Chapter 1

Lipofuscin Accumulation into and Clearance from Retinal Pigment Epithelium Lysosomes: Physiopathology and Emerging Therapeutics

Marcelo M. Nociari, Szilard Kiss and Enrique Rodriguez-Boulan

Abstract

Photoreceptors undergo a constant renewal of their light sensitive outer segments (POSs). In the renewal process, 10% of the POS mass is daily phagocytized by the adjacent retinal pigment epithelium (RPE). POS contain vast amounts of 11-cis retinal and all-trans-retinal, two highly reactive vitamin A aldehydes that spontaneously dimerize into lipid bisretinoids (LBs) and accumulate into RPE lysosomes during phagocytosis. As LBs are refractory to lysosomal hydrolases and RPE cells do not divide, this accumulation is irreversible and results in the formation of lipofuscin granules. Lipofuscin accumulation is toxic for RPE cells through a variety of light-dependent and light-independent mechanisms. Beyond a threshold, RPE cells die resulting in secondary loss of overlying photoreceptors. Currently, there are no effective treatments for retinal disorders associated with genetic or age-associated LB accumulation, such as Stargardt disease and age-related macular degeneration (AMD). Thus, there is a great need for medical interventions. Here, we discuss the current understanding of lipofuscin’s pathogenicity and the status of different strategies under development to promote LB elimination from RPE lysosomes.

Keywords: lipofuscin, Stargardt, age-related macular degeneration (AMD), bisretinoids, retinal pigment epithelium (RPE), cyclodextrins, cellular clearance, TFEB, lysosome

1. Introduction

To understand the origin and consequences of the lysosomal accumulation of lipofuscin in the eye, a basic knowledge of retinal function and organization is required.
1.1. The retinal pigment epithelium (RPE) in vertebrate’s eyes

Light entering the eye gets refracted by the cornea and lens on the neural retina, where photoreceptors (PR) convert photons into a cascade of chemical and electrical events that propagate to second-order (horizontal, bipolar, and amacrine cells) and third-order (ganglion cells) retinal neurons, which distribute this information to various visual centers of the brain through the fibers of the optic nerve. The bodies of PR cells, rods and cones, display three sectors (Figure 1): the outer segment, filled with stacks of disks densely packed with light-sensitive photopigment; the inner segment, filled with genetic, biosynthetic, and metabolic organelles.

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**Figure 1.** Basic structure of retina’s inverted architecture. Light travels through ganglion cells (GC) as well as second-order neurons comprising bipolar (Bi), amacrine (A), horizontal (H), and Muller (M) cells, the retina. RPE localizes between the choroid capillaries and the cone (C) and rod (R) photoreceptors. Photoreceptors outer segments (OS), inner segments (IN), and synaptic terminals (ST).
(nucleus, endoplasmic reticulum, Golgi complex, ribosomes, mitochondria); and the synaptic terminal that connects with bipolar neurons of the retina. In vertebrates, the retina is inverted in the sense that light passes through secondary and tertiary neuronal layers in the inner retina before reaching the rods and cones in the outer retina (Figure 1). Photoreceptors are metabolically very active cells that require large amounts of nutrients and generate massive amounts of waste. Provision of nutrients and removal of waste are critical support tasks performed by the retinal pigment epithelium (RPE), a monolayer of cuboidal cells tightly opposed to the photoreceptors through a narrow subretinal space. The multifunctional RPE cells constitute the blood-outer retina barrier that controls the movement of nutrients, ions, water, gases, and wastes between the photoreceptors and underlying choroidal blood vessels [1], absorbs excess light through their melanin granules, performs segments of the visual cycle that regenerates the visual pigment, performs daily phagocytosis of photoreceptor outer segments, and produces trophic factors necessary for photoreceptor survival [2]. It may be rationalized that a fundamental objective of the inverted retina design is to bring photoreceptors in close contact with RPE, a key relationship for the integrity of the retina.

1.2. Role of RPE in visual-pigment regeneration

In 1967, George Wald was awarded the Nobel Prize for revealing the essential role of Vitamin A for vision [3]. Vitamin A entirely is derived from the diet. A critical function of RPE cells is to supply the vitamin A-derived chromophore, 11-cis retinal, required for the light-sensing function of visual pigments to photoreceptor cells. Visual pigments are G-protein receptors (opsins) covalently linked to 11-cis-retinal. Opsins cannot respond to light by themselves but need their prosthetic group, 11-cis-retinal that undergoes cis-trans isomerization upon illumination. The absorption characteristics of 11-cis retinals, in different pigments, are determined by the opsins. Humans have four types of visual pigments: rhodopsin, expressed by rod photoreceptors, which is sensitive to dim light and provides black-and-white vision; L-opsin, M-opsin, and S-opsin expressed by cone photoreceptors sensitive to red, green, and blue color lights, respectively [4]. When light strikes the visual pigments, it promotes isomerization of 11-cis retinal into all-trans retinal (ATR), which in turn promotes the structural rearrangement of opsin into an active conformation that initiates phototransduction (Figure 2).

To recover photosensitivity, opsin must be regenerated by releasing all-trans retinal and binding new 11-cis retinal. Released all-trans retinal is pumped out of the disks into the cytosol by a photoreceptor specific ATP-binding transporter (ABCA4) and reduced to all-trans-retinol by all-trans-retinal dehydrogenases (RDH8 and RDH12). All-trans-retinol diffuses into the RPE where it is esterified by lecithin:retinol acyltransferase (LRAT) to all-trans-retinyl esters, which are stored in retinosomes. All-trans-retinyl esters are isomerized by 65-kDa RPE-specific protein (RPE65) to 11-cis retinol, which is oxidized to 11-cis retinal before traveling back to the photoreceptors outer segment where it is again conjugated to an opsin to form new, functional visual pigment. These series of metabolic steps, by which all-trans-retinal is converted to regenerate the visual pigments, constitute the classical visual cycle [5]. As described above, the RPE performs a number of critical steps in the classical visual cycle that provides 11-cis retinal to rods and cones. There is also a cone-specific visual cycle [6], which...
is RPE independent, that will not be discussed here. The RPE can also generate 11-cis retinal from vitamin A captured via its transmembrane transporter Stra6 from the choroidal circulation. Another important source of 11-cis retinal is the retinosomes, i.e., intracellular lipid droplet deposits of all-trans-retinyl esters in the cytoplasm of RPE cells (Figure 2).

1.3. Lipid-bisretinoid (LB) biogenesis

Vitamin A aldehydes (retinaldehydes) are highly reactive molecules capable of forming adducts with biological amines without the need for a catalyst [7]. In the disks of photoreceptor outer segments (POSs), retinaldehyde concentrations are relatively high, due to the \textit{all-trans-retinal} released by photo-transduction and the \textit{11-cis-retinal} conveyed for the regeneration of visual pigments (Figure 2). Not surprisingly, POS’ retinaldehydes tend to covalently react with the amine group of phosphatidyl-ethanolamine (PE) to form N-retinylidene-PE (NRPE), which reacts with the second molecule of retinal to produce \textit{lipid-bisretinoids} (LBs). Thus, LBs are a family of adducts, all structurally related, that derive from the condensation of two retinaldehydes with one PE molecule [8, 9].
1.4. Photoreceptor renewal

Because of their task in vision and proximity to the fast flowing choroidal capillaries, photoreceptors are continually exposed to high doses of radiant energy and oxygen, which makes them prone to photo-oxidative damage. To secure long (decades) of useful life, under these demanding conditions, photoreceptors undergo a daily renewal process wherein the most distal tips of their POS, comprising the ~100 oldest disks, are removed and equivalent number are basally produced to maintain constant outer segment length [10]. This cellular renewal process has a circadian rhythm. The rods shed POS most vigorously in the morning, whereas cones shed more vigorously at the onset of darkness [11]. The enormous amount of waste daily generated by this process is cleared by the adjacent RPE cells. In the mammalian eye, one RPE cell serves approximately 40 photoreceptor cells, each of which sheds ~7% of its mass per day. RPE engulfs and degrades POS fragments via a receptor-mediated phagocytic process similar to that involved in macrophage-mediated removal of apoptotic cells [12, 13]. This is an impressive metabolic task for RPE, since each cell must ingest and digest ~4000 disks before the next phagocytic load. Thus, RPE is one of the most active phagocytic cells in the body. Because RPE cells do not divide, they must completely dispose this daily material to avoid POS components buildup in their lysosomes.

1.5. RPE lipofuscin accumulation

“Lipofuscin” is the generic name given to subcellular material that accumulates with age within the lysosomal compartment of a variety of postmitotic cells and is characterized by its golden-orange autofluorescent emission. Very few compounds of animal origin exhibit fluorescent emissions in the lipofuscin's region of the spectrum [14]. A fairly rigid structure with highly conjugated double bond system is necessary for such fluorescence because, accumulation of lipofuscin is considered an universal biomarker of aging, as it is also referred to as “age pigment”. Lipofuscins are resistant to degradation by lysosomes, proteasomes, and are not evidently exocytosed. Hence, their accumulation appears irreversible in cells that do not divide. Most lipofuscins stain positive for proteins, lipids, and carbohydrates [15]. Their exact composition varies among tissues but most commonly contains a large proportion of incompletely degraded proteins [16]. The RPE is one of the tissues with the largest buildups of lipofuscin. RPE lipofuscin increases with age in all healthy eyes [17, 18]. It localizes in lysosomal bodies of the RPE [19] and can occupy ~ 20% of the cytoplasmic space by 80 years of age [20].

In order to illuminate the cellular processes responsible for the formation of RPE lipofuscin, several groups attempted to analyze RPE-lipofuscin's chemical composition. Eldred and Katz [21] were the first to isolate the fluorescent pigments of the RPE lipofuscin. Spectroscopy and mass spectroscopy analyses of lyophilized chloroform extracts of RPE cells from healthy donors of different ages revealed that the most common fluorophore was a lipid-bisretinoid [22], N-retinylidene-N-retinylethanolamine also called A2E [23]. Protocols for in vitro synthesis of A2E as well as its incorporation into lysosomes of cultured RPE were developed, allowing to model RPE lipofuscin accumulation in vitro [24, 25]. Further efforts to isolate and characterize the remaining chromophores in the chloroform extracts from RPE lipofuscin granules, yielded additional LBs, including A2-GPE (A2-glycero-phospho-ethanolamine),...
A2-DHP-PE (A2-dihydropyridine-phosphatidyl-ethanolamine), all-trans-retinal dimer (ATRD), and all-trans-retinal dimer phosphatidyl-ethanolamine (ATRD-PE) (Figure 3) as well as several higher molecular weight hydrophobic polymers derived from the reaction between A2E and its oxidation products [26–29].

Of note, the chloroform-insoluble fraction of the RPE lipofuscin, which represents 70% of its dry weight, was not analyzed in these studies. Thus, to fully characterize this fraction, Schutt et al. performed a proteome analysis of sucrose-purified RPE granules [30]. They identified 65 abundant cellular proteins, which included structural, metabolic, mitochondrial, chaperone, transmembrane, and signaling transduction proteins. Many of these proteins were modified by reactive carbonyl compounds (4-hydroxynonenol and malonyldialdehyde) and exhibited advanced glycation end products (AGEs) [31]. A second study by Warburton et al. [32] identified 41 proteins, most of which included phagosomal, lysosomal, and photoreceptor proteins (including rhodopsin) in agreement with the notion that RPE lipofuscin was mainly a buildup of undigested POS material. Surprisingly, only 12 proteins (11%) of Warburton’s list were common with the 65 proteins identified by Schutt et al. This discrepancy probably results from variations in the purity of sucrose-isolated granules [33] and from the fact that lipofuscin proteins are microheterogeneous in size due to abundant oxidative modifications while contaminant proteins are intact and therefore, run as well-focused spots. In a third study, Ng et al. [34] analyzed the composition of highly purified RPE-lipofuscin granules devoid of

![Figure 3. LBs found in RPE lipofuscin. R1, R2 are fatty acids with 14 to 22 carbons and 0 to 6 double bonds.](image-url)
membranes and reported that the luminal material was 98% lipids, mostly LBs [35]. Taking all this information into account, the current concept is that RPE lipofuscin originates from LBs in photoreceptors and is transferred to RPE lysosomes during POS phagocytosis. This model is supported by animal studies that show that accumulation of lipofuscin in the RPE only occurs if (i) there is a supply of 11-cis-retinal to synthesize visual pigments, as RPE65−/− mice display no lipofuscin [36] and (ii) there is phagocytosis of POS, since no accumulation of lipofuscin is detectable in phagocytosis-defective animals. In healthy individuals, LB formation occurs slowly because the concentrations of retinaldehydes are relatively low, thereby, taking many years to generate significant amounts of LBs. In contrast, in individuals with mutations in ABCA4, the formation of LBs is dramatically accelerated [37].

1.6. Cellular toxicity caused by RPE lipofuscin accumulation

In retinal diseases associated with the accumulation of LBs in RPE lysosomes, vision loss is the result of the death of photoreceptor cells secondary to the functional impairment of RPE. Cell culture experiments have shown that lysosomal accumulation of LBs can cause RPE cell death [38]. However, how exactly lipofuscin accumulation disrupts RPE performance and viability is not fully understood. The variety of LB-elicited toxic mechanisms proposed so far (Figure 4) and their investigation as potential pharmacological targets are discussed below.

1.6.1. Phototoxicity

In vitro data with both, whole lipofuscin granules [39] or individual bisretinoids (A2E [40], all-trans-retinal dimer [27] and A2-GPE [41]) loaded into lysosomes have shown that LBs...
sensitize RPE cells to light exposure. Cellular photosensitivity is proportional to the amount of LBs accumulated [42] and the wavelength, with a maximum at 430 nm (blue light), which coincides with the excitation spectrum of the LBs [43]. The absorption of blue photons by the LBs’ extended double bond conjugated system, in the presence of oxygen, leads to the formation of oxidized LB species [44, 45] that after repetitive oxidative attacks become fragmented into far reaching, highly reactive, carbonyl bearing small molecules [43, 44, 46–48]. These fragments promote cell damage by forming Schiff base adducts with free amine groups in lysosomal hydrolases, nucleotides, phospholipids, lipids, proteins [49], DNA [50], proteasomes [51], and molecules in extracellular retinal deposits (drusen), which could trigger local innate and adaptive immune responses [52, 53]. Interestingly, healthy mice immunized with Schiff base adducts found in the AMD lesions, developed AMD-like retinal pathology [54]. There is also in vivo evidence indicating that RPE lipofuscin undergoes photodegradation in the eye. Ueda et al [55] showed that ABCA4−/− animals were more susceptible to light damage than WT animals and that in both groups, older animals carrying larger amounts of LBs were also more susceptible. More recently Ref. [56] showed that RPE-lipofuscin photodegradation takes place in mouse eyes under standard ambient illumination. Specifically, they found that WT and ABCA4−/− mice reared in constant darkness contained 45 and 62% more LBs in the RPE than their respective 12-h cyclic light-reared controls. In addition, ABCA4−/− mice who received vitamin E, a potent inhibitor of LB oxidation [44], displayed 54% more LBs than controls. Studies in humans, using fluorescence microscopy for quantifying lipofuscin, and MALDI-IMS (high-resolution matrix assisted laser desorption-ionization imaging mass spectrometry) for detecting A2E showed that lipofuscin fluorescence colocalized with A2E only in the darkest zones of the retina [57]. When the same technology was applied to the eyes of ABCA4−/− mice, lipofuscin fluorescence, and A2E colocalized 100% [58]. Since RPE is exposed to higher levels of illumination in eyes from diurnal than nocturnal species, these data suggest that A2E is much more photooxidized into MALDI-IMS unidentifiable fluorescent derivatives in human eyes than in mice eyes. However, how much LB photooxidation contributes to retinal pathology is an open question. There is a large amount of clinical trial data on the use of antioxidants (lutein, zeaxanthin, and vitamins C and E) supplementation (alone or in combination) to prevent or delay retinal degeneration. A Cochrane meta-analysis performed on four large, high-quality–randomized clinical trials involving a total of 65,250 participants, without signs of AMD at baseline showed no effect of antioxidant therapy for preventing the onset of retinal degeneration per se [59]. Another Cochrane review meta-analysis [60] involving data from 13 randomized clinical trials, including two large trials, the AREDS1/2 and the Vitamin E Intervention in Cataract and Age-Related Maculopathy study, and 11 smaller (20–400 participants) randomized trials were performed to decide whether antioxidants can slow progression of retinal damage in patients with established AMD. The AREDS1/2 shows that long-term, high-dose supplementation with vitamin E (400 IU), vitamin C (500 mg), beta-carotene (15 mg), zinc (80 mg), and copper (2 mg) reduced the risk of progression to geographic atrophy AMD by 8% in only a subgroup of patients with intermediate AMD at baseline. The other 11 trials demonstrate little evidence for the effectiveness of antioxidant therapy for preventing either visual loss or AMD progression. In summary, treatments with antioxidants have shown very modest efficacy at preventing or stopping the progression of lipofuscin-associated retinal degenerations. Indeed, patients with mutations
in ABCA4 (Stargardt-1, CRD, and RP) gene are not cured by high-doses of antioxidants [61]. This may indicate that scavenging reactive oxygen species is not the best approach to halt LB-driven damage. Alternatively, LB-photooxidation could be damaging through its propensity to activate the complement system [62]. In support of this idea, there is a histologic evidence of complement deposition in drusen of retinas with AMD [63] and animal studies show that overexpression of inhibitors of complement protects retinas of mice with elevated LB content [64]. Furthermore, genetic polymorphism in genes encoding complement factor H (CFH), CFB component C2, CFI, and complement components 2, 3 and 7 has been associated with elevated risk for LB-driven retina disease. Initially, there was a tremendous excitement to test complement inhibitors in the eye. Out of a dozen tested, only 1 molecule, Lampalizumab (Genentech), has made it into phase 3 clinical trials. In the phase 2 clinical test, Lampalizumab decreased the rate of growth of the geographic atrophy area, especially those with CFI polymorphisms [65]. Two world-wide multi-center prospective phase 3 clinical trials, which are enriched with CFI subjects, are now fully enrolled and results are expected in early 2018.

1.6.2. Inactivation of lysosome-dependent degradative processes

Because RPE cells are the most active postmitotic phagocytes in the body, they heavily rely on the fitness of their degradative machinery to operate. Indeed, a high baseline of autophagic activity level has been detected in the RPE and photoreceptors [66, 67], which were further enhanced during periods of POS phagocytosis [68]. Digestion of rhodopsin is also necessary for adaptation of rods to changes in light intensity [69]. Chemical or genetic inhibition of autophagy in RPE cells increased accumulation of undigested material and reduced cell viability [70]. Deletion of the autophagy inducer gene RB1CC1 in rodent RPE caused severe retinal degeneration, underlining the importance of basal autophagy [71]. Histological examination of retinas from Stargardt and AMD subjects revealed massive accumulation of lysosomal material similar to lipofuscin in the apical regions of RPE cells and of extruded extracellular deposits (drusen and pseudo-drusen) that support the idea of a defect in the recycling of endocytic and autophagic cargoes [72, 73]. In vitro experiments in which exogenous A2E was loaded in the lysosomes of cultured RPE cells, as surrogate of lipofuscin accumulation, show also a significant impairment in the digestion of phagocytized POS [74, 75] and autophagocytized proteins [76], implying lysosome-dependent degradative pathways are a primary point of attack by LB accumulation. The mechanism by which LBs mediate these inhibitions is not fully understood yet. Measurements of lysosomal protease, lipidase, glycosidase, nucleases, sulfatase, and phosphatase activities in homogenates of RPE revealed that A2E does not inhibit lysosomal activity by direct interaction with the hydrolases [77]. Lysosomes-containing A2E seems to have increased pH [74]. Bergmann et al. [76], working with purified lysosomes, provided evidence that A2E inhibits the vacuolar H(+)−ATPase (v-ATPase). v-ATPase is a transmembrane lysosomal protein in charge of maintaining the acidic environment within the lysosomes. Because acidic conditions are a prerequisite for the activity of lysosomal hydrolases, A2E-induced increase of lysosomal pH would explain, in part, its effect on lysosomal functions and autophagy [78]. Consistently, restoration of acidic pH in RPE lysosomes has shown promising results at improving lysosomal dependent
degradative processes [79]. Furthermore, v-ATPase, together with mTORC1 complex, Rag GTPases, Ragulator, and Rheb, is an essential component of the lysosome nutrient-sensing (LYNUS) complex [80]. Under conditions of plenty of food, the v-ATPase complex senses luminal amino acids [81] and recruits mTORC1 to the lysosomal surface where it gets activated by phosphorylation [82]. Active mTORC1 complex is the main kinase negatively controlling autophagy and lysosomal biogenesis. When v-ATPase is inhibited by starvation, mTORC1 is released from the lysosome, becomes immediately inactive by dephosphorylation, and can no longer inhibit autophagy or TFEB nuclear translocation [83, 84]. The latter, by increasing lysosomal number, trafficking, hydrolase content, initiation of autophagy [85, 86], and lipid catabolism [87], facilitates the rapid degradation of a variety of substrates. How A2E inhibition of v-ATPase affects these cascades is not yet understood. Few studies have characterized the status of endogenous mTORC1 and TFEB in the RPE [88–90] and no enough data are available for LB-loaded RPE. A likely scenario is that TFEB activation by LBs provides a first line of defense that is insufficient to address accumulating autophagosomes containing partially degraded POS. However, in the absence of such lysosomal stress response, the RPE might succumb even faster. This model would explain experimental data showing that A2E induced a concentration- and time-dependent protective autophagic response in RPE cell cultures. [91]. Clinical trials using rapamycin, a mTORC1 specific inhibitor, to treat advanced stages of AMD showed no positive results [92, 93].

1.6.3. Lysosomal membrane permeabilization (LMP)

The A2E molecule contains a central pyridinium ring that houses permanently positive amine nitrogen and two long hydrophobic polyene arms. A similar structure is shared by other LBs, including A2-GPE, A2PE, and their isomers. Instead, A2-DHP-PE, all-trans-retinal (ATRD), all-trans-retinal dimer-E (ATRD-E), and all-trans-retinal dimer-PE (ATRD-PE) have non-charged ring cores, although ATRD-E and ATRD-PE have protonable nitrogens that confer them with amphipathic character at low pH. Amphipathic LBs have the potential to intercalate into membranes [22, 94]. Schutt et al [95] investigated the destabilizing effects of A2E on purified lysosomes by measuring the release of luminal β-hexosaminidase to the supernatant. Concentrations as low as 2 μm induced leakage, whereas plasma membranes were insensitive to much higher concentrations. In support of the idea that amphipathic LBs cause lysosomal membrane permeabilization (LMP) is the observation that RPE cells loaded with lipofuscin granules or A2E into their lysosomes undergo significant LMP [40]. Multiple mechanisms can be responsible for A2E mediating LMP. A2E can act as a surfactant and cause direct membrane damage. De and Sakmar [94] found that A2E-induced leakage of liposomes at concentrations of 200–300 μm. LMP could also be the result of A2E crystallization within lysosomes, which might cause inflammation by activating a multimolecular signaling complex of the innate immune system, the NLRP3 inflammasome, resulting in a caspase-1–mediated activation and secretion of mature IL1β family cytokines [96, 97]. Relevantly, A2E accumulation induces NALP3-mediated secretion of mature IL1β [98]. Fluorescence staining of lipofuscin revealed a membrane bound autofluorescent granule with the bulk of A2E in the lumen rather than in the membrane. Atomic force microscopy shows the core of the granule comprises of solid mini aggregates [99]. Accordingly, we observed A2E (MW 592 Da) in aqueous media...
cannot cross 0.10 micron pore size filters with molecular weight cutoff of 300,000 Da. This retention was due to size exclusion as A2E passed through 3 micron filters of the same material (Figure 5).

Toxicity of lipofuscin could also involve other less-studied mechanisms, including mitochondrial poisoning, as it has been shown that lysosomal A2E progressively leaks into the mitochondrial compartment [100], where it destabilizes the membrane [101] and inhibits oxidative phosphorylation [102], derail of cholesterol trafficking [103], activation of Retinoid Acid Receptor (RAR)-dependent VEGF secretion in RPE [104, 105], and inhibition of RPE-65 isomerase activity, which limits the RPE supply of 11-cis retinal [106]. In summary, although numerous mechanisms of toxicity elicited by pathologic accumulation of LBs in RPE lysosomes have been proposed, no viable therapeutic options have resulted yet from targeting them. Hence, strategies to reduce LB accumulation from RPE have been further investigated.

2. Strategies to reduce lipofuscin accumulation in RPE cells

Alternative strategies to mitigate the cytotoxic effects of LBs involve preventing their accumulation. Two approaches have been pursued (1) to prevent de novo formation of LB and (2) to remove previously accumulated LB.

2.1. Strategies that prevent de novo formation of LBs

Long-term restriction of vitamin A intake has been shown to reduce retinaldehyde levels in RPE but is not a therapeutic option, since it causes night blindness and systemic hypovitaminosis [107]. In 2005, Radu et al. [108] showed that oral administration of a synthetic form of vitamin A (fenretinide), already in use against cancer, acne, cystic fibrosis, rheumatoid arthritis, and psoriasis, could competitively block RBP4 transport of vitamin A from the

Figure 5. LBs form crystal-like aggregates in aqueous environment. (A) Turbidity of A2E dilutions in water versus 0.1 M βCD. (B) Passage through 0.10 micron pore filters (MW cutoff of 300,000 Da) of LBs in water or βCD solutions. (C) Autofluorescent detection of A2E (MW 592 Da) and ATRD (600 Da) in pre and post filtrates. Retention was due to size exclusion since they crossed 3 micron filters of identical material (not shown).
blood to the RPE. Oral fenretinide produced mild reversible skin dryness and night blindness. However, in 2011, a phase-2 study on 225 AMD patients failed to show beneficial effects. Oral emixustat hydrochloride is a synthetic nonretinoid reversible inhibitor of the RPE65 enzyme, which converts all-trans-retinyl to 11-cis-retinal, a rate-limiting reaction of the visual cycle. This drug showed minimal toxicity in phase-1 trials and effectively reduced photoreceptor response to light, consistent with its mechanism of action. However, in May 2016, the results from the phase 2b/3 SEATTLE study did not show any significant difference in retinal degenerative rate or visual acuity changes. Oral deuterated vitamin A (ALK-001), is vitamin A modified by replacing hydrogen with deuterium, a safe, nonradioactive isotope. Deuterated vitamin A has lower tendency to spontaneously dimerize into LBs. Long-term, oral administration of ALK-001 to ABCA4−/− reduced the accumulation of lipofuscin and A2E by 70 and 80%, respectively [109]. Assessment of the retina electric response to light signals (electroretinogram) revealed that ALK-001 treatment prevented the gradual loss of visual function observed in the ABCA4−/− mouse. Safety phase-1 clinical trials have been completed but phase-2 is ongoing. It is too early to know whether ALK-001 will be beneficial for Stargardt or AMD patients.

Oral aldehyde traps (VM200, Vision Medicines) constitute of new drugs that react with retinaldehydes forming reversible Schiff bases and thus, reducing the available levels of free aldehydes with cellular amine groups. In preclinical studies, VM200 preserved retinal structure and function of mice retinas in a dose-dependent manner [7]. Safety phase-1 is in progress but there is no effectiveness data in humans.

In summary, none of the visual cycle modulators have made it out of the nearly half a dozen phase 1 and phase 2 clinical trials, so far. They all cause significant night blindness that limit their use. Although they all seem very effective at slowing down the formation of new LBs in animals, they lack effect on previously accumulated LB, which may explain why fenretinide or emixustat did not benefit patients already diagnosed with AMD. Likewise in animal models, where these drugs are given preventively for long periods, humans may need to take them early, i.e., much before the clinical manifestations appear, and for life.

2.2. Strategies for removing previously accumulated LB

Potentially, their main advantage over agents that prevent accumulation of LBs is that they might be administered to patients with Stargardt or AMD who display large buildup of LBs. Most of these strategies are in preclinical stage.

2.2.1. Oral soraprazan (Katairo GmbH)

In 2012, Schraermeyer’s group reported successful elimination of lipofuscin from RPE cells in monkey retinas after 1 year of oral administration of the drug. Researchers showed that lipofuscin granules were expelled by RPE cells toward their basolateral side and were cleared by macrophages recruited to the area [110]. Although soraprazan is known to reversibly block the potassium binding site of the gastric H+/K+ ATPase proton pump, the precise mechanism by which it causes clearance of LB deposits is unknown.
2.2.2. Enzymatic degradation of LBs

Because LBs are refractory to degradation by lysosomal hydrolases, several groups searched for exogenous enzymes with LB destroying activity. Horseradish peroxidase (HRP) was the first one identified [111]. It catalyzes the oxidative cleavage of the polyene–arms of LBs. To test the effectiveness of HRP, cultures of RPE cells preloaded with LBs underwent an enzyme replacement therapy-like treatment with HRP. The efficiency of the clearance was low but the major problem was the considerable amount of highly toxic reactive molecules released as a by-product of the HRP-mediated oxidation of LBs [112]. It was more recently reported in Ref. [113] that neutrophil myeloperoxidase (MPO) catalyzes the in vitro degradation of A2E. The authors delivered MPO to lysosomes of RPE cells via mannose-6-phosphate (M6P) receptor. M6P-MOP exhibited a half-life of 10 h in the lysosomes and degraded lysosomal A2E in, but also disrupted lysosomal acidification and triggered lysosomal stress, manifested by the nuclear translocation of TFEB that eventually led to cell death. Thus, the strategy of eliminating LBs with peroxidases seems to be limited by the inherent associated release of detrimental reactive species, which would be equivalent to try to clear LBs by photooxidation.

2.2.3. Beta-cyclodextrins (β-CDs)

βCDs are membrane-impermeant cyclic sugars made of seven glucose residues. They contain a nonpolar central cavity that is capable of accommodating hydrophobic ligands and a hydrophilic outer surface that makes them soluble in water [114]. Several FDA-approved cyclodextrins are currently used to improve the delivery of lipophilic drugs. We demonstrated that βCDs form soluble complexes with LBs [115] (Figure 5). In silico modeling predicted 2:1 βCD-A2E complex, where one βCD accommodates per arm of LB. We also observed that βCDs reduced the content of A2E from polarized RPE monolayers on Transwell filter cultures and from RPE in the eyes of mice that accumulate massive amounts of lysosomal LBs [116] indicating that βCD treatment can eliminate not only A2E, but also the complex LB mixes found in RPE lipofuscin. The mechanisms by which βCDs induce clearance of lysosomes’ content in RPE is yet to be determined but could potentially be optimized to develop a novel therapeutic approach to clear LB-buildups. The mechanism of cholesterol removal by βCDs is one of the best characterized. Likewise LBs, cholesterol forms soluble inclusion complexes with βCDs. In normal cells, cholesterol is more abundantly present in plasma membrane, common recycling endosome and trans-Golgi complex [117]. Removal of cholesterol from these membranes, requires high concentrations (5–10 mM) and prolonged times because βCDs, which have an ~8 Å deep cavity, must form stacked dimers (improbable event) to remove 18 Å long cholesterols out of the lipid bilayer and shelter them from the water [118]. In cells with Niemann Pick Type-C defect, i.e., with inactivating mutations in either NPC1 or NPC2 genes, that code for two intra-lysosomal lipid transporter proteins, cholesterol is, also, aberrantly accumulated within lysosomes. Removal of lysosomal cholesterol buildups requires lower concentrations (0.1–1.0 mM) and shorter incubation times with βCD [119]. Furthermore, sulfo-butyl-ether-βCD (Captisol®), a βCD derivative that cannot form stacked dimers and that therefore cannot solubilize membrane cholesterol, can still reduce lysosomal buildups [120]. The model for βCD-mediated removal of lysosomal cholesterol proposes that
βCD enters lysosomes by endocytosis, where it binds free cholesterol in the lumen and shuttle it to the limiting lysosomal membrane [121, 122]. From there, cholesterol is transferred, by a not fully characterized trafficking machinery, that probably involves points of membrane contact between organelles and cholesterol binding proteins [123], to the ER, plasma membrane, peroxisomes, and mitochondria [124, 125]. In the absence of extracellular cholesterol acceptor molecules, the stoichiometric analysis of βCD clearance provides no evidence of cholesterol release to the media but rather indicates a rapid metabolic processing within the cytosolic compartment [126, 127]. In the case of LBs, if βCDs clearance works similarly, then it would be important to see what putative LB-transport system acts thereafter to ship LBs for degradation [85, 128] or to expel them from the cell. Confirmation of such operating trafficking pathway could represent an important advance to identify pharmacological targets for the elimination of lysosomal LBs.

In a mice model of atherosclerosis [129], βCDs have shown effective removal of cholesterol crystals from macrophage foam cells. The mechanism in this case seems to be mostly mediated by the execution of a LXR (liver-X-receptor) dependent transcriptional program response that enhanced the efflux and degradation of cholesterol and reduced inflammation.

Administration of βCD also lowered the levels of amyloid-β in an animal model of Alzheimer Disease [130] and from drusen deposits in animal models of Stargardt disease [131]. The mechanism in these cases is less clear but it seems to be transcriptionally controlled. Accordingly, it will be important to determine if βCDs trigger a transcriptional program that primes RPE cells to eliminate its lysosomal content, independently on whether they form soluble complex with the wasted material. Similarly, fibroblasts from patients with ceroid-lipofuscinosisis, the most common cause of neurodegeneration of children in the United States, and cellular or animal models with misfolded α-synuclein accumulation were cleared by βCDs [132, 133]. The mechanism in these cases seemed to be mediated by TFEB [86, 134]. The pathway responsible for βCD activation of TFEB is not defined and is induced by millimolar doses of cyclodextrins. βCDs appear to induce autophagy [135] and exocytosis of lysosomes [136]. Finally, overexpression of activated TFEB has been demonstrated to ameliorate pathology in late-onset neurodegenerative diseases such as Parkinson, Huntington and Alzheimer, as well as in models of spinal and bulbar muscular atrophy and to clear deposits in lysosomal storage disorders (LSDs) [83, 86, 137–139]. Thus, it will be important to determine whether TFEB is necessary or if it can synergistically contribute to the clearance of RPE lipofuscin.

3. Conclusions

Buildup of lipofuscin in RPE lysosomes often evolves into irreversible damage of overlying photoreceptors. This is a common event in individuals with mutations in ABCA4 gene and is believed to underlie the progression of age-related lesions in AMD people, the most common cause of blindness in the elderly population. Unfortunately, the great majority of patients diagnosed with this problem have no therapeutic options available. Analysis of RPE lipofuscin, identified as major components lipid bisretinoids (LBs), sub-products of the spontaneous dimerization of retinaldehydes produced during the visual cycle. Targeting LBs secondary
complications with oral antioxidants, inhibitors of complement or autophagy inducers provided no or little beneficial effect. The recent failures in clinical trials with visual cycle modulators, which prevent \textit{de novo} formation of LBs may reflect the incapacity of these drugs to stop degeneration once LB-accumulation has been established, which is probably the case for most individuals with clinical symptoms. Hence, the development of novel strategies to permanently remove previously accumulated lipofuscin is an urgent medical need. The characterization of LBs as the core-components of RPE lipofuscin has permitted to rationally develop strategies to remove them from RPE cells in the laboratory. This, combined with an improved understanding of the molecular pathways that govern autophagy and stimulate cellular clearance might allow in the near future, to develop improved therapies for retinal degenerations resulting from genetic or age-related retinal lipofuscin accumulation.

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**Author details**

Marcelo M. Nociari*, Szilard Kiss and Enrique Rodriguez-Boulan*

*Address all correspondence to: mnociari@med.cornell.edu and boulan@med.cornell.edu

Department of Ophthalmology, Weill Cornell Medicine, Margaret Dyson Vision Research Institute, Cornell University, New York, NY, USA

**References**

[1] Bok D. The retinal pigment epithelium: a versatile partner in vision, J. Cell Sci. 1993;189 LP-195

[2] Streilein JW, Ma N, Wenkel H, Fong Ng T, Zamiri P. Immunobiology and privilege of neuronal retina and pigment epithelium transplants. Vision Research. 2002;42:487-495

[3] Wald G. The molecular basis of visual excitation. Nature. 1968;219:800-807

[4] Fu Y. Phototransduction in rods and cones. In: Webvision: The Organisation of the Retina and Visual System. 2010: pp. 1-47

[5] Palczewski K. JBC Papers in Press. Published on November 10, 2011 as Manuscript R111.301150 The latest version is at http://www.jbc.org/cgi/doi/10.1074/jbc.R111.301150. 2011. DOI: 10.1074/jbc.R111.301150
[6] Wang JS, Kefalov VJ. The cone-specific visual cycle. Progress in Retinal and Eye Research. 2011;30:115-128. DOI: http://dx.doi.org/10.1016/j.preteyeres.2010.11.001

[7] Maeda A, Golczak M, Chen Y, Okano K, Kohno H, Shiose S, Ishikawa K, Harte W, Palczewska G, Maeda T, Palczewski K. Primary amines protect against retinal degeneration in mouse models of retinopathies. Nature Chemical Biology. 2012;8:170-178. DOI: 10.1038/nchembio.759

[8] Boyer NP, Higbee D, Currin MB, Blakeley LR, Chen C, Ablonczy Z, Crouch RK, Koutalos Y. Lipofuscin and N-retinylidene-N-retinylethanolamine (A2E) accumulate in retinal pigment epithelium in absence of light exposure: Their origin is 11-cis-retinal. Journal of Biological Chemistry. 2012;287:22276-22286. DOI: 10.1074/jbc.M111.329235

[9] Wu Y, Li J, Yao K. Structures and biogenetic analysis of lipofuscin bis-retinoids. Biomedical & Biotechnology. 2013;14:763-773. DOI: 10.1631/jzus.B1300051

[10] Young RW. The renewal of photoreceptor cell outer segments. Journal of Cell Biology. 1967;33:61-72

[11] McMahon DG, Iuvone PM, Tosini G. Circadian organization of the mammalian retina: From gene regulation to physiology and diseases. Progress in Retinal and Eye Research. 2014;0:58-76. DOI: 10.1016/j.preteyeres.2013.12.001

[12] Kim J, Zhao H, Martinez J, Doggett TA, Alexander V, Tang PH, Ablonczy Z, Chan CC, Zhou Z, Green R, Ferguson TA. Non-canonical autophagy promotes the visual cycle. Cell. 2014;154:365-376. DOI: 10.1016/j.cell.2013.06.012.

[13] Mao Y, Finnemann SC. Regulation of phagocytosis by Rho GTPases. Small GTPases. 2015;6:1-11. DOI: 10.4161/21541248.2014.989785

[14] Croce AC, Bottiroli G. Autofluorescence spectroscopy and imaging: A tool for biomedical research and diagnosis. European Journal of Histochemistry. 2014;58:2461. DOI: 10.4081/ejh.2014.2461

[15] Farin S, Porta EA. Sequential histochemical studies of neuronal lipofuscin in human cerebral cortex from the first to the ninth decade of life. Archives of Gerontology and Geriatrics. 2002;34:219-231

[16] Brunk UT, Terman A. Lipofuscin: Mechanisms of age-related accumulation and influence on cell function. Free Radical Biology and Medicine. 2002;33:611-619. DOI: 10.1016/S0891-5849(02)00959-0

[17] Delori FC, Dorey CK, Staurenghi G, Arend O, Goger DG, Writer JJ. In vivo fluorescence of the ocular fundus exhibits retinal pigment epithelium lipofuscin characteristics. Investigative Ophthalmology & Visual Science. 1995;36:718-729

[18] Weirer JJ, Delori FC, Wing GL, Fitch KA. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. Investigative Ophthalmology & Visual Science. 1986;27:145-152
[19] Parish CA, Hashimoto M, Nakanishi K, Dillon J, Sparrow J. Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium. Proceedings of the National Academy of Sciences of United States of America. 1998;95:14609-14613

[20] Feeney-Burns L, Hilderbrand ES, Eldridge S. Aging human RPE: Morphometric analysis of macular, equatorial, and peripheral cells. Investigative Ophthalmology & Visual Science. 1984;25:195-200

[21] Eldred GE, Katz MI. The autofluorescent products of lipid peroxidation may not be lipofuscin-like. Free Radical Biology & Medicine. 1989;7:157-163. DOI: 10.1016/0891-5849(89)90007-5

[22] Eldred GE, Lasky MR. Retinal age pigments generated by self-assembling lysosomotropic detergents. Nature. 1993;361:724-726

[23] Ren RX, Sakai N, Nakanishi K. Total synthesis of the ocular age pigment A2-E: A convergent pathway. Journal of the American Chemical Society. 1997;7863:3619-3620

[24] Sparrow JR, Kim SR, Wu Y. Experimental approaches to the study of A2E, a bisretinoid lipofuscin chromophore of retinal pigment epithelium. Methods in Molecular Biology. 2010;652:315-327. DOI: 10.1007/978-1-60327-325-1_18

[25] Boulton ME. Studying melanin and lipofuscin in RPE cell culture models. Experimental Eye Research. 2014;126:61-67. DOI: 10.1016/j.exer.2014.01.016

[26] Kiser PD, Golczak M, Palczewski K. Chemistry of the retinoid (visual) cycle. Chemical Reviews. 2013;114:194-232. DOI: 10.1021/cr400107q

[27] Kim SR, Jang YP, Jockusch S, Fishkin NE, Turro NJ, Sparrow JR. The all-trans-retinal dimer series of lipofuscin pigments in retinal pigment epithelial cells in a recessive Stargardt disease model. Proceedings of the National Academy of Sciences of United States of America. 2007;104:19273-19278. DOI: 10.1073/pnas.0708714104

[28] Sparrow JR, Gregory-Roberts E, Yamamoto K, Blonska A, Ghosh SK, Ueda K, Zhou J. The bisretinoids of retinal pigment epithelium. Progress in Retinal and Eye Research. 2012;31:121-135. DOI: 10.1016/j.preteyeres.2011.12.001

[29] Murdaugh LS, Dill AE, Dillon J, Simon JD, Gaillard ER. Age-related changes in rpe lipofuscin lead to hydrophobic polymers. In: Studies on Retinal and Choroidal Disorders. 2012:pp. 113-139. DOI: 10.1007/978-1-61779-606-7

[30] Schutt F, Ueberle B, Schno M, Holz FG, Kopitz J. Proteome analysis of lipofuscin in human retinal pigment epithelial cells. FEBS Letters. 2002;528:217-221

[31] Schutt F, Bergmann M, Holz FG, Kopitz J. Proteins modified by malondialdehyde, 4-hydroxynonenal , or advanced glycation end products in lipofuscin of human retinal pigment epithelium. Investigative Ophthalmology & Visual Science. 2017;44:3663-3668. DOI: 10.1167/iovs.03-0172
[32] Warburton S, Southwick K, Hardman RM, Secrest AM, Grow RK, Xin H, Woolley AT, Burton GF, Thulin CD. Examining the proteins of functional retinal lipofuscin using proteomic analysis as a guide for understanding its origin. Molecular Vision. 2005;11:1122-1134

[33] Brunet S, Thibault P, Gagnon E, Kearney P, Bergeron JJM, Desjardins M. Organelle proteomics: Looking at less to see more. Trends in Cell Biology. 2003;13:629-638. DOI: 10.1016/j.tcb.2003.10.006

[34] Ng K, Gugiu B, Renganathan K, Davies MW, Gu X, Crabb JSWSW, Kim SR, Rózanowska MB, Bonilha VL, Rayborn ME, Salomon RG, Sparrow JR, Boulton ME, Hollyfield JG, Crabb JSWSW, Malgorzata B. Retinal pigment epithelium lipofuscin proteomics. Molecular & Cellular Proteomics. 2008;7:1397-1405. DOI: 10.1074/mcp.M700525-MCP200

[35] H.E. Bazan, N.G. Bazan, L. Feeney-Burns, E.R. Berman, Lipids in human lipofuscin-enriched subcellular fractions of two age populations. Comparison with rod outer segments and neural retina., Invest. Ophthalmol. Vis. Sci. 1990;31:1433-1443

[36] Katz ML, Redmond TM. Effect of Rpe65 knockout on accumulation of lipofuscin fluorophores in the retinal pigment epithelium. Investigative Ophthalmology & Visual Science. 2001;42

[37] Molday RS, Zhong M, Quazi F. The role of the photoreceptor ABC transporter ABCA4 in lipid transport and Stargardt macular degeneration. Biochimica et Biophysica Acta. 2009;1791:573-583. DOI: 10.1016/j.bbalip.2009.02.004

[38] Mihai DM, Washington I. Vitamin A dimers trigger the protracted death of retinal pigment epithelium cells. Cell Death & Disease. 2014;5:e1348. DOI: 10.1038/cddis.2014.314

[39] Rózanowska M, Jarvis-Evans J, Korytowski W, Boulton M, Burke J, Sarna T. Blue light-induced reactivity of retinal age pigment. Journal of Biological Chemistry. 1995:18825-18830

[40] Schu F, Davies S, Holz FG, Boulton ME. Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. Investigative Ophthalmology & Visual Science. 2000;41:2303-2308

[41] Yamamoto K, Yoon KD, Ueda K, Hashimoto M, Sparrow JR. A novel bisretinoid of retina is an adduct on glycerophosphoethanolamine. Investigative Ophthalmology & Visual Science. 2011;52:9084-9090. DOI: 10.1167/iovs.11-8632

[42] Sparrow JR, Nakanishi K, Parish CA. The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. Investigative Ophthalmology & Visual Science. 2000;41:1981-1989

[43] Boulton M, Dontsov A, Jarvis-Evans J, Ostrovsky M, Svistunenko D. Lipofuscin is a photoinducible free radical generator. Journal of Photochemistry & Photobiology B. 1993;19:201-204

[44] Sparrow JR, Zhou J, Ben-Shabat S, Vollmer H, Itagaki Y, Nakanishi K. Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE. Investigative Ophthalmology & Visual Science. 2002;43:1222-1227
[45] Ben-shabat S, Itagaki Y, Jockusch S, Sparrow JR, Turro NJ, Nakanishi K. Formation of a nonaoxirane from A2E, degeneration, and evidence of singlet oxygen involvement. Angewandte Chemie (International Ed. in English). 2002;41:814-817

[46] Washington I, Jockusch S, Itagaki Y, Turro NJ, Nakanishi K. Superoxidation of bis-retinoids. Angewandte Chemie (International Ed. in English). 2005;44:7097-7100. DOI: 10.1002/anie.200501346

[47] Yoon KD, Yamamoto K, Ueda K, Zhou J, Sparrow JR. A novel source of methylglyoxal and glyoxal in retina: Implications for age-related macular degeneration. PLoS One. 2012;7:e41309. DOI: 10.1371/journal.pone.0041309

[48] Wang Z, Keller LMM, Dillon J, Gaillard ER, Oxidation of A2E results in the formation of highly reactive aldehydes and ketones. Photochemistry and Photobiology. 2006;82:1251-1257. DOI: 10.1562/2006-04-01-RA-864

[49] Yoon KD, K Yamamoto, J Zhou, JR Sparrow, Photo-products of retinal pigment epithelial bisretinoids react with cellular thiols, 2011 1839-1849

[50] Sparrow JR, Zhou J, Cai B. DNA is a target of the photodynamic effects elicited in A2E-laden RPE by blue-light illumination. Investigative Ophthalmology & Visual Science. 2003;44:2245. DOI: 10.1167/iovs.02-0746

[51] Zhang X, Zhou J, Fernandes AF, Sparrow JR, Pereira P, Taylor A, Shang F. The proteasome: A target of oxidative damage in cultured human retina pigment epithelial cells. Investigative Ophthalmology & Visual Science. 2008;49:3622-3630. DOI: 10.1167/iovs.07-1559

[52] J. Zhou, K. Ueda, J. Zhao, J.R. Sparrow, Correlations between Photodegradation of Bisretinoid Constituents of Retina and Dicarbonyl Adduct Deposition *, J. Biol. Chem. 2015;290:27215-27227

[53] Kanda A, Abecasis G, Swaroop A. Inflammation in the pathogenesis of age-related macular degeneration. British Journal of Ophthalmology. 2008;92:448-450. DOI: 10.1136/bjo.2007.131581

[54] Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL. Oxidative damage-induced inflammation initiates age-related macular degeneration. Nature Medicine. 2008;14:194-198. DOI: 10.1038/nm1709

[55] Wu L, Ueda K, Nagasaki T, Sparrow JR, Light damage in Abca4 and Rpe65rd12 mice. Investigative Ophthalmology & Visual Science. 2014;55:1910-1918. DOI: 10.1167/iovs.14-13867

[56] K. Ueda, J. Zhao, H.J. Kim, J.R. Sparrow, H. Jin, J.R. Sparrow, Photodegradation of retinal bisretinoids in mouse models and implications for macular degeneration., Proc. Natl. Acad. Sci. U. S. A. 2016;113:6904-6909

[57] Ablonczy Z, Higbee D, Anderson DM, Dahrouj M, Grey AC, Gutierrez D, Koutalos Y, Schey KL, Hanneken A, Crouch RK. Lack of correlation between the spatial distribution of
A2E and lipofuscin fluorescence in the human retinal pigment epithelium. Investigative Ophthalmology & Visual Science. 2013;54:5535-5542. DOI: 10.1167/iovs.13-12250

[58] Grey AC, Crouch RK, Koutalos Y, Schey KL, Ablonczy Z. Spatial localization of A2E in the retinal pigment epithelium. Investigative Ophthalmology & Visual Science. 2011;52:3926-3933. DOI: 10.1167/iovs.10-7020

[59] J.R. Evans, J.G. Lawrenson, K.S. Henshaw, J.G. Lawrenson, Antioxidant vitamin and mineral supplements for preventing age-related macular degeneration, Cochrane Libr. 2012;11:CD000254

[60] Evans JR, Lawrenson JG. Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. Cochrane Database Systems Reviews. 2012;11:CD000254. DOI: 10.1002/14651858.CD000254.pub3

[61] Downie LE, Keller PR. Degeneration : Research evidence in practice. Optometry and Vision Science. 2014;91:821-831

[62] Zhou J, Kim SR, Westlund BS, Sparrow JR. Complement activation by bisretinoid constituents of RPE lipofuscin. Investigative Ophthalmology & Visual Science. 2009;50:1392-1399. DOI: 10.1167/iovs.08-2868

[63] Bradley DT, Zipfel PF, Hughes AE. Complement in age-related macular degeneration: A focus on function. Eye. 2011;25:683-693. DOI: 10.1038/eye.2011.37

[64] T.L. Lenis, S. Sarfare, Z. Jiang, M.B. Lloyd, D. Bok, R.A. Radu, Complement modulation in the retinal pigment epithelium rescues photoreceptor degeneration in a mouse model of Stargardt disease., Proc. Natl. Acad. Sci. U. S. A. 2017;114:3987-3992

[65] Hanus J, Zhao F, Wang S. Current therapeutic developments in atrophic age-related macular degeneration. 2016:122-127. DOI: 10.1136/bjophthalmol-2015-306972

[66] Chen Y, Sawada O, Kohno H, Le YZ, Subauste C, Maeda T, Maeda A. Autophagy protects the retina from light-induced degeneration. Journal of Biological Chemistry. 2013;288:7506-7518. DOI: 10.1074/jbc.M112.439935

[67] Mitter SK, Rao HV, Qi X, Cai J, Sugrue A, Dunn WA, Grant MB, Boulton ME. Autophagy in the retina: A potential role in age-related macular degeneration, In: LaVail MM, Ash JD, Anderson RE, Hollyfield JG, Grimm C (Eds.), Retinal Degenerative Diseases. Springer US: Boston, MA;2012: pp. 83-90. DOI: 10.1007/978-1-4614-0631-0_12

[68] Yao J, Jia L, Shelby SJ, Ganios AM, Feathers K, Thompson DA, Zacks DN. Circadian and noncircadian modulation of autophagy in photoreceptors and retinal pigment epithelium:circadian and noncircadian modulation of autophagy. Investigative Ophthalmology & Visual Science. 2014;55:3237-3246

[69] Remé CE, Wolfrum U, Imsand C, Hafezi F, Williams TP. Photoreceptor autophagy: Effects of light history on number and opsin content of degradative vacuoles. Investigative Ophthalmology & Visual Science. 1999;40:2398-2404
[70] Mitter SK, Song C, Qi X, Mao H, Rao H, Akin D, Lewin A, Grant M, Dunn W, Ding J, Bowes Rickman C, Boulton M. Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. Autophagy. 2014;10:1989-2005. DOI: 10.4161/auto.36184

[71] Yao J, Jia L, Khan N, Lin C, Mitter SK, Boulton ME, Dunaief JL, Klionsky DJ, Guan JL, Thompson DA, Zacks DN. Deletion of autophagy inducer RB1CC1 results in degeneration of the retinal pigment epithelium. Autophagy. 2015;11:939-953. DOI: 10.1080/15548627.2015.1041699

[72] Eagle RC Jr., Lucier AC, Bernardino VB Jr., Yanoff M. Retinal pigment epithelial abnormalities in fundus flavimaculatus: A light and electron microscopic study. Ophthalmology. 1980;87:1189-1200

[73] T. Ach, E. Tolstik, J.D. Messinger, A. V Zarubina, R. Heintzmann, C.A. Curcio, Lipofuscin Redistribution and Loss Accompanied by Cytoskeletal Stress in Retinal Pigment Epithelium of Eyes With Age-Related Macular Degeneration, Invest. Ophthalmol. Vis. Sci. 2015;56:3242-3252

[74] Holz FG, Schütt F, Kopitz J, Eldred GE, Kruse FE, Völcker HE, Cantz M. Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of lipofuscin. Investigative Ophthalmology & Visual Science. 1999;40:737-743

[75] Vives-bauza C, Anand M, Shirazi AK, Magrane J, Gao J, Vollmer-snarr HR, Manfredi G, Finnemann SC, Shiraz AK, Shirazi AK, Magrane J, Gao J, Vollmer-snarr HR, Manfredi G, Finnemann SC. The age lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells. Journal of Biological Chemistry. 2008;283:24770-24780. DOI: 10.1074/jbc.M800706200

[76] M. Bergmann, F. Schütt, F.G. Holz, J. Kopitz, Inhibition of the ATP-driven proton pump in RPE lysosomes by the major lipofuscin fluorophore A2-E may contribute to the pathogenesis of age-related macular degeneration, FASEB J. 2004;8:562-564

[77] Bermann M, Schütt F, Holz FG, Kopitz J. Does A2E, a retinoid component of lipofuscin and inhibitor of lysosomal degradative functions, directly affect the activity of lysosomal hydrolases?. Experimental Eye Research. 2001;72:191-195. DOI: 10.1006/exer.2000.0949

[78] Yamamoto A, Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, Tashiro Y. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. Cell Structure and Function. 1998;23:33-42. DOI: 10.1247/csf.23.33

[79] Guha S, Liu J, Baltazar G, Laties AM, Mitchell CH. Rescue of compromised lysosomes enhances degradation of photoreceptor outer segments and reduces lipofuscin-like autofluorescence in retinal pigmented epithelial cells. Advances in Experimental Medicine and Biology. 2014;801:105-111. DOI: 10.1007/978-1-4614-3209-8_14
[80] Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H+-ATPase. Science (80-.). 2011;334:678-683. DOI: 10.1126/science.1207056

[81] Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, Sabatini DM. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science (80-.). 2008;320:1496-1501

[82] Benjamin D, Hall MN. mTORC1: turning off is just as important as turning on. Cell. 2014;156:627-628. DOI: http://dx.DOI.org/10.1016/j.cell.2014.01.057

[83] Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, Gennarino VA, Di Malta C, Donaudy F, Embrione V, Polishchuk RS, Banfi S, Parenti G, Cattaneo E, Ballabio A. A gene network regulating lysosomal biogenesis and function. Science (80-.). 2009;325:473-478

[84] Palmieri M, Impey S, Kang H, di Ronza A, Pelz C, Sardiello M, Ballabio A,. Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. Human Molecular Genetics. 2011;20:3852-3866. DOI: 10.1093/hmg/ddr306

[85] Settembre C, Fraldi A, Medina DL, Ballabio A, Signals from the lysosome: A control centre for cellular clearance and energy metabolism. Nature Reviews Molecular Cell Biology. 2013;14:283-296. DOI: 10.1038/nrm3565

[86] Medina DL, Fraldi A, Bouche V, Annunziata F, Mansueto G, Spamanato C, Puri C, Pignata A, Martina JA, Sardiello M, Palmieri M, Polishchuk R, Puertollano R, Ballabio A. Transcriptional activation of lysosomal exocytosis promotes cellular clearance. Developmental Cell. 2011;21:421-430. DOI: 10.1016/j.devcel.2011.07.016

[87] Ghosh A, Pahan K. PPARa in lysosomal biogenesis: A perspective. Pharmacological Research. 2016;103:144-148. DOI: 10.1016/j.phrs.2015.11.011

[88] Yu B, Xu P, Zhao Z, Cai J, Sternberg P, Chen Y. Subcellular distribution and activity of mechanistic target of rapamycin in aged retinal pigment epithelium dysregulated mTORC1 signaling in aged RPE. Investigative Ophthalmology & Visual Science. 2014;55:8638-8650

[89] Chen Y, Wang J, Cai J, Sternberg P. Altered mTOR signaling in senescent retinal pigment epithelium. Investigative Ophthalmology & Visual Science. 2010;51:5314-5319. DOI: 10.1167/iovs.10-5280

[90] Martina JA, Diab HI, Lishu L, Jeong-A L, Patange S, Raben N, Puertollano R. The nutrient-responsive transcription factor TFE3 promotes autophagy, lysosomal biogenesis, and clearance of cellular debris. Science Signaling. 2014;7:ra9. DOI: 10.1126/scisignal.2004754

[91] Zhang J, Bai Y, Huang L, Qi Y, Zhang Q, Li S, Wu Y, Li X. Protective effect of autophagy on human retinal pigment epithelial cells against lipofuscin fluorophore A2E: implications for age-related macular degeneration. Cell Death & Disease. 2015;6:e1972. DOI: 10.1038/cddis.2015.330
[92] Wong WT, Dresner S, Forooghian F, Glaser T, Doss L, Zhou M, Cunningham D, Shimel K, Harrington M, Hammel K, Cukras CA, Ferris FL, Chew EY. Treatment of geographic atrophy with subconjunctival sirolimus: Results of a phase I/II clinical trial subconjunctival sirolimus for treatment of GA. Investigative Ophthalmology & Visual Science. 2013;54:2941-2950

[93] Rodríguez-Muela N, Koga H, García-Ledo L, de la Villa P, de la Rosa EJ, Cuervo AM, Boya P. Balance between autophagic pathways preserves retinal homeostasis. Aging Cell. 2013;12:478-488. DOI: 10.1111/acel.12072

[94] De S, Sakmar TP. Interaction of A2E with model membranes. Implications to the pathogenesis of age-related macular degeneration. Journal of General Physiology. 2002;120:147-157. DOI: 10.1085/jgp.20028566

[95] Schutt F, Bergmann M, Holz FG, Kopitz J. Isolation of intact lysosomes from human RPE cells and effects of A2-E on the integrity of the lysosomal and other cellular membranes. Graefe’s Archive for Clinical and Experimental Ophthalmology. 2002;240:983-988. DOI: 10.1007/s00417-002-0558-8

[96] Rajamäki K, Lappalainen J, Oörni K, Välimäki E, Matikainen S, Kovanen PT, Eklund KK, Va E, Matikainen S, Petri T, Rajama K, Eklund KK. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: A novel link between cholesterol metabolism and inflammation. PLoS One. 2010;5:e11765. DOI: 10.1371/journal.pone.0011765

[97] Grebe A, Latz E. Cholesterol crystals and inflammation. Current Rheumatology Reports. 2013;15:313. DOI: 10.1007/s11926-012-0313-z

[98] Anderson OA, Finkelstein A, Shima DT. A2E induces IL-1β production in retinal pigment epithelial cells via the NLRP3 inflammasome. PLoS One. 2013;8:e67263. DOI: 10.1371/journal.pone.0067263

[99] Haralampus-grynaviski NM, Lamb LE, Clancy CMR, Skumatz C, Burke JM, Sarna T, Simon JD. Spectroscopic and morphological studies of human retinal lipofuscin granules. Proceedings of the National Academy of Sciences. 2003;100:3179-3184

[100] Schutt F, Bergmann M, Holz FG, Dithmar S, Volcker HE, Kopitz J. Accumulation of A2-E in mitochondrial membranes of cultured RPE cells. Graefe’s Archive for Clinical and Experimental Ophthalmology. 2007;245:391-398. DOI: 10.1007/s00417-006-0376-5

[101] Repnik U, Hafner Česen M, Turk B. Lysosomal membrane permeabilization in cell death: Concepts and challenges. Mitochondrion. 2014;19:49-57. DOI: 10.1016/j.mito.2014.06.006

[102] Vives-bauza C, Anand M, Shiraz AK, Shirazi AK, Magrane J, Gao J, Vollmer-snarr HR, Manfredi G, Finnemann SC. The age lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells. Journal of Biological Chemistry. 2008;283:24770-24780. DOI: 10.1074/jbc.M800706200
[103] Lakkaraju A, Finnemann SC, Rodriguez-Boulan E. The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. Proceedings of the National Academy of Sciences of the United States of America. 2007;104:11026-11031. DOI: 10.1073/pnas.0702504104

[104] Iriyama A, Fujiki R, Inoue Y, Takahashi H, Tamaki YY, Takezawa S, Takeyama K, Jang WD, Kato S, Yanagi Y. A2E, a pigment of the lipofuscin of retinal pigment epithelial cells, is an endogenous ligand for retinoic acid receptor. Journal of Biological Chemistry. 2008;283:11947-11953. DOI: 10.1074/jbc.M708989200

[105] Iriyama A, Inoue Y, Takahashi H, Tamaki Y, Jang W, Yanagi Y. A2E, a component of lipofuscin, is pro-angiogenic in vivo. Journal of Cellular Physiology. 2009;220:469-475. DOI: 10.1002/jcp.21792

[106] Moiseyev G, Nikolaeva O, Chen Y, Farjo K, Takahashi Y, Ma J. Inhibition of the visual cycle by A2E through direct interaction with RPE65 and implications in Stargardt disease. Proceedings of the National Academy of Sciences of the United States of America. 2010;107:17551-17556

[107] Singer JR, Bakall B, Gordon GM, Reddy RK. Treatment of vitamin A deficiency retinopathy with sublingual vitamin A palmitate. Documenta Ophthalmologica 2016;132:137-145. DOI: 10.1007/s10633-016-9533-2

[108] Radu RA, Han Y, Bui TV, Nusinowitz S, Bok D, Lichter J, Widder K, Travis GH, Mata NL. Reductions in serum vitamin A arrest accumulation of toxic retinal fluorophores: A potential therapy for treatment of lipofuscin-based retinal diseases. Investigative Ophthalmology & Visual Science. 2005;46:4393-4401

[109] Charbel Issa P, Barnard AR, Herrmann P, Washington I, MacLaren RE. Rescue of the Stargardt phenotype in Abca4 knockout mice through inhibition of vitamin A dimerization. Proceedings of the National Academy of Sciences. 2015;112:8415-8420. DOI: 10.1073/pnas.1506960112

[110] Julien S, Schraermeyer U. Lipofuscin can be eliminated from the retinal pigment epithelium of monkeys. Neurobiology of Aging. 2012;33:2390-2397. DOI: 10.1016/j.neurobiolaging.2011.12.009

[111] Wu Y, Zhou J, Fishkin N, Rittmann BE, Sparrow JR. Enzymatic degradation of A2E, a retinal pigment epithelial lipofuscin bisretinoid. Journal of the American Chemical Society 2011;133:849-857. DOI: 10.1021/ja107195u

[112] Sparrow JR, Zhou J, Ghosh SK, Liu Z. Bisretinoid degradation and the ubiquitin-proteasome system. Advances in Experimental Medicine and Biology. 2014;801:593-600. DOI: 10.1007/978-1-4614-3209-8_75

[113] Yogalingam G, Lee AR, Mackenzie DS, Maures TJ, Rafalko A, Prill H, Berguig G, Hague C, Christianson T, Bell SM, LeBowitz JH. Cellular uptake and delivery of Myeloperoxidase to lysosomes promotes lipofuscin degradation and lysosomal stress in retinal cells. Journal of Biological Chemistry. 2017. DOI: 10.1074/jbc.M116.739441
[114] Stella VJ, He Q. Cyclodextrins. Toxicologic Pathology. 2008;36:30-42. DOI: 10.1177/0192623307310945

[115] Nociari MM, Lehmann GL, Perez Bay AE, Radu RA, Jiang Z, Goicochea S, Schreiner R, Warren JD, Shan J, Adam de Beaumais S, Ménand M, Sollogoub M, Maxfield FR, Rodriguez-Boulan E. Beta cyclodextrins bind, stabilize, and remove lipofuscin bisretinoids from retinal pigment epithelium. Proceedings of the National Academy of Sciences of United States of America. 2014;111:E1402-E1408. DOI: 10.1073/pnas.1400530111

[116] Maeda A, Maeda T, Golczak M, Palczewski K. Retinopathy in mice induced by disrupted all-trans-retinal clearance. Journal of Biological Chemistry. 2008;283:26684-26693. DOI: 10.1074/jbc.M804505200

[117] Mondal M, Mesmin B, Mukherjee S, Maxfield FR. Sterols are mainly in the cytoplasmic leaflet of the plasma membrane and the endocytic recycling compartment in CHO cells. Molecular Biology of the Cell. 2009;20:581-588. DOI: 10.1091/mbc.E08-07-0785

[118] López CA, de Vries AH, Marrink SJ. Computational microscopy of cyclodextrin mediated cholesterol extraction from lipid model membranes. Scientific Reports. 2013;3:1-6. DOI: 10.1038/srep02071

[119] Vance, Jean E., and Barbara Karten. “Niemann-Pick C Disease and Mobilization of Lysosomal Cholesterol by Cyclodextrin.” Journal of Lipid Research 2014;55:1609-1621. PMC. Web. 24 May 2017

[120] Yancey PG, Rodrigueza WV, Kilsdonk EPC, Stoudt GW, Johnson WJ, Phillips MC, Rothblat GH. Cellular cholesterol efflux mediated by cyclodextrins: Demonstration of kinetic pools and mechanism of efflux. Journal of Biological Chemistry. 1996;271:16026-16034. DOI: 10.1074/jbc.271.27.16026

[121] Rosenbaum AI, Zhang G, Warren JD, Maxfield FR. Endocytosis of beta-cyclodextrins is responsible for cholesterol reduction in Niemann-Pick type C mutant cells. Proceedings of the National Academy of Sciences of the United States of America. 2010;107:5477-5482. DOI: 10.1073/pnas.0914309107

[122] Mondjinou YA, McCauliff LA, Kulkarni A, Paul L, Hyun SHH, Zhang Z, Wu Z, Wirth M, Storch J, Thompson DH. Synthesis of 2-hydroxypropyl-β-cyclodextrin/pluronic-based polyrotaxanes via heterogeneous reaction as potential Niemann-Pick type C therapeutics. Biomacromolecules. 2013;14:4189-4197. DOI: 10.1021/bm400922a

[123] Chu BB, Liao YC, Qi W, Xie C, Du X, Wang J, Yang H, Miao HH, Li BL, Song BL. Cholesterol transport through lysosome-peroxisome membrane contacts. Cell. 2015;161:291-306. DOI: 10.1016/j.cell.2015.02.019

[124] Neufeld EB, Cooney AM, Pitha J, Dawidowicz EA, Dwyer NK, Pentchev PG, Blanchette-Mackie EJ. Intracellular trafficking of cholesterol monitored with a cyclodextrin. Journal of Biological Chemistry. 1996;271:21604-21613

[125] Maxfield FR, Wüstner D. Analysis of cholesterol trafficking with fluorescent probes. Methods in Cell Biology. 2012;108:367-393. DOI: 10.1016/B978-0-12-386487-1.00017-1
[126] Taylor AM, Liu B, Mari Y, Liu B, Repa JJ. Cyclodextrin mediates rapid changes in lipid balance in Npc1−/− mice without carrying cholesterol through the bloodstream. Journal of Lipid Research. 2012;53:2331-2342. DOI: 10.1194/jlr.M028241

[127] Rothblat GH, de la Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC. Cell cholesterol efflux: Integration of old and new observations provides new insights. Journal of Lipid Research. 1999;40:781-796. DOI: 10.1074/jbc.271.27.16026

[128] Ghosh A, Jana M, Modi K, Gonzalez FJ, Sims KB, Berry-Kravis E, Pahan K. Activation of peroxisome proliferator-activated receptor α induces lysosomal biogenesis in brain cells: Implications for lysosomal storage disorders. Journal of Biological Chemistry. 2015;290. DOI: 10.1074/jbc.M114.610659

[129] Zimmer S, Grebe A, Bakke SS, Bode N, Halvorsen B, Ulas T, Skjelland M, De Nardo D, Labzin LI, Kerksiek A, Hempel C, Heneka MT, Hawxhurst V, Fitzgerald ML, Trebicka J, Bjorkhem I, Gustafsson JA, Westerterp M, Tall AR, Wright SD, Espevik T, Schultze JL, Nickenig G, Lutjohann D, Latz E. Cyclodextrin promotes atherosclerosis regression via macrophage reprogramming. Science Translational Medicine. 2016;8:333ra50. DOI: 10.1126/scitranslmed.aad6100

[130] Yao J, Ho D, Calingasan NY, Pipalia NH, Lin MT, Beal MF. Neuroprotection by cyclodextrin in cell and mouse models of Alzheimer disease. Journal of Experimental Medicine. 2012;209:2501-2513. DOI: 10.1084/jem.20121239

[131] Hoh Kam J, Lynch A, Begum R, Cunea A, Jeffery G. Topical cyclodextrin reduces amyloid beta and inflammation improving retinal function in ageing mice. Experimental Eye Research. 2015;135:59-66. DOI: 10.1016/j.exer.2015.03.023

[132] Bar-On P, Rockenstein E, Adame A, Ho G, Hashimoto M, Masliah E. Effects of the cholesterol-lowering compound methyl-beta-cyclodextrin in models of alpha-synucleinopathy. Journal of Neurochemistry. 2006;98:1032-1045. DOI: 10.1111/j.1471-4159.2006.04017.x

[133] Song W, Wang F, Lotfi P, Sardiello M, Segatori L. 2-Hydroxypropyl-β-cyclodextrin promotes TFEB-mediated activation of autophagy: Implications for therapy. Journal of Biological Chemistry. 2014;289:0-27. DOI: 10.1074/jbc.M113.506246

[134] Song W, Wang F, Savini M, Ake A, di Ronza A, Sardiello M, Segatori L. TFEB links autophagy to lysosomal biogenesis. Human Molecular Genetics. 2013;22:1994-2009. DOI: 10.1093/hmg/ddt052

[135] Cheng J, Ohsaki Y, Tauchi-Sato K, Fujiita A, Fujimoto T. Cholesterol depletion induces autophagy. Biochemical and Biophysical Research Communications. 2006;351:246-252. DOI: 10.1016/j.bbrc.2006.10.042

[136] Chen FW, Li C, Ioannou YA. Cyclodextrin induces calcium-dependent lysosomal exocytosis. PLoS One. 2010;5:e15054. DOI: 10.1371/journal.pone.0015054

[137] Kilpatrick K, Zeng Y, Hancock T, Segatori L. Genetic and chemical activation of TFEB mediates clearance of aggregated α. Synuclein. 2015;1-21. DOI: 10.1371/journal.pone.0120819
Spampanato C, Feeney E, Li L, Cardone M, Lim JA, Annunziata F, Zare H, Polishchuk R, Puertollano R, Parenti G, Ballabio A, Raben N. Transcription factor EB (TFEB) is a new therapeutic target for Pompe disease. EMBO Molecular Medicine. 2013;5:691-706. DOI: 10.1002/emmm.201202176

Tsunemi T, Ashe TD, Morrison BE, Soriano KR, Au J, Roque RAV, Lazarowski ER, Damian VA, Masliah E, La Spada AR. PGC-1α rescues Huntington’s disease proteotoxicity by preventing oxidative stress and promoting TFEB function. Science Translational Medicine. 2012;4:142ra97. DOI: 10.1126/scitranslmed.3003799
