

# Chapter 14

## Neuroprotection for Photoreceptors

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**Abstract** Progressive dysfunction and death of photoreceptors is the major cause of loss of vision in most retinal diseases. Many studies investigating photoreceptor protection have used animal models, and some of the results have been implemented clinically. These include responsible gene applications, applying neurotrophic factors or antioxidants and blocking or preventing specific death signal transduction. Retinal prosthesis or appropriate cell transplantations have also been reported at the end stage of photoreceptor death. Conventional strategies for neuroprotection using neurotrophic factors or antioxidants have attempted to strengthen the cellular metabolism against variable stresses. However, not every application is well tolerated, although some clinical applications are ongoing. Recent understanding of photoreceptor cell death has led to targeting cell death initiation or blocking its execution. These include correcting the amount of intracellular cGMP, modification of epigenetic processes, and prevention of some proteolytic enzyme activity, such as calpains. Further, another approach from the aspect of the drug delivery system has also been developed. An improved design of photoreceptor protection will require a better understanding of the photoreceptor neurodegenerative mechanisms.

**Keywords** Cell death mechanism • Drug delivery • Neurotrophic factors • Photoreceptor protection • Proteolytic enzymes

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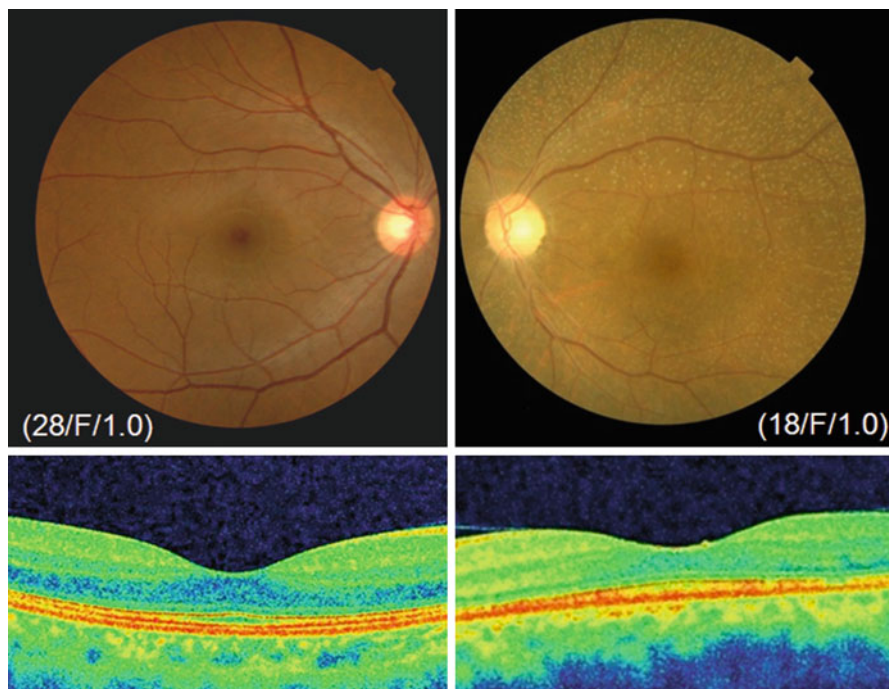
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## 14.1 Introduction

Retinal disease is the main cause for blindness. Retinal disease includes age-related macular degeneration (AMD), diabetic retinopathy, and retinitis pigmentosa (RP). In these diseases, photoreceptor degeneration is a hallmark of severity. Photoreceptor cell death has been studied extensively in retinal detachment [1] and inherited retinal disease by using suitable animal models. Retinitis pigmentosa (RP) is the leading cause of incurable inherited blindness and is caused by mutations in many different genes that cause the death of rod photoreceptors. More than 60 causative genes have been identified (<http://www.sph.uth.tmc.edu/retnet/sum-dis.htm>). During the course of rod photoreceptor cell death, a marked increase of reactive oxygen species (ROS) due to reduced oxygen utilization [2] or a decrease of neurotrophic factors from the rods [3] was reported to be the main cause of gradual cone cell death. In the retinal detachment animal model, photoreceptor cell death was reported to start at approximately 12 h after detachment, and this may cause a loss and/or delay in the recovery of visual acuity if the detachment covered the area of cone-rich macular region [4]. Because clinically significant vision loss is associated with this deterioration of cone function, the prevention of cone loss during retinal degeneration is one of the targets for retinal degeneration therapy [5].

## 14.2 Structure of Photoreceptors

Human photoreceptors include rods and cones. Rods comprise 95 % of the photoreceptors in the humans and play role in scotopic vision, while cones are responsible for photopic vision and increase in cell density towards the center of the macula [6]. The photoreceptors have unique shapes, consisting of an outer segment (OS), an inner segment (IS), a cell body, and a synaptic terminal where neurotransmission occurs. Visual pigments called opsins are involved in the OS and participate as the trigger of phototransduction, namely conversion of light into electrochemical messages. Disorganization of this signal transduction results in a major impact on quality of life, and the prevention of this cell death has important implications. Recent advances in diagnostic methods, such as optical coherence tomography (OCT), improve the evaluation of these structures (Fig. 14.1). OCT has become the gold standard for assessing anatomical abnormalities in retinal diseases. In particular, a recent advanced model of adaptive optics-OCT images the cell density of the macula. RP patients, even with good visual acuity, may show a disrupted arrangement of cones when using this advanced method, while conventional OCT cannot distinguish the difference in macular structure [7]. Advances in clinical equipment may provide new insights into photoreceptor death.



**Fig. 14.1** Optical coherence tomography (*OCT*) has become the gold standard for assessing anatomical abnormalities in retinal diseases, especially for the macular area. RP patients with good visual acuity (18-year-old woman in the right column) show a relatively reserved macular structure, although the outer segment (*OS*)/inner segment (*IS*) line was disrupted and was not observed outside of the macula

### 14.3 Mechanism of Photoreceptor Cell Death

In the course of cell death in higher organisms, including humans, a variety of morphologies have been identified so far. In 1960, Lockshin and Williams [8] reported a series of cell death and proposed the term programmed cell death. From the difference in cell morphology at the time of cell death, this programmed cell death is classified as type 1 (apoptosis), type 2 (cell death with autophagy), or type 3 (necrosis).

#### 14.3.1 Apoptosis, Autophagy, and Necrosis

Kerr and coworkers reported a specific form of cell death as apoptosis [9]. The cell death was controlled genetically, and proteins of the B-cell lymphoma 2 (*BCL2*) families are well-described key regulators involved in this mechanism. The caspase

family of cysteine protease is regulated by these proteins [10], and they are divided into proapoptotic and antiapoptotic proteins, which, together, make the “life-or-death decision” for the cell [11]. However, recent studies showed that caspase activity is not the sole mediator of photoreceptor cell death, although it may be activated during photoreceptor degeneration [1].

Cell death with autophagy is Type 2 programmed cell death. Cells, when exposed to stress such as amino acid starvation or the accumulation of abnormal protein, show phospholipid gathered along with the abnormal protein and autophagy begins. The intracellular lysosomes and autophagosomes cause membrane fusion, followed by breakdown of the abnormal proteins. Thus, the autophagy phenomenon is believed to allow the temporary avoidance of cell damage against some stresses, such as starvation. However, the avoidance of cell damage by autophagy cannot be maintained during longer periods of starvation. Punzo and coworkers reported results of starvation experiments and showed that the nonautonomous cone death in retinitis pigmentosa may be a result of the starvation of cones [12]. Apoptotic and autophagic pathways have been reported to share common pathways [13] and may coexist in cells [14].

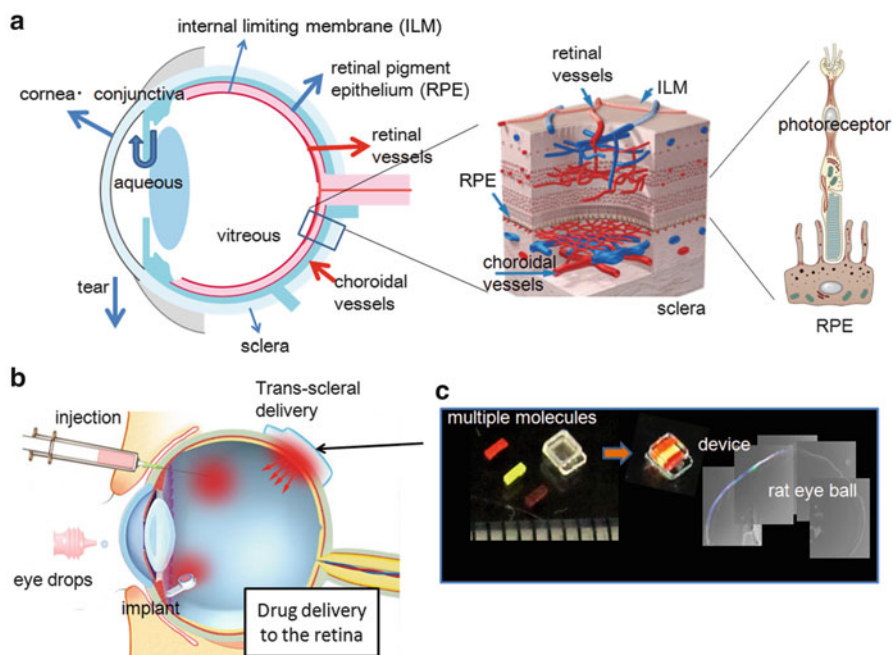
Traditionally, necrosis has been considered an uncontrolled process of cell death. However, this status has regulated components in certain circumstances. Recent studies revealed that death receptor-induced necrosis is mediated by the activation of receptor-interacting protein 1 (RIP1) [15], which is regulated by RIP3-dependent phosphorylation of RIP kinase [16]. In photoreceptor necrosis, tumor necrosis factor (TNF)-induced cellular necrosis was reported in interphotoreceptor retinoid-binding protein (IRBP) ( $-/-$ ) mice [17]. The study also showed that, in addition to apoptosis, RIP kinase-mediated necrosis contributes strongly to cone and rod degeneration in *Irbp* $^{-/-}$  retina. Nakazawa and coworkers also reported that TNF- $\alpha$  plays a critical role in RD-induced photoreceptor degeneration [18].

### 14.3.2 Detection of Cell Death

These death features were reported originally from morphological analysis, and transmission electron microscopy (TEM) was best for the morphological detection of the cell death [19]. Biochemical analyses to detect cell death have also been developed [20]. However, each cell death type, either apoptosis, autophagy, or necrosis, has common and/or similar features, and some biological reactions show redundancy during the photoreceptor death process. Therefore, many authors have used these methods in combination and examined the results carefully.

## 14.4 Strategy for Photoreceptor Protection

There are several reasons for delay in the development of the photoreceptor cell death prevention. These are in part due to an incomplete understanding of the regulation of photoreceptor cell death as described above. Cell death is not only apoptosis, but necrosis and autophagy have been reported to be important factors, and these events may occur together. Another major reason for the delay in treatment options is the structure of the retina and its related tissues, including the blood-retinal barrier (BRB) (Fig. 14.2). The development of novel therapeutic strategies to overcome these challenges will require the use of suitable animal models [21].



**Fig. 14.2** Retina is protected by many tissue barriers (a), which is why development of retinal therapy has been difficult. Recently, we reported a novel trans-scleral drug delivery device placed on the sclera that consisted of a drug-releasing semipermeable membrane and impermeable membranes acting as the drug reservoir (b). The device facilitates a sustained one-way and multiple drug release (c)

### ***14.4.1 Gene Therapy***

Many methods have been reported to rescue photoreceptor cells from death, such as neurotrophic factor application, cell transplantation, and genetic replacement [22]. Since mutation in many different genes can generate inherited retinal degeneration and almost all patients lose rods, genetic replacement has been discussed as an option to only some patients [22]. Gene replacement therapy for Leber congenital amaurosis type 2 (LCA2) has been applied in five patients with LCA2 using adeno-associated virus type 2 (AAV2). Three-year follow-up results were reported recently, and the data showed a statistically significant improvement of best-corrected visual acuity between baseline and 3 years (maximum visual acuity was achieved around 6 months and persisted) [23]. Although the natural history of the disease may not be altered by the therapy [24], some gene applications may show promising results. Recently, an interesting application has reported by converting adult rods into cones via knockdown of the rod photoreceptor determinant Nrl [25]. The lineage made the cells resistant to the mutations in rod-specific genes and prevented photoreceptor loss in mice. New methods associated with genetic application may provide breakthroughs to the difficulties identified so far.

### ***14.4.2 Neuroprotection by Neurotrophic Factors***

Neuroprotection is a mutation-independent approach to protect photoreceptor cells. Neurotrophic factors have been used to enhance cell survival in retinas undergoing cell death from a wide variety of insults. Neurotrophic factors that promote cell growth, differentiation, survival, and function of specific nerve cell populations were identified during neuroscience investigations. The discovery of nerve growth factor (NGF) by Rita Levi-Montalcini in the 1950s was an important event in modern neuronal cell biology [26]. Neurotrophic factors are families of proteins that promote neuron survival, growth, and development. These neurotrophic factors include neurotrophins (NGF, brain-derived neurotrophic factor (BDNF), NT-3, NT-4/5), the serine protease inhibitor (srpin) family (pigment epithelium-derived factor (PEDF)), and neuropoietic cytokines (ciliary neurotrophic factor (CNTF)) [27]. Continuous BDNF expression in double transgenic mice showed significant photoreceptor protection [28]. CNTF caused a delay of retinal degeneration in several retinal degeneration animal models [29]. Further, amyotrophic lateral sclerosis (ALS) trials using subcutaneous CNTF injection that showed no differences between the placebo and treatment groups and the delivery methods of CNTF were reconsidered and applied for patients with RP using CNTF-expressing intraocular implants for cone protection [30]. Although a phase III clinical trial has been reported for RP patients using this intraocular implant, evaluation of the clinical results has not been completed [31]. Both BDNF and CNTF execute its photoreceptor effects via Müller glial cells, suggesting the presence of secondary

effects from the glial cells [32]. Glial cell line-derived neurotrophic factor (GDNF) [3] was also considered and examined as a candidate for photoreceptor neuroprotection.

### ***14.4.3 Neuroprotection by Antioxidants***

The retina has the highest metabolic rate by weight in the body [33], and it is always exposed to ROS. Thus, the redox-regulating system is important for retinal survival [34]. Antioxidants have been used to increase the resistance of neurons to oxidative stress, and they are considered as another avenue for neuroprotection in animal models [35]. Increased stress is found not only in photoreceptor cell death but also in other diseases, such as glaucoma [36]. Animal experiments and clinical trials have been reported. These included vitamin A alone or in combination with vitamin E, docosahexaenoic acid (DHA), or lutein and were shown to be partially effective [37]. Berson and coworkers reported a slower decline of visual acuities in patients with RP who were taking vitamin A and consumed a diet rich in omega-3 fatty acids [38]. Nonetheless, only minor and highly variable protective effects have been observed in these patients [39]. This phenomenon may be due to the diversity of experimental designs in regard to treatment time, dosage, and the variable background of the patients. Recently, very interesting results were published by Jin and coworkers [40]. They constructed patient-specific rod photoreceptor cells using induced pluripotent stem (iPS) cells derived from each patient's fibroblasts. Cells with specific mutations exhibited different responses to vitamin E. Their study may clarify the pathogenic mechanism induced by different gene mutations and suggest strategies of future treatment for patients with different genetic backgrounds.

### ***14.4.4 Blocking Cell Death by the Regulation of cGMP***

Conventional strategies for neuroprotection using neurotrophic factors or antioxidants have sought to strengthen the cellular metabolism against variable stresses as determined by analysis of cell survival. However, neuroprotection using neurotrophic factors or antioxidants as described above is not sufficient to prevent photoreceptor cell death [41]. Therefore, recent approaches have attempted to prevent cell death by targeting the initiation of cell death or blocking its execution. These have been considered differentially from the stages of photoreceptor cell death: early stage for the accumulation of cGMP, intermediate stage for epigenetic processes, and end stage for excessive activation of proteolytic enzymes such as calpains [39].

cGMP, which is produced by retinal guanylate cyclase and hydrolyzed by phosphodiesterase-6 (PDE6), plays an important role in signal phototransduction [42]. High cGMP levels have been reported in a murine animal model of *rd1*, which

showed an abnormal activity of phosphodiesterase (PDE) due to gene mutation. Evidence suggests that cGMP accumulation is not specific to *rd1* and is observed in many other inherited retinal degeneration animal models during the course of retinal degeneration [43]. The correlation between very low levels of cGMP and chick photoreceptor cell death suggests that an inadequate level of cGMP may cause photoreceptor degeneration [44]. cGMP targets cyclic nucleotide-gated (CNG) ion channels, which mediate the influx of  $\text{Ca}^{++}$ , and cGMP-dependent protein kinase (PKG). PKG activates the transcription factor cyclic AMP response element-binding protein (CREB), which has antiapoptotic pathway activity [45]. Targeting of these signals for photoreceptor protection may require more extensive studies.

#### ***14.4.5 Blocking Cell Death by Epigenetic Modification***

Epigenetic factors, such as methylation, acetylation, deacetylation, and poly-ADP-ribosylation, influence histone modification and translation and have been recognized as important events for cell death and survival [46]. Typical enzymes that control these activities are DNA methyltransferases, histone acetyltransferases (HATs), histone deacetylases (HDACs), and poly-ADP-ribose polymerase (PARP). Inhibition of HDAC activity increases the cell survival rate and is suspected to be an upstream molecule that reduces PARP activation [47]. Thus, a PARP inhibitor also showed reduced photoreceptor cell death in some animal model [48]. Interestingly, related studies also revealed epigenetic modification of DNA repair, termed abortive mitosis [49]. The degenerating photoreceptors incorporate bromodeoxyuridine, which is usually observed in DNA replication. The antioxidant molecules described above may reduce the DNA oxidation and show reduced epigenetic effects.

#### ***14.4.6 Blocking Cell Death by Blocking Proteolytic Activity***

At the end stage of photoreceptor cell death, proteolytic degradation of the cellular components has been investigated. Well-established components of proteolytic degradation are calpains and calpastains. Calpains are a family of  $\text{Ca}^{++}$ -activated cysteine proteases and execute their activity by dimers of an 80 kDa catalytic subunit and a 30 kDa regulatory subunit. Elevated levels of cellular  $\text{Ca}^{++}$  activate calpain activity and lead to cell death of not only photoreceptor cells [50] but also ganglion cells [51]. Calpastain activity correlates with the reduced neuronal cell death [52]. However, contradictory results have been reported [53], and the elucidation of the precise mechanism for cell survival and cell death related with these molecules is required for clinical application.



Increased of calcium ion concentrations inside neural cells results in the activation of  $\text{Ca}^{++}$ -dependent proteolysis, hydrolysis, lipid peroxidation, and ROS production [54]. These results lead to the neuronal cell death and prevention of these events has been considered. The activation of  $\text{K}^+$  channels in nerve cells effectively regulates cell membrane potentials. Large-conductance voltage- and  $\text{Ca}^{++}$ -dependent  $\text{K}^+$  (max-K or BK) channels react to increased intracellular  $\text{Ca}^{++}$  and membrane depolarization and show rapid hyperpolarization of the membrane and reduce voltage-dependent  $\text{Ca}^{++}$  influx [55]. Activation of this channel has been reported to rescue many neuronal cells [56, 57]. Isopropyl unoprostone, a prostaglandin metabolite analog that has been used clinically as an anti-glaucomatous agent, has been shown to protect photoreceptors against oxidative stress- and light-induced retinal damage in rats [58] through BK channel regulation [59]. A pilot study using topical unoprostone twice a day in patients with retinitis pigmentosa demonstrated significant improvement in central 2° retinal sensitivity [60].

#### ***14.4.7 Advanced Stages of Retinal Prosthesis and Cell Transplantations***

Retinal prosthesis and cell transplantations may be an option for the restoration of vision, because once photoreceptor cell loss occurs, there are no effective treatments to restore sight. However, electronic devices have been used for the replacement of dead photoreceptor cells. The devices were placed in the cortex, suprachoroidal space, and epiretinal and subretinal spaces. These devices convert images into electric signals using the remaining neuronal cells [61–63].

Cell transplantation offers a promising approach for treatment at the end stages of retinal degenerative diseases. The techniques of transplanting different types of cells have advanced rapidly in the past 30 years, and the results have suggested that transplantation may be a useful approach to treat some retinal diseases. Some of the transplanted cells produce neurotrophic factors to support photoreceptor cells [64]. Photoreceptor cell generation and transplantation from both mouse and human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have been reported. Thus, cell transplantation provides another promising strategy for photoreceptor functional rescue [65, 66].

### **14.5 Development of Delivery Tools**

Recent progress in the treatment for some retinal diseases, such as AMD, allows administration of some drugs by intravitreal injection [67]. Intravitreal injections of neurotrophic factors have also been shown to rescue degenerating photoreceptor cells in animals [68]. However, several problems limit their clinical usefulness,

such as delivering the neurotrophic factor to the appropriate site and the short half-life of the neurotrophic factors. Intravitreal sustained delivery of some neurotrophic factors has been shown to rescue retinal cells [27]. Although the surgical procedure was reported to be tolerable, the implants were set in the vitreous. Treatments inside the eyeball may induce adverse side effects, such as retinal detachment and infection [69]. In contrast, we reported our novel trans-scleral drug delivery device placed on the sclera that consisted of a drug-releasing semipermeable membrane and impermeable membranes acting as the drug reservoir. Because of the nonbiodegradable and one-way release nature of the device, we were able to achieve sustained release of the drug to the retina. This type of drug delivery system can also be designed to release multiple drugs [70] (Fig. 14.2).

## 14.6 Conclusion

Photoreceptor cell death is the major cause of loss of vision in most retinal diseases. So far, many studies have attempted to achieve photoreceptor cell protection by various molecules. However, not every application was well tolerated, although some types of applications (phase 2 or 3) are ongoing. Improved photoreceptor protective treatments require a better understanding of photoreceptor neurodegenerative mechanisms. In addition, improvement of molecule delivery systems (not only drugs, but also genes) may allow new insights into strategies for photoreceptor protection.

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