Ho JA, Hsu SI, Wu LC (2015) Autophagic Regulation of Retinal Pigment Epithelium Homeostasis. Pigmentary Disorders 2: 227. doi:10.4172/2376-0427.1000227

Copyright: © 2015 Chang YJ, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
in human drusen materials [20], and involves in the regulation of lysosome-mediated degradation in RPE [21]. Nuci rats with mutant Cryba1 gene (crystallins beta A1) form deposits between RPE and lysosome-mediated degradation in RPE [21]. Nuc1 rats with mutant in human drusen materials [20], and involves in the regulation of lysosome-mediated degradation in RPE [21]. Nuci rats with mutant Cryba1 gene (crystallins beta A1) form deposits between RPE and lysosome-mediated degradation in RPE [21]. Nuc1 rats with mutant

Autophagy maintains cellular homeostasis through nutrient recycling by lysosomal degradation pathway. This catabolism process induced by a spectrum of stimuli involves the engulfment of damaged organelles and cellular debris, and the adaption of nutrient and energy demand changes. Defects in autophagy are implicated in cancers and neurodegenerative syndromes such as Parkinson’s disease (PD), Alzheimer’s disease (AD), and amyotrophic lateral sclerosis (ALS). Autophagy asserts the functions of RPE and retina [7]. In order to respond stimuli including light damage, oxidative stress, and mitochondrial malfunctions, autophagy associated molecules have been indicated to be highly expressed in ocular cells [23-25]. Macropatholysis plays an essential role in ARPE-19 cells to respond stimuli [24]. However, accumulated substances like oxidized lipids and lipofuscin, a toxic aging pigment derived from residues of lysosome incompletely digested POS [26], mainly composed of biretinooid metabolites of vitamin A (A2E) [27], interfere with the function of autophagy leading to cell dysfunction and disease progression [28] (Figure 2). Lipofuscin biretinoids last in RPE once formed and are causes of blinding retinal diseases. A2E inhibits autophagic clearance in RPE [29]. These cone-shaped lipids shift cholesterol from membrane bilayers and accumulate cholesterol in RPE lysosomes and late endosomes [30], which restrains the activity of autophagy since autopahgosome-lysosome interactions are modulated by membrane cholesterol levels [31-33]. Previous investigations indicate that lipofuscin are hard to be cleared, however, recent study reveals that these biretinooids could be removed to treat macular degeneration and Stargardt disease [34]. Evidence indicates that lipofuscin can be removed from monkey PEP (36 of 48 monkeys) by treating with molecules belonging to tetrahydropyridoethers class [35]. Additionally, β-cyclodextrin (β-CD), consisted of seven D-glucose units, eliminates lipofuscin biretinoids of RPE cells in vitro and eye cups of Abca4/Rdh8 double knock-out mice [36].

**mTOR Regulation of Autophagy in RPE**

The mammalian target of rapamycin (mTOR), an evolutionarily-conserved serine/threonine kinase, regulates the balance between cell growth and autophagy in response to external stimuli including stress, growth factors, and nutritional status [37]. The activity of autophagy is regulated by mTOR (Figure 3). The inhibition of mTOR initiates a series of actions including partial dephosphorylation of ATG13 (AuTophaGy13), recruitment of RB1CC1 (RB1-inducible coiled-coil 1) complex, and activation of ULKs (unc-51-like kinases) to activate autophagy [38]. The key mediator, ULK1/2-ATG13-RB1CC1 complex,

**Table 1:** Autophagy related studies in RPE.
reduces senescence in cultured RPE cells [49]. Additionally, the eye lens detrimental effects, which preserves photoreceptor functions [48] and animal studies [47]. Rapamycin therefore averts mTOR-mediated activates autophagy through the inhibition of mTORC1 activity in blocks ULK1 signaling in RPE cells. It is indicated that rapamycin biogenesis by modulation of lysosomal membrane potential, and mTORC1 impairs autophagy through the inhibition of auto-lysosomal RLBP1), and for phagocytosis of POS (MERTK) [46]. The activation of for conversion of all-trans-retinal to 11-cis-retinal (RPE65, LRAT, and retards the production of RPE-characteristic proteins such as enzymes of mTORC1 has also been observed in RPE degeneration [46] and thickened RPE in respond to stress. Interestingly, increased activation of mTORC1 promotes cell growth when energy/nutrients are sufficient, or regulates biosynthetic programs under stress. The suppression of mTORC1 activity is key to induce autophagy when nutrients are deprived. AKT/mTOR signaling mediates cell proliferation in RPE [43,44]. The activation of mTORC1 by growth factors or by mTORC2-activated AKT elicits the biosynthesis of proteins and lipids through S6K-regulated phosphorylation of rpS6 (ribosomal protein S6) and SREBP-regulated lipogenesis [45]. RPE cells undergo mTOR-mediated hypertrophy and dedifferentiation in responding to oxidative stress to increase cellular biomass and form thickened RPE in respond to stress. Rapamycin therefore averts mTOR-mediated detrimental effects, which preserves photoreceptor functions [48] and reduces senescence in cultured RPE cells [49]. Additionally, the eye lens protein, CRYBA1/βA3/A1-crystallin, is critical to preserve the function of lysosome. It binds to V0 subunits of V-ATPase, a component of the mTORC1 pathway, to maintain normal lysosomal activity, and of course the activity of autophagy [50].

**Mitochondrial Dynamics in Retina**

Mitochondria are highly dynamic organelles. They generate energy via oxidative phosphorylation, and continually proceed repetitive cycles of fusion and fission to rearrange inter-organelle lipids and contents. Mitochondrial dynamics, namely fission, fusion, movement along cytoskeletal tracks, and mitophagy, regulate mitochondria properties of morphology, biogenesis, redistribution and mitochondrial DNA inheritance [51]. The rate of fission and fusion that determines the length of mitochondria and the closeness of formed network is fluctuated by metabolic conditions and environmental stimuli [52]. Mitochondrial fission is mediated by a phosphorylation and S-nitrosylation activated accompanied with other proteins such as ATG14, BECN1, PIK3C3, PIK3R4, and UVRAG regulate the formation of double-membrane autophagosomes [39]. LC3, a ubiquitin-like protein, facilitates autophagic proteolysis via ubiquitin and SQSTM1 (sequestosome 1,p62) binding sites [40]. The impairment of autophagy in RPE cells can result in cellular degeneration, aggregation-prone protein accumulation, and cell death. SQSTM1 accumulation in perinuclear aggregates or inclusion bodies due to autophagy failure has been reported in neurodegenerative diseases [41]. Decreased autophagic activity in RPE cells has been indicated as a cause of dursen formation, which may lead to the development of AMD [42]. RPE cells express two types of TOR multiprotein complexes, mTORC1 and mTORC2, executing distinct cell activities. The activation of mTORC1 promotes cell growth when energy/nutrients are sufficient, or regulates biosynthetic programs under stress. The suppression of mTORC1 activity is key to induce autophagy when nutrients are deprived. AKT/mTOR signaling mediates cell proliferation in RPE [43,44]. The activation of mTORC1 by growth factors or by mTORC2-activated AKT elicits the biosynthesis of proteins and lipids through S6K-regulated phosphorylation of rpS6 (ribosomal protein S6) and SREBP-regulated lipogenesis [45]. RPE cells undergo mTOR-mediated hypertrophy and dedifferentiation in responding to oxidative stress to increase cellular biomass and form thickened RPE in respond to stress.

**Figure 2:** Dysregulated heterophagy in aged RPE cells is associated with the accumulation of lipofuscin. Incomplete digestion of POS accelerates the accumulation of vitamin A metabolites (lipofuscin) within aged RPE cells lysosomes and the deposits of lipid-protein complexes (drusen) between Bruch’s membrane and RPE cells. These aggregates and deposits collaborate with microenvironmental and genetic factors to disturb normal functions of the RPE cells, leading to photoreceptor dysfunction and macular degeneration. Impaired lysosomal POS clearance increases lipofuscin trapped in lysosome that increases detrimental oxidative stress. Inefficient autophagic flux weakens lysosomal function, which increases exocytosis of damaged proteins and activates drusen formation-associated inflammassomes.

**Figure 3:** Regulation of mTOR pathway in RPE cells. mTORC1 is involved in protein synthesis, lipid synthesis and protein degradation in RPE cells. mTORC1 classically controls protein synthesis via the phosphorylation of several translation regulators, such as 4EBP1 and S6K. mTORC1 inhibits the activity of 4EBP1 through phosphorylation, which blocks the inhibitory effects of 4EBP1 on eIF4A leading to the initiation of translation. S6K1 phosphorylates ribosomal protein S6 (pS6) to up-regulate translation initiation and elongation as well. Moreover, mTORC1 positively regulates SREBPs and PPARγ, lipid metabolism-associated transcription factors, via the phosphorylation of S6K. SREBPs, a downstream factor of phosphorylated S6K, trigger the expression of numerous genes, such as fatty acid and cholesterol synthesis. However, S6K also activates Lpin-1 which exhibits suppression effects on SREBPs transcription in lipid synthesis. Activated mTORC1 complexes block the activities of ULK1 and PI3K3C3 complexes resulting in the suppression of the activation of phosphatidylinositol triphosphate (Pip3) synthesis. This reduces the formation of autophagosomes through the decrease of LC3-PE associated autophagophores induction in RPE cells. mTORC2 regulates autophagy via Akt pathway to mediate the function of mTORC1 in RPE cells. mTORC2 signaling pathways regulate cell growth and blockades apoptosis via activates several kinases such as serine- and glucocorticoid-induced protein kinase 1 (SGK1) and protein kinase C (PKC).
cytosolic GTPase protein, dynamin-related protein (Drp1) [53], which facilitate fission through the interaction with tail-anchored outer mitochondrial membrane protein, MFF [54]. In yeast, the recruitment of Drp1 is through mitochondria outer membrane protein, Fis1 [55]. Mitochondrial fusion require GTPase proteins, optic atrophy type 1 (OPA1) for outer, and mitofusins (Mfn1/2) for inner mitochondrial membrane fusion, respectively [52]. The manipulation of the activities of Drp1 and OPA1/Mfn would lead to elongated and fragmented mitochondria, respectively. These dynamic changes ultimately influence the fate of cells [53,56]. Intraocular pressure (IOP) promotes mitochondria fission. Elevated IOP levels reduce OPA1 mRNA levels in optic nerve head, which results in mitochondrial morphology changes (shortened length, boosted cristae numbers) in an animal model of glaucoma [57,58]. The survival of ganglion cells under glaucomatous stress (increased IOP) could be attributed to the up-regulated OPA1 expression [59].

Mitophagy, a selective degradation of damaged mitochondria by autophagy, functions to maintain quality control of organelles [60]. Dysfunction of mitochondria has been linked to the progression of AMD and other neurodegenerative diseases such as PD, AD, and Huntington’s disease (HD) [61-67]. Defective mitochondria are labeled and engulfed by isolation membrane followed by fusion with lysosomes [68].

Damaged and fragmented mitochondria in RPE resulted from intense light-induced high level of oxidative stress make cells vulnerable to cell death stimuli due to the depletion in ATP and the release of cytochrome c, which activate caspases and initiate apoptosis [51]. Prominent mitochondrial changes induced by oxidative stress in aged RPE cells (primary cells from donors at age >60-year-old) are observed in comparison with those of mid- (48-60-year-old) and young- (9-20-year-old) aged ones. These changes in number, size, shape, matrix density, cristae architecture, and membrane integrity correlate well with reduced ATP level, mitochondrial membrane potential (Δψm), cytosolic Ca++), and increased mitochondrial (Ca++) [69]. It is intriguing that mitochondrial heat shock protein 70 (HSP70) is critical in the aging of mitochondria. The expression of this protein in RPE cells is suppressed under oxidative conditions. Besides, progerialike phenotypes is shown in HSP70 knockdown C. elegans [70].

### Chaperone-mediated Autophagy in Retina

Chaperone-mediated autophagy (CMA) is a process for protein degradation to constituent amino acids in most cells (Figure 4). Cytosolic materials can be engulfed through macroautophagy. Autophagosomes fuse with multivesicular bodies (MVB) or directly with lysosomes for degradation. Cytosolic proteins with specific pentapeptide motif (e.g. KFERQ) are recognized by a chaperone, heat shock cognate protein of 70 KDa (HSC70), to be delivered to lysosomal receptor, lysosome-associated membrane protein type 2A (LAMP-2A) for direct translocation across membrane for degradation [71]. Multimerized LAMP-2A forms a translocation complex to facilitate the penetration of bound substrate proteins. Unfolded substrate proteins are subsequently translocated assisted by a luminal chaperone for complete degradation. Unique characteristics of CMA are the selectivity on specific protein and the unnecessary of formation of vesicles for lysosomal degradation. The HSC70 recognizes a pentapeptide motif in amino acid (AA) sequence of substrate proteins. The pentapeptide consists 1) an invariant AA, a glutamine (Q) residue; 2) a positively charged AA (lysine, K or arginine, R residues) at either ends of the sequence (beginning or terminal ends), 3) a hydrophobic AA (phenylalanine, F, valine, V, leucine, L, or isoleucine, I residues), 4) a negatively charged AA (glutamic acid, E, or aspartic acid, D, residues), and 5) charged AA (either positively or negatively charged residues) [72]. The feature of CMA motif is to generate charged AA sequence. Post-translational modifications (phosphorylation and acetylation) change the charges of AA, which can modulate the subsequent chaperone binding [73]. The interplay of CMA and macroautophagy has been addressed that these two autophagic regulations are in a bidirectional relationship. Blockage of CMA promotes macroautophagy [74]; upregulation of CMA compromises macroautophagy [75]. However, this cross talk seems to be cell-type-dependent. Recently, evidence indicates that the autophagic cross talk in retinal cells is not bidirectional [76]. Reduced macroautophagy activity is found in aged retina, which coincided with elevated levels of CMA. Inhibition of macroautophagy in retinal cells provokes significant CMA in vitro and in vivo as well. However, downregulation of CMA failed to elicit increased macroautophagy, suggesting a uni-direction between these two autophagic pathways and a specific feature of visual lost in aging. This finding might reflect the observations of oxidative stress-induced RPE cell death that oxidative stress-induced suppression of HSP70 impairs the activity of CMA, which fails to trigger macroautophagy, resulting in subsequent induction of cell death.

Figure 4: Degradation of damaged organelles and misfolded proteins via macroautophagy and chaperone-mediated autophagy. Damaged mitochondria can be degraded by macroautophagy through the formation of autophagosomes, which can directly fuse with lysosome or indirectly through MVB. Peptides with KFERQ motif can be degraded by chaperone-mediated autophagy. Cytosolic HSC70 interacts with target peptides, and translocates peptides into lysosome through LAMP-2A. HSC70- KFERQ peptide complex can enter MVB through microautophagy.

---

**Citation:** Chang YJ, Ho JA, Hsu SI, Wu LC (2015) Autophagic Regulation of Retinal Pigment Epithelium Homeostasis. Pigmentary Disorders 2: 227. doi:10.4172/2376-0427.1000227
Regulation of Autophagy in AMD Treatments

Age-related macular degeneration (AMD or ARMD) is the leading cause of severe vision loss in older adults. AMD occurs when the small central portion of the retina field (macula) is deteriorated. Risk factors including aging, genetic background, smoking, unhealthy diet, obesity, high blood pressure, hypercholesterolemia and arteriosclerosis, induce the syndromes of AMD [38]. There are two main types, dry and wet forms, of macular degeneration. The mostly common seen is dry AMD (nonexudative), which affects 90% of the people who have this vision disorder. Cellular debris (drusen) deposits between the retina and the choroid (in Bruch’s membrane) causing atrophy and scarring to retina result in this type of AMD. With the accumulation of drusen, vision distortion appears. In the late stage, a thinning of the light-sensitive layer of retinal pigment epithelial cell (RPE) proceeds leading to retinal atrophy or cell death. On the other hand, the wet form of macular degeneration (exudative) is characterized by the growth of abnormal blood vessels from choroid, also known as choroidal neovascularization. Vision distortion, blind spots, and loss of vision on the central vision are caused by hemorrhage caused by blood vessel leakage of exudate and fluid into retinal.

Currently, there is no available medical or surgical treatment for the cure of AMD. Nevertheless, evidence shows that some treatments against oxidative stress and in avoidance of lipofuscin accumulation potentiate the prevention of severe vision loss or delay the progression of AMD [77]. Autophagic dysregulation has been linked to neurodegenerative diseases including AMD [78]. The regulation of autophagy is optimum for normal degenerative processes and prevention of AMD development. However, there is no consensus of opinions that whether the inhibition or acceleration of autophagy would be efficient in AMD treatments [79].

The stage of developing autophagic pathways autophagy inhibitors such as wortmannin, 3-methyladenine (3-MA) suppress autophagy through the inhibition of class III PtdIns3K (PI3K), which is an mTOR activator. Blocking autophagy in early stage by 3-MA in developing retinal neuroepithelium results in abnormal retinal tissue formation and function [80]. The use of these inhibitors reveals the cellular protective role of autophagy in neurodegenerative diseases [81,82].

Likewise, the effects of late stage autophagy inhibitors demonstrate similar protective role of autophagic in retina as those of early stage inhibitors. Late stage autophagy inhibitors, such as fluorouracil, bafilomycin A1, impair the fusion of autophagolysosomes with lysosomes in autophago-lysosomal pathway. Chloroquine (CQ) is reportedly to inhibit autophagy in lung, colon and breast cancer [83-85]. This antimalarial drug induces lipid accumulation and hinders phagocytosis in ARPE-19 cells, implicating lysosomal dysfunction in macular degeneration [22]. However, excessive autophagy has been indicated to be involved in retinopathy or other visual disorder [86]. TAM, a nonsteroidal estrogen receptor antagonist against breast cancer [87], can induce macula-involved retinopathy [88,89], possibly through the triggering of Zn(II) associated autophagy. Moreover, dry AMD undergoes degeneration without cellular proliferation, whereas wet AMD involves choroidal endothelial cell proliferation. Rapamycin is a VEGF (vascular endothelial growth factor) inhibitor. It restricts choroidal neovascularization (CNV) through interfering with the activity of VEGF-A [90]. Rapamycin is then beneficial for wet AMD treatment [91], although several off-target effects have been addressed [79].

In contrast to promote autophagy, blockage of autophagy may be beneficial to AMD. Reduction of lipofuscin accumulation is achieved by an agonist of SHT1a receptor, 8-hydroxy-2-(dipropylamino) tetralin (8-OH DPAT), which protects retina from degeneration by reducing autophagy- and photo-oxidative stress-derived lipofuscin accumulation in cultured ARPE-19 cells, possibly through the stimulation of mTOR phosphorylation, which decreases the induction of autophagy [92]. It seems that appropriate manipulation of autophagy is critical in maintaining the normal function of retina.

Conclusions

Autophagy is critical to the normal function of retina. Dysfunction of lysosome resulted from microenvironmental and genetic factors such as lipofuscin/drusen accumulation, chronic inflammation, and mTOR activation, impairs autophagy leading to AMD progression. Although there is no effective treatments for AMD, properly manipulates autophagic flux would be pharmacologic strategy to treat degenerative diseases. Autophagy-regulating kinases such as mTOR and AMPK are potential candidates for direct or adjunctive treatments of RPE dysfunction or AMD progression.

Acknowledgments

This work is supported by the grants of Ministry of Science and Technology (R.O.C) 103-2113-M-260-002-MY2, and Taichung Veterans General Hospital- National Chi Nan University, TCVGH-NGNU1047905 and TCVGH-NGNU1037901.

References

1. Strauss O (2005) The retinal pigment epithelium in visual function. Physiol Rev 85: 845-881.
2. Boya P, Reggiori F, Codogno P (2013) Emerging regulation and functions of autophagy. Nat Cell Biol 15: 713-720.
3. Mizushima N, Levine B, Cuervo AM, Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. Nature 451: 1069-1075.
4. Akerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 11: 545-555.
5. Cuervo AM, Wong E (2014) Chaperone-mediated autophagy: roles in disease and aging. Cell Res 24: 92-104.
6. Kaushik S, Cuervo AM (2012) Chaperone-mediated autophagy: a unique way to enter the lysosome world. Trends Cell Biol 22: 407-417.
7. Kim JY, Zhao H, Martinez J, Doggett TA, Kolesnikov AV, et al. (2013) Noncanonical autophagy promotes the visual cycle. Cell 154: 365-376.
8. Finnemann SC, Bonilha VL, Marmorstein RD, Rodriguez-Boulan E (1997) Phagocytosis of rod outer segments by retinal pigment epithelial cells requires alpha(v)beta5 integrin for binding but not for internalization. Proc Natl Acad Sci 94: 12932-12937.
9. Guo F, Ding Y, Caberoxoy N, Alvarado G, Wang F, et al. (2015) ABCF1 extrinsically regulates retinal pigment epithelial cell phagocytosis. Mol Biol Cell 26: 2311-2320.
10. Yao J, Jia L, Khan N, Lin C, Mitler SK, et al. (2015) Deletion of autophagy inducer RB1CC1 results in degeneration of the retinal pigment epithelium. Autophagy 11: 939-953.
11. Subriti A, Toropainen E, Ramsay E, Akerfelt T, Nojima S, et al. (2013) Oxidative stress protection by exogenous delivery of rhHsp70 chaperone to the retinal pigment epithelium (RPE), a possible therapeutic strategy against RPE degeneration. Pharm Res 32: 211-221.
12. Zhang J, Tripathi DN, Jing J, Alexander A, Kim J, et al. (2015) ATF4 functions at the peroxosome to induce phoxophy in response to ROS. Nat Cell Biol 17: 1259-1269.
13. Toyofuku T, Nojima S, Ishikawa T, Takamatsu H, Tsujimura T, et al. (2012) Endosomal sorting by Semaphorin 4A in retinal pigment epithelium supports photoreceptor survival. Genes Dev 26: 816-829.
14. Lee SY, Oh JS, Rho JH, Jeong NY, Kwon YH, et al. (2014) Retinal pigment epithelial cells undergoing mitotic catastrophe are vulnerable to autophagy inhibition. Cell Death Dis 5: e1303.
15. Maeda A, Maeda T, Golczak M, Palczewski K (2008) Retinopathy in mice induced by disrupted all-trans-retinal clearance. J Biol Chem 283: 26684-26693.

16. Chen Y, Sawada O, Kohno H, Le YZ, Subauste C, et al. (2013) Autophagy protects the retina from light-induced degeneration. J Biol Chem 288: 7506-7518.

17. Feeney-Burns L, Eldred GE (1983) The fate of the phagosome: conversion to 'age pigment' and impact in human retinal pigment epithelium. Trans Ophthalmol Soc UK 103: 416-421.

18. Ferguson TA, Green DR2 (2014) Autophagy and phagocytosis converge for better vision. Autophagy 10: 165-167.

19. Zigler JS Jr, Zhang C, Grebe R, Sehrawat G, Hackett L Jr, et al. (2011) Mutation in the PAF1A3/A-crystallin gene impairs phagosome degradation in the retinal pigmented epithelium of the rat. J Cell Sci 124: 523-531.

20. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, et al. (2002) Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci U S A 99: 14682-14687.

21. Sinha D, Valapala M, Shang P, Hose S, Grebe R, et al. (2015) Lysosomes: Regulators of autophagy in the retinal pigmented epithelium. Exp Eye Res. 1-10.

22. Chen PM, Gombart ZJ, Chen JW (2011) Chloroquine treatment of ARPE-19 cells leads to lysosome dilation and intracellular lipid accumulation; possible implications of lysosomal dysfunction in macular degeneration. Cell Biosci 9: 1-10.

23. Kunchithapautham K, Rohrer B (2007) Apoptosis and autophagy in photoreceptors exposed to oxidative stress. Autophagy 3: 433-441.

24. Remé CE, Wolfrum U, Imsand C, Hafezi F, Williams TP (1999) Photoreceptor autophagy: effects of light history on number and opsin content of degradative vacuoles. Invest Ophthalmol Vis Sci 40: 2398-2404.

25. Wang AL, Lukas TJ, Yuan M, Du N, Tso MO, et al. (2009) Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. PLoS One 4: e4160.

26. Kennedy CJ, Lakkaraju A, Finnemann SC, Rodriguez-Boulan E (2007) The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. Proc Natl Acad Sci U S A 104: 11026-11031.

27. Toops KA, Tan LX, Jiang Z, Radu RA, et al. (2014) Beta cyclodextrins bind, stabilize, and remove lipofuscin bisretinoids from retinal pigment epithelium. Proc Natl Acad Sci U S A 111: 14102-14107.

28. Sparrow JR, Boulton M (2005) RPE lipofuscin and its role in retinal pathobiology. Exp Eye Res 80: 595-606.

29. Lakkaraju A, Finnemann SC, Rodriguez-Boulan E (2007) The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. Proc Natl Acad Sci U S A 104: 11026-11031.

30. Natsume M, Palmisano AM, Shen Q, Kawajiri S (2013) Mechanisms of mitochondrial fission and fusion. Cold Spring Harb Perspect Biol 5.

31. van der Bieke AM, Sheng Q, Kajiwara S (2013) Mechanisms of mitochondrial fission and fusion. Cold Spring Harb Perspect Biol 5.

32. Cho DH, Nakamura T, Lipton SA (2010) Mitochondrial dynamics in cell death and neurodegeneration. Cell Mol Life Sci 67: 3435-3447.

33. Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, et al. (2011) MF is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. J Cell Biol 191: 1141-1158.

34. Okamoto K, Shaw JM (2005) Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. Annu Rev Genet 39: 503-536.

35. Westermann B (2010) Mitochondrial fusion and fission in cell life and death. Nat Rev Mol Cell Biol 11: 872-884.

36. Ju WK, Liu Q, Kim KY, Lindsay JD, et al. (2007) Elevated hydrostatic pressure triggers mitochondrial fission and decreases cellular ATP in differentiated RGC-5 cells. Invest Ophthalmol Vis Sci 48: 2145-2151.

37. Ju WK, Kim KY, Lindsay JD, Angert M, Duong-Polk KK, et al. (2008) Intracellular pressure elevation induces mitochondrial fission and triggers OPA1 release in glaucomatous optic nerve. Invest Ophthalmol Vis Sci 49: 4903-4911.

38. Ju WK, Kim KY, Duong-Polk KK, Lindsay JD, Ellisman MH, et al. (2010) Increased optic atrophy type 1 expression protects retinal ganglion cells in a mouse model of glaucoma. Mol Vis 16: 1331-1342.

39. Chen H, Chan DC (2009) Mitochondrial dynamics—fusion, fission, movement, and mitophagy—in neurodegenerative diseases. Hum Mol Genet 18: R169-176.

40. D’Cruz PM, Yasumura D, Weir J, Matthes MT, Abderrahim H, et al. (2000) Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. Hum Mol Genet 9: 845-851.

41. Gail A, Li Y, Thompson DA, Weir J, Orth U, et al. (2000) Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. Nat Genet 26: 270-271.
63. Doorey CK, Wu G, Ebenstein D, Garsd A, Weller J (1989) Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. Invest Ophthalmol Vis Sci 30: 1691-1699.

64. Green WR, Enger C (1993) Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman Lecture. Ophthalmology 100: 1519-1535.

65. Beatty S, Koh H, Philip M, Henson D, Boulton M (2000) The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 45: 115-134.

66. Duniaef JL, Denteche T, Ying GS, Mlaim AH (2002) The role of apoptosis in age-related macular degeneration. Arch Ophthalmol 120: 1435-1442.

67. Winkler BS, Boulton ME, Gottsch JD, Sternberg P (1999) Oxidative damage and age-related macular degeneration. Mol Vis 5: 32.

68. Youle RJ, Narender DP (2011) Mechanisms of mitophagy. Nat Rev Mol Cell Biol 12: 9-14.

69. He Y, Ge J, Burke JM, Myers RL, Dong ZZ, et al. (2010) Mitochondria impairment correlates with increased sensitivity of aging RPE cells to oxidative stress. J Ocul Biol Dis Infor 3: 92-108.

70. Kimura KI, Tanaka N, Nakamura N, Takano S, Ohkuma S (2007) Knockdown of mitochondrial heat shock protein 70 promotes progeria-like phenotypes in caenorhabditis elegans. J Biol Chem 282: 5910-5916.

71. Cuervo AM, Knecht E, Terlecky SR, Dice JF (1995) Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. Am J Physiol 269: C1200-1208.

72. Dice JF (1990) Peptide sequences that target cytosolic proteins for lysosomal proteolysis. Trends Biochem Sci 15: 305-309.

73. Lv L, Li D, Zhao D, Lin R, Chu Y, et al. (2011) Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. Mol Cell 42: 719-730.

74. Massey AC, Follenzi A, Kiffin R, Zhang C, Cuervo AM (2008) Early cellular changes after blockage of chaperone-mediated autophagy. Autophagy 4: 442-456.

75. Kaushik S, Massey AC, Mizushima N, Cuervo AM (2008) Constitutive activation of chaperone-mediated autophagy in cells with impaired macroautophagy. Mol Biol Cell 19: 2179-2192.

76. Rodriguez-Muela N, Koga H, Garcia-Ledo L, de la Villa P, de la Rosa EJ, et al. (2013) Balance between autophagic pathways preserves retinal homeostasis. Aging Cell 12: 478-488.

77. Payne AJ, Kaja S, Naumchuk Y, Kunikuknju N, Kouten P5 (2014) Antioxidant drug therapy approaches for neuroprotection in chronic diseases of the retina. Int J Mol Sci 15: 1865-1886.

78. Mitter SK, Song C, Qi X, Mao H, Rao H, et al. (2014) Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. Autophagy 10: 1989-2005.

79. Kaamiranta K, Sinha D, Blasiak J, Kauppinen A, Veréc Z, et al. (2013) Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration. Autophagy 9: 973-984.

80. Mellén MA, de la Rosa EJ, Boya P (2008) The autophagic machinery is necessary for removal of cell corpses from the developing retina and neuroepithelium. Cell Death Differ 15: 1279-1290.

81. Kim SH, Munemasa Y, Kwong JM, Ahn JH, Mareninov S, et al. (2008) Activation of autophagy in retinal ganglion cells. J Neurosci Res 86: 2943-2951.

82. Wu YT, Tan HL, Shui G, Baurv C, Huang Q, et al. (2010) Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition of class I and III phosphoinositide 3-kinase. J Biol Chem 285: 10850-10861.

83. Wang Y, Peng RO, Li DD, Ding Y, Wu XQ, et al. (2011) Chloroquine enhances the cytotoxicity of topotecan by inhibiting autophagy in lung cancer cells. Chin J Cancer 30: 690-700.

84. Choi JH, Yoon JS, Won YW, Park BB, Lee YY (2012) Chloroquine enhances the chemotherapeutic activity of 5-fluorouracil in a colon cancer cell line via cell cycle alteration. APIMS 120: 597-604.

85. Maycotte P, Aylal S, Cummings GT, Thorburn J, Morgan MJ, et al. (2012) Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. Autophagy 8: 200-212.

86. Cho KS, Yoon YH, Choi JA, Lee SJ, Koh JY (2012) Induction of autophagy and cell death by tamoxifen in cultured retinal pigment epithelial and photoreceptor cells. Invest Ophthalmol Vis Sci 53: 5344-5353.

87. Jensen EV, Jordan VC (2003) The estrogen receptor: a model for molecular medicine. Clin Cancer Res 9: 1980-1989.

88. Paganini-Hill A, Clark LJ (2000) Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen. Breast Cancer Res Treat 64: 165-176.

89. Sadowski B, Kriegbaum C, Apfelstedt-Sylla E (2001) Tamoxifen side effects, age-related macular degeneration (AMD) or cancer associated retinopathy (CAR)? Eur J Ophthalmol 11: 309-312.

90. Dejneka NS, Kuroki AM, Fosnot J, Tang W, Tolentino MJ, et al. (2004) Systemic rapamycin inhibits retinal and choroidal neovascularization in mice. Mol Vis 10: 964-972.

91. Nishikawa T, Tsuno NH, Okaji Y, Sunami E, Shuno Y, et al. (2010) The inhibition of autophagy potentiates anti-angiogenic effects of sulphoraphane by inducing apoptosis. Angiogenesis 13: 227-238.

92. Thampil P, Rao HV, Mitter SK, Cai J, Mao H, et al. (2012) The 5HT1a receptor agonist 8-Oh DPAT induces protection from lipofuscin accumulation and oxidative stress in the retinal pigment epithelium. PLoS One 7: e34468.