Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Pharmaceutical therapies targeting autophagy for the treatment of age-related macular degeneration

Kirstan A. Vessey[1,2], Andrew I. Jobling[1], Ursula Greferath[1] and Erica L. Fletcher[1]

Abstract

Age-related macular degeneration (AMD) is a major cause of irreversible vision loss in the elderly. Although new therapies have recently emerged, there are currently no ways of preventing the development of the disease. Changes in intracellular recycling processes. Changes in intracellular recycling processes, called autophagy, lead to debris accumulation and cellular dysfunction in AMD models and AMD patients. Drugs that enhance autophagy hold promise as therapies for slowing AMD progression in preclinical models; however, more studies in humans are required. While a definitive cure for AMD will likely hinge on a personalized medicine approach, treatments that enhance autophagy hold promise for slowing vision loss.

Addresses

[1] Department of Anatomy and Physiology, The University of Melbourne, VIC 3010, Australia

[2] School of Science and Technology, The University of New England, NSW 2350, Australia

Corresponding author: Fletcher, Erica L. (e.fletcher@unimelb.edu.au)

Current Opinion in Pharmacology 2024, **76**:102463

This review comes from a themed issue on **Ocular Pharmacology (2024)**

Edited by **Claudio Bucolo**, **Sanjoy K. Bhattacharya** and **Chiara M. Eandi**

For complete overview about the section, refer Ocular Pharmacology (2024)

Available online 23 May 2024

https://doi.org/10.1016/j.coph.2024.102463

1471-4892/

Introduction

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness among individuals aged over 50 years, impacting nearly 30% of those over 80 years [1–4]. Advanced disease is characterized by central vision loss, hindering reading, driving, and facial recognition and in severe cases, it can lead to complete legal blindness, significantly impacting independence and quality of life [2,3]. AMD is characterized by the progressive degeneration of the photoreceptors, which are the light-sensitive neurons of the retina, as well as their supporting cells, known as the retinal pigment epithelium (RPE) [3,5]. Diagnosis is based on the presence and size of extracellular deposits called drusen, which accumulate between the RPE and the choroidal blood supply [3,5]. Additionally, pigmentary abnormalities and the presence of abnormal waste deposits known as lipofuscin within RPE cells are also indicators. Late-stage AMD is typically classified into two groups: dry or geographic atrophy (GA) and wet or neovascular AMD (nAMD) [4–6]. The majority of late-stage AMD cases are of the atrophic (GA) form and are characterized by loss of the patches of RPE and overlying photoreceptors, while the remaining late-stage cases of nAMD exhibit the growth of abnormal vessels beneath the RPE or within the retina [6]. While vision loss occurs over time in the atrophic form, it is accelerated in nAMD due to the leakage and bleeding of the abnormal vessels into the macula, resulting in macular edema, direct photoreceptor damage, and eventually scarring [6,7]. Currently, there are no medical treatments for early or intermediate AMD and there are no treatments that completely arrest late-stage AMD progression, but there are some therapies that show some benefit in slowing late-stage disease. In this review, we will consider the current treatment options that are approved for slowing the progression of late-stage AMD and focus on how novel treatments targeting metabolism and the intracellular recycling pathway, autophagy, may have additional therapeutic potential.

Current treatments for late-stage age-related macular degeneration

Neovascular age-related macular degeneration : Anti-vascular endothelial growth factor agents

Over recent years, one of the great success stories in the treatment of late-stage AMD has been the use of anti-VEGF agents to treat nAMD [8]. Anti-VEGF therapies, such as ranibizumab (Lucentis), aflibercept (Eylea), faricimab (Beovu), and bevacizumab (Avastin), have demonstrated remarkable efficacy in inhibiting the progression of nAMD [8–10]. By blocking VEGFA, these treatments reduce abnormal blood vessel growth, leakage, and subsequent retinal damage [8]. Clinical trials have consistently shown that anti-VEGF treatments can improve visual acuity and quality of life for most patients with nAMD over the short term, up to five years [8]. Recent evidence indicates that some of the newer anti-VEGF drugs may display superior properties, e.g. brolucizumab may require fewer injections and may provide improved visual gains [9]. Faricimab targets VEGF and also angiopoietin 2, (Ang2) a potent neovascular signaling molecule [10]. Faricimab is relatively recently approved, and so a head-to-head comparison of the advantages of anti-VEGF/Ang2 relative to stand-alone anti-VEGF molecules is yet to be completely determined [10].

However, despite this success, some challenges are associated with anti-VEGF therapies. Patients with nAMD often require life-long monthly or bimonthly intravitreal injections, which can be burdensome for both patients and healthcare providers. Compliance with this treatment regimen can be challenging and expensive, leading to suboptimal outcomes. Additionally, there have not been many follow-up studies showing efficacy over the longer term (>5 years treatment). One study has carefully considered the effects of ranibizumab over 7 years [11]. It was found that, compared with baseline measurements, almost half of those treated with anti-VEGF therapies retained stable vision, but unfortunately, around thirty percent experienced visual acuity decline [11]. Notably, nearly 40% of the eyes in the study did not develop fibrotic lesions, indicating a deviation from the typical disease course characterized by retinal destruction and the formation of disciform scars [11]. These findings imply that anti-VEGF therapy may play a role in preventing or delaying the end stages of AMD, where the disease progression slows down or reaches a plateau. However, while anti-VEGF therapy is a success story for some, more than half of patients go on to lose vision and develop fibrosis at the seven-year time point. Other studies also suggest that many of these patients develop retinal scarring and GA two to five years after initiating treatment [12–14]. Thus, additional novel therapies targeting fibrosis and modes of treatment that do not involve regular intraocular injections are greatly needed to improve outcomes for patients with nAMD.

Geographic atrophy: Anti-complement 3 (C3) and anti-C5 agents

In a recent and exciting event for the field, the Federal Drug Administration (FDA) in the Unites States approved SYFOVRE™ (pegcetacoplan injection) as the first treatment for GA in 2023 [15,16]. This drug, like those targeting VEGF, requires an intraocular injection monthly or every two months. The therapy is an anti-C3 agent that blocks the innate immune system complement cascade product C3, with the aim to inhibit inflammatory-driven damage in the eye [15]. Positive results were reported from a phase 2 clinical study, which showed at 12 months of monthly or every other month (EOM) treatment, the growth of the atrophy lesion area was reduced by 33–45% relative to sham-injected eyes [15]. However, despite this, reduction in lesion area, there were no improvements in visual acuity, nor the low-light visual acuity deficit [15]. Furthermore, two recent phase three clinical trials have also found a modest but significant reduction in atrophic lesion growth relative to control groups: OAKs study, 24% (monthly) and 25% (EOM); Derby study, 36% (monthly) to 29% (EOM) [16–18]. While pegcetacoplan treatment shows promise, the long-term effects are yet to be determined. As a source of some concern, pegcetacoplan seems to be associated with increased rates of nAMD; 12% when administered monthly, 7% when administered EOM and 3% in the control group by 2 years, suggesting careful patient selection and monitoring is required to avoid adverse consequences [16,18].

In the same year, September 2023, a therapy targeting the next stage of the complement cascade, C5, avacincaptad pegol (called IZERVAY), was also approved for use by the FDA [19–21]. Like pegcetacoplan, avacincaptad pegol showed reduced growth of the area of atrophy relative to sham injected eyes following 12 months of monthly treatment [20]. While the drug was well tolerated with no serious adverse events, more work is required to confirm the safety and efficacy of this treatment in slowing vision loss [20]. Other clinical trials targeting inflammation in the eye were also released recently in the Lancet in 2024 [22]. Results from a phase 2 clinical trial, blocking microglial action using oral minocycline showed initial promise, however, at the end failed to slow GA lesion enlargement over 2 years and showed no improvement in vision [22].

Heading forward, like the anti-VEGF therapies for nAMD, pegcetacoplan, and avacincaptad pegol require regular intraocular injection, and so will pose challenges for both patients and healthcare providers. Ensuring patient compliance with this demanding schedule will be difficult and costly, potentially resulting in suboptimal treatment outcomes. Overall, it is exciting that novel drugs for GA treatment have FDA approval for the first time, however it is still unknown how widely accepted they will be in clinical practice and how efficacious they will be in the broader population over time.

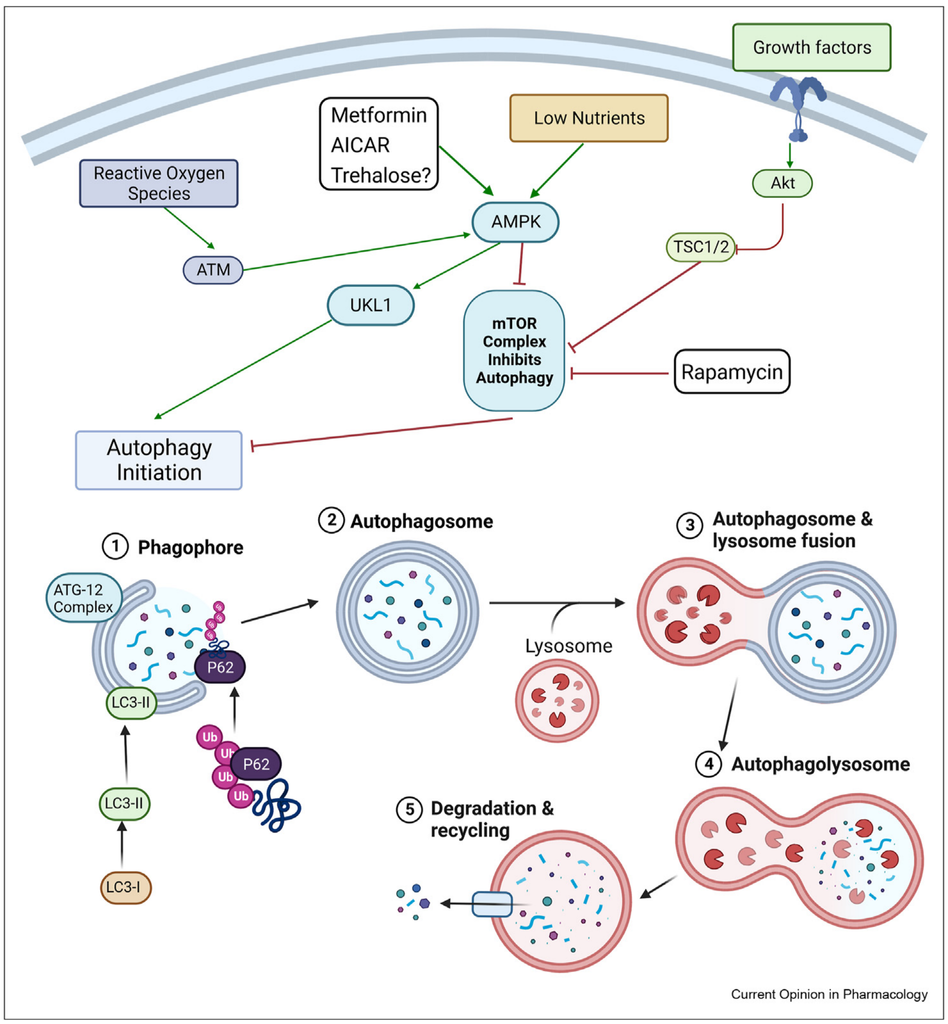
Despite these promising advances in therapies, there are no drugs that slow the progression of vision loss in early to late-stage disease, nor is there a treatment that completely arrests late-stage disease. Consequently, there is still an urgent need for new effective treatments. This is reflected in the number of potential treatments currently undergoing clinical assessment with around 2250 registered clinical trials for AMD (ClinicalTrials.gov) as of April 2024. Thus, the hope for a new treatment for late-stage AMD is high. One pathway that is currently under investigation is the intracellular recycling pathway of autophagy. The remainder of this review will focus on the role of autophagy in AMD development and treatments targeting this pathway in the treatment of AMD.

Autophagy: A cellular process for self-rejuvenation

Autophagy, derived from the Greek words, “auto” meaning self and “phagy” meaning eating, is a highly conserved cellular process that allows cells to recycle and degrade their own components [23,24 for comprehensive reviews]. It is an essential mechanism for maintaining cellular homeostasis, adapting to cellular stress, and promoting cell survival. Autophagy is an umbrella term that encompasses three main specific forms: macroautophagy, microautophagy, and chaperone-mediated autophagy [23]. Each type of autophagy involves distinct mechanisms and contribute to different cellular functions; however, this review will focus solely on macroautophagy, hereafter called autophagy, which is the most extensively studied and well-understood form of autophagy (Figure 1).

The process of cellular waste management via autophagy involves several stages [23,24]. First, a membrane structure called the phagophore forms in the cytoplasm, which elongates and engulfs portions of the cytoplasm, including damaged organelles, misfolded proteins, and

Figure 1



Schematic of the molecular pathways that initiate and direct autophagy. Autophagy initiators: growth factor pathways are indicated in green; low-nutrient pathways are indicated in yellow; reactive oxygen species pathways are indicated in purple and intersecting pathways are indicated in blue. Drug treatments that modify autophagy initiation that are considered in this review are circled in black with a white background. The green arrows indicate pathway upregulation; red T-line arrows indicate pathway down-regulation. After autophagy is initiated, contents for degradation are (steps 1–2) enveloped in a double-membraned autophagosome. (Step 3) This fuses with a lysosome, which contains degradation enzymes and lowers the pH. (Step 4–5) The contents are degraded and returned to the cytoplasm for re-use. Acronyms: microtubule-associated protein 1A/1B-light chain 3, LC3; AuTophaGy-related, ATG; 5′ adenosine monophosphate-activated protein kinase, AMPK; ataxia telangiectasia mutated, ATM; protein kinase B, AKT; mechanistic target of rapamycin kinase, mTOR; ukelele, UKL; tuberous sclerosis complex subunit, TSC; sequestosome 1 SQSTM1, P62; ubiquitin, Ub. This illustration was started from a Biorender Template and content and stylistic modifications were made.

intracellular pathogens targeted for degradation. This elongated membrane then seals to form a double-membraned autophagosome, which encapsulates the waste within its inner compartment. Subsequently, the autophagosome fuses with a lysosome, forming a structure known as an autophagolysosome. Lysosomes are acidic and contain hydrolytic enzymes, such as proteases and lipases, which degrade the contents of the autophagosome, releasing breakdown products that can be reused by the cell. The degradation products, such as amino acids, fatty acids, and nucleotides are then released into the cytoplasm and made available for cellular processes, including energy production and biosynthesis [23,24]. This self-renewal process serves as a cellular survival mechanism in times of nutrient scarcity and stress conditions, allowing cells to survive in unfavorable conditions, and promoting their adaptation to environmental changes. Moreover, autophagy plays a vital role in cellular quality control by eliminating damaged organelles, misfolded proteins, and toxic aggregates, thereby maintaining cellular homeostasis.

Autophagy is regulated by a complex network of signaling proteins, driven by growth factor signaling, low nutrient levels, and reactive oxygen species ([24,25]; Figure 1). A couple of the major regulators of autophagy are mammalian target of rapamycin (mTOR), and 5' adenosine monophosphate-activated protein kinase (AMPK), which act as negative and positive regulators of autophagy, respectively [24,25]. When nutrients and growth factors are abundant, mTOR is active and inhibits autophagy. However, during nutrient deprivation or in response to cellular stress, AMPK is stimulated and mTOR is inhibited, triggering autophagy initiation [24,25]. Additionally, autophagy is dependent on the microtubule-associated protein 1A/1B-light chain 3 (LC3). During autophagy, the cytosolic form of LC3, LC3-I, is converted to LC3-II through a two-step process [26] involving cleavage of LC3-I and exposure to a phosphatidylethanolamine lipid, resulting in the formation of LC3-II. This lipidation step allows LC3-II to associate with the autophagosome membrane, where it plays a critical role in cargo sequestration and autophagosome formation. Other autophagy-related proteins ATG12 complex (ATG5, ATG7, ATG10, ATG12, ATG16L1) ensures the proper formation [26] and maturation of autophagosomes, and help in preventing the premature fusion of the autophagosome with lysosomes [27]. Monitoring the conversion of LC3-I to LC3-II and the subcellular localization of these autophagy proteins provides insights into autophagic activity [26].

The importance of autophagy in health and disease has garnered significant attention in recent years. Dysfunctional autophagy has been implicated in various human diseases, including neurodegenerative disorders, cancer, and metabolic disorders [28,29], as well as AMD [30]. Defects in the autophagy pathway can lead to the accumulation of toxic protein aggregates, impaired organelle turnover, and altered cellular metabolism, contributing to disease pathogenesis [28,30]. Modulating autophagic activity through lifestyle interventions, such as calorie restriction and exercise, or through pharmacological interventions has shown beneficial effects for the treatment of various diseases, including cancer, metabolic disorders, and neurodegenerative conditions [31], and holds promise for the treatment of AMD.

Noncanonical autophagy in the retinal pigment epithelium

The RPE plays an important role in maintaining the health of the retina and its death and dysfunction is a critical driver of AMD. The RPE enlists an unusual, noncanonical form of autophagy, which may contribute to intracellular recycling dysfunction and disease progression in AMD [32,33]. The RPE is a monolayer of terminally differentiated epithelial cells that lies between the neural retina and choroidal blood supply [[34,35] for comprehensive reviews of RPE function]. In addition to providing nutrients and oxygen from the choroidal blood supply to the neural retina, removing waste products from the retina, and generating the outer blood retinal barrier; RPE cells are one of the most phagocytic postmitotic cells found in the body. One RPE cell is in contact with 30–50 photoreceptors and carries out diurnal phagocytosis of shed photoreceptor outer segments, allowing recycling of vitamin A and fatty acids through the visual cycle and lipid cycling [36]. One molecular pathway that is used to ensure active engulfment and processing of outer segments involves LC3-associated phagocytosis (LAP), which is a noncanonical form of autophagy [32,33]. LAP can be assessed by detecting the close association of LC3 immunoreactivity with rhodopsin-positive phagosomes, and this process competes with the traditional autophagy-lysosomal pathway [32]. This may render the RPE particularly sensitive to dysfunction in autophagy processing pathways.

Autophagy is impaired in the retinal pigment epithelium in aging and age-related macular degeneration

With age, RPE cells upregulate autophagy to enhance the renewal of cellular components. Age-related increases in autophagosome numbers and expression of autophagy proteins have been observed in the RPE of mice and human donor specimens [37,38]. Despite upregulation of autophagy, in the mouse eye, RPE cells are lost with age, and while the number of LC3-positive autophagosomes increase, the number of lysosomes available for processing does not increase, suggesting that autophagy-lysosomal function is diminished in the RPE with age [37]. Further assessment of protein expression important for autophagy processes indicates that AMPKα, the energy and stress sensor, is upregulated in the aged mouse RPE [37]. In the RPE, AMPKα has been found to play a crucial role in maintaining a balance between autophagy and photoreceptor outer segment phagosome processing. Increased expression of AMPKα upregulates autophagy and downregulates phagocytosis to prioritize resources for the more vital autophagy pathway [33]. This suggests there is a slowing of photoreceptor outer segment processing with age [37]. Finally, intracellular waste proteins tagged by Protein 62, a 62 kDa protein, also known as sequestosome-1 (P62), accumulates when autophagy-lysosomal processes are impaired in the RPE and retina [39] and is increased in the RPE of aged mice [37]. Together, these data suggest that processing of intracellular waste is reduced in the aging RPE, potentially contributing to cell failure with age.

In AMD, autophagy flux is significantly reduced in the RPE of human donors [40]. There is an accumulation of cytoplasmic debris, lipids, and undigested protein aggregates that are tagged for autophagic degradation, indicating that autophagy is dysregulated in the RPE of patients with AMD [38,40]. There is also evidence that failure to degrade mitochondria via the autophagy pathway, reduced mitophagy, mitochondrial dysfunction, and RPE death in AMD in humans [41]. Studies using mouse models with conditional deletions of autophagy pathway genes, ATG5 and ATG7 in the RPE indicate the importance of autophagy in RPE function and AMD pathogenesis. These mice show age-dependent degeneration of the RPE, characterized by pigmentation defects and RPE atrophy, which are hallmarks of late-stage AMD [42]. Additionally, rodent models of AMD, such as apolipoprotein E4 (ApoE4) mice fed a high-fat diet and apolipoprotein E (ApoE) null animals exhibit impaired autophagy in aged animals and RPE changes consistent with early AMD [38,43]. Failure in autophagy pathways results in the accumulation of intracellular waste and contributes directly to RPE cell dysfunction and loss in mouse models of AMD and humans with AMD.

Impaired autophagy in the RPE has also been associated with the promotion of inflammation, which may further contribute to RPE cell dysfunction and death. In coculture experiments with bone marrow-derived macrophages, RPE cells with dysfunctional autophagy increase the secretion of inflammatory caspase-1, a marker for NLRP3 inflammasome activation, and proinflammatory cytokine, interleukin-1-beta (IL-1β) [44]. This suggests that defective autophagy in the RPE can activate the inflammasome cascade, driving macrophage activation, potentially contributing to RPE cell death in AMD [44]. In summary, impaired autophagy leads to the accumulation of cellular waste RPE degeneration, and inflammation, all of which contribute to the development and progression of AMD. Understanding the role of autophagy in RPE function and AMD pathogenesis may provide insights into potential therapeutic strategies for this sight-threatening disease.

Treatments targeting autophagy as a therapy for age-related macular degeneration

Treatments targeting enhanced autophagy have been investigated in animal models and humans as a therapy for AMD and as antiaging drugs more generally [45]. While caloric restriction has long been known to increase longevity [46,47], finding drugs to target these pathways to enhance autophagy without modifying diet has been a holy grail of anti-aging research. Drugs that stimulate the AMPK pathway and those that directly inhibit the mTOR pathway have been the subject of the most research for longevity and slowing pathology associated with AMD. These treatments will be reviewed below (Figure 1).

mTOR inhibitor, rapamycin/sirolimus

Rapamycin, also known as sirolimus, is an autophagy enhancer [48,49]. It is a macrolide antibiotic that was originally developed as an antifungal agent and is a potent inhibitor of mTORC1; it is also well known for its anti-inflammatory effects [49]. In cell culture and animal models, rapamycin has been found to be beneficial in increasing longevity [50], but also in slowing pathological manifestations of AMD. With regards to AMD specifically, in RPE cell culture studies, rapamycin was found to attenuate induced premature senescence and cell death caused by glutathione [51]. In a light-damage model of AMD in mice, rapamycin was found to improve rod photoreceptor survival and function by enhancing autophagy and partially by inhibiting inflammation [52]. As a potential treatment for nAMD, rapamycin has also been found to inhibit angiogenesis by decreasing VEGF production and attenuating the endothelial cell response to VEGF [53,54]. One mechanism determined from the RPE-endothelial coculture model, is that rapamycin reduces VEGF production by the RPE, which in turn reduces endothelial cell sprouting and angiogenesis [55]. In a rodent model of pathological retinal neovascularization, rapamycin treatment was found to reduce new vessel growth [56,57]. Similarly, in the laser-induced rodent model of nAMD, rapamycin was found to inhibit retinal and choroidal neovascularization, suggesting a protective effect [56]. These animal studies suggest rapamycin may show therapeutic potential in the treatment of AMD, both GA and nAMD.

In contrast to these preclinical studies, however, in humans, there is currently little persuasive data indicating the efficacy of rapamycin for the treatment of AMD. Rapamycin's utility in human studies has been limited by its low solubility and the need for intravitreal injections. For the treatment of GA, in a phase 2 clinical trial, monthly administration of rapamycin did not result in altered rates of GA progression [58]. Patients were treated for two years and no improvement in GA lesion size or best corrected visual acuity was observed. However, issues with the drug formulation may have contributed to this, as patients were averse to the treatment due to a “drug depot” effect, and additionally, there was an increase in sterile endophthalmitis, above that expected from other drug intravitreal injections, e.g. anti-VEGFs, which caused the early termination of the study [58]. With respect to the treatment of nAMD, rapamycin has also been used in conjunction with anti-VEGF therapy, aflibercept [59]. However, in a small clinical trial, intravitreal rapamycin with adjunct aflibercept administered every 2 months over 36 months (n = 10 control vs n = 10 treated) did not improve either central subfield thickness or best corrected visual acuity. The authors did note that the treatment appeared to have potential anatomical benefits as a treatment for persistent, exudative AMD; however, this requires further investigation with a larger cohort to better understand the potential risks and benefits [59]. However, overall, there is currently limited evidence for the use of rapamycin in the treatment of AMD in the clinic.

AMP-activated protein kinase stimulators: Metformin and 5-amino-4-imidazolecarboxamide ribonucleoside

AMPK regulators such as metformin and 5-amino-4-imidazolecarboxamide ribonucleoside (AICAR) can modulate autophagy via inhibition of mTOR; however, the AMPK pathway can also initiate autophagy independently of mTOR via the ukelele (ULK) pathway (Figure 1, [60]). Thus, treatments targeting this energy-sensing regulator have alternate molecular pathways to enhance autophagy, which may be of value in enhancing longevity and in the treatment of AMD. Like rapamycin, AMPK enhancers have been found to increase longevity in a range of preclinical models [61−63]) and for metformin in humans too [64].

Metformin

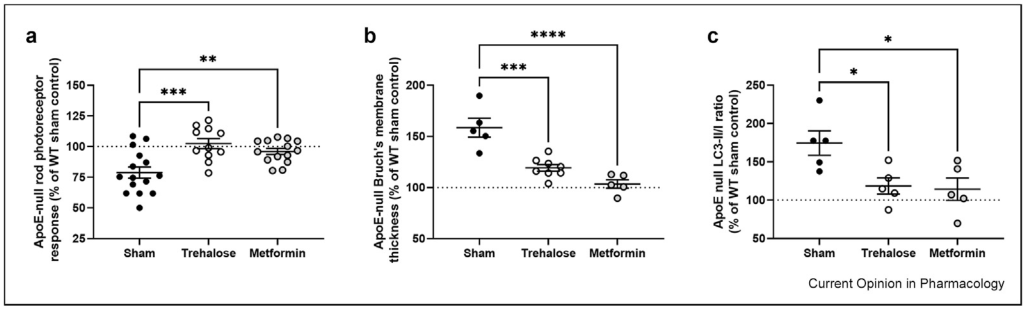
Metformin was initially extracted from the plant *Galega officinalis* (French lilac) [65]. Unlike rapamycin, it is water-soluble and orally bioavailable. Metformin has been used as a glucose-lowering medication in humans for more than 60 years. Of all the antidiabetic medications, metformin is now the recommended first-line treatment for type 2 diabetes according to the American Diabetes Association (ADA) guidelines. However, the uses of this drug are constantly being expanded to include cancer, obesity, nonalcoholic fatty liver disease, and metabolic syndrome [65]. In the treatment of AMD, in a range of RPE cell culture models, including RPE derived from inducible pluripotent stem cells (iPSCs) from AMD patients, metformin treatment has been found to aid metabolism and slow RPE damage and death, suggesting the potential for slowing AMD progression [66−69]. Consistent with these cell culture findings, in mice lacking ApoE, which display an early AMD phenotype of reduced retinal function and thickening of Bruch’s membrane with age, metformin was found to enhance retinal function and reduce Bruch’s membrane thickening ([43]; Figure 2). In these animals, metformin was found to enhance autophagy in the retina and RPE by targeting AMPK, but the effects on autophagy were generally independent of the regulation of the mTOR complex [43].

Recent evidence from humans also suggests that metformin may have therapeutic potential in reducing the chance of development of AMD [70−72] and maybe in slowing AMD progression. Following a quantitative meta-analysis of a range of retrospective analyses, from 9 suitable studies including 1,427,074 individuals with diabetes, metformin was found to be associated with a decreased odds ratio of developing AMD (OR 0.63; 95% CI: 0.46−0.86; p = 0.004) independent of other predisposing variables [71]. Despite this, at the present time there are no published clinical trials; however, “Metformin for the Minimization of Geographic Atrophy Progression in Patients with AMD (METforMIN)” is listed on the clinical trials registry as of June 2023. The results to date for this phase 2 clinical trial have been presented in abstract summary form at the Association for Research in Vision and Ophthalmology (ARVO) conference in 2023 [73]. Briefly, this trial was a single-blind, randomized, evaluation of the safety and efficacy of metformin use to decrease GA progression in nondiabetic patients with dry AMD. While 66 patients were recruited, only 21 metformin-treated and 23 monitored control patients remained at study end. Patients were treated from recruitment to 18 months and assessed at 24 months, and no serious adverse events related to metformin use were reported [73]. At this time point metformin did not alter GA area or best corrected visual acuity compared to monitored controls. However, for low luminance best corrected visual acuity, and the metformin group showed mild to no loss of vision $-1.6 \pm$ (2.0 SD) versus $-5.8 \pm$ (1.9 SD) for the monitored control; however, these changes were not significant (p = 0.13) [73]. While not statistically significant, these results show some promise, as low-luminance vision is a critical indicator of AMD progression; however, due to the low number of participants, the treatment paradigm (ceasing of treatment at 18 months and assessment at 24 months), further studies are warranted, especially on early-intermediate AMD patients.

5-amino-4-imidazolecarboxamide ribonucleoside

5-amino-4-imidazolecarboxamide ribonucleoside (AICAR) was first proposed in 1995 to be a cell-permeable AMP analog or mimetic, where it was found

Figure 2



Thirteen-month-old mice lacking ApoE have reduced retinal function, increased Bruch's membrane thickness, and reduced autophagy, which is ameliorated by treatment for eight months with either trehalose or metformin in the drinking water. C57blk6J control and ApoEnull mice were given standard drinking water (sham), trehalose (3 g/kg/day) or metformin (0.4 g/kg/day) from 5 months to 13 months of age. **(a)** Rod photoreceptor response was evaluated using the electroretinogram to assess retinal function. In ApoEnull mice, photoreceptor function was reduced, and this was restored to control levels by 8 months of treatment with either trehalose or metformin. **(b)** Bruch's membrane thickness was evaluated using ultrathin resin sections and transmission electron microscopy. Bruch's membrane thickening was observed in ApoEnull, consistent with an AMD phenotype, and this was ameliorated by treatment with either trehalose or metformin. **(c)** LC3-I and LC3-II levels were evaluated from retinal samples using western blot. In ApoEnull mice, there was a relative increase in the LC3-II/I ratio compared to controls suggesting a slowing of autophagy. Both trehalose and metformin restored the LC3-II/I ratio in ApoEnull retina to control levels. The original methods and data are presented in Ref. [43]. For the purposes of this review, the ApoEnull sham and treatment group data have been reanalyzed and regraphed for presentation as a percentage of the C57blk6J control sham group (100%, dotted line). Individual data points are presented, along with the mean and standard error of the mean. One way ANOVA with post-hoc significance of * $p < 0.05$ is shown.

to activate AMPK energy pathways in intact hepatic cells [74]. While AICAR is water-soluble, it is not orally bioavailable and requires administration via intraperitoneal or intravitreal injection in animal studies. Since it was initially proposed, AICAR has been used in a variety of studies, ranging from metabolism and exercise physiology to cancer [75]. In RPE cell culture, AICAR has been found to enhance the autophagy clearance of P62-tagged intracellular waste and promote cell survival [76]. Furthermore, in RPE derived from iPSCs of GA AMD patients, AICAR was found to generally improve metabolism and mitochondrial function [67,77]. In mice lacking ApoE and fed a high-fat diet, which a preclinical model of AMD and AICAR was found to upregulate RPE expression of the ATP-binding cassette (ABC) transporters, ABCA1 and ABCG1, which are essential for the formation of high-density lipoprotein (HDL) and lipid transport; it was also found to reduce Bruch's membrane lipid deposits and thickness [78]. These findings suggest that the use of AICAR in the treatment of slowing AMD progression may be beneficial; however, to date, there are no clinical trials reported in the literature or registered, and so the potential efficacy of this treatment in humans is unknown.

Autophagy enhancer: Trehalose

Trehalose (α,α-trehalose) is a disaccharide formed by a 1,1 linkage of two D-glucose molecules. It is water-soluble, bioavailable, very stable, and not easily hydrolyzed by acid or cleaved by α-glucosidase [79]. It is found primarily as an energy source in a variety of species, including plants, algae, fungi, yeasts, bacteria, and insects. In higher vertebrates, such as mammals, trehalose is not produced and is only obtained from the diet [79]. The sources in the modern human diet are limited, but for example, a high source is commercially grown mushrooms that can contain 8–17% (w/w) of trehalose. In mammals, trehalose is broken down into two glucose molecules in the small intestine by the enzyme and trehalase; however, due to this relatively late step in the digestive processing of trehalose, consumption results in a reduced absorption rate and consequently reduced glycemia, insulinemia, and oxidation rate in energy metabolism at rest and during exercise [80,81]. In cultured human RPE, trehalose has been shown to enhance basal autophagy and lysosomal clearance increasing the expression of cathepsin D; it also promotes cell survival after oxidative stress insult [82]. In cultured RPE derived from iPSCs of AMD patients with GA, trehalose was found to enhance autophagy in a mechanism that was independent of AMPK modulation and to ultimately improve metabolism and mitochondrial function [67,77]. Consistent with these cell culture findings in mice lacking ApoE, trehalose provided in the drinking water was found to enhance autophagy in the retina and RPE (Figure 2). However, unlike the previous culture-based study, this in vivo study showed trehalose enhanced autophagy by targeting pMAPK-14 and AMPK, acting independently of regulating the mTOR complex [43]. Overall, trehalose was found to enhance retinal function and reduce Bruch's membrane thickening in aged mice lacking ApoE mice relative to untreated controls [43]. These findings suggest that the use of trehalose in the diet may be useful in slowing AMD progression; however, like AICAR, to date there are no clinical trials reported in the literature or registered, and so the potential efficacy of this treatment for humans is unknown.

Conclusions

AMD is a leading cause of irreversible vision loss among individuals aged over 50 years. Currently, there are no medical treatments for early or intermediate AMD, and there are no treatments that completely arrest late-stage AMD progression. While the currently approved anti-VEGF and anti-C3 treatment options show some capacity for slowing the progression of late-stage AMD, the need for intravitreal injections, poor patient compliance, and limited efficacy means that more treatments are greatly needed. Novel therapies targeting metabolism and autophagy may have additional therapeutic potential. In particular, repurposing of the antidiabetic drug metformin shows great promise as a general therapy to slow vision loss in AMD. However, a true cure for AMD is still many years away and will likely rely on individualized medicine. In the meantime, drugs targeting autophagy may slow vision loss, if only for a short duration, could be important steppingstones to improving patient quality of life.

Author roles

Kirstan A. Vessey: Conceptualization, Data curation, Visualization, Funding acquisition, Roles/Writing - original draft.

Ursula Greferath: Writing - review & editing.

Andrew I. Jobling: Writing - review & editing.

Erica L. Fletcher: Conceptualization, Funding acquisition, Writing - review & editing.

Funding

This work was supported by the National Health and Medical Research Council [Award number: National Health and Medical Research Council; APP2011200]; National Health and Medical Research Council [National Health and Medical Research Council: Synergy Grant; APP1181010]; The Rebecca L. Cooper Medical Research Foundation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to acknowledge the technical and intellectual input of students who contributed to this work, Jessica Ma and Josephine Wong.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

1. Hassell JB, Lamoureux EL, Keeffe JE: **Impact of age related macular degeneration on quality of life**. *Br J Ophthalmol* 2006, **90**:593–596.

2. Rattner A, Nathans J: **Macular degeneration: recent advances and therapeutic opportunities**. *Nat Rev Neurosci* 2006, **7**: 860–872.

3. Fleckenstein M, Keenan TDL, Guymer RH, Chakravarthy U, Schmitz-Valckenberg S, Klaver CC, Wong WT, Chew EY: **Age-related macular degeneration**. *Nat Rev Dis Prim* 2021, **7**:31.

4. Guymer RH, Campbell TG: **Age-related macular degeneration**. *Lancet* 2023, **401**:1459–1472.

5. Miller JW: **Age-related macular degeneration revisited–piecing the puzzle: the LXIX Edward Jackson memorial lecture**. *Am J Ophthalmol* 2013, **155**:1–35 e13.

6. Spaide RF, Jaffe GJ, Sarraf D, Freund KB, Sadda SR, Staurenghi G, Waheed NK, Chakravarthy U, Rosenfeld PJ, Holz FG, *et al.*: **Consensus nomenclature for reporting neovascular age-related macular degeneration data: consensus on neovascular age-related macular degeneration nomenclature study group**. *Ophthalmology* 2020, **127**: 616–636.

7. Tenbrock L, Wolf J, Boneva S, Schlecht A, Agostini H, Wieghofer P, Schlunck G, Lange C: **Subretinal fibrosis in neovascular age-related macular degeneration: current concepts, therapeutic avenues, and future perspectives**. *Cell Tissue Res* 2022, **387**:361–375.

8. Solomon SD, Lindsley K, Vedula SS, Krzystolik MG, Hawkins BS: **Anti-vascular endothelial growth factor for neovascular age-related macular degeneration**. *Cochrane Database Syst Rev* 2019, **3**, CD005139.

9. Yeom H, Kwon HJ, Kim YJ, Lee J, Yoon YH, Lee JY: **Real-world study to evaluate the efficacy and safety of intravitreal brolucizumab for refractory neovascular age-related macular degeneration**. *Sci Rep* 2023, **13**, 11400.

10. Nair AA, Finn AP, Sternberg Jr P: **Spotlight on faricimab in the treatment of wet age-related macular degeneration: design, development and place in therapy**. *Drug Des Dev Ther* 2022, **16**:3395–3400.

11. Rofagha S, Bhisitkul RB, Boyer DS, Sadda SR, Zhang K, Group S-US: **Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP)**. *Ophthalmology* 2013, **120**: 2292–2299.

12. Daniel E, Toth CA, Grunwald JE, Jaffe GJ, Martin DF, Fine SL, Huang J, Ying GS, Hagstrom SA, Winter K, *et al.*: **Risk of scar in the comparison of age-related macular degeneration treatments trials**. *Ophthalmology* 2014, **121**:656–666.

13. Grunwald JE, Pistilli M, Daniel E, Ying GS, Pan W, Jaffe GJ, Toth CA, Hagstrom SA, Maguire MG, Martin DF, *et al.*: **Incidence and growth of geographic atrophy during 5 Years of comparison of age-related macular degeneration treatments trials**. *Ophthalmology* 2017, **124**:97–104.

14. McBain VA, Kumari R, Townend J, Lois N: **Geographic atrophy in retinal angiomatous proliferation**. *Retina* 2011, **31**: 1043–1052.

15. Liao DS, Grossi FV, El Mehdi D, Gerber MR, Brown DM, Heier JS, Wykoff CC, Singerman LJ, Abraham P, Grassmann F, *et al.*: **Complement C3 inhibitor pegcetacoplan for geographic atrophy secondary to age-related macular degeneration: a randomized phase 2 trial**. *Ophthalmology* 2020, **127**:186–195.

16. Heier JS, Lad EM, Holz FG, Rosenfeld PJ, Guymer RH, Boyer D, Grossi F, Baumal CR, Korobelnik JF, Slakter JS, *et al.*: **Pegcetacoplan for the treatment of geographic atrophy secondary to age-related macular degeneration (OAKS and DERBY): two multicentre, randomised, double-masked, sham-controlled, phase 3 trials**. *Lancet* 2023, **402**:1434–1448.

17. Goldberg R, Heier JS, Wykoff CC, Staurenghi G, Singh RP, Steinle N, Boyer DS, Mones J, Holz FG, Bliss C, *et al.*: **Efficacy of intravitreal pegcetacoplan in patients with geographic atrophy (GA): 12-month results from the phase 3 OAKS and DERBY studies**. *Invest Ophthalmol Vis Sci* 2022, **63**:1500. 1500.

18. Appels-Media-Release: SYFOVRE® (pegcetacoplan injection): *Continued to demonstrate increasing treatment effects over 3 Years in patients with geographic atrophy (GA).* 2023.

19. Csaky KG, Miller JML, Martin DF, Johnson MW: **Drug approval for the treatment of geographic atrophy: how we got here and where we need to go**. *Am J Ophthalmol* 2024, **263**: 231–239.

20. Khanani AM, Patel SS, Staurenghi G, Tadayoni R, Danzig CJ, Eichenbaum DA, Hsu J, Wykoff CC, Heier JS, Lally DR, *et al.*: **Efficacy and safety of avacincaptad pegol in patients with geographic atrophy (GATHER2): 12-month results from a randomised, double-masked, phase 3 trial**. *Lancet* 2023, **402**: 1449–1458.

21. Jaffe GJ, Westby K, Csaky KG, Mones J, Pearlman JA, Patel SS, Joondeph BC, Randolph J, Masonson H, Rezaei KA: **C5 inhibitor avacincaptad pegol for geographic atrophy due to age-related macular degeneration: a randomized pivotal phase 2/3 trial**. *Ophthalmology* 2021, **128**:576–586.

22. Keenan TDL, Bailey C, Abraham M, Orndahl C, Menezes S, Bellur S, Arunachalam T, Kangale-Whitney C, Srinivas S, Karamat A, *et al.*: **Phase 2 trial evaluating minocycline for geographic atrophy in age-related macular degeneration: a nonrandomized controlled trial**. *JAMA Ophthalmol* 2024, **142**: 345–355.

23. Mizushima N, Komatsu M: **Autophagy: renovation of cells and tissues**. *Cell* 2011, **147**:728–741.

24. Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, Cecconi F, Choi AM, Chu CT, Codogno P, Colombo MI, *et al.*: **Molecular definitions of autophagy and related processes**. *EMBO J* 2017, **36**:1811–1836.

25. Kroemer G, Marino G, Levine B: **Autophagy and the integrated stress response**. *Mol Cell* 2010, **40**:280–293.

26. Yoshii SR, Mizushima N: **Monitoring and measuring autophagy**. *Int J Mol Sci* 2017, **18**.

27. Kaur J, Debnath J: **Autophagy at the crossroads of catabolism and anabolism**. *Nat Rev Mol Cell Biol* 2015, **16**:461–472.

28. Menzies FM, Fleming A, Rubinsztein DC: **Compromised autophagy and neurodegenerative diseases**. *Nat Rev Neurosci* 2015, **16**:345–357.

29. Mulcahy Levy JM, Thorburn A: **Autophagy in cancer: moving from understanding mechanism to improving therapy responses in patients**. *Cell Death Differ* 2020, **27**:843–857.

30. Zhang ZY, Bao XL, Cong YY, Fan B, Li GY: **Autophagy in age-related macular degeneration: a regulatory mechanism of oxidative stress**. *Oxid Med Cell Longev* 2020, **2020**, 2896036.

31. Towers CG, Thorburn A: **Therapeutic targeting of autophagy**. *EBioMedicine* 2016, **14**:15–23.

32. * Kim JY, Zhao H, Martinez J, Doggett TA, Kolesnikov AV, Tang PH, Ablonczy Z, Chan CC, Zhou Z, Green DR, *et al.*: **Noncanonical autophagy promotes the visual cycle**. *Cell* 2013, **154**:365–376.

A noncanonical form of autophagy is used by the RPE to ensure active engulfment and processing of photoreceptor outer segments involves LC3-associated phagocytosis (LAP). LAP can be assessed by detecting close association of LC3 immunoreactivity with rhodopsin-positive phagosomes in the RPE and this process competes with the traditional autophagy-lysosomal pathway.

33. Qin S, De Vries GW: **alpha2 but not alpha1 AMP-activated protein kinase mediates oxidative stress-induced inhibition of retinal pigment epithelium cell phagocytosis of photoreceptor outer segments**. *J Biol Chem* 2008, **283**:6744–6751.

34. Boulton M, Dayhaw-Barker P: **The role of the retinal pigment epithelium: topographical variation and ageing changes**. *Eye (Lond)* 2001, **15**:384–389.

35. Strauss O: **The retinal pigment epithelium in visual function**. *Physiol Rev* 2005, **85**:845–881.

36. Young RW: **The renewal of photoreceptor cell outer segments**. *J Cell Biol* 1967, **33**:61–72.

37. * Ma JYW, Greferath U, Wong JHC, Fothergill LJ, Jobling AI, Vessey KA, Fletcher EL: **Aging induces cell loss and a decline in phagosome processing in the mouse retinal pigment epithelium**. *Neurobiol Aging* 2023, **128**:1–16.

In 22-month-old mice, age-related increases in autophagosome numbers and expression of autophagy proteins were observed in the RPE relative to 3-month-old animals. Despite upregulation of autophagy, the number of lysosomes available for processing did not increase, suggesting that autophagy-lysosomal function is diminished in the RPE with age.

38. Mitter SK, Song C, Qi X, Mao H, Rao H, Akin D, Lewin A, Grant M, Dunn Jr W, Ding J, *et al.*: **Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD**. *Autophagy* 2014, **10**:1989–2005.

39. * von Eisenhart-Rothe P, Grubman A, Greferath U, Fothergill LJ, Jobling AI, Phipps JA, White AR, Fletcher EL, Vessey KA: **Failure of autophagy-lysosomal pathways in rod photoreceptors causes the early retinal degeneration phenotype observed in Cln6nclf mice**. *Invest Ophthalmol Vis Sci* 2018, **59**:5082–5097.

Cln6 mutant mice, which have a lysosomal storage dysfunction that leads to neuronal death, display increases in intracellular waste and early photoreceptor death, highlighting the importance of the autophagy-lysosomal pathway in maintaining retinal health.

40. * Golestaneh N, Chu Y, Xiao YY, Stoleru GL, Theos AC: **Dysfunctional autophagy in RPE, a contributing factor in age-related macular degeneration**. *Cell Death Dis* 2017, **8**, e2537.

In the RPE of human donors with AMD, autophagy flux is significantly reduced. There is an accumulation of cytoplasmic debris, lipid, and undigested protein aggregates that are tagged for autophagic degradation, indicating that autophagy is dysregulated in the RPE of patients with AMD.

41. * Datta S, Cano M, Satyanarayana G, Liu T, Wang L, Wang J, Cheng J, Itoh K, Sharma A, Bhutto I, *et al.*: **Mitophagy initiates retrograde mitochondrial-nuclear signaling to guide retinal pigment cell heterogeneity**. *Autophagy* 2023, **19**:966–983.

This study showed that failure to degrade mitochondria via the autophagy pathway (reduced mitophagy) causes mitochondrial dysfunction and RPE death in AMD in humans.

42. * Zhang Y, Cross SD, Stanton JB, Marmorstein AD, Le YZ, Marmorstein LY: **Early AMD-like defects in the RPE and retinal degeneration in aged mice with RPE-specific deletion of Atg5 or Atg7**. *Mol Vis* 2017, **23**:228–241.

Mouse models with conditional deletion of autophagy pathway genes, ATG5 and ATG7 in the RPE show age-dependent degeneration of the RPE, characterized by pigmentation defects and RPE atrophy and can be used as a preclinical model of AMD

43. * Vessey KA, Jobling AI, Tran MX, Wang AY, Greferath U, Fletcher EL: **Treatments targeting autophagy ameliorate the age-related macular degeneration phenotype in mice lacking APOE (apolipoprotein E)**. *Autophagy* 2022, **18**:2368–2384.

Aged mice lacking ApoE display an early AMD phenotype and this process can be slowed by treatment to enhance autophagy with metformin or trehalose.

44. Liu J, Copland DA, Theodoropoulou S, Chiu HA, Barba MD, Mak KW, Mack M, Nicholson LB, Dick AD: **Impairing autophagy in retinal pigment epithelium leads to inflammasome activation and enhanced macrophage-mediated angiogenesis**. *Sci Rep* 2016, **6**, 20639.

45. Madeo F, Tavernarakis N, Kroemer G: **Can autophagy promote longevity?** *Nat Cell Biol* 2010, **12**:842–846.

46. McCay CM, Crowell MF, Maynard LA: **The effect of retarded growth upon the length of life span and upon the ultimate body size**. 1989 *Nutrition* 1935, **5**:155–171. ; discussion 172.

47. Most J, Tosti V, Redman LM, Fontana L: **Calorie restriction in humans: an update**. *Ageing Res Rev* 2017, **39**:36–45.

48. Blagosklonny MV: **Rapamycin for longevity: opinion article**. *Aging (Albany NY)* 2019, **11**:8048–8067.

49. Camardo J: **The Rapamune era of immunosuppression 2003: the journey from the laboratory to clinical transplantation**. *Transplant Proc* 2003, **35**:18S–24S.

50. Sharp ZD, Strong R: **The role of mTOR signaling in controlling mammalian life span: what a fungicide teaches us about longevity**. *J Gerontol A Biol Sci Med Sci* 2010, **65**:580–589.

51. Sun Y, Zheng Y, Wang C, Liu Y: **Glutathione depletion induces ferroptosis, autophagy, and premature cell senescence in retinal pigment epithelial cells**. *Cell Death Dis* 2018, **9**:753.

52. Kunchithapautham K, Coughlin B, Lemasters JJ, Rohrer B: **Differential effects of rapamycin on rods and cones during light-induced stress in albino mice**. *Invest Ophthalmol Vis Sci* 2011, **52**:2967–2975.

53. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, *et al.*: **Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor**. *Nat Med* 2002, **8**:128–135.

54. Nakahara T, Morita A, Yagasaki R, Mori A, Sakamoto K: **Mammalian target of rapamycin (mTOR) as a potential therapeutic target in pathological ocular angiogenesis**. *Biol Pharm Bull* 2017, **40**:2045–2049.

55. Stahl A, Paschek L, Martin G, Gross NJ, Feltgen N, Hansen LL, Agostini HT: **Rapamycin reduces VEGF expression in retinal pigment epithelium (RPE) and inhibits RPE-induced sprouting angiogenesis in vitro**. *FEBS Lett* 2008, **582**:3097–3102.

56. Dejneka NS, Kuroki AM, Fosnot J, Tang W, Tolentino MJ, Bennett J: **Systemic rapamycin inhibits retinal and choroidal neovascularization in mice**. *Mol Vis* 2004, **10**:964–972.

57. Yagasaki R, Nakahara T, Ushikubo H, Mori A, Sakamoto K, Ishii K: **Anti-angiogenic effects of mammalian target of rapamycin inhibitors in a mouse model of oxygen-induced retinopathy**. *Biol Pharm Bull* 2014, **37**:1838–1842.

58. Gensler G, Clemons TE, Domalpally A, Danis RP, Blodi B, Wells 3rd J, Rauser M, Hoskins J, Hubbard GB, Elman MJ, *et al.*: **Treatment of geographic atrophy with intravitreal sirolimus: the age-related eye disease study 2 ancillary study**. *Ophthalmol Retina* 2018, **2**:441–450.

59. Rowe LW, Minturn RJ, Burgett LA, Bracha P, Maturi RK: **Intravitreal sirolimus with adjunct aflibercept versus aflibercept monotherapy for persistent, exudative age-related macular degeneration: a pilot study**. *Int J Retina Vitreous* 2023, **9**:1.

60. Kim J, Kundu M, Viollet B, Guan KL: **AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1**. *Nat Cell Biol* 2011, **13**:132–141.

61. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, Gomes AP, Ward TM, Minor RK, Blouin MJ, *et al.*: **Metformin improves healthspan and lifespan in mice**. *Nat Commun* 2013, **4**:2192.

62. Castillo K, Nassif M, Valenzuela V, Rojas F, Matus S, Mercado G, Court FA, van Zundert B, Hetz C: **Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons**. *Autophagy* 2013, **9**:1308–1320.

63. Pallauf K, Rimbach G, Rupp PM, Chin D, Wolf IM: **Resveratrol and lifespan in model organisms**. *Curr Med Chem* 2016, **23**: 4639–4680.

64. Campbell JM, Bellman SM, Stephenson MD, Lisy K: **Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis**. *Ageing Res Rev* 2017, **40**:31–44.

65. Zhou J, Massey S, Story D, Li L: **Metformin: an old drug with new applications**. *Int J Mol Sci* 2018, **19**.

66. Dang KR, Wu T, Hui YN, Du HJ: **Newly-found functions of metformin for the prevention and treatment of age-related macular degeneration**. *Int J Ophthalmol* 2021, **14**:1274–1280.

67. * Ebeling MC, Geng Z, Stahl MR, Kapphahn RJ, Roehrich H, Montezuma SR, Ferrington DA, Dutton JR: **Testing mitochondrial-targeted drugs in iPSC-RPE from patients with age-related macular degeneration**. *Pharmaceuticals* 2022, **15**.

This study tested a range of drugs to enhance autophagy, including trehalose, metformin and AICAR on RPE derived from iPSCs of AMD patients, finding that the results varied depending on the source of RPE iPSC, but that in general the drugs enhanced autophagy and metabolism to improve cellular function

68. Zhao X, Liu L, Jiang Y, Silva M, Zhen X, Zheng W: **Protective effect of metformin against hydrogen peroxide-induced oxidative damage in human retinal pigment epithelial (RPE) cells by enhancing autophagy through activation of AMPK pathway**. *Oxid Med Cell Longev* 2020, **2020**, 2524174.

69. Qu S, Zhang C, Liu D, Wu J, Tian H, Lu L, Xu GT, Liu F, Zhang J: **Metformin protects ARPE-19 cells from glyoxal-induced oxidative stress**. *Oxid Med Cell Longev* 2020, **2020**, 1740943.

70. Brown EE, Ball JD, Chen Z, Khurshid GS, Prosperi M, Ash JD: **The common antidiabetic drug metformin reduces odds of developing age-related macular degeneration**. *Invest Ophthalmol Vis Sci* 2019, **60**:1470–1477.

71. * Holtz JK, Thinggaard BS, Grauslund J, Subhi Y: **Association between oral metformin use and the risk of age-related macular degeneration: a systematic review with meta-analysis**. *Acta Ophthalmol* 2023, **101**:595–605.

Recent evidence from humans suggests that metformin may have therapeutic potential in reducing the chance of development of AMD. Following a quantitative meta-analysis, metformin was found to be associated with a decreased odds ratio of developing AMD independent of other predisposing variables

72. Moir J, Hyman MJ, Gonnah R, Flores A, Hariprasad SM, Skondra D: **The association between metformin use and new-onset ICD coding of geographic atrophy**. *Invest Ophthalmol Vis Sci* 2024, **65**:23.

73. Shen LL, Keenan J, Chahal N, Taha AT, Saroya J, Ma CJ, Sun HM, Yang D, Flaxel CJ, Fawzi AA, *et al.*: **METformin for the MINimization (METforMIN) of geographic atrophy progression in patients with non-exudative age-related macular degeneration – a randomized trial**. *Invest Ophthalmol Vis Sci* 2023, **64**:5057. 5057.

74. Corton JM, Gillespie JG, Hawley SA, Hardie DG: **5-aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells?** *Eur J Biochem* 1995, **229**:558–565.

75. Visnjic D, Lalic H, Dembitz V, Tomic B, Smoljo T: **AICAr, a widely used AMPK activator with important AMPK-independent effects: a systematic review**. *Cells* 2021, **10**.

76. Viiri J, Amadio M, Marchesi N, Hyttinen JM, Kivinen N, Sironen R, Rilla K, Akhtar S, Provenzani A, D'Agostino VG, *et al.*: **Autophagy activation clears ELAVL1/HuR-mediated accumulation of SQSTM1/p62 during proteasomal inhibition in human retinal pigment epithelial cells**. *PLoS One* 2013, **8**, e69563.

77. Fisher CR, Ebeling MC, Geng Z, Kapphahn RJ, Roehrich H, Montezuma SR, Dutton JR, Ferrington DA: **Human iPSC- and primary-retinal pigment epithelial cells for modeling age-related macular degeneration**. *Antioxidants (Basel)* 2022, **11**.

78. Kim YK, Hong HK, Yoo HS, Park SP, Park KH: **AICAR upregulates ABCA1/ABCG1 expression in the retinal pigment epithelium and reduces Bruch's membrane lipid deposit in ApoE deficient mice**. *Exp Eye Res* 2021, **213**, 108854.

79. Richards AB, Krakowka S, Dexter LB, Schmid H, Wolterbeek AP, Waalkens-Berendsen DH, Shigoyuki A, Kurimoto M: **Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies**. *Food Chem Toxicol* 2002, **40**:871–898.

80. van Can JG, Ijzerman TH, van Loon LJ, Brouns F, Blaak EE: **Reduced glycaemic and insulinemia responses following trehalose ingestion: implications for postprandial substrate use**. *Br J Nutr* 2009, **102**:1395–1399.

81. Jentjens RL, Jeukendrup AE: **Effects of pre-exercise ingestion of trehalose, galactose and glucose on subsequent metabolism and cycling performance**. *Eur J Appl Physiol* 2003, **88**:459–465.

82. Abokyi S, Shan SW, To CH, Chan HH, Tse DY: **Autophagy upregulation by the TFEB inducer trehalose protects against oxidative damage and cell death associated with NRF2 inhibition in human RPE cells**. *Oxid Med Cell Longev* 2020, **2020**, 5296341.