

Mitochondrial dysfunction in glaucomatous degeneration

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Abstract

• Glaucoma is a kind of optic neuropathy mainly manifested in the permanent death of retinal ganglion cells (RGCs), atrophy of the optic nerve, and loss of visual ability. The main risk factors for glaucoma consist of the pathological elevation of intraocular pressure (IOP) and aging. Although the mechanism of glaucoma remains an open question, a theory related to mitochondrial dysfunction has been emerging in the last decade. Reactive oxygen species (ROS) from the mitochondrial respiratory chain are abnormally produced as a result of mitochondrial dysfunction. Oxidative stress takes place when the cellular antioxidant system fails to remove excessive ROS promptly. Meanwhile, more and more studies show that there are other common features of mitochondrial dysfunction in glaucoma, including damage of mitochondrial DNA (mtDNA), defective mitochondrial quality control, ATP reduction, and other cellular changes, which are worth summarizing and further exploring. The purpose of this review is to explore mitochondrial dysfunction in the mechanism of glaucomatous optic neuropathy. Based on the mechanism, the existing therapeutic options are summarized, including medications, gene therapy, and red-light therapy, which are promising to provide feasible neuroprotective ideas for the treatment of glaucoma.

• **KEYWORDS:** glaucoma; mitochondrial dysfunction; oxidative stress; mitochondrial dynamics; mitophagy; neuroprotection

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INTRODUCTION

Glaucoma is a group of neurodegenerative diseases^[1] marked by the irreversible death of retina ganglion cells (RGCs), characteristic optic atrophy, and loss of visual fields^[2]. At present, glaucoma has become the leading cause of permanent blindness all around the world^[3]. A comprehensive assessment from 2014 anticipated that there will be 111.8 million glaucoma patients worldwide in 2040^[4]. Based on whether the anterior chamber angle is open or not and whether the intraocular pressure (IOP) is raised, glaucoma can be roughly divided into three categories: open angle glaucoma (OAG), angle-closure glaucoma (ACG) and normal tension glaucoma (NTG)^[5]. In light of the similarities between glaucoma and primary mitochondrial optic neuropathies^[6-7], numerous studies have concentrated on the impact of mitochondrial dysfunction in the course of glaucoma, which is the main topic of our review as well.

Although glaucoma has been a threat to human health for many years, the exact pathogenesis and contributing factors of glaucoma remain an open question to be elucidated. On the one hand, it is acknowledged that the primary risk factor for glaucoma is pathologic elevated IOP^[8]. The stability of IOP is kept by the aqueous humor generated by the ciliary body. When aqueous humor is generated too much or outflow is blocked, the IOP will rise progressively. Increased IOP causes mechanical stress and injury to RGCs and the optic nerve head (ONH), particularly the lamina cribrosa and surrounding tissues^[9]. Damaged optic neuronal axons under prolonged stress result in poor transport, impeding the retrograde movement of essential trophic factors from the brainstem to the RGCs^[10]. Meanwhile, a decrease in the transfer of oxygen and nutrients to the retina and optic nerve is generated as a result of blood vessel compression brought on by high IOP, which causes the production of reactive oxygen species (ROS) and oxidative damage^[11].

Aging is another key factor affecting the progression of glaucoma^[8]. Although there are significant differences in glaucoma prevalence of different races, it increases substantially with age^[12]. What's more, our group has shown that mitochondrial

DNA (mtDNA) integrity, the copy number of mtDNA, fusion mediators, mitophagy, and genes related to antioxidation all show a downward trend with the increase of age in the retina of zebrafish models^[13]. Additionally, due to the increasing need for light-emitting diodes (LEDs) and digital screens, light damage is a significant factor in ocular aging. Exposure to certain wavelengths or intensities of light for a certain time can result in serious damage to the retina^[14-15], which may facilitate the development of age-related ocular disorders. Other risk factors^[16], including thin corneas, positive family history, and ethnicity, are also connected with the pathogenesis of glaucoma.

Currently, IOP is the sole variable that can be controlled and is proven to be effective in glaucoma treatment^[17]. However, the degeneration of RGCs and axons is still progressing after strict control of IOP^[7]. Therefore, finding the exact pathological mechanisms of glaucoma and developing specific treatment options are urgently needed. Here we review the significance of preserving healthy mitochondrial function for the retina and optic nerve and how mitochondrial dysfunction affects the pathogenesis of glaucoma. Then we summarize current and potential therapeutic options, particularly mitochondrial-related ones. The therapies aimed at improving mitochondrial function at the cellular or molecular level may offer new hope for research and clinical utility to delay the process of glaucoma.

Normal Mitochondrial Functions in the Retina and Optic Nerve

Energy and reactive oxygen species In normal situations, the light passing through the pupil is sensed by retinal photoreceptor cells^[18]. The signal will be transmitted by a series of retinal cells and finally by RGCs to the visual cortex. The axons of RGCs forming the optic nerve cross the lamina cribrosa and are wrapped in myelin sheaths post the lamina cribrosa^[19]. Instead of carrying out saltatory conduction, unmyelinated prelaminar and laminar RGC axons transmit electronic signals by high-density voltage-sodium channels and Na⁺-K⁺ adenosine triphosphate (ATP) pumps^[19]. Because of this special structure, the neurons and optic nerve require oxidative phosphorylation (OXPHOS) to produce large quantities of ATP to keep the electrochemical gradients for transmitting signals^[20]. Corresponding to this condition, a physiological rise in the number of mitochondria is found in the unmyelinated RGC axons^[21]. Mitochondria are important sites for producing energy in the form of ATP^[22]. Meanwhile, it participates in cell apoptosis, the balance between the production and removal of active substances, and the regulation of intracellular calcium absorption and release^[23-24]. As the primary energy source of most cells, the mitochondrial OXPHOS system includes two essential components – the electron transport chain (ETC) and ATP synthases (Figure 1). Five protein complexes helping

complete the process of OXPHOS are located on the inner membrane of mitochondria^[25]. Electrons from niacinamide adenine dinucleotide (NADH) or reduced flavin adenine dinucleotide (FADH₂) are sequentially transferred to oxygen through the complexes, while protons are pumped into the space between the inner and outer membranes to create a proton concentration gradient^[26]. When these protons return to the mitochondrial matrix, ATP is produced by ATP synthase^[26]. The stable production of ATP in mitochondria is the basis for the normal operation of various cellular functions.

It is worth mentioning that the mitochondrial respiratory chain provides sufficient energy but also produces reactive oxygen or nitrogen species (ROS/RNS; Figure 1), such as hydrogen peroxide (H₂O₂), hydroxyl radical (OH), the anion radical (O²⁻) and nitric oxide radical^[27-28]. Complex I and III of the ETC are the most active sites for the generation of ROS^[29]. Despite the fact that ROS and RNS, which is generated by the reaction of nitric oxide and ROS, can act as critical second messengers in many signaling processes^[7,30], excessive reactive species can oxidize DNA and various biological molecules, representing the primary cause of damage to cellular structures^[30]. There are a lot of antioxidants that fight against oxidation inside cells, such as glutathione peroxidases, superoxide dismutase, and catalases^[31], which can alleviate the harmful effects resulting from reactive species. Nevertheless, under some situations and damage, the production of reactive substances surpasses the capacity of the cellular antioxidant mechanism^[28]. This leads to the state of oxidative stress, which is regarded as an imbalance between oxidants and antioxidants that tends to favor oxidation, resulting in destroy of redox signaling and/or molecular damage^[27,32]. Oxidative stress can irreparably damage mitochondrial and cellular functions.

Mitochondrial Quality Control As byproducts of mitochondrial energy production, ROS can cause mtDNA mutations and interfere with protein folding and structure. Therefore, a sophisticated quality control mechanism needs to be developed to remove damaged components and biosynthesize new macromolecules^[33]. In this regard, it is essential to mention mitophagy as well as mitochondrial dynamics - fusion and fission. Mitophagy, called the selective autophagy of mitochondria^[34], is a mechanism that eliminated damaged mitochondria and is precisely mediated by PTEN-induced putative kinase 1 (Pink1) and Parkin^[35]. In addition, the morphology and connectivity of the mitochondrial network are controlled by the processes of mitochondrial fusion and fission, depending on the demand for specific biological needs^[36-37]. Fusing together several mitochondria to produce a single mitochondrion is an example of fusion while fission refers to the process of breaking apart a single large mitochondrion into many smaller ones^[38].

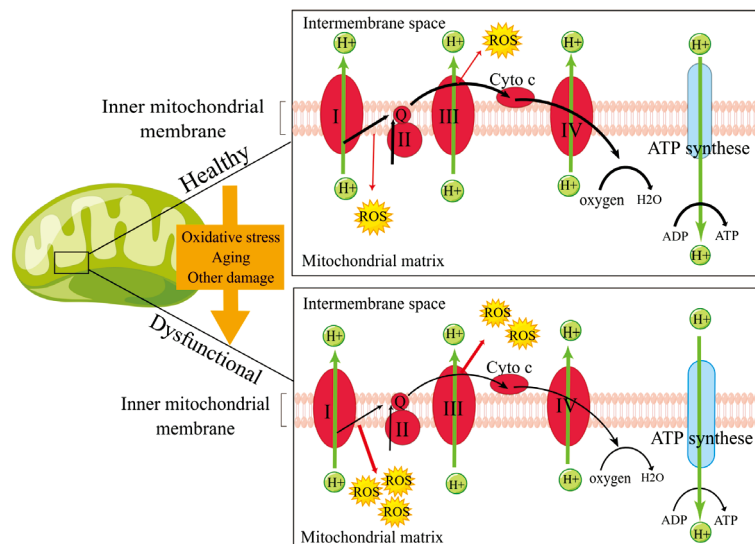


Figure 1 Electron transport chains in healthy and dysfunctional mitochondria The ETC is made up of four different protein complexes: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome reductase), and complex IV (cytochrome oxidase). Electrons that originate from NADH or FADH₂ are finally transported to oxygen after passing through complex I or complex II, coenzyme Q, complex III, cytochrome c, and complex IV. During the process, oxygen can react with electrons in advance to produce ROS. In healthy mitochondria, ROS can be well removed while in dysfunctional mitochondria, the antioxidant capacity of mitochondria and the electron transport efficiency are reduced, resulting in excessive ROS and causing oxidative damage to mitochondria^[32]. ETC: Electron transport chain; ROS: Reactive oxygen species; Cyto c: Cytochrome c; Q: Coenzyme Q. Created by Figdraw.

Mitochondria constantly undergo the cycle of fusion and fission, so they come in a variety of shapes^[39]. Fusion mediated by optic atrophy 1 (Opa1) and mitochondrial fusion proteins 1/2 (Mfn1/Mfn2) can dilute mutant mtDNA with non-mutant mtDNA for mitigating DNA damage^[40]. In an obvious fusion event, two mitochondria collide end to end and fuse at the site of the collision^[39]. The outer membrane of mitochondria fuses first and the inner mitochondrial membrane (IMM) fuses as well. Meanwhile, the mitochondrial contents mix and spread throughout the new mitochondria. In addition, there is a course called the “kiss-and-run fusion” event^[41]. One mitochondrion does not undergo an obvious structural combination, but exchanges matrix contents with another during brief encounters^[41]. Therefore, the significance of mitochondrial fusion is not only morphological regulation but also content exchange. As for the fission event, it contributes to biosynthesizing new mitochondria and also to removing dysfunctional mitochondria^[42-43]. How this dual fate happens has intrigued scientists. New studies find that when cells are about to proliferate, mitochondria tend to undergo midzone fission^[44]. However, before most peripheral fission, which is mediated by dynamin-related protein 1 (Drp1) and fission protein 1^[45], mitochondrial membrane potential and proton motive force are reduced, while ROS and calcium levels are increased. The cellular changes suggest that mitochondrial dysfunction may drive mitochondria to undergo peripheral fission to remove damaged mitochondria^[44]. Although it is not clear how to keep the balance between fusion and fission,

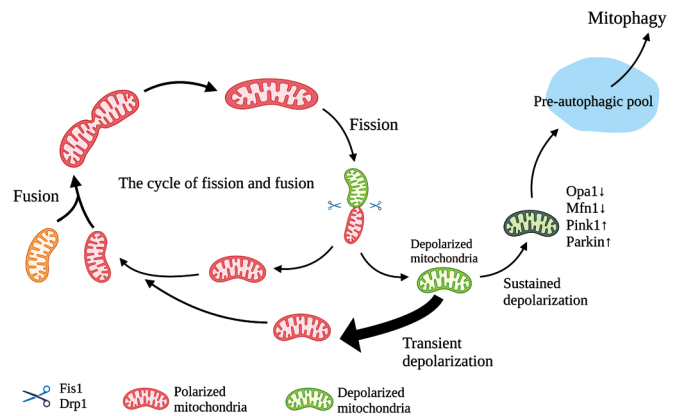


Figure 2 A schematic illustration of the relationship between mitochondrial fission, fusion, and mitophagy Mitochondria are continuously recycled between mitochondrial fission and fusion events. Mitochondrial fusion mechanisms dilute the effects of mutation by fusing mutated mtDNA (orange) with non-mutated mtDNA. When the damage exceeds a certain limit, mitochondrial fission occurs, in which mitochondria depolarize. Subsequently, these mitochondria either return to normal membrane potential (thick arrow) and continue the cycle, or remain depolarized^[42]. However, sustained depolarization will trigger Opa1 cleavage and Mfn1 reduction, along with Pink1/Parkin accumulation. Eventually, these mitochondria are cleared by mitophagy after remaining in the pre-autophagic pool for several hours. mtDNA: Mitochondrial DNA; Opa1: Optic atrophy 1; Mfn1: Mitofusin-1; Pink 1: PTEN-induced kinase 1. Created by Biorender.

it has been found that fission usually occurs shortly after fusion^[42] (Figure 2). After a fission event, some mitochondria

remain in a state of constant depolarization suggesting dysfunction. Such mitochondria are solitary for several hours in the pre-autophagic pool and then cleared by mitophagy^[42]. After undergoing a brief period of depolarization, healthy mitochondria return to normal membrane potential and continue the cycle of fusion and fission^[42].

Mitochondrial Dysfunction in Glaucoma Mitochondrial dysfunction means that mitochondria are not able to carry out normal functions. Because of the high-energy demand, it is essential that the neurons in the retina and optic nerve have the appropriate number of mitochondria and that these mitochondria are functioning properly^[23]. The aspects of mitochondrial dysfunction can be described by crosstalk among oxidative stress, membrane depolarization, mtDNA damage, reduction of ATP synthesis, cristae decomposition, and defective mitophagy^[11,35], all of which can be reflected in the course of glaucoma. Here we mainly summarize mitochondrial dysfunction into three major blocks for review: mtDNA damage, oxidative stress, and defective mitochondrial quality control.

Mitochondrial DNA damage Importantly, mtDNA is close to the location of the mitochondrial respiratory chain and not safeguarded by protective proteins^[32], making it exceptionally susceptible to oxidative damage caused by ROS. Hence, the rate of mutation in mitochondrial DNA is significantly higher than that of nuclear DNA^[35]. ROS can cause single-strand breaks (SSBs), double-strand breaks (DSBs), or oxidized bases^[46], resulting in mtDNA mutations. A previous study has shown that the integrity and volume of mtDNA gradually decline with age on account of the accumulation of oxidative damage from ROS^[47]. The extent of the damage can be measured by the level of a biomarker called 8-hydroxy-2'-deoxyguanosine (8-OH-dG)^[48], which indicates oxidative damage to DNA. The contents of 8-OH-dG in the aqueous humor of primary open angle glaucoma (POAG) patients are considerably greater than that of the healthy group^[49]. Besides, the elevated level of 8-OH-dG was discovered in the trabecular meshwork (TM) of POAG patients, which suggests that the damage to mtDNA is not confined to the retina but is widespread in the eyes.

What's more, a previous study showed that 27 different novel mtDNA nonsynonymous mutations are identified in POAG patients, among which 22 mutations could be pathogenic^[50]. Although mtDNA encodes only about 1% of mitochondrial proteins, it contains protein subunits involved in OXPHOS^[35]. Mutations of related genes or defects in components of OXPHOS could contribute to mitochondrial dysfunction. For instance, mutations in the mitochondrially encoded cytochrome B (MT-CYB), which encodes cytochrome b in Complex III, can cause defects in Complex III and disrupts

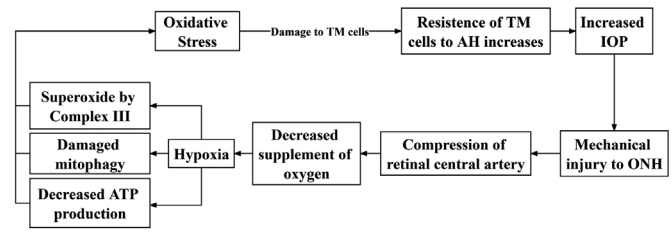


Figure 3 A schematic diagram of the relationship between oxidative stress and high IOP Excessive ROS can damage the TM cells, greatly increasing their resistance to aqueous humor and making persistent high IOP. High IOP causes mechanical injury to the optic nerve and compresses the retinal central artery, resulting in reduced blood flow and oxygen deprivation. Hypoxia reduces ATP production and stimulates the generation of superoxide by complex III. It also results in decreased mitophagy, which further intensifies oxidative stress. IOP: Intraocular pressure; ROS: Reactive oxygen species; TM: Trabecular meshwork; ONH: Optic nerve head.

the production of ATP^[51]. In TM cells, the complex I inhibitor Rotenone could increase the generation of ROS and induce the release of cytochrome c, finally leading to oxidative stress and cell apoptosis^[52]. Thus, damaged mtDNA can cause negative consequences for ROS production and clearance, ATP production, and other mitochondrial functions^[47].

Oxidative stress Oxidative stress is an essential component in the course of glaucoma, particularly in the occurrence of mitochondrial dysfunction. According to the present studies, oxidative stress can be a cause and/or a concomitant result of mitochondrial dysfunction. The specific mechanisms need to be further explored and summarized, which is the focus of discussion in this section.

Intraocular hypertension and oxidative stress Based on the previous studies, the abnormal structure and function of TM cells could be a crucial reason for increased IOP in primary OAG^[48] (Figure 3). Due to the fact that the TM has a lower level of antioxidant defense than both the cornea and iris, TM cells are the most vulnerable tissues to oxidative damage in the anterior chamber^[53]. The imbalance between oxidation and antioxidation in aqueous humor leads to the increase of ROS, which lowers the adherence of TM cells to the extracellular matrix and stimulates the pathway of nuclear factor kappa-B (NF-κB) in addition to damaging the mtDNA of TM endothelial cells, eventually leading to the collapse, fusion and enlargement of TM cytoskeleton^[48]. NF-κB is a kind of transcription factor whose constant activation can induce the expression of proinflammatory factors^[54], aggravating the inflammation responses in glaucoma. All of these changes deteriorate the abnormal resistance of TM cells to aqueous humor drainage, giving rise to progressively increasing IOP. The gradual rise in IOP causes mechanical injury to the ONH and surrounding tissues^[55], thereby compressing the central

retinal artery. Vascular dysregulation interferes with the self-regulatory function of ocular perfusion, so the eyes can no longer maintain a constant blood flow. This impaired blood flow to the eye results in a decreased supply of oxygen to the retina. Vascular dysfunction allows ocular structures, including the retina and TM, to undergo repeated reperfusion injury and unstable oxygen supply^[56], exacerbating oxidative stress and poor availability of energy within cells. During glaucoma, RGC axons primarily rely on glycolysis to compensate for deficient mitochondrial function, but glycolysis is not sufficient to salvage the metabolic vulnerability caused by glaucoma^[57]. When the retina is hypoxic-ischemic, three types of nitric oxide synthases are produced: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS)^[58]. Nitric oxide (NO) produced by nNOS and iNOS is involved in cytotoxic effects, causing neuronal and axon damage^[59]. Meanwhile, NO can trigger multiple pathways through different mediators resulting in cell death, such as the transcription of apoptotic proteins and the influx of Ca^{2+} ^[60-61]. And NO competes with oxygen for cytochrome c oxidase, leading to reduced mitochondrial cytochromes and other REDOX centers in the respiratory chain^[62]. Under hypoxia, before reaching cytochrome c, electrons from the REDOX center escape and interact with O_2 to form superoxide anion radicals^[20,62]. Prolyl hydroxylases (PHD), which is necessary for the first step of the ubiquitination and degradation process of hypoxia-inducible factor 1 α (HIF-1 α), can be inhibited by ROS, thus triggering the stabilization of HIF-1 α , which in conjunction with HIF-1 β activates downstream transcription^[63]. One previous study demonstrates that HIF-1 α does increase in glaucomatous eyes compared to healthy people^[64]. Hypoxia can also disrupt the dynamic balance between the mitochondrial fission-fusion cycle, causing it to wobble toward the fission tendency and resulting in excessive fragmentation of the network of mitochondria^[65]. In terms of specific molecular mechanisms, the Opa1 released under hypoxia would be converted to S-Opa1. Excessive cumulation of S-Opa1 would inhibit the activity of fusion^[65-66]. Hypoxia-induced increased mitochondrial fission can divide dysfunctional mitochondria from the network and eliminate them through mitophagy^[67], perhaps as an endogenous rescue response to mitochondrial damage. In the review of Jassim *et al*^[20], long-term hypoxia can cause mitochondrial dysfunction and hinder the mitophagy induction process, amplifying the buildup of damaged mitochondria, perhaps because the damage to mitochondria caused by hypoxia exceeds the ability to repair.

Aging and oxidative stress Typically, aging causes the degradation of the antioxidant system and the reduction of mitochondrial efficiency, resulting in the formation of aging mitochondria, thus increasing ROS and oxidative stress, ultimately causing cell apoptosis^[68]. In glaucoma, aging can

lead to reduced tolerance of RGCs and optic nerve to various stimuli and pressure, eventually resulting in the degeneration and loss of RGCs^[7]. It has been demonstrated that as people age, the contents of nicotinamide adenine dinucleotide (NAD⁺; a crucial component in REDOX reactions) and glutathione in the retina gradually decrease, compelling RGCs more susceptible to high IOP^[69]. This age-related vulnerability is also observed in other neurodegenerative diseases^[7], suggesting that neurons are indeed unusually sensitive to cellular changes related to aging. The phenomenon of oxidative stress is a significant characteristic of aging^[7], suggesting a synergistic relationship between the two. Many metabolites crucial for the metabolism of mitochondria and preventing damage from the damage of oxidative stress are discovered to decrease with age in the optic neurons of DBA/2J mice, which is a classical model of inherited and age-related glaucoma^[70]. RGCs gradually switch to fatty acid metabolism after a period of mitochondrial stress and metabolite depletion^[69]. The beta-oxidation of fatty acid can drive the production of ROS^[71], causing damage to several molecules. Additionally, advanced glycation end products (AGEs), which have been observed in the retina and optic disc of glaucomatous eyes, are formed in aging retinas under oxidative stress. Large accumulations of AGEs can form protein aggregates and produce more ROS to exacerbate the state of oxidative stress^[7].

Our team's prior research^[13] has demonstrated that in the aging retinas of zebrafish, the increased demand for nutrients and energy activates mTOR, which encourages the fission of mitochondria to increase the production of ATP and make up for the deterioration in mitochondrial function brought on by aging. In the long term, however, a sustained increase in fission and a constant decrease in fusion can have a detrimental effect, exacerbating the aging process. Accumulation of excess ROS was also found in the retinas of aging zebrafish, resulting in oxidative damage that destroys mtDNA. Meanwhile, because mitophagy is inhibited by activated mTOR, unhealthy and damaged mitochondria are not eliminated in time. Thus, the aging process of the eye is accompanied by a series of changed molecules related to the dysfunction of mitochondria, which plays a pathogenic role in neurodegenerative diseases, including glaucoma.

Additionally, light-induced damage is another important aspect of ocular aging. Various lights that people expose to in daily life may cause an increasing accumulation of damage in the eyes, particularly blue light (310-450 nm) coming from LED light and electronic devices^[72]. With short wavelength and strong penetration, blue light can arrive at the mitochondria of the retina^[73] and be absorbed by the ETC components containing flavin and cytochrome^[74], inducing photochemical effects. Moreover, blue light enables mitochondria to decrease

ATP synthesis and boost ROS production^[75-76], suggesting that the injury caused by blue light is closely related to oxidative stress. In R28 cells, 411 nm blue light significantly increased the content of superoxide free radicals in mitochondria^[75]. Blue light also contributes to the fragmentation of DNA^[75] and the stimulation of caspase-3 and Bcl-associated X-protein^[76], causing apoptotic neuronal death. Researchers believe that daily intake of blue light does not harm normal neurons because they have mechanisms to maintain homeostasis^[15]. But in glaucoma, mitochondrial homeostasis has been disrupted due to various factors, so optic neurons are more vulnerable to blue light^[15,77].

Other factors and oxidative stress Calcium is significant in fundamental cell metabolism and signaling, interacting with systems participating in the control of ROS^[78]. Calcium from the endoplasmic reticulum or cytoplasm can reach the membrane gap and the matrix of mitochondria, regulating different proteins and enzymes depending on where they are located^[79]. Abnormal calcium influx may be triggered when certain injuries occur, such as disruption of the integrity of the mitochondria membrane or inhibition of calcium ATPase activity^[78]. Constant overload of calcium opens mitochondrial permeability transition pores, promoting mitochondria to release ROS and calcium into the cytosol, eventually leading to mitochondrial dysfunction^[80]. Moreover, ROS itself can restrain the activity of calcium ATPase in neurons^[81], aggravate the disorder of intracellular calcium homeostasis, and generate more ROS. With the age rising, various metabolites and oxidative stress work together to gradually impair calcium homeostasis, leading to the degeneration of ocular cells^[82].

It is believed that the buildup of neurotoxic amounts of glutamate has a significant relationship to oxidative stress^[11], which is a common feature of many neurodegenerative disorders^[83]. Glutamate is one of the primary neurotransmitters in the mammalian central nervous system^[83]. Glutamate toxicity is mediated mainly by glutamate binding with N-methyl-D-aspartate (NMDA) receptors, which are distributed throughout the brain^[84]. According to research, one single injection of 20 nmol/L NMDA into the vitreous body of adult rats led to the death of 70% of RGCs in the retina^[84]. Higher level of glutamate are found in the vitreous of patients with glaucoma, which induces a compensatory mechanism producing more glutamine synthetase (GS)^[85]. Although GS can regulate the physiological concentration of glutamate, they are extremely sensitive to oxidative damage^[86]. ROS modifies GS, preventing glutamate in the retina from being converted to a non-toxic form. Excessive glutamate stimulates NMDA receptors for the long term, subsequently causing the influx of Ca²⁺^[87] and cell death.

Oxidative stress and related factors mentioned in this section

are dynamic, overlapping, and diverse. In many cases, it is even hard to tell causes from effects. Mitochondria, as the main source of ROS, naturally become the core concept of the hypothesis that oxidative stress drives glaucoma pathogenesis.

Defective mitochondrial quality control Mitochondria possess a specific macroautophagy mechanism known as mitophagy, which exists to eliminate dysfunctional mitochondria and retain a well-functioning mitochondrial population^[88]. Mitophagy is of vital importance for the normal functioning of mitochondria, which involves two reasons^[89]: 1) Mitochondria are the main origin of ROS and are extremely susceptible to the damage of oxidation. 2) Defective mitochondria could produce more ROS, releasing cytochrome c to induce cell apoptosis^[90]. The process of mitophagy is strictly controlled by Pink1 and Parkin. Pink1 encoded by nuclear DNA is a serine/threonine kinase, participating in the upstream of a cytosolic protein Parkin^[91]. Normally, Pink1 is primarily located in the IMM. Then Pink1 is translocated by mitochondrial translocases to the cytosol and degraded by the proteasomes^[91]. Under this condition, Pink1 is unable to ubiquitinate outer mitochondrial membrane (OMM) proteins and Parkin. Nevertheless, in damaged mitochondria, Pink1 is not degraded by proteasomes but is transported to OMM to accumulate. Subsequently, Pink1 phosphorylates the ubiquitin of Parkin and initiates the activity of Parkin. Protein substrates are ubiquitinated by Parkin, and autophagosomes are recruited to degrade the mitochondria^[91]. More and more studies have shown that impaired mitophagy can worsen the state of mitochondrial dysfunction in glaucoma^[92], and induction of mitophagy is expected to protect RGC against neurodegeneration of glaucoma^[93]. Hu and coworkers demonstrated that upregulation of Parkin by mitophagy in primary cultures RGCs that were subjected to glutamate excitotoxicity can be improved by overexpressing Opa1, achieving the goal of RGC protection^[94]. In addition, a recent study reported that iron chelator deferiprone, a mitophagy inducer, can increase mitophagy and decrease cell apoptosis to protect RGCs. Together, coordinating the level of mitophagy may be the key to protecting RGCs and axons from glaucomatous degeneration.

A crucial aspect of mitochondrial quality management is the cycle between mitochondrial dynamics - fusion and fission, as well as mitophagy, especially within the optic nerve with high energy requirements. Mediated by Drp1, mitochondrial fission aims at mitochondrial separation and further asymmetric separation, resulting in the production of normal and damaged organelles^[95]. The latter is eventually removed by mitophagy^[95]. During oxidative stress, accumulated ROS accounts for a decrease in mitochondrial output, so mitochondria need to increase fission and decrease fusion to compensate for the

decline in function^[13]. What's more, the imbalance of fusion and fission in cells plays a bridge between the elevated IOP and mitochondrial dysfunction in the degeneration of glaucoma^[92]. Ju *et al*^[96] have shown that high IOP can induce mitochondrial fission and cristae depletion in RGCs in glaucomatous DBA/2J mice, resulting in mitochondrial dysfunction. Recent research described a possible molecular mechanism of mitochondrial division induced by ocular hypertension. In RGCs of DAB/2J mice, high IOP causes a decrease in the apolipoprotein A-I binding protein (AIBP) expression, which increases mitochondrial fission and reduces ATP production^[97]. Similarly, in glaucomatous DAB/2J mice, high IOP can significantly increase the dephosphorylation of Drp1 at serine 637, which enhances the fragmentation of mitochondria and neuronal cell apoptosis^[98].

Mitochondrial fusion mediated by Opa1 and Mfn1/2 can promote mitochondrial biogenesis and network for mitochondrial integrity via distributing mitochondrial proteins and expediting the complementation of normal and mutated mtDNA^[99]. Nguyen *et al*^[100] have discovered that Opa1 deficiency can decrease the level of superoxide dismutase 2 (SOD2) and increase the NMDA receptors, leading to glutamate excitotoxicity and oxidative stress. Moreover, Opa1 deficiency also causes the loss of RGCs and increased mitochondrial fission in the optic nerve^[100]. Excessive proteolytic transformation of L-Opa1 to S-Opa1 is observed in the fragmentation and dysfunction of mitochondria^[101]. Increased expression of Opa1 and the ratio of L-Opa1/S-Opa1 protect RGCs from ischemia-induced damage^[102]. Interestingly, the maintenance of mitochondrial cristae structure^[39] and inhibition of cell apoptosis by blocking the release of cytochrome c are two functions of Opa1 that are unrelated to the control of fusion^[103]. Besides, it has been shown that overexpression of Mfn2 can increase the fusion process of mitochondrial and elevate the level of respiration^[104]. Collectively, it can be concluded that maintaining the stable operation of mitophagy and mitochondrial dynamics is essential for the improvement of glaucomatous degeneration.

Optic Neuroprotective Strategies Related to Mitochondrial Function Traditional antiglaucoma medications, such as cholinergic drugs, alpha-2 adrenergic agonists, prostaglandin analogues, and beta-blockers, reduce IOP by blocking the generation of aqueous humor and increasing its efflux^[105]. Nevertheless, some of these drugs have been found to protect the optic nerve from dying by increasing neurotrophic factor expression, regulating the release of glutamate and binding to receptors^[106-107]. It can be seen that in addition to reducing IOP, traditional drugs also have the ability to regulate several factors and processes concerning mitochondria.

Given the importance of oxidative stress in glaucoma,

antioxidants may be a good choice to achieve therapeutic benefits, especially before irreversible damage occurs. There have been numerous studies that have applied antioxidants, including several Vitamins, coenzyme Q10, astaxanthin, ginkgo biloba, omega 3/6 and resveratrol, to animal models of glaucoma to see how effective the drugs are^[108]. Dietary vitamin E deficiency increases RGC loss and lipid peroxidation in surgically-induced rat models of glaucoma, indicating that vitamin E can act as an adjunct therapy for glaucoma^[109]. A study published in 2021 showed that nicotinamide (vitamin B3), the precursor of NAD, can effectively prevent metabolic disorders induced by ocular hypertension^[110]. The normal operation and survival of neurons depend on NAD, a REDOX cofactor. When nicotinamide was administered to elderly D2 mice, scientists found that low doses of niacinamide (550 mg/kg-d) inhibited the buildup of mitochondria with abnormal cristae, limited DNA damage, and prevented the expression of most age-related genes within RGCs. At higher doses (2000 mg/kg-d), about 93% of the eyes had no damage to the optic nerve. This study opens the unexpected possibility of long-term use of nicotinamide in the precaution and treatment of glaucoma. As a natural antioxidant, resveratrol is an agonist of silent information regulators (SIRT6), which are widely distributed in the retina of mice, rats, and humans^[111]. Our previous work has demonstrated that in the ischemia/reperfusion models, resveratrol can block the Bax-caspase-3-dependent apoptotic pathway^[112] to display an anti-apoptotic role, increase the expression of Opa1 and adjust the L-Opa1/S-Opa1 ratio^[102]. It can also activate the AMPK/SIRT1/PGC-1 α pathway and decrease mTOR signaling, promoting mtDNA quality *via* decreased mitochondrial fragmentation and increased mitophagy^[13]. Similarly, previous studies by our group elaborate that oral administration of naringenin can increase the mitochondrial fusion protein 2 and rescue the age-related downregulation of autophagy, improving the visual function and retinal structure^[38]. Hence, antioxidants have displayed a strong potential to delay the progression of glaucomatous degeneration in experimental glaucoma models. Apart from testing antioxidants for glaucoma in animals, several antioxidants are already in clinical trials, which could speed up their use in humans. Ginkgo biloba as a Chinese medicinal plant has been undergoing clinical trials because of its antioxidant and neuroprotective properties. Ginkgo biloba supplementation can significantly provide a neuroprotective role for RGCs under hypoxia^[113]. In a study published in 2018, patients with glaucoma took dietary supplements made up of vitamins, minerals, omega 3, and plant extracts including Ginkgo biloba. After a month, patients were observed to have significantly improved blood flow at the retinal level and reduced pressure in the retinal central artery, suggesting an

improvement in the disease^[114]. All of these results indicate that antioxidants have a strong potential in the treatment of glaucoma in both animal models and humans, which are expected to provide more feasible strategies for the drug treatment of glaucoma. Currently, they can be used as adjunct drugs in combination with conventional glaucoma drugs to improve patient outcomes.

Another important thing to consider when using antioxidants is that effective antioxidants are often hydrophobic and less water-soluble^[115]. Therefore, it is important to take necessary measures to improve drug availability for improving drug efficacy. For instance, probucol is a kind of bisphenol compound with anti-inflammatory and antioxidant properties, which can remove free radicals, inhibit lipid oxidation and reduce cell apoptosis^[116]. Although probucol is the most insoluble hydrophobic antioxidant, researchers are able to significantly improve the solubility and bioavailability of the drug by embedding, modifying or adding excipients, which provides a precedent for the application of similar properties of antioxidants^[116-118].

In addition to the antioxidants mentioned above, drugs that can directly increase mitochondrial activity by targeting mitochondria are being explored and developed. Mitochondria has specific characteristics used for targeting, such as high mitochondrial membrane and particular components of IMM^[119]. The antioxidant lipophilic cations SkQ1, cationic plastoquinone derivatives, can penetrate the membrane of mitochondria and quench ROS to reduce the oxidative damage of polyunsaturated fatty acids in mitochondrial cardiolipin^[120]. SkQ1 blocks necrosis caused by ROS and also slows down the development of experimental glaucoma in rabbits^[121-122], which seems to be a promising strategy but needs more evidence for clinical trials. Additionally, penetrating peptides that are likely to directly interact with cardiolipin in the IMM^[123] could neutralize ROS and prevent cardiolipin from oxidation, inhibiting ROS-related mitochondrial apoptosis^[124]. Administration of Szeto-Schiller peptide 31 (SS-31) in models of glaucomatous rats can dramatically suppress cytochrome c release, boost B-cell lymphoma-2 (Bcl-2) expression, and downregulate the Bcl-2-associated X (Bax) proteins. It is encouraging that SS-31, the first of the penetrating peptides, is already undergoing multiple trials to examine its effectiveness and security in treating mitochondrial diseases^[125-126], especially for glaucoma. Other potential antioxidants targeting mitochondria have been identified to provide neuroprotection for neurodegenerative diseases^[127].

Other than medication, mitochondria dynamics-associated gene therapies can introduce a new gene into patients to treat diseases at the genetic level. As adeno-associated viruses (AAV) rarely undergo genome integration and are not related

to human diseases, they are widely studied as potential vectors for gene therapy^[128]. It has been shown that delivering *Opal in vivo via AAV2* can increase the expression of the *Opal* gene and protect RGC against glaucomatous neurodegeneration^[129]. What's more, Drp1 inhibition can be achieved by *in vivo* delivery of dominant negative *Drp1 K38A* mutant (Drp1^{K38A}), which can protect RGCs by reducing oxidative stress and mitochondria fission^[92]. Although there are still many aspects to be considered in gene therapy, it is clear that it offers new hope for mitochondrial dynamics-related treatment for neurodegeneration of glaucoma.

Furthermore, non-invasive red light (650-800 nm) can be absorbed by the cytochrome of Complex IV, accelerating ATP generation^[15] and reducing the damage of oxidative stress^[23]. The release of NO induced by red light can increase the blood flow of ONH, strengthening the transport of O₂ and nutrients to RGC axons^[15]. From the data of the literature, red light across the pupil of rats can enhance the function and survival of RGCs^[130]. Red-light therapy can be achieved with a special lens that converts ultraviolet light from the sun into extra red light^[131], which offers a convenient, noninvasive, and painless potential treatment for glaucoma. The limitation of red-light therapy is that the details of the underlying biochemical mechanisms remain to be explored. Meanwhile, due to the differences in technical methods and various factors, no consensus paradigm for treatment has emerged^[132], which confines the complete establishment of red-light therapy. However, it has become a public consensus to try to use good light sources for the eyes in daily life (Table 1).

Encouraging achievements continue to emerge in the neuroprotective treatment of glaucoma, but some challenges cannot be ignored. Most of the antioxidants found are only complementary drugs to glaucoma, which need to be in combination with conventional drugs. What's more, they still need to go through repeated trials and final approval before becoming a true adjuvant for glaucoma. Some drugs are only discovered to have possible molecular mechanisms for the treatment of glaucoma but have not been translated into clinical trials. Furthermore, added mitochondria-targeting drugs need to be developed to achieve a more precise therapeutic effect against mitochondrial dysfunction. Therefore, how to effectively protect RGCs and other cells involved in glaucoma remains a great challenge for scientists and clinicians.

CONCLUSION

To sum up, mitochondria are central for cells to perform a range of biological activities. Mitochondrial dysfunction promotes the development of glaucoma *via* mtDNA mutations, oxidative stress, and defective mitochondrial quality control. Scientists have identified a variety of potential neuroprotective options for improving mitochondrial function, such as

Table 1 Summary of current and potential treatments for glaucoma mentioned

Drugs or actions	Effects
Traditional antiglaucoma medications ^[105]	
Prostaglandin analogs	Increase uveoscleral and/or trabecular outflow
Beta-blockers	Decrease AH production
Alpha-adrenergic agonists	Decrease production; Increase outflow; Decrease episcleral venous pressure
Parasympathomimetic agents	Increase trabecular outflow
Rho kinase inhibitors	Decrease production; Increase outflow; Decrease episcleral venous pressure
Carbonic anhydrase inhibitors	Decrease AH production
Hyperosmotic agents	Dehydration of vitreous
Antioxidants	
Vitamin E ^[109]	Vitamin E deficiency increases RGC death and lipid peroxidation
Vitamin B3 ^[110]	Reduce the degree of increased IOP; Prevent metabolic disorders caused by ocular hypertension; Protect the optic nerve from damage
Resveratrol ^[13,102,112]	Anti-apoptosis; Increase the expression of Opa1; Decreased mitochondrial division; Increased mitophagy; Reduce IOP
Naringenin ^[38]	Improve retinal structure and visual function
Ginkgo biloba ^[114]	Improve blood flow; Reduce the pressure of the retinal central artery
Drugs targeting mitochondria	
SkQ1 ^[120]	Penetrate the membrane of mitochondria; Quench ROS; Block necrosis caused by ROS
Szeto-Schiller peptide 31 ^[125-126]	Suppress cytochrome c; Increase Bcl-2; Decrease Bax proteins
Gene therapy	
Opa1 ^[129]	Increase Opa1; Protect RGCs against neurodegeneration
Drp1 inhibition ^[92]	Reduce oxidative stress and mitochondria fission
Non-invasive therapy	
Red-light therapy ^[15,23]	Improve blood flow; Increase transport of oxygen and nutrients; Increase ATP production

AH: Aqueous humor; ROS: Reactive oxygen species; IOP: Intraocular pressure; Opa1: Optic atrophy 1; Drp1: Dynamin-related protein 1.

antioxidants, drugs targeting mitochondrial components, mitochondrial dynamics-associated gene therapy, and red-light therapy. Although most are still in the stage of animal or clinical trials, they still offer new insights into the adjuvant treatment of glaucoma. In conclusion, with the continuous exploration of the pathogenesis of glaucoma, the treatment system of glaucoma will be more abundant and complete, which will bring huge benefits to glaucoma patients, their families, and society.

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REFERENCES

1 Maddineni P, Kasetti RB, Patel PD, Millar JC, Kiehlbauch C, Clark AF, Zode GS. CNS axonal degeneration and transport deficits at the optic nerve head precede structural and functional loss of retinal ganglion cells in a mouse model of glaucoma. *Mol Neurodegener* 2020;15(1):48.

2 Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet* 2004;363(9422):1711-1720.

3 Kang JM, Tanna AP. Glaucoma. *Med Clin North Am* 2021;105(3):493-510.

4 Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology* 2014;121(11):2081-2090.

5 Młynarczyk M, Falkowska M, Micun Z, Obuchowska I, Kochanowicz J, Socha K, Konopińska J. Diet, oxidative stress, and blood serum nutrients in various types of glaucoma: a systematic review. *Nutrients* 2022;14(7):1421.

6 Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res* 2004;23(1):53-89.

7 Lee S, Van Bergen NJ, Kong GY, Chrysostomou V, Waugh HS, O'Neill EC, Crowston JG, Trounce IA. Mitochondrial dysfunction in glaucoma and emerging bioenergetic therapies. *Exp Eye Res* 2011;93(2):204-212.

8 Tezel G. Molecular regulation of neuroinflammation in glaucoma: current knowledge and the ongoing search for new treatment targets. *Prog Retin Eye Res* 2022;87:100998.

9 Quigley HA, Addicks EM, Green WR, Maumenee AE. Optic nerve damage in human glaucoma. II. The site of injury and susceptibility to damage. *Arch Ophthalmol* 1981;99(4):635-649.

10 Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma. *JAMA* 2014;311(18):1901.

- 11 Duarte JN. Neuroinflammatory mechanisms of mitochondrial dysfunction and neurodegeneration in glaucoma. *J Ophthalmol* 2021;2021:4581909.
- 12 Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006;90(3):262-267.
- 13 Wang N, Luo ZW, Jin M, Sheng WW, Wang HT, Long XY, Wu Y, Hu PP, Xu H, Zhang X. Exploration of age-related mitochondrial dysfunction and the anti-aging effects of resveratrol in zebrafish retina. *Aging* 2019;11(10):3117-3137.
- 14 Organisciak DT, Vaughan DK. Retinal light damage: mechanisms and protection. *Prog Retin Eye Res* 2010;29(2):113-134.
- 15 Osborne NN, Núñez-Álvarez C, del Olmo-Aguado S, Merrayo-Lloves J. Visual light effects on mitochondria: the potential implications in relation to glaucoma. *Mitochondrion* 2017;36:29-35.
- 16 Lee SSY, MacKey DA. Glaucoma—risk factors and current challenges in the diagnosis of a leading cause of visual impairment. *Maturitas* 2022;163:15-22.
- 17 Schuster AK, Erb C, Hoffmann EM, Dietlein T, Pfeiffer N. The diagnosis and treatment of glaucoma. *Dtsch Arztebl Int* 2020;117(13):225-234.
- 18 Zhang TZ, Fan B, Chen X, Wang WJ, Jiao YY, Su GF, Li GY. Suppressing autophagy protects photoreceptor cells from light-induced injury. *Biochem Biophys Res Commun* 2014;450(2):966-972.
- 19 Ruiz-Pesini E, Emperador S, López-Gallardo E, Hernández-Ainsa C, Montoya J. Increasing mtDNA levels as therapy for mitochondrial optic neuropathies. *Drug Discov Today* 2018;23(3):493-498.
- 20 Jassim AH, Inman DM, Mitchell CH. Crosstalk between dysfunctional mitochondria and inflammation in glaucomatous neurodegeneration. *Front Pharmacol* 2021;12:699623.
- 21 Barron MJ, Griffiths P, Turnbull DM, Bates D, Nichols P. The distributions of mitochondria and sodium channels reflect the specific energy requirements and conduction properties of the human optic nerve head. *Br J Ophthalmol* 2004;88(2):286-290.
- 22 Abate M, Festa A, Falco M, Lombardi A, Luce A, Grimaldi A, Zappavigna S, Sperlongano P, Irace C, Caraglia M, Misso G. Mitochondria as playmakers of apoptosis, autophagy and senescence. *Semin Cell Dev Biol* 2020;98:139-153.
- 23 Osborne NN, Núñez-Álvarez C, Joglar B, del Olmo-Aguado S. Glaucoma: focus on mitochondria in relation to pathogenesis and neuroprotection. *Eur J Pharmacol* 2016;787:127-133.
- 24 Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. *Cell* 2006;125(7):1241-1252.
- 25 Nolfi-Donagan D, Braganza A, Shiva S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biol* 2020;37:101674.
- 26 Zhao RZ, Jiang S, Zhang L, Yu ZB. Mitochondrial electron transport chain, ROS generation and uncoupling. *Int J Mol Med* 2019;44(1):3-15.
- 27 Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol* 2015;4:180-183.
- 28 Zhao J, Wang S, Zhong W, Yang B, Sun LX, Zheng YJ. Oxidative stress in the trabecular meshwork. *Int J Mol Med* 2016;38(4):995-1002.
- 29 Chen CL, Zhang LW, Jin ZC, Kasumov T, Chen YR. Mitochondrial redox regulation and myocardial ischemia-reperfusion injury. *Am J Physiol Cell Physiol* 2022;322(1):C12-C23.
- 30 Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. *Cell Death Differ* 2015;22(3):377-388.
- 31 Cáceres-Vélez PR, Hui F, Hercus J, Bui B, Jusuf PR. Restoring the oxidative balance in age-related diseases—An approach in glaucoma. *Ageing Res Rev* 2022;75:101572.
- 32 Chrysostomou V, Rezanian F, Trounce IA, Crowston JG. Oxidative stress and mitochondrial dysfunction in glaucoma. *Curr Opin Pharmacol* 2013;13(1):12-15.
- 33 Pickles S, Vigié P, Youle RJ. Mitophagy and quality control mechanisms in Mitochondrial maintenance. *Curr Biol* 2018;28(4):R170-R185.
- 34 Onishi M, Yamano K, Sato M, Matsuda N, Okamoto K. Molecular mechanisms and physiological functions of mitophagy. *EMBO J* 2021;40(3):e104705.
- 35 Skeie JM, Nishimura DY, Wang CL, Schmidt GA, Aldrich BT, Greiner MA. Mitophagy: an emerging target in ocular pathology. *Invest Ophthalmol Vis Sci* 2021;62(3):22.
- 36 Miettinen TP, Björklund M. Mitochondrial function and cell size: an allometric relationship. *Trends Cell Biol* 2017;27(6):393-402.
- 37 Rambold AS, Pearce EL. Mitochondrial dynamics at the interface of immune cell metabolism and function. *Trends Immunol* 2018;39(1):6-18.
- 38 Chen GP, Zeng L, Yan F, Liu JL, Qin MQ, Wang FF, Zhang X. Long-term oral administration of naringenin counteracts aging-related retinal degeneration via regulation of mitochondrial dynamics and autophagy. *Front Pharmacol* 2022;13:919905.
- 39 Chan DC. Mitochondrial dynamics and its involvement in disease. *Annu Rev Pathol* 2020;15:235-259.
- 40 Tam ZY, Gruber J, Halliwell B, Gunawan R. Mathematical modeling of the role of mitochondrial fusion and fission in mitochondrial DNA maintenance. *PLoS One* 2013;8(10):e76230.
- 41 Liu XG, Weaver D, Shirihai O, Hajnóczky G. Mitochondrial ‘kiss-and-run’: interplay between mitochondrial motility and fusion-fission dynamics. *EMBO J* 2009;28(20):3074-3089.
- 42 Twig G, Elorza A, Molina AJA, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 2008;27(2):433-446.
- 43 Burman JL, Pickles S, Wang CX, Sekine S, Vargas JNS, Zhang Z, Youle AM, Nezhich CL, Wu XF, Hammer JA, Youle RJ. Mitochondrial fission facilitates the selective mitophagy of protein aggregates. *J Cell Biol* 2017;216(10):3231-3247.
- 44 Kleele T, Rey T, Winter J, Zaganelli S, Mahecic D, Perreten Lambert H, Ruberto FP, Nemir M, Wai T, Pedrazzini T, Manley S. Distinct fission signatures predict mitochondrial degradation or biogenesis. *Nature* 2021;593(7859):435-439.

- 45 Shi WJ, Tan C, Liu C, Chen D. Mitochondrial fission mediated by Drp1-Fis1 pathway and neurodegenerative diseases. *Rev Neurosci* 2022;34(3):275-294.
- 46 Quan Y, Xin YG, Tian GE, Zhou JT, Liu XJ. Mitochondrial ROS-modulated mtDNA: a potential target for cardiac aging. *Oxid Med Cell Longev* 2020;2020:9423593.
- 47 Chistiakov DA, Sobenin IA, Revin VV, Orekhov AN, Bobryshev YV. Mitochondrial aging and age-related dysfunction of mitochondria. *Biomed Res Int* 2014;2014:238463.
- 48 Nita M, Grzybowski A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. *Oxid Med Cell Longev* 2016;2016:3164734.
- 49 Sorkhabi R, Ghorbanihaghjo A, Javadzadeh A, Rashtchizadeh N, Moharrery M. Oxidative DNA damage and total antioxidant status in glaucoma patients. *Mol Vis* 2011;17:41-46.
- 50 Abu-Amero KK, Morales J, Bosley TM. Mitochondrial abnormalities in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 2006;47(6):2533-2541.
- 51 Faro VL, Nolte IM, Ten Brink JB, Snieder H, Jansonius NM, Bergen AA. Mitochondrial genome study identifies association between primary open-angle glaucoma and variants in *MT-CYB*, *MT-ND4* genes and haplogroups. *Front Genet* 2021;12:781189.
- 52 He Y, Leung KW, Zhang YH, Duan S, Zhong XF, Jiang RZ, Peng Z, Tombran-Tink J, Ge J. Mitochondrial complex I defect induces ROS release and degeneration in trabecular meshwork cells of POAG patients: protection by antioxidants. *Invest Ophthalmol Vis Sci* 2008;49(4):1447-1458.
- 53 Izzotti A, Saccà SC, Longobardi M, Cartiglia C. Sensitivity of ocular anterior chamber tissues to oxidative damage and its relevance to the pathogenesis of glaucoma. *Invest Ophthalmol Vis Sci* 2009;50(11):5251-5258.
- 54 Li GR, Luna C, Liton PB, Navarro I, Epstein DL, Gonzalez P. Sustained stress response after oxidative stress in trabecular meshwork cells. *Mol Vis* 2007;13:2282-2288.
- 55 Kwok S, Pan MQ, Hazen N, Pan XL, Liu J. Mechanical deformation of peripapillary retina in response to acute intraocular pressure elevation. *J Biomech Eng* 2022;144(6):061001.
- 56 Pinazo-Durán MD, Gallego-Pinazo R, García-Medina JJ, et al. Oxidative stress and its downstream signaling in aging eyes. *Clin Interv Aging* 2014;9:637-652.
- 57 Jassim AH, Fan Y, Pappenhagen N, Nsiah NY, Inman DM. Oxidative stress and hypoxia modify mitochondrial homeostasis during glaucoma. *Antioxid Redox Signal* 2021;35(16):1341-1357.
- 58 Kaur C, Sivakumar V, Foulds WS. Early response of neurons and glial cells to hypoxia in the retina. *Invest Ophthalmol Vis Sci* 2006;47(3):1126-1141.
- 59 Kaur C, Foulds WS, Ling EG. Hypoxia-ischemia and retinal ganglion cell damage. *Clin Ophthalmol* 2008;2(4):879-889.
- 60 Mishra OP, Ashraf QM, Delivoria-Papadopoulos M. Phosphorylation of cAMP response element binding (CREB) protein during hypoxia in cerebral cortex of newborn piglets and the effect of nitric oxide synthase inhibition. *Neuroscience* 2002;115(3):985-991.
- 61 Zubrow AB, Delivoria-Papadopoulos M, Ashraf QM, Fritz KI, Mishra OP. Nitric oxide-mediated Ca²⁺/calmodulin-dependent protein kinase IV activity during hypoxia in neuronal nuclei from newborn piglets. *Neurosci Lett* 2002;335(1):5-8.
- 62 Solaini G, Baracca A, Lenaz G, Sgarbi G. Hypoxia and mitochondrial oxidative metabolism. *Biochim Biophys Acta BBA Bioenerg* 2010;1797(6-7):1171-1177.
- 63 Hamanaka RB, Chandel NS. Mitochondrial reactive oxygen species regulate hypoxic signaling. *Curr Opin Cell Biol* 2009;21(6):894-899.
- 64 Tezel G, Wax MB. Hypoxia-inducible factor 1alpha in the glaucomatous retina and optic nerve head. *Arch Ophthalmol* 2004;122(9):1348-1356.
- 65 Wang SY, Tan J, Miao YY, Zhang Q. Mitochondrial dynamics, mitophagy, and mitochondria-endoplasmic reticulum contact sites crosstalk under hypoxia. *Front Cell Dev Biol* 2022;10:848214.
- 66 Ge YF, Shi XJ, Boopathy S, McDonald J, Smith AW, Chao LH. Two forms of Opa1 cooperate to complete fusion of the mitochondrial inner-membrane. *eLife* 2020;9:e50973.
- 67 Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. *Science* 2012;337(6098):1062-1065.
- 68 Vatner SF, Zhang J, Oydanich M, Berkman T, Naftalovich R, Vatner DE. Healthful aging mediated by inhibition of oxidative stress. *Ageing Res Rev* 2020;64:101194.
- 69 Williams PA, Harder JM, Foxworth NE, Cochran KE, Philip VM, Porciatti V, Smithies O, John SWM. Vitamin B3 modulates mitochondrial vulnerability and prevents glaucoma in aged mice. *Science* 2017;355(6326):756-760.
- 70 Libby RT, Anderson MG, Pang IH, Robinson ZH, Savinova OV, Cosma IM, Snow A, Wilson LA, Smith RS, Clark AF, John SWM. Inherited glaucoma in DBA/2J mice: pertinent disease features for studying the neurodegeneration. *Vis Neurosci* 2005;22(5):637-648.
- 71 Schönfeld P, Reiser G. Why does brain metabolism not favor burning of fatty acids to provide energy? Reflections on disadvantages of the use of free fatty acids as fuel for brain. *J Cereb Blood Flow Metab* 2013;33(10):1493-1499.
- 72 Jaadane I, Boulenguez P, Chahory S, Carré S, Savoldelli M, Jonet L, Behar-Cohen F, Martinsons C, Torriglia A. Retinal damage induced by commercial light emitting diodes (LEDs). *Free Radic Biol Med* 2015;84:373-384.
- 73 Tao JX, Zhou WC, Zhu XG. Mitochondria as potential targets and initiators of the blue light hazard to the retina. *Oxid Med Cell Longev* 2019;2019:6435364.
- 74 Ouyang XL, Yang J, Hong ZX, Wu YD, Xie YF, Wang GH. Mechanisms of blue light-induced eye hazard and protective measures: a review. *Biomed Pharmacother* 2020;130:110577.

- 75 Knels L, Valtink M, Roehlecke C, Lupp A, de la Vega J, Mehner M, Funk RHW. Blue light stress in retinal neuronal (R28) cells is dependent on wavelength range and irradiance. *Eur J Neurosci* 2011;34(4):548-558.
- 76 Wood JPM, Lascaratos G, Bron AJ, Osborne NN. The influence of visible light exposure on cultured RGC-5 cells. *Mol Vis* 2007;14:334-344.
- 77 Lascaratos G, Ji D, Wood JPM, Osborne NN. Visible light affects mitochondrial function and induces neuronal death in retinal cell cultures. *Vis Res* 2007;47(9):1191-1201.
- 78 Rohowetz LJ, Kraus JG, Koulen P. Reactive oxygen species-mediated damage of retinal neurons: drug development targets for therapies of chronic neurodegeneration of the retina. *Int J Mol Sci* 2018;19(11):3362.
- 79 Rossi A, Pizzo P, Filadi R. Calcium, mitochondria and cell metabolism: a functional triangle in bioenergetics. *Biochim Biophys Acta BBA Mol Cell Res* 2019;1866(7):1068-1078.
- 80 He Y, Ge J, Tombran-Tink J. Mitochondrial defects and dysfunction in calcium regulation in glaucomatous trabecular meshwork cells. *Invest Ophthalmol Vis Sci* 2008;49(11):4912-4922.
- 81 Zaidi A, Michaelis ML. Effects of reactive oxygen species on brain synaptic plasma membrane Ca^{2+} -ATPase. *Free Radic Biol Med* 1999;27(7-8):810-821.
- 82 Wojda U, Salinska E, Kuznicki J. Calcium ions in neuronal degeneration. *IUBMB Life* 2008;60(9):575-590.
- 83 Iovino L, Tremblay ME, Civiero L. Glutamate-induced excitotoxicity in Parkinson's disease: the role of glial cells. *J Pharmacol Sci* 2020;144(3):151-164.
- 84 Lotery AJ. Glutamate excitotoxicity in glaucoma: truth or fiction? *Eye (Lond)* 2005;19(4):369-370.
- 85 Bagnis A, Izzotti A, Centofanti M, Saccà SC. Aqueous humor oxidative stress proteomic levels in primary open angle glaucoma. *Exp Eye Res* 2012;103:55-62.
- 86 Tezel G, Yang XJ, Cai J. Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma. *Invest Ophthalmol Vis Sci* 2005;46(9):3177-3187.
- 87 Song AJ, Cho GW, Vijayakumar KA, Moon C, Ang MJ, Kim J, Park I, Jang CH. Neuroprotective effect of valproic acid on salicylate-induced tinnitus. *Int J Mol Sci* 2021;23(1):23.
- 88 Lou GF, Palikaras K, Lautrup S, Scheibye-Knudsen M, Tavernarakis N, Fang EF. Mitophagy and neuroprotection. *Trends Mol Med* 2020;26(1):8-20.
- 89 Twig G, Shirihai OS. The interplay between mitochondrial dynamics and mitophagy. *Antioxid Redox Signal* 2011;14(10):1939-1951.
- 90 Crompton M. The mitochondrial permeability transition pore and its role in cell death. *Biochem J* 1999;341(2):233-249.
- 91 Cowan K, Anichtchik O, Luo SQ. Mitochondrial integrity in neurodegeneration. *CNS Neurosci Ther* 2019;25(7):825-836.
- 92 Ju WK, Perkins GA, Kim KY, Bastola T, Choi WY, Choi SH. Glaucomatous optic neuropathy: Mitochondrial dynamics, dysfunction and protection in retinal ganglion cells. *Prog Retin Eye Res* 2022:101136.
- 93 Dai Y, Hu XX, Sun XH. Overexpression of parkin protects retinal ganglion cells in experimental glaucoma. *Cell Death Dis* 2018;9(2):88.
- 94 Hu XX, Dai Y, Zhang R, Shang KT, Sun XH. Overexpression of optic atrophy type 1 protects retinal ganglion cells and upregulates parkin expression in experimental glaucoma. *Front Mol Neurosci* 2018;11:350.
- 95 Zorov DB, Vorobjev IA, Popkov VA, Babenko VA, Zorova LD, Pevzner IB, Silachev DN, Zorov SD, Andrianova NV, Plotnikov EY. Lessons from the discovery of mitochondrial fragmentation (fission): a review and update. *Cells* 2019;8(2):175.
- 96 Ju WK, Kim KY, Lindsey JD, Angert M, Duong-Polk KX, Scott RT, Kim JJ, Kukhazov I, Ellisman MH, Perkins GA, Weinreb RN. Intraocular pressure elevation induces mitochondrial fission and triggers OPA1 release in glaucomatous optic nerve. *Invest Ophthalmol Vis Sci* 2008;49(11):4903-4911.
- 97 Choi SH, Kim KY, Perkins GA, Phan S, Edwards G, Xia YN, Kim J, Skowronska-Krawczyk D, Weinreb RN, Ellisman MH, Miller YI, Ju WK. AIBP protects retinal ganglion cells against neuroinflammation and mitochondrial dysfunction in glaucomatous neurodegeneration. *Redox Biol* 2020;37:101703.
- 98 Edwards G, Perkins GA, Kim KY, Kong Y, Lee Y, Choi SH, Liu YJ, Skowronska-Krawczyk D, Weinreb RN, Zangwill L, Strack S, Ju WK. Loss of AKAP1 triggers Drp1 dephosphorylation-mediated mitochondrial fission and loss in retinal ganglion cells. *Cell Death Dis* 2020;11(4):254.
- 99 Legros F, Lombès A, Frachon P, Rojo M. Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *MBoC* 2002;13(12):4343-4354.
- 100 Nguyen D, Alavi MV, Kim KY, Kang T, Scott RT, Noh YH, Lindsey JD, Wissinger B, Ellisman MH, Weinreb RN, Perkins GA, Ju WK. A new vicious cycle involving glutamate excitotoxicity, oxidative stress and mitochondrial dynamics. *Cell Death Dis* 2011;2(12):e240.
- 101 Lee H, Smith SB, Yoon Y. The short variant of the mitochondrial dynamin OPA1 maintains mitochondrial energetics and cristae structure. *J Biol Chem* 2017;292(17):7115-7130.
- 102 Pang YL, Qin MQ, Hu PP, Ji KB, Xiao RH, Sun N, Pan XH, Zhang X. Resveratrol protects retinal ganglion cells against ischemia induced damage by increasing Opa1 expression. *Int J Mol Med* 2020;46(5):1707-1720.
- 103 Frezza C, Cipolat S, Martins de Brito O, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De Strooper B, Scorrano L. OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell* 2006;126(1):177-189.
- 104 Chen H, Chomyn A, Chan DC. Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *J Biol Chem* 2005;280(28):26185-26192.
- 105 Gedde SJ, Vinod K, Wright MM, Muir KW, Lind JT, Chen PP, Li TJ, Mansberger SL, American Academy of Ophthalmology Preferred Practice Pattern Glaucoma Panel. Primary open-angle glaucoma preferred practice pattern[®]. *Ophthalmology* 2021;128(1):P71-P150.

- 106 Semba K, Namekata K, Kimura A, Harada C, Mitamura Y, Harada T. Brimonidine prevents neurodegeneration in a mouse model of normal tension glaucoma. *Cell Death Dis* 2014;5(7):e1341.
- 107 Wood JPM, Schmidt KG, Melena J, Chidlow G, Allmeier H, Osborne NN. The β -adrenoceptor antagonists metipranolol and timolol are retinal neuroprotectants: comparison with betaxolol. *Exp Eye Res* 2003;76(4):505-516.
- 108 Garcia-Medina JJ, Rubio-Velazquez E, Lopez-Bernal MD, Cobo-Martinez A, Zanon-Moreno V, Pinazo-Duran MD, Del-Rio-Vellosillo M. Glaucoma and antioxidants: review and update. *Antioxidants (Basel)* 2020;9(11):1031.
- 109 Ko ML, Peng PH, Hsu SY, Chen CF. Dietary deficiency of vitamin E aggravates retinal ganglion cell death in experimental glaucoma of rats. *Curr Eye Res* 2010;35(9):842-849.
- 110 Tribble JR, Otmani A, Sun SS, et al. Nicotinamide provides neuroprotection in glaucoma by protecting against mitochondrial and metabolic dysfunction. *Redox Biol* 2021;43:101988.
- 111 Luo HD, Zhou M, Ji KB, Zhuang JJ, Dang WJ, Fu SY, Sun T, Zhang X. Expression of sirtuins in the retinal neurons of mice, rats, and humans. *Front Aging Neurosci* 2017;9:366.
- 112 Luo HD, Zhuang JJ, Hu PP, Ye W, Chen SS, Pang YL, Li NF, Deng C, Zhang X. Resveratrol delays retinal ganglion cell loss and attenuates gliosis-related inflammation from ischemia-reperfusion injury. *Invest Ophthalmol Vis Sci* 2018;59(10):3879-3888.
- 113 Labkovich M, Jacobs EB, Bhargava S, Pasquale LR, Ritch R. Ginkgo biloba extract in ophthalmic and systemic disease, with a focus on normal-tension glaucoma. *Asia Pac J Ophthalmol (Phila)* 2020;9(3):215-225.
- 114 Harris A, Gross J, Moore N, Do T, Huang A, Gama W, Siesky B. The effects of antioxidants on ocular blood flow in patients with glaucoma. *Acta Ophthalmol* 2018;96(2):e237-e241.
- 115 Lau M, Giri K, Garcia-Bennett AE. Antioxidant properties of probucol released from mesoporous silica. *Eur J Pharm Sci* 2019;138:105038.
- 116 Cai ZY, Fu MD, Liu K, Duan XC. Therapeutic effect of Keap1-Nrf2-ARE pathway-related drugs on age-related eye diseases through anti-oxidative stress. *Int J Ophthalmol* 2021;14(8):1260-1273.
- 117 Mooranian A, Zamani N, Mikov M, Goločorbin-Kon S, Stojanovic G, Arfuso F, Kovacevic B, Al-Salami H. Bio micro-nano technologies of antioxidants optimised their pharmacological and cellular effects, *ex vivo*, in pancreatic β -cells. *Nanotechnol Sci Appl* 2020;13:1-9.
- 118 Tale S, Purchel AA, Dalsin MC, Reineke TM. Diblock terpolymers are tunable and pH responsive vehicles to increase hydrophobic drug solubility for oral administration. *Mol Pharm* 2017;14(11):4121-4127.
- 119 Zinovkin RA, Zamyatnin AA. Mitochondria-targeted drugs. *Curr Mol Pharmacol* 2019;12(3):202-214.
- 120 Antonenko YN, Avetisyan AV, Bakeeva LE, et al. Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 1. Cationic plastoquinone derivatives: synthesis and *in vitro* studies. *Biochemistry (Mosc)* 2008;73(12):1273-1287.
- 121 Yang XJ, Ge J, Zhuo YH. Role of mitochondria in the pathogenesis and treatment of glaucoma. *Chin Med J (Engl)* 2013;126(22):4358-4365.
- 122 Neroev VV, Archipova MM, Bakeeva LE, et al. Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 4. Age-related eye disease. SkQ1 returns vision to blind animals. *Biochemistry (Mosc)* 2008;73(12):1317-1328.
- 123 Birk AV, Liu SY, Soong Y, Mills W, Singh P, Warren JD, Seshan SV, Pardee JD, Szeto HH. The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. *J Am Soc Nephrol* 2013;24(8):1250-1261.
- 124 Zhao KS, Zhao GM, Wu DL, Soong Y, Birk AV, Schiller PW, Szeto HH. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J Biol Chem* 2004;279(33):34682-34690.
- 125 Chavez JD, Tang XT, Campbell MD, Reyes G, Kramer PA, Stuppard R, Keller A, Zhang HL, Rabinovitch PS, Marcinek DJ, Bruce JE. Mitochondrial protein interaction landscape of SS-31. *Proc Natl Acad Sci U S A* 2020;117(26):15363-15373.
- 126 Pang Y, Wang C, Yu L. Mitochondria-targeted antioxidant SS-31 is a potential novel ophthalmic medication for neuroprotection in glaucoma. *Med Hypothesis Discov Innov Ophthalmol* 2015;4(3):120-126.
- 127 Bonda DJ, Wang XL, Perry G, Nunomura A, Tabaton M, Zhu XW, Smith MA. Oxidative stress in Alzheimer disease: a possibility for prevention. *Neuropharmacology* 2010;59(4-5):290-294.
- 128 Mendell JR, Al-Zaidy SA, Rodino-Klapac LR, Goodspeed K, Gray SJ, Kay CN, Boye SL, Boye SE, George LA, Salabarria S, Corti M, Byrne BJ, Tremblay JP. Current clinical applications of *In Vivo* gene therapy with AAVs. *Mol Ther* 2021;29(2):464-488.
- 129 Ju WK, Kim KY, Duong-Polk KX, Lindsey JD, Ellisman MH, Weinreb RN. Increased optic atrophy type 1 expression protects retinal ganglion cells in a mouse model of glaucoma. *Mol Vis* 2010;16:1331-1342.
- 130 Del Olmo-Aguado S, Núñez-Álvarez C, Osborne NN. Red light of the visual spectrum attenuates cell death in culture and retinal ganglion cell death *in situ*. *Acta Ophthalmol* 2016;94(6):e481-e491.
- 131 Li L, Sahi SK, Peng MY, Lee EB, Ma L, Wojtowicz JL, Malin JH, Chen W. Luminescence- and nanoparticle-mediated increase of light absorption by photoreceptor cells: converting UV light to visible light. *Sci Rep* 2016;6:20821.
- 132 Fitzgerald M, Hodgetts S, Van Den Heuvel C, Natoli R, Hart NS, Valter K, Harvey AR, Vink R, Provis J, Dunlop SA. Red/near-infrared irradiation therapy for treatment of central nervous system injuries and disorders. *Rev Neurosci* 2013;24(2):205-226.