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Autophagy in Ocular Pathophysiology

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Abstract

Autophagy is an evolutionarily conserved intracellular recycling pathway that is indispensable for cellular quality control. Dysfunctional autophagy has been associated with several neurodegenerative, myodegenerative, infectious, and cancerous disorders. Autophagic processes are not only important for cellular maintenance in the retina but also intimately involved with phagocytosis and the very core of retinal visual process. Additionally, excessively upregulated autophagy may culminate into a cell death modality, which may be detrimental to the non-dividing cells of various eye segments. Major advances have been made in understanding the role and fate of autophagy in different ocular tissue layers. In this chapter, we summarize the current understanding of autophagy in the eye in the context of development, aging, and disease. We also speculate on the putative therapeutic strategies where autophagy may be incorporated to treat oculopathies.

Keywords: retinal degeneration, RPE, photoreceptor, lens, cornea, retinal ganglion cells, phagocytosis, lipofuscin, autophagic flux, lysosomes, non-canonical autophagy, diurnal rhythm, circadian, cell death, aging, neovascularization, glaucoma, diabetic retinopathy, macular degeneration

1. Introduction

As a housekeeping cellular degradative and recycling process, autophagy is indispensable for the maintenance of ocular physiology. Since the early 2000s, research in understanding the mechanism and role of autophagy in development and disease has received a tremendous boost [1]. A rapidly growing wealth of data, focused on the diverse role of autophagy in ocular development, physiology, or disease, has enabled researchers to begin understanding this complex process with the hope of manipulating it as a therapeutic tool in treating a myriad of disorders that often lead to loss of visual acuity or complete blindness [2, 3].

The eye has a complex anatomy (**Figure 1A**), with a plethora of specialized cells working together to create the visual perception [4]. Almost all cells in the developed eye have some common characteristics; they have high metabolic rates; are highly differentiated and are either post-mitotic or slowly dividing [5–10]. In addition, owing to the high blood perfusion rates, the eye is an oxygen-rich organ that, along with stressors like UV radiation and visible light, provides a highly oxidative microenvironment leading to cellular damage [7, 11, 12]. In order to combat this onslaught of oxidative damage, cells require not only effective antioxidant defense mechanisms but also cellular repair both at the organellar and macromolecular levels. Autophagy (referring mainly to macroautophagy), along with proteasomal degradation and DNA repair mechanisms, provides this critical housekeeping service to almost every cell type in the eye from the cornea in the anterior part of the eye to the retina/choroid in the posterior [3].

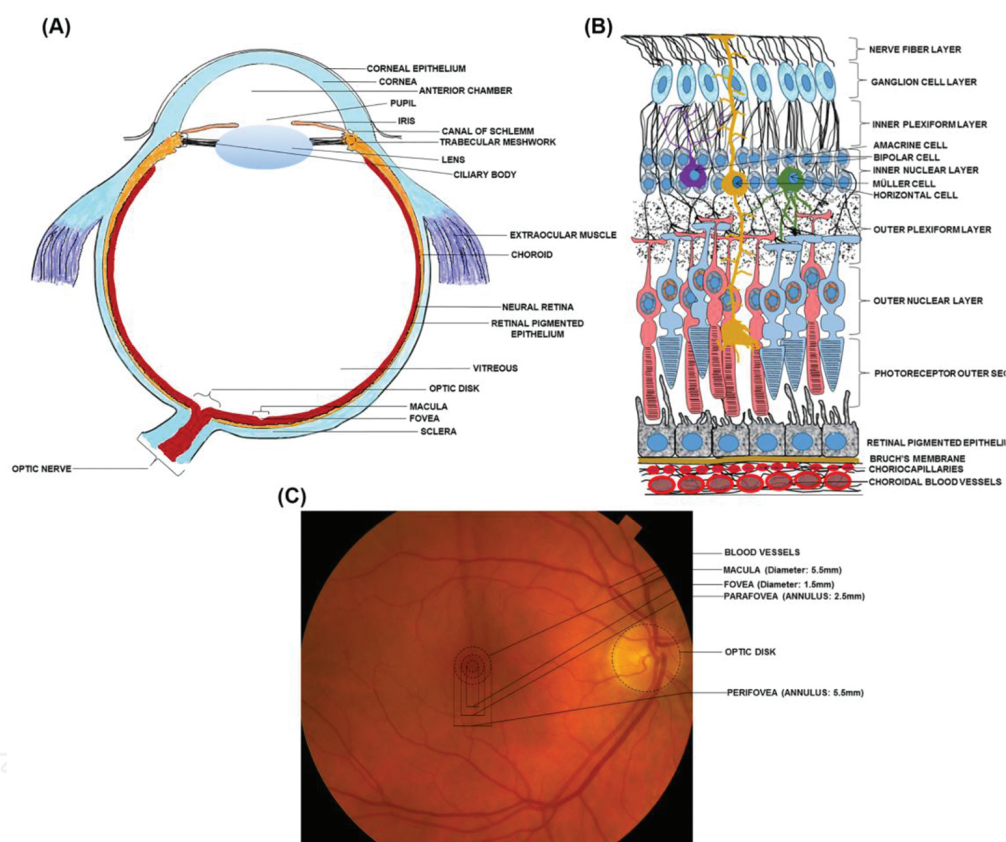


Figure 1. (A) Basic anatomy of the eye: principal ocular tissue components are shown. (B) Diagrammatic cross section of the retina. (C) Fundus image of a healthy adult eye (kindly provided by Dr. Yang Sun, MD, PhD, Department of Ophthalmology, IU School of Medicine, Indianapolis) with no ocular disease history is shown. The ganglion cell axons exit the eye at the optic disk, which is a 'blind spot' due to the complete absence of photoreceptors. The macula is tightly packed with photoreceptors and is critical for central vision. The fovea is a small pit-like area within the macula highly enriched with cone photoreceptors.

Perhaps, the most convincing evidence of the importance of autophagic activity in the eye is the preferential expression of autophagic proteins in the ocular cells and diurnal variation in expression of autophagic protein expression in the retina [13, 14]. As early as 1977, Reme et al. showed increased autophagosome formation in the inner segments of the photoreceptor cells

3 hours after maximum photoreceptor disk shedding in the rat retina [15, 16]. Diurnal variation in autophagosome formation rates was shown to be strongly dependent on light and amplitudes were severely dampened in animals kept under constant darkness [17, 18]. Recent reports have shown autophagic activity in the retinal pigmented epithelium (RPE) to be strongly correlated to the phagocytosis of photoreceptor outer segments (POS) underlining the importance of autophagy not just in being a housekeeping process but as an essential component of RPE function [19, 20].

Because of its dual role in cell survival and death, autophagy has often been referred to as a 'double-edged sword' [21, 22]. As a degradative and recycling pathway, autophagy is essential for sequestration and digestion of toxic waste that could otherwise lead to loss of cell function and eventually lead to cell death. Autophagy (specifically macroautophagy) remains the only known process by which damaged cellular organelles as large as the mitochondria can be digested and recycled [23]. Metabolites generated from autophagic digestion and recycling serve as essential components for new macromolecules and organelles. Because of the ability of this process to be upregulated when the cell is subjected to stress such as nutrient starvation, oxidative stress, hypoxia, and growth factor depletion, autophagy can be thought of as an adaptive process that can meet the energy demand under unfavorable conditions [24].

Due to the generally non-dividing nature of many of the constituent cells of the eye, most reports on autophagy in the eye have concluded it to be a necessary cytoprotective mechanism that prevents the accumulation of cellular damage and inflammation over the lifetime of an individual [25, 26]. However, reduced autophagic efficiency is implicated in a number of ocular pathologies such as age-related macular degeneration (AMD), glaucoma, diabetic retinopathy (DR), photoreceptor degeneration, and ocular infections. Because of its 'destructive self-eating' nature, when autophagic activity exceeds a certain threshold or duration, it may actually promote cellular demise. Moreover, autophagy can 'cross talk' with other cell death modalities like apoptosis to influence overall cell fate [26]. It is thus critical to understand the mechanism and functional role of autophagy in specific cells of the eye before autophagic modulation be incorporated in ocular therapeutic strategies.

In this chapter, we summarize the overall understanding of the role of autophagy in development and normal aging of the eye. We then describe the aspects of autophagy with respect to ocular diseases.

2. Autophagy in the healthy and aging eye

There is increasing evidence that autophagy plays a critical role in ocular development and homeostasis. Developmentally, the vertebrate eye derives from coordinated interactions between neuroepithelium, surface ectoderm, and extraocular mesenchyme (originating from neural crest and mesoderm) [27]. The major development of the eye occurs between the 3rd and 10th weeks of fetal development with the initial formation of the optic vesicles followed by the gradual formation of the lens and the optic nerve [27–30]. Development of the lens requires maturation of lens fibers by degrading the mitochondria, nuclei, Golgi apparatus, and

endoplasmic reticulum to create the transparent organelle-free zone to allow passage of light into the ocular chamber (reviewed in [31]). During development, the hyaloid artery supplies the lens with much needed nutrition and eventually its distal end degrades in the inner vitreous of the eye bulb, while the proximal end becomes the central retinal artery [32]. While the pigmented layer and the retina form from the outer and inner layers of the posterior (4/5th) optic cup, respectively, iris and the ciliary body are formed from the anterior (1/5th) region. The sclera and choroid are formed from the mesenchyme on the outer side of the optic cup. The primary and secondary lens fibers form the lens. The vitreous humor is a gel-like substance formed from the mesenchymal cells of the neural crest (reviewed in [33]). In the normal human eye, the photoreceptors continue to mature after birth. Foveal cone photoreceptor cell size shrinks (from 7.5 to 2 μm diameter), while cell density increases (18–42/100 μm) until 3 years of age [34, 35].

Programmed cell death plays a crucial role in neuroretinal development [36]. Autophagic proteins AMBRA1 and Beclin1 are strongly expressed in chicken embryonic (E5) neural retina [37]. Autophagy supplies ATP to energize the externalization of phosphatidyl serine on the dying cell surface, an essential step in the clearance of cell corpses from the developing retinal neuroepithelium. Pharmacological inhibition (3-Methyladenine) of autophagy increases TUNEL-positive apoptotic cells [38]. It will be interesting to investigate the role of autophagy in the development of mammalian neuroretinal cells. Autophagy has also been shown to reduce cell size in other cell types [39–41]. Antagonistic mTOR and autophagic pathways control activity of YAP/TAZ transcription factors, thereby influencing cell size and proliferation [42]. Differential cellular signaling to modulate autophagy and mTOR in both dividing and differentiated photoreceptors during pre- and postnatal cone photoreceptor enrichment in the macular fovea is an area hitherto unexplored.

Autophagic vacuoles engulfing mitochondria were reported in the lens in 1984 [43, 44]. Autophagy was the expected pathway of choice for the developing lens' fiber cells to degrade cell organelles to create the organelle-free zone (reviewed in [45]). Autophagosomes were reported in both differentiating primary and secondary lens fiber cells [43, 46]. However, during the embryonic period, deletion of either Atg5 or Pik3c3 genes in mice did not affect lens organelle clearance [43, 46]. Costello et al. put forward an alternative hypothesis that since both Atg5- and Pik3c3-independent autophagy have been reported and that mutation in autophagy gene FYCO1 causes autosomal recessive congenital cataract, the role of autophagy (and mitophagy) cannot be ruled out in organelle clearance [47–50]. ATG5-independent non-canonical autophagy has also been implicated in the mitochondrial clearance required during metabolic reprogramming of induced pluripotent cells (iPSC) [51]. Perhaps a simpler approach, where overall lens autophagic flux is inhibited (possibly by inhibiting lysosomal fusion), needs to be adopted to confirm that organelle clearance in developing lens is not dependent on autophagy. Furthermore, it remains to be seen if the various proteolytic mechanisms active during lens fiber differentiation can compensate autophagic deficiency (for further reading, please refer [30]).

The adult eye is enclosed in the outer fibrous tunic, composed of the sclera (posterior 5/6th of the eye bulb) and cornea (transparent anterior part) (**Figure 1A**). The middle layer is known

as the vascular tunic (or uvea) comprising of the choroid, ciliary body, and iris. The ciliary body supports the lens and controls the shape of the lens with the ciliary muscle. The innermost layer is the retina with ten distinct layers. Moving in a direction from the inside of the eye, these layers are arranged as (1) the inner limiting membrane; (2) the nerve fiber layer; (3) the ganglion cell layer; (4) the inner plexiform layer; (5) the inner nuclear layer; (6) the outer plexiform layer; (7) the outer nuclear layer; (8) the outer limiting membrane; (9) the photoreceptor (rods and cones) layer; and (10) the retinal pigmented epithelium (RPE) (**Figure 1B**) [52]. The innermost layers of the neural retina comprise of different classes of neuronal cells such as the ganglion cells, the Müller, horizontal, bipolar, amacrine cells, and the photoreceptor cells (rods and cones). Together, these cells constitute a complex network of visual sensory synapses that communicate the visual signals to the brain via the central nervous system.

The extraocular muscles (EOMs) control eye directional movement and eyelid movements and contain cells that are likely to accumulate mitochondrial damage during aging, resulting in slower eye muscle movements [53–55]. McMullen et al. reported that autophagy was severely impaired in 18- and 30-month-old compared with 6-month old Fisher 344-Brown Norway rat EOMs supported by their observation of decline in LC3, ATG5, and ATG7 [56].

The cornea, being a non-keratinized epithelial surface, requires to be kept moist by tear secretions from the lacrimal, meibomian (or tarsal) glands, and the conjunctival goblet cells [57, 58]. Basal autophagy-lysosomal activity in the constituent fibroblasts (also known as keratocytes) of the corneal stroma is critical for the clearance of transforming growth factor β -induced protein (TGF- β Ip) (discussed in detail in next section along with other corneal abnormalities) [59].

The lacrimal glands produce the aqueous components of the tears (i.e., lacritin, lysozymes, lactoferrin, lipocalin, secretory immunoglobulin A (IgA) and complements), which protect the cornea against a large number of infectious agents (reviewed in [60]). The meibomian glands produce the lipid components of tears called meibum consisting of a variety of esters and fatty acids that prevent evaporation of the tears from the conjunctiva (reviewed in [58]). The mucous secretion from the conjunctival goblet cells allows for even distribution of the tears over the conjunctival surface (reviewed in [61]).

Earliest data on autophagic activity in the acinar cells of the lacrimal glands showed dramatic buildup of autophagosomes upon treatment with vinblastine (microtubule inhibitor that blocks autophagosome–lysosome fusion), strongly suggesting the existence of basal autophagy [62, 63]. Autophagy (along with apoptosis) is upregulated in response to inflammation induced in BALB/c mice lacrimal glands, resulting in acinar cell death. It remains to be investigated whether this phenomenon is critical for tissue repair and remodeling post-inflammation injury [64]. The tear component glycoprotein, lacritin, has been reported to protect *in vitro*-cultured corneal epithelial cells under inflammatory cytokine-induced stress via upregulation of autophagic flux [65–67]. Lacritin-stimulated acetylation of transcription factor FOXO3a, followed by acetylated FOXO3a-ATG101 coupling and coupling of stress-acetylated FOXO1 with ATG7, is critical for this autophagic response [68].

The earliest publication on autophagy in the conjunctiva described autophagic structures in guinea pigs [69].

The trabecular meshwork (TM) is located in the iridocorneal junction of the eye and is responsible for draining the aqueous humor from the eye via the anterior chamber (**Figure 1B**) [70]. Porcine TM cells under hypoxic conditions show increased autophagy, perhaps as a response to increase reactive oxygen species (ROS) [71]. TM cells when chronically exposed to oxidative stress tend to develop lysosomal basification and membrane permeabilization owing to the increased lysosomal iron content. Although autophagic activity is elevated in oxidatively stressed TM cells, the levels of ATG5, ATG7, and ATG12 are significantly reduced [72, 73]. This paradoxical observation hints at the possibility of the existence of active and potentially novel non-canonical autophagic pathways that may use another enzymatic network to modify LC3. *Ex vivo* human trabecular meshwork, cells collected from healthy donor eyes, displays an increase in both oxidative DNA damage (8-hydroxy-2-deoxyguanosine) and autophagic activity markers (increased LC3II/I ratio and reduced p62/SQSTM1) in older donors [74]. However, *in vitro* data have failed to establish autophagy as either an inducer of senescence or an inhibitor. Trehalose-induced autophagy and oxidative stress-induced senescence-associated- β -galactosidase (SA- β -gal) activity, while rapamycin treatment did not show the same effect [73].

The iris modulates the amount of light entering the retina by controlling the size and diameter of the pupil. The iris contains pigmented epithelial cells that have the same origin as RPE and contain melanosomes [75]. *In vitro*-cultured newt iris epithelial cells dedifferentiated to lens cells and this process was accompanied by the sequestration of the melanosomes, ribosomes, and multivesicular bodies (MVB) in autophagic vesicles [76]. Autophagy has not been widely studied in the iris. However, since the iris-pigmented epithelial cells and RPE have some common features like melanosome content as well as phagocytic ability, it is expected that they would have high metabolic rates making them susceptible to accumulating cellular damage [77, 78]. Therefore, autophagy, among other housekeeping processes, must be at an efficient level in order to exacerbate this cellular damage. Consistent with this hypothesis, Petrovski et al. have reported an increase in basal LC3 levels in iris sections from aging human cadaver eyes [79]. Interestingly, the authors observed LC3 expression in mouse iris sections. They also reported an increase in basal LC3 levels in the non-pigmented aqueous humor producing epithelium of the ciliary body (connection between iris and choroid) of aging human cadaver eyes and hypothesized that autophagy might play a key role in the maintenance of intraocular pressure (IOP) in the aging eye. Similar LC3 expression was observed in mouse ciliary body [79]. One must however exercise caution before interpreting autophagic protein expression in any ocular tissue. We and others have observed significant oscillations of autophagic protein expression in the retinal layers of rodents [19]. Recent reports show oscillatory expression of circadian clock genes *Bmal1*, *Clock*, *Cry1*, *Cry2*, *Per1*, and *Per2* in the irisciliary body of C57BL/6 mice with significant correlation with diurnal IOP variations over a 12-h/12-h light/dark cycle [80]. *Per1*-deficient mice show increased susceptibility to neuronal injury after cerebral ischemia and one of the key reasons behind this has been hypothesized to be a dramatic attenuation of autophagic activity [81]. Therefore, before conclusions are made about the

absence of expression of autophagic proteins in the iris of mice, diurnal/circadian studies on autophagic protein expression must be conducted.

During normal aging, basal LC3 levels are elevated in the lens of human cadaver eyes [79]. Analysis of autophagic gene expression by a combined approach of microarray, qRT-PCR, and Western blotting revealed as many as 42 autophagy-related genes in microdissected human lens epithelium and fiber cells (age range: 47–69 years) providing convincing evidence of autophagy in the lens [82, 83]. Two independent reports suggest the critical role of autophagy in maintaining lens homeostasis in mutant models of $\alpha\beta$ -crystallin (R120G) and α A-crystallin (R120A) (discussed in detail in the next section) [84, 85].

The mean retinal thickness of the human eye reduces by about 0.53 μm annually with concurrent loss of macular thickness, implying that there is a significant cell loss in the retina even with no pathology [86, 87]. It remains a challenge to researchers to determine whether changes in cell biology of the retinal cells that trigger onset of disease are different from the normal aging process. The nerve fiber layer shows substantial thinning over time (nearly 150/ mm^2 during an average lifetime) due to the significant loss of retinal ganglion cells (RGCs) that make up the ganglion cell layer of the retina and convey visual signals from the photoreceptor layer to the optic nerve that constitutes of RGC axons and glial cells [88–91]. The optic nerve also suffers some detrimental changes during aging due to the loss of ganglionic axons [92]. The neuroretinal rim area reduces at a rate of 0.28–0.39% annually, while the optic cup area and the vertical cup diameter start to increase especially after the third decade of life [93]. Apoptosis is considered the primary cell death mode for RGC loss [94–96]. Reports suggest that autophagy may promote RGC survival after optic nerve axotomy in mice [97]. Atg5, 7 and 12, LC3, and Beclin-1 expression is elevated in the mouse RGCs up to 7 days after optic nerve injury [98]. It has been suggested that autophagic flux impairment in the RGC axons may lead to age-associated changes in the optic nerve [99]. We will elaborate the role of autophagy in terms of RGC and disease in Section 3.

Photoreceptor density decreases at a rate of 0.2–0.4% annually with a greater degree of rod cell loss than cones causing reduced dark adaptation in aged individuals [100, 101]. This loss is mostly in the peripheral and the parafoveal retina rather than at the fovea (**Figure 1C**) [102]. We have previously shown strong expression of Atg9 and LC3 in the ganglion cell layer, retinal vessels, a subpopulation of the inner nuclear layer, the outer nuclear layer of rods and cones, and the RPE [103]. Deletion of Beclin1 or Atg7 or mitophagy-specific Parkin gene in mice causes severe retinal degeneration along with accumulation of abnormal mitochondria [104].

The RPE monolayer consists of perhaps the most multifunctional cells of the eye. The RPE has a plethora of functions such as phagocytosis of photoreceptor outer segments, renewal of chromophores in visual transduction cycle, supplying nutrients from the choroidal side to the photoreceptors, ion, and metabolite exchange and light absorption (reviewed in [7]). Like the entire retina, the RPE is also prone to age-associated decline in function and vitality and accumulate massive cell damage even during normal aging [104, 105]. Additionally, the RPE has to combat light and reactive oxygen species induced damage not just to its own cellular components but also to those of the photoreceptors. Aging changes in the RPE layer is not uniform across the retina [105, 106]. It appears that the peripheral cell area increases while that

at the central retina declines. Cell density decreases with increasing distance from the fovea, but the foveal RPE cell density is relatively very stable. Surprisingly, the aged non-diseased macula shows a population of apoptotic RPE [107]. Both the RPE and photoreceptors are highly metabolic and a healthy pool of mitochondria is required to meet this energy demand. There is a significant reduction in the number of healthy mitochondria and extensive damages to mitochondrial cristae, and matrices are observed [108]. A number of publications together have shown RPE and photoreceptors expressing autophagy proteins (p62, LC3, ATG7, ATG9, and Beclin1) in both human cadaver and rodent model retina sections [13, 14, 19, 103, 109]. Furthermore, as mentioned earlier, diurnal oscillations of autophagic proteins and autophagosomes in the RPE/photoreceptor layers confirm a functional role of autophagy that is integrally linked to POS phagocytosis [19, 110, 111]. Autophagic digestion of rhodopsin light pigment in rod photoreceptors is also necessary for adaptation to changes in light intensity (3–200-lx) [110]. Lentiviral shRNA-mediated silencing of autophagic genes (Beclin1 and ATG7) or 3-Methyadenine (3-MA)-mediated autophagy inhibition in human RPE in cell culture increases susceptibility to oxidative stress with compromised mitochondria, increased lipofuscin, and reduced cell viability [13]. Deletion of RB1CC1 in rodent RPE caused severe retinal degeneration underlining the importance of basal autophagy in the RPE [109]. Levels of autophagic protein such as ATG7, ATG9, and LC3 increase with aging in the retinal layers including RPE and photoreceptors in both human cadaver donor and c57Bl/6 mouse retina sections [13].

Non-canonical LC3-associated phagocytosis (LAP), dependent on Atg5 and Beclin1 but independent of the autophagy pre-initiation complex consisting of Ulk1/Atg13/Fip200, was reported to be critical for degradation of POS and renewal of retinoids required for chromophore synthesis for optimal visual function (**Figure 2**) [111, 112]. Melanoregulin, a 28 KDa membrane-associated protein is critical for lysosomal hydrolase activity in the RPE as well as for RILP-p150Glued complex-mediated retrograde melanosome transport via actin filaments in melanocytes [113, 114]. Frost et al. demonstrated a diurnal variation in melanoregulin expression in the RPE and its distinct association with the ATG5-dependent LAP [111]. Loss of melanoregulin causes accumulation of phagosomes and lipofuscin in the RPE with elevated cathepsin-D secretion that could injure not only the RPE but also the adjacent ocular layers [111, 114]. Furthermore, ROS generated from NADPH oxidase activity resulting from the delayed clearance of all-trans retinal (activated visual chromophore of the visual transduction cycle) shows severe RPE cytotoxicity [104]. LC3 association with phagosomes is signaled by elevated NADPH oxidase activity in other ‘professionally’ phagocytic cells like macrophages [115]. Park2 (mitophagy receptor protein) and LC3 activity are indispensable for RPE defense against all-trans retinal induced cytotoxicity [104]. It is now evident that while basal rate canonical autophagy is critical for quality control and stress adaptation, non-canonical forms of autophagy where some but not all components and mechanisms of the canonical form participate, supports the very core of retinal visual function.

The ocular vasculature has several indispensable functions including supply of oxygen and nutrients to the ocular components; transportation of ions and metabolites; circulation of immune-surveillant cells; and exclusion of pro-inflammatory cytokines and molecular toxins

[116]. The study of autophagic flux and its role in ocular vascular endothelial physiology is still at rudimentary stages. However, recently it has been reported that conditional deletion of endothelial Atg7 in ApoE^{-/-} mice results in accumulation of oxidized LDL within the RPE and choroidal vascular endothelium of the eye underscoring the importance of autophagy in vascular lipid homeostasis [117]. Conflicting opinions exist regarding the role of autophagy in angiogenesis most likely due to the different tissue source of the endothelial cells under study. Lee et al. have recently reported that Beclin1 deficiency leads to increased hypoxia-induced angiogenesis in human pulmonary artery endothelial cells [118]. On the other hand, in bovine aortic endothelial cells, Du et al. suggest that autophagy promotes angiogenesis and elevated ROS levels [119]. Autocrine vascular endothelial growth factor (VEGF) suppresses autophagy in human umbilical vascular endothelial cells (HUVECs) to maintain cell viability. The role of $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins has long been implicated in retinal neovascularization [120, 121]. $\alpha v\beta 5$ integrins act downstream of VEGF activating focal adhesion kinases (FAKs) that are critical for cell migration [122]. Recent reports suggest a critical role of autophagy in restricting integrin activity and thus inhibiting cell migration [123]. Autophagy receptor NBR1 has been shown to be a specific cargo receptor for targeting focal adhesion components to the lysosome for degradation [124].

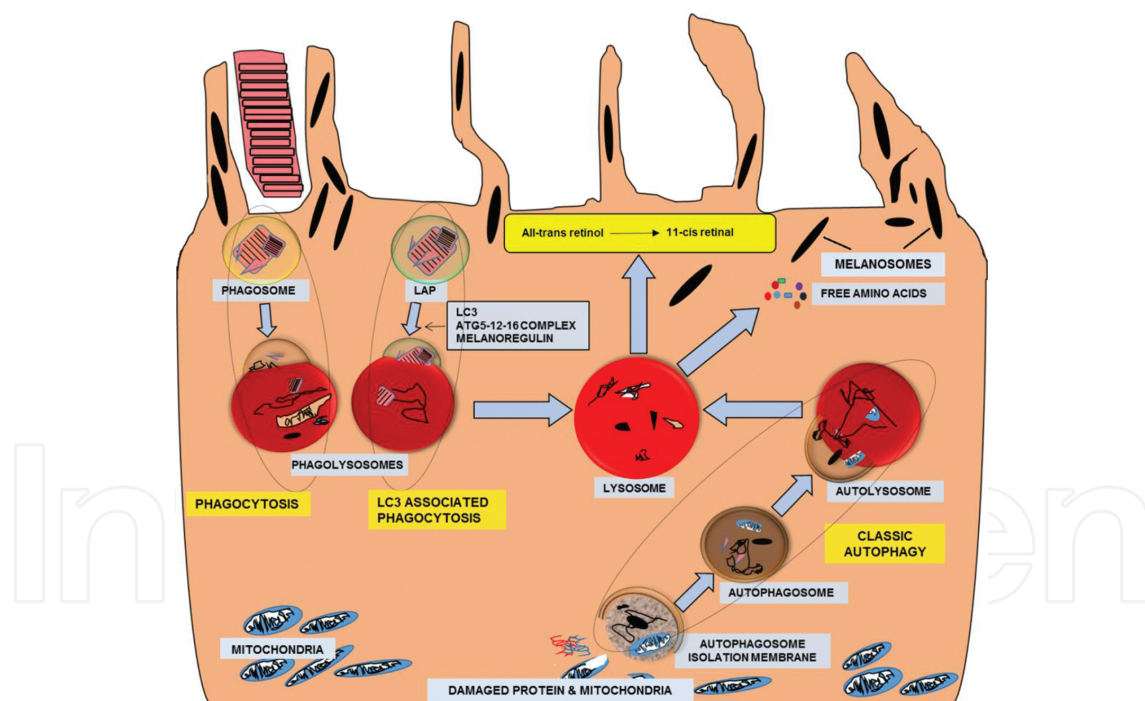


Figure 2. Classic and non-canonical LC3-associated phagocytosis (LAP) in the RPE: basal autophagy is essential in the RPE to maintain organelle and protein quality. Phagocytosis of ingested outer segments may be mediated by autophagy components LC3, ATG5-12-16 complex, and delivery of the phagosome to the lysosome is dependent on these proteins underlining the existence of a non-canonical autophagic pathway in the eye that supports RPE phagocytic function. Furthermore, melanoregulin (MREG) facilitates LC3 recruitment to the phagosomes. Phagocytosis is essential for renewal of all-trans retinol to 11-cis retinal visually active chromophore that is sent to the photoreceptors for enabling the visual cycle. Hence, while basal canonical autophagy is essential for basic housekeeping of the RPE, non-canonical autophagy supports at least in part the visual cycle and photoreceptor disk processing.

These mechanisms need to be reinvestigated in retinal endothelial cells in order to elucidate the role of autophagy in maintenance of retinal vasculature. Inhibiting autophagy in the RPE *in vitro* elevates the levels of pro-angiogenic intercellular adhesion molecule (ICAM), stromal cell-derived factor (SDF-1) and VEGF A in response to challenge by lipofuscin component A2E [125]. Therefore, retinal vascular stability may not only be influenced by autophagy in the vascular endothelial cells but also by the cross talk from adjacent cell layers.

One of the inevitable consequences of oxidative damage in the aging retina is the accompanying inflammatory response and elevated levels of damage-associated molecular patterns (DAMP) [126]. Although the eye was considered immune privileged for a long time, immunocompetent cells like the monocyte-derived cells, microglia, dendritic cells, and perivascular macrophages have been detected in the retina [127–130]. Ample evidence suggests that the inflammation observed during normal tissue aging is an adaptive response and the word coined for this inflammation is ‘para-inflammation’ [129, 131–133]. Para-inflammation is required for retinal tissue homeostasis plays a crucial role in tissue repair/remodeling, but when para-inflammation becomes chronic or progresses to destructive inflammation, retinal damage and pathology may ensue (reviewed in [134]). As mentioned earlier, the aging retina shows an increase in apoptotic cells. However, several reports have recently indicated that other cell death modalities like autophagy and necrosis may also exist in the eye that may become particularly active in retinal degenerative conditions [135, 136]. While apoptosis restricts the release of inflammatory danger signals, late-stage apoptosis and necrosis can initiate DAMP-mediated inflammation. Autophagy, at least in the early stages, has been considered a protective response that suppresses inflammatory signals [137]. Shi et al. showed the activation of autophagy by sterile inflammation (NLRP3- and AIM2-mediated inflammation) limited caspase-1-mediated maturation of IL-1 β and IL-18 [138]. Impairing autophagy in RPE leads to not only inflammasome activation but also macrophage-mediated angiogenesis [139–141]. The age pigment, lipofuscin, is a common feature of many post-mitotic cells throughout the body and is largely derived from autophagic removal of damaged organelles [142]. Lipofuscin accumulation occurs in an age-dependent manner in both photoreceptor cells and the RPE. In both cell types, lipofuscin is derived at least in part via autophagy of damaged organelles (e.g., mitochondria) [142], but the situation is more complicated in the RPE where (a) lipofuscin is also an inevitable consequence of phagocytosis of spent photoreceptor outer segments [143] and (b) phagocytosis is linked to a non-canonical autophagy pathway [111, 112]. Lipofuscin is both a cause and consequence of oxidative stress and oxidative stress-mediated accumulation of lipofuscin increases dramatically in the RPE when autophagy is pharmacologically inhibited [13].

Considerable cross talk exists between apoptosis and autophagy. p53 and Bcl-2 family proteins and calpain have been classically considered as apoptotic proteins but can also modulate autophagy [144–146]. For example, Beclin1 is cleaved by caspase upon depletion of IL-3 in Ba/F3 cells leading to inactivation of autophagy and release of proapoptotic cytochrome c from the mitochondria [147]. Direct cleavage of ATG3 (a ubiquitin-like-conjugating enzyme involved in autophagosome biogenesis) by activated caspase-8 can lead to inhibition of autophagy and cell death [148]. Yet other reports show autophagy prevents necrosis by

reducing metabolic stress [149]. Although cross talk between cell death pathways needs to be confirmed in ocular cells, it is safe to assume that autophagy in the aging eye plays a critical role in maintaining balance between the cell death modalities to avoid a pathological scenario. Arrested autophagic flux by lysosomal disruption enhances buildup of ubiquitinated protein aggregates and cell death under oxidative stress that cannot be prevented by apoptotic caspase inhibitor (zVAD-FMK) [150].

3. Autophagy in ocular disease

3.1. Autophagy in congenital ocular abnormalities

Several congenital deformities of the eye occur such as coloboma, congenital glaucoma, congenital cataracts, congenital detached retina, partially persistent iridopupillary membrane, persistent hyaloid artery, microphthalmia and Peter's anomaly, Leber's Congenital Amaurosis [151–156]. While the cause of most of these diseases is rooted deep in mutation of genes such as PAX2, PAX6, CYP1B1, GLC3A, GLC3B, GLC3C, FOXC1, CEP290, CRB1, GUCY2D, RPE65, several reports suggest autophagy may be compromised in some of these diseases [157]. Persistent hyaloid artery and persistent hyperplastic primary vitreous (PHPV) could result from incomplete involution of the hyaloid vessel [32, 158]. Studies have shown that hypoxic conditions, as seen in the developing eye, increase autophagic activity in vascular endothelial cells. Hypoxia plays a major role in triggering the hyaloid vessel regression and activation of autophagy seems to enhance hyaloid regression in the developing eye [159]. Recessive mutations in EPG5 cause a rare inherited congenital multisystem disorder called Vici syndrome with defective systemic autophagy [160]. EPG5, the human homolog of the *Caenorhabditis elegans* autophagy gene *epg-5*, encodes a key protein (ectopic P-granules autophagy protein 5) that regulates the formation of autolysosomes [160]. Retinal hypopigmentation and bilateral cataracts are among the chief manifestations of this disorder, once again suggesting the importance of autophagy in retinal development (reviewed in [157]). Mutations in WDR45 (also known as WIP14) cause a rare biphasic X-chromosome-linked disorder called beta-propeller protein-associated neurodegeneration (BPAN) [161, 162]. WDR45 interacts with ATG2 and ATG9 that regulate lipid and membrane supplies for autophagosome formation and elongation [163–165]. A subset of BPAN patients has optic nerve atrophy suggesting a possibility that defective autophagy may be part of the disease etiology [166]. Congenital eye disorders typically emerge from underlying genetic mutations, which may often prove difficult to manage therapeutically. Understanding the effect of these genes on cell biology leading to the disease may, in some cases, provide a better therapeutic avenue.

Autophagy is an integral part of developmental cell biology that is coordinated by a vast network of genes [167]. Although still at rudimentary stages, research on the role and fate of autophagy in ocular development must be intensified in the search of more promising therapies in debilitating congenital eye disorders.

3.2. Conjunctiva

Several topical eye ointments contain benzalkonium chloride (BAC) as a preservative that has been shown to induce caspase-independent cell death in a conjunctival cell line, was reversible by autophagy induction [168].

3.3. Cornea

The consequences of corneal infection can be devastating with corneal scars that would require corneal transplant [20]. *Toxoplasma gondii* and herpes simplex virus-1 (HSV-1) are two most common pathogens that can directly infect the cornea. Alternately, HSV-1 can infect the cornea indirectly via first infecting the oral mucosa [169]. In some extremely infectious cases, HSV-1 infections may cause stromal keratitis leading to blindness and is the leading cause of corneal blindness globally [170]. A key virulence mechanism of HSV-1 is to hijack and inhibit autophagy in the host cell via the binding of Beclin1 with the viral protein ICP34.5 [171, 172]. Additionally, ICP34.5 can inhibit autophagy induction by inhibiting the antiviral eIF2 α kinase-signaling pathway (including PKR and eIF2 α) [173]. Contradicting opinions exist as to whether autophagy promotes HSV-1 infection or inhibits it. Petrovski et al. recently showed that autophagy was induced in HSV-1-infected rabbit corneal cell survival against apoptosis [174]. Yakoub et al. showed that HSV-1 does not increase autophagic activity in the cell but basal autophagy is required for it to infect the host cells [175] and pharmacological induction of autophagy in host cells suppresses HSV-1 infection [176]. Yet in another independent study, Alexander et al. reported no effect on HSV-1 replication in autophagy-deficient host mouse fibroblast cells [177]. It seems that the experimental model of choice from one research team to another may affect the final outcome of autophagy on HSV-1 in the infected host cell.

T. gondii infection in the cornea is a classic example where an invading pathogen disrupts cellular endosomal-lysosomal fusion and therefore prevents itself from degradation by lysosomal enzymes [178]. Defects in the CD40 pathway that activates macrophages to eliminate *T. gondii* cause ocular toxoplasmosis, suggesting autophagy may prevent *T. gondii*-mediated infection [179, 180].

Granular Corneal Dystrophy 2 (GCD2), an autosomal dominant disorder caused by mutation R124H in the transforming growth factor β -induced gene (TGFB1) on chromosome 5q31, shows dramatic accumulation of mutant transforming growth factor β -induced protein (TGF- β 1p) in autophagosomes and/or lysosomes of corneal fibroblasts [59]. Autophagy is activated, but the rate of autophagic degradation is not sufficient to inhibit the accumulation of the aberrant protein or polyubiquitinated proteins that are also digested in part by autophagy [59, 181].

3.4. Trabecular meshwork

Defects in fluid drainage by the TM can lead to elevated IOPs and eventually cause irreversible damage to the optic nerve leading to glaucoma. Glaucoma is manifested with loss of peripheral vision leading eventually to complete blindness [182–184]. Both elevated IOP and biaxial TM stretching have been independently shown to promote autophagosome formation [185, 186]. Additionally, aging TM is subjected to both hypoxic and highly oxidative conditions that cause

increased ROS production and accumulation of non-degradable material along with lipofuscin in lamina cribrosa as well as TM cells [187]. In both cell types, autophagic flux is severely impaired contributing to glaucoma pathogenesis [73, 188]. Autophagy seems to be protective from apoptotic caspase signals in TM cells [189].

3.5. Optic nerve

Optic nerve damage is a commonality in all glaucoma subtypes [190]. Other retinal neuropathies such as optic neuritis, hereditary optic atrophy, and traumatic injury may also lead to degeneration of retinal ganglion cell axons in the optic nerve [191]. Optineurin overexpression in retinal ganglion cells (RGC-5) *in vitro* was found to be beneficial via its stimulatory effect on autophagy [192]. However, some reports contradict this hypothesis suggesting that autophagy may, in chronic hypertensive glaucoma models, be neuropathic to the optic nerve [99, 186]. This disagreement may arise from variations in disease stage and models under investigation (reviewed in [191]).

3.6. Lens

As mentioned in the earlier section, there still seems to be considerable debate over the role of autophagy in digestion of organelles of differentiating lens fiber cells to create the organelle-free zone. However, even the reports that argue against the role of autophagy in organelle clearance suggest that it is indispensable for lens quality control. Morishita et al. showed that *Pik3kc3/VPS34* deletion in mouse caused congenital lens defects including cataract and that in *ATG5* deletion mice lens, the lens developed age-related cataracts although not congenital cataracts [46]. Mutations in *FYCO1* (facilitates microtubule-dependent directional transport of autophagosome vesicles) show severe autosomal-recessive congenital cataracts in patients [48, 193]. $\alpha\beta$ -Crystallin mutation (R120G) in hereditary cataract mouse model causes concurrent increase in autophagosome fractional volumes and p62-positive aggregates in lens suggesting impaired autophagic flux that leads to increased lens opacity [194]. Similar results were also observed in a hereditary mutant double knock-in (*R49C^{+/+}*) mouse model where autophagic flux also seemed impaired [85]. Recently, an ESCRT-III subunit *CHMP4B* has been proposed to be involved in autophagosomal clearance of extranuclear DNA and chromatin [195]. *CHMP4B* mutation is associated with autosomal dominant posterior polar cataract formation [196].

3.7. Retina

Many studies have investigated the implications of autophagy in retinal degenerative diseases [12–14, 103, 109, 125, 150, 194, 197, 198]. Age-related macular degeneration (AMD) is an aging-associated neuropathy that affects primarily the photoreceptors and RPE in the macula resulting in loss of peripheral vision and eventual legal blindness [199]. Early in AMD pathology, sub-RPE deposits known as drusen are observed on Bruch's membrane (BM) by funduscopy. There are two types of AMD: 'dry AMD' characterized by geographic atrophy (GA) and 'wet AMD' characterized by neovascularization. Although a heterogeneous disease, the key reason behind the pathology is the increased susceptibility of the RPE to oxidative

stress [200, 201]. Diseased retina shows a significantly greater extent of damaged organelles (mitochondria, peroxisome, melanosomes, etc.) and protein aggregates compared to age-matched healthy retina [108]. The overall accumulation of damaged organelles and macromolecules suggests a collapse of overall antioxidant and proteolytic capacity of the RPE that sets up the stage for disease [202]. Not surprisingly, autophagy has been shown to be severely impaired in AMD retinas of both human cadaver eyes compared with age-matched donors as well as in AMD mouse models [13, 14, 198]. *In vivo* deletion of autophagic gene RB1CC1 in mouse retina results in retinal degeneration that shares many features with AMD disease [109]. RPE cells *in vitro* accumulate lipofuscin and greater loss of mitochondrial activity and membrane integrity under oxidative stress when autophagy is inhibited [13, 125, 203]. Autofluorescent lipofuscin in the RPE destabilizes the lysosome and is a hallmark of RPE senescence that has been widely implicated in AMD [204, 205]. Lysosomal destabilization leads to a severely impeded autophagic flux that has been recently suggested in dry AMD where a higher accumulation of p62 was observed in the foveomacular regions of AMD patient retinas compared with age-matched donors [14]. Interestingly, as mentioned earlier, autophagy inhibition in lipofuscin chromophore A2E-laden RPE results in elevated levels of several pro-inflammatory and pro-angiogenic factors that suggest a possible role of autophagy in both dry and wet forms of AMD [125].

Diabetic retinopathy (DR) is a retinal complication characterized by pericyte loss, microvascular instability, blood retinal barrier (BRB) leakage, and abnormalities in the retinal vasculature [206, 207]. Since pericyte loss is a key feature of DR, the effect of autophagy was investigated in a combination mouse model of diabetes and hypercholesterolaemia. The authors showed that autophagy promoted pericyte survival under mild stress but under chronic stress conditions resulted in pericyte death [208]. This may be considered as a perfect example of the dual role of autophagy both as a protector and as a destructive pathway. Extravascular oxidized low-density lipoprotein (LDL) has been reported to be damaging to the BRB and to cause apoptotic pericyte loss [209]. Du et al. suggested that oxidized LDL may cause RPE injury by excessive oxidative stress, ER stress, autophagy, and apoptosis [210]. High glucose (30 mM) conditions in the RPE result in higher levels of p62 and LC3 accompanied by an increase in the number of autophagosomes [211]. This increase in autophagosomes is possibly to accommodate for the increased ROS damage sustained by the mitochondria, but it needs to be determined whether autophagic flux is reduced as lysosomal pH is reported to be elevated under high glucose conditions [212]. As described earlier, circadian rhythmicity and diurnal variations in expression amplitudes of autophagic proteins is a prominent feature of the retina. Disruption of the peripheral clock has been reported in DR pathology affecting cellular processes such as regulation of inflammation and lipid metabolism [213–216]. Our unpublished data show dramatic phase-shift and amplitude dampening of key autophagic proteins in the retina of rodent models of diabetes (manuscript under preparation). It remains to be elucidated how disruption of diurnal rhythm dysregulates the normal balance between retinal cell metabolism and autophagy, which contributes to DR pathology.

Photoreceptor degeneration is widely observed both in AMD as well as in retinitis pigmentosa (RP). The latter is a highly heterogeneous disease with hereditary mutations in multiple gene loci

[217]. Both caspase-dependent and caspase-independent pathways are involved in photoreceptor cell death in RP [218–220]. rd/rd mouse, the rds/rds mouse, and the light-damage model in albino mice show several elements of the autophagic pathway to be upregulated. This induction seemed secondary to an increase in oxidative stress markers, suggesting that autophagy may be upregulated specifically to remove damaged photoreceptors [221].

Inherited lysosomal storage disease Niemann–Pick type C (NPC) disease is caused by mutations in genes NPC 1 and 2 [222]. LC3 and autophagosomal numbers are elevated in the ganglion cell layer of Balb/cNctr-Npc1m1N/J mouse model possibly because of disruption of autophagic flux and reduced degradation of autophagosomes in the lysosome [223].

3.8. Retinal detachment

Retinal detachment has a number of causes and could be rhegmatogenous or may due to other causes such as traumatic brain injury, severe myopia, retinal tear, or vascular abnormalities frequently encountered in diabetic retina and hypertension [224–227]. In rodent models, retinal detachment induced by subretinal injection of 1% hyaluronic acid resulted in a rapid increase in autophagic activity 3 days after insult. However, 7 days post-injury the autophagic response declined with a simultaneous rise in calpain activity resulting in photoreceptor cell death. Calpain inhibition resulted in increased autophagy and prolonged the survival of photoreceptors [228]. Furthermore, activating autophagy in the same model in Fas-dependent manner inhibited apoptotic death of photoreceptors [229]. Unpublished results suggest hypoxia (increased Hif1 α and Hif2 α protein levels) induced by the retina-RPE separation is a key inducer of autophagy *in vivo*. In an independent study, Dong et al. confirmed the increase in autophagy 3 days after retinal detachment induction. They also showed induction of necroptotic cell death as seen by increased RIP kinase activation [230]. Although they concluded that autophagic and necroptotic cell death can be blocked by the use of necrostatin, it must be considered that all procedures were done at 3 days post-injury when autophagy is at its peak. It would be interesting to see whether necroptosis is still active at 5 days post-injury when autophagy has subsided.

3.9. Uvea

The uvea (consisting of the choroid and the ciliary body) may be affected in some disease conditions such as uveitis and uveal melanoma. Autoantigen-induced experimental autoimmune uveitis (EAU) in Lewis rats shows an increased autophagic activity in infiltrating T lymphocytes that was required for disease recurrence [231].

Uveal melanoma results from malignant tumors arising from melanocytes in the uvea and is the most common intraocular cancer [232]. Mutations in GNAQ and GNA11 genes contribute to a majority of uveal melanoma cases [233, 234]. Ambrosini et al. showed that mutant GNAQ promoted AKT activation via phosphorylation and deletion of mutant GNAQ upregulated AMP kinase-dependent autophagic cell death in primary choroidal uveal melanoma cell lines [235].

3.10. Autophagy in ocular inflammation

Inflammation is an unavoidable phenomenon of aging. Elevated inflammation in the eye contributes to disease pathologies including uveitis, diabetic retinopathy, or maculopathies [236, 237]. As discussed earlier, chronic inflammation in diseased eye is destructive and detrimental to ocular health compared with para-inflammatory immune surveillance that responds to, and repairs, localized tissue injuries. In AMD, drusen deposits play a major role in eliciting inflammation via both the inflammasomes and the complement pathway [238]. While mild upregulation of NLRP3 inflammasome has been shown to be protective, accumulation of lipofuscin, drusen, and damaged mitochondrial DNA have all been implicated in pathological upregulation of inflammasome activity [239]. Furthermore, complement pathway element C5a has been shown to prime the RPE cell for upregulating NLRP3 inflammasome activity in response to light-induced damage [240].

Ocular tissue	Autophagic role	Disease implications	References
Lens	Critical for proteolytic digestion and lens quality control. Role in lens organelle clearance during eye development controversial.	Disrupted autophagy due to <i>FYCO1</i> mutations leads to and autosomal recessive congenital cataract. <i>CHMP4B</i> mutation leads to autosomal dominant posterior polar cataract. Hereditary cataract mouse models show disruption of autophagic flux.	Costello et al. [47] Chen et al. [48] Wignes et al. [194] Shiels et al. [196]
Cornea	Cellular housekeeping and defense against infectious pathogens	Insufficient autophagic degradation leads to accumulation of TGF-βIp in autolysosomes. <i>T. gondii</i> disrupts cellular endosomal-lysosomal fusion machinery as a part of the infectious process.	Choi et al. [251, 252, 254]
Optic nerve	Promotes cell survival in RGC post-optic nerve axotomy. Optineurin mediated RGC <i>in vitro</i> survival dependent on autophagy stimulation. Alternative opinions exist of autophagy effecting cell death in chronic hypertensive model of glaucoma.	Some BPAN patients have optic nerve atrophy possibly due to defective autophagy.	Park et al, [186] Gregory et al. [166] Sternberg et al. [189]
Trabecular meshwork	Autophagy upregulated under hypoxia, elevated IOP and biaxial TM stretching. Oxidative stress increases autophagy but ATG5, ATG7, and ATG12 reduced.	Disruption of endoplasmic reticulum autophagy may be a key feature of myocilin accumulation seen in a majority of glaucoma cases.	Pulliero et al. [74] Porter et al. [185] McElnea et al. [187]

Ocular tissue	Autophagic role	Disease implications	References
	Autophagic markers increase during aging in donor eyes.		
Ocular vasculature	Critical for proteolytic and lipid homeostasis. Potential regulator of vascular stability by prevention of angiogenesis. Autophagy essential for hyaloid regression and clearance during development.	Diabetic Retinopathy: Accumulation of extravascular oxidized LDLs due to disrupted autophagy leads to blood-retina barrier damage and causes pericyte loss.	Torisu et al. [117] Lee et al. [118] Kim et al. [159] Fu et al. [208] Wu et al. [209]
Retina	Diurnal modulation of Autophagic proteins. Non-canonical LC3 associated Autophagy is essential for phagocytosis is critical for degradation of POS. Basal autophagy indispensable for maintenance of RPE and photoreceptor homeostasis. Restricts sterile inflammation in the retina	AMD: Autophagy initiation and flux are disrupted in human donors and mouse models for AMD. Diabetic Retinopathy: Disrupted autophagy may cause pericyte loss.	Kim et al. [112] Yao et al. [19, 109]; Mitter et al. [13] Viiri et al. [14] Fu et al. [208] Liu et al. [139]
Uvea	Autophagy promotes cellular survival	Uveal Melanoma: Inhibition of autophagy may be effective in Uveal melanoma therapy	Ambrosini et al. [232]

Table 1. Description of role of autophagy along with disease implications in various ocular tissues.

Autophagy plays a critical role in controlling NLRP3 inflammasome activity in the retina. Several *in vitro* experiments show dramatic upregulation of inflammasome activity in RPE and secretion of IL-1 β after autophagic flux inhibition [139–141] [203]. Wortmannin inhibits autophagy by inhibiting class III PI3Kinase [241]. Intravitreal injection of wortmannin inhibits autophagy *in vivo* in mouse retina-induced inflammasome activity and CCR2+ monocyte-derived macrophage augmentation that promotes angiogenesis [139]. Although the role of autophagy in activating and augmenting the retinal complement pathway needs to be deeply investigated, evidence does exist of C3- and CD63-positive deposits on Bruch’s membrane in aged mice possibly as a result of increased autophagy and exocytosis [197] (see **Table 1**).

4. Autophagy as a therapeutic target in ocular pathology

Since autophagy is a pathway with a dual role in cell maintenance as well as cell death; multiple stages such as initiation, maturation, and lysosomal fusion; and cross talk with multiple cellular pathways, its manipulation is a challenging therapeutic option in diseases. Neverthe-

less, autophagy has received special attention in cancer, metabolic, neurodegenerative, and infectious diseases [242–245]. Since overall proteolytic capacity is attenuated in a majority of ocular diseases, autophagy modulation must be incorporated in current therapeutic regimens to achieve a better outcome.

Treatment strategies for immature cataracts have been sought after for more than a century [245, 246]. Topical solutions with antioxidants glutathione, cysteine ascorbate, L-taurine, riboflavin, and 2% N-acetyl carnosine showed some promise in reducing immature cataracts [247]. Including autophagy stimulation may improve this treatment strategy. Posterior capsule opacification, a common post-surgical complication of mature cataracts, results from the remnant lens fibers and epithelial cells that proliferate and damage the new lens implant [248]. Laser capsulotomy surgery although usually successful may in rare occasions give rise to retinal detachment and is also extremely challenging to execute when treating congenital cataracts in younger patients [249]. It is not known whether autophagy (as well as other mechanisms) supports cell survival of the remnant lens epithelial cells. Pharmacologically stimulating cell death may involve autophagy that either promotes or inhibits survival of these cells.

As mentioned earlier, removal of TGF- β 1p deposits is a focus in granular corneal dystrophy 2 (GCD2) research. Lithium, which has shown some success in removing these deposits from *in vitro*-cultured corneal fibroblasts from GCD2 patients, has also been shown to induce autophagy as a part of its cytoprotective mechanism [250, 251]. Since TGF- β 1p accumulates in autolysosomes, autophagy stimulation by lithium or by rapamycin and melatonin as suggested in another study must be explored as a treatment strategy in this disease (please refer to Choi et al. for more details) [59, 252–254].

Non-infectious uveitis treatment with subconjunctival injections of rapamycin as an immunosuppressive agent has shown promise in clinical studies with patients showing improved visual acuity and reduced vitreous haze with no noticeable adverse effects [255]. Mechanistic studies may reveal that at least a part of this immune suppressive ability of rapamycin may be credited to autophagy stimulation.

Antitumor activity is seen in combinatorial therapy involving mTOR inhibition and autophagy inhibition with hydroxychloroquine has been shown to restrict melanoma and these treatments are currently under phase-1 trial [256, 257]. Such treatment strategies may be adopted in treatment of uveal melanoma although the fact that chloroquine may induce cataract formation, demands that careful dose-response studies be conducted to ensure no adverse effects [258, 259].

Autophagic degradation is attenuated in AMD. Lipofuscin accumulation in the disease has been shown to perturb the lysosomes that have serious implications on RPE health [142, 260–262]. Lysosomal activity disruption affects both autophagic flux and phagocytosis [263]. Hence, putative therapies should first focus on restoring lysosomal activity to improve degradation of existing autophagosomes. Rapamycin administration to senescence-accelerated OXYS rats improved the RPE morphology in the retina [264]. Clinical trials using rapamycin to treat GA in advanced stages of dry AMD showed ‘no positive anatomic or functional effects’ [265]. The

treatment failure may partly be attributed partly to the fact that the intervention may have been attempted at a time when the disease was well underway with well-developed AMD lesions. An earlier intervention in addition to stimulating lysosomal activity may produce better results.

To include autophagy in ocular therapeutic strategy for better treatment outcomes, the following aspects must be considered. (1) Stimulating autophagy initiation: Several pharmacological activators of autophagy have been identified for possible therapeutic treatments. Rapamycin and its analogs (CCI-779, RAD001 and AP23573) act via inhibiting the mTOR pathway. Metformin mediates AMP kinase activity which stimulates autophagy initiation. Yet other drugs such as lithium and valproic acid have been identified that stimulate autophagy induction. Studies using rapamycin or resveratrol have shown promising results in treatment of cardiac hypertrophy [266, 267]. Clearance of α -synuclein and polyQ mutant Huntingtin aggregates has also resulted from using rapamycin in Parkinson's and Huntingtin disease, respectively [268–270]. Also, small molecule enhancers of rapamycin have also been reported that show positive results in neuroprotection. However, whether stimulation of autophagy would be at all beneficial in retinal diseases perspective depends significantly on the status of lysosomal machinery at the stage of the disease when intervention is attempted. Stimulating autophagosome biogenesis when lysosomes are destabilized will not alleviate the cytotoxic burden resulting from damaged protein aggregates. (2) Stimulating lysosomal activity: An effective strategy to clear aggregate proteins may be attempted by improving lysosomal activity and thereby increasing (or restoring) the autophagic flux. Transcription Factor EB (TFEB) is considered a 'master regulator of autophagy' and drives the expression of several autophagy and lysosomal genes including p62, Atg9b, LC3B, Wipi1, and Lamp1 [271]. Gene therapy with TFEB in mouse model of hepatic disease improved clearance of protein aggregation and rescued alpha-1-anti-trypsin deficiency [272]. High efficiency gene transfers have been achieved to specific retinal layers previously with different adeno-associated virus (AAV) serotypes. TFEB gene transfer may dramatically improve lysosomal biogenesis and overall autophagic flux in the RPE and may be of particular importance in AMD therapeutic strategies.

5. Summary and future directions

The autophagic machinery consists of a fine-tuned complex network of genes whose mysteries are still being unraveled by researchers. Autophagy research in the eye so far has established it as an essential housekeeping pathway indispensable for ocular homeostasis. While therapeutic strategies to regulate autophagy in ocular diseases are still in rudimentary stages, promising results from initial trials have raised hope of autophagic modulation moving gradually from bench to clinic. The challenge lies in modulation of autophagy to the levels required in the particular disease scenario, that is do we want cell death in malignant conditions or just to restore autophagy to levels where it can not only clear cellular waste but also effectively reverse inflammation and contain cell death signals.

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