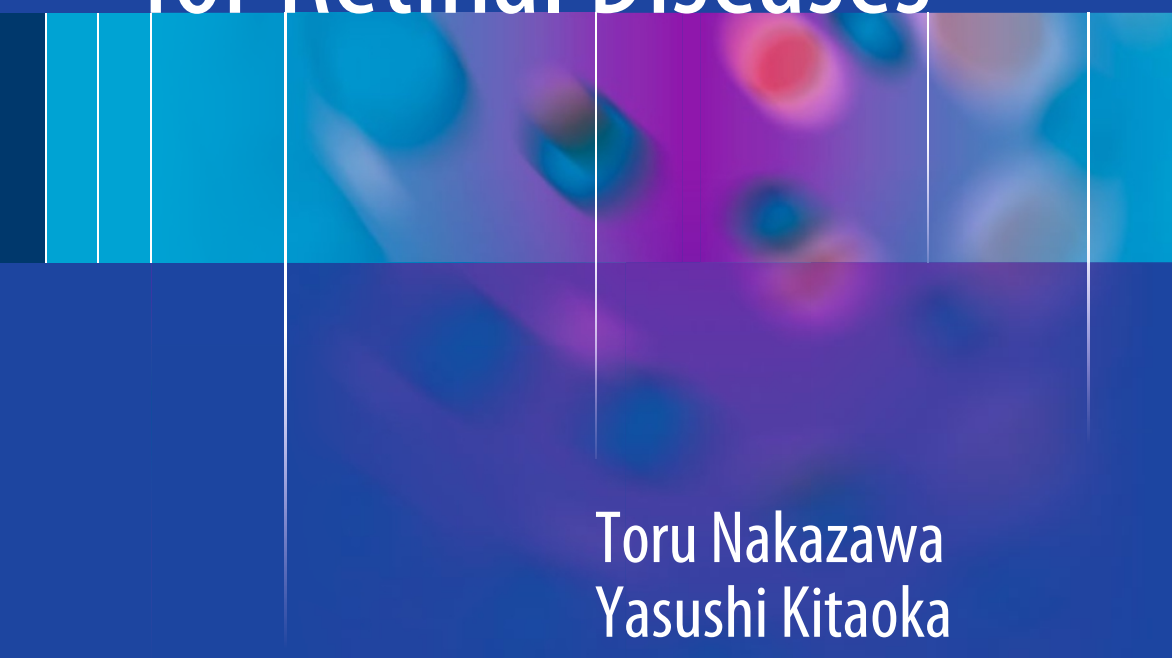
Neuroprotection and Neuroregeneration for Retinal Diseases



Toru Nakazawa
Yasushi Kitaoka
Takayuki Harada
Editors

Springer

Neuroprotection and Neuroregeneration for Retinal Diseases

Toru Nakazawa • Yasushi Kitaoka
Takayuki Harada
Editors

Neuroprotection and Neuroregeneration for Retinal Diseases

Springer

Editors
Toru Nakazawa
Department of Ophthalmology
Tohoku University Graduate
School of Medicine
Sendai, Miyagi, Japan

Yasushi Kitaoka
Department of Ophthalmology
St. Marianna University
School of Medicine
Kawasaki, Japan

Takayuki Harada
Visual Research Project
Tokyo Metropolitan
Institute of Medical Science
Tokyo, Japan

ISBN 978-4-431-54964-2 ISBN 978-4-431-54965-9 (eBook)
DOI 10.1007/978-4-431-54965-9
Springer Tokyo Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014942841

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

In the past, major causes of blindness were infection and cataracts. At the time, these conditions were thought to be incurable and inflicted debilitating effects on the quality of life. However, advances in pharmacology and surgery have changed this situation remarkably, and these conditions are now treatable and preventable. Instead of infection and cataracts, the majority of causes of blindness in developed countries in the last few decades have become retinal or optic nerve degeneration. These are the current challenges we must face, and development of strategies for protection of retinal cells and the optic nerve with drugs is urgently required. Currently, many preclinical reports on neuroprotection are available, and they have revealed a number of potential therapeutic targets although they have not yet supplied sufficient data for use in clinical settings. However, while there has been a significant increase in understanding of novel or detailed cell death mechanisms, which is useful for identifying novel targets, research into gene therapy using several neurotrophic factors for protection of retinal cells is progressing. These developments are providing hope for patients whose sight is affected by retinal degeneration.

Most recently, work by the Nobel Prize winner Shinya Yamanaka has attracted much attention and inspired many researchers in the medical field. It has encouraged regenerative therapy by cell transplantation with induced pluripotent stem (iPS) cells, and this technology will benefit many people suffering from diseases that require replacement of affected cells and tissues. Application of this strategy in the ophthalmic field has a promising future, and indeed, safety trials for the use of iPS cells in patients with age-related macular degeneration (AMD) will be starting soon. Stimulation of neuroprotection and neuroregeneration will provide effective treatment for retinal and optic nerve degeneration, and one day these conditions will be curable as cataracts now are. To this end, research into retinal neuroprotection and neuroregeneration has provided some convincing results so far and this field is advancing rapidly. Therefore, it is essential for clinicians and researchers

of ophthalmology to keep up-to-date with the latest information from preclinical and clinical studies, although it is not very easy to do so. The aim of this book is to summarize the recent progress in neuroprotection and neuroregeneration of the retina and optic nerve in both preclinical and clinical aspects of ophthalmology.

It has been estimated that glaucoma will affect more than 80 million individuals worldwide by 2020, with at least 6–8 million people becoming bilaterally blind. Glaucoma is a leading global cause of irreversible blindness and is perhaps the most prevalent of all neurodegenerative diseases. Thus, we devote a large section to glaucoma (Part I, Chaps. 1–13). There are many aspects of this disease and potential therapeutic targets. Part I of the book describes the pathological mechanisms and retinal ganglion cell death, including glutamate neurotoxicity, calcium signaling, and oxidative stress. It also provides information on the roles of glial cells and microglial cells, a new form of necrosis, and axonal flow disturbance and degeneration, together with possible therapeutic strategies for glaucoma.

On the other hand, AMD is the biggest cause of blindness in the United States and European countries. Epidemiological studies have revealed that AMD has many aggravating factors including smoking, light toxicity, and oxidative stress, and it is known that intake of vitamins and lutein, which are antioxidants, shows preventive effects against this condition. Moreover, wet-type macular degeneration, which is associated with neovascularization, is commonly treated with intraocular injection of drugs targeting vascular endothelial growth factor (VEGF). Major advances in treatment strategies are being achieved in this field. In some types of AMD and retinitis pigmentosa, the retinal pigment epithelium (RPE) is disturbed, and thus investigations from cell–biological aspects, such as studies on protection of photoreceptors and RPE and interaction between photoreceptors–RPE, are discussed in Part II (Chaps. 14–18).

Furthermore, mechanisms underlying retinal detachment, uveitis, and optic neuritis, as well as neuroprotection in each disease, are discussed in Part III (Chaps. 19–23). Like glaucoma and AMD, these conditions affect many people and cause debilitating effects on the quality of life. In addition to neuroprotection, use of candidates other than iPS cells, such as endothelial progenitor cells (EPC), for retinal regeneration and optic nerve regeneration research provides good prospects for the future. For example, novel strategies exploiting neuroinflammation and stimulating cytoskeletal organization in growth cones are noteworthy.

This book was mainly written by up-and-coming ophthalmologists who are specialized in retinal diseases and are devoted to both clinical and preclinical research. However, each chapter is written in such a way that beginners in the field can understand the complex contexts. We believe that the contents of this book will appeal to a wide variety of readers with an interest in ophthalmic research and will be particularly useful to ophthalmologists in training and also to medical students and scientists who are outside the field. Some readers may wish to skip certain specialized areas at first, but that is not a problem. It is more important for one to enjoy reading the parts that are of interest or that are one's favorite topic.

On the other hand, we hope that those who are busy ophthalmic specialists can acquire the latest preclinical and clinical knowledge in a short time from *Neuroprotection and Neuroregeneration for Retinal Diseases*.

Sendai, Miyagi, Japan — Toru Nakazawa
Kawasaki, Japan — Yasushi Kitaoka
Tokyo, Japan — Takayuki Harada

Contents

Part I
Neuroprotection for Glaucoma

Chapter 1
Molecular Architecture of Glutamate Signaling Pathway in Glaucomatous Optic Neuropathy

Yasunari Munemasa

Abstract Glutamate is the predominant excitatory neurotransmitter in the central nervous system. Excessive concentrations of glutamate have been reported in various neurological diseases, including glaucomatous optic neuropathy (GON). Glutamate excitotoxicity triggered by overstimulation of *N*-methyl-D-aspartate (NMDA)-type glutamate receptors may contribute to retinal ganglion cell (RGC) degeneration in glaucoma and other retinal neuronal cell death in ischemic insult including diabetic retinopathy. Neuroprotective effects with the blockage of overstimulated NMDA receptors were found in several previous studies in RGC degeneration models, such as the axotomy, ischemia, and laser-induced high intraocular pressure models. Although there is a great deal of evidence for elevated glutamate in GON, a clinical trial of memantine, an NMDA receptor antagonist, was unsuccessful. Thus, excitotoxicity is not recognized as a primary factor in GON, although glutamate leaking from dying/dead RGCs or compromised glia may contribute to the secondary death of neighboring RGCs via excessive activation of NMDA receptors during the development of glaucoma. Therefore, appropriate modulation of NMDA receptor-mediated retinal excitotoxicity remains to be elucidated and may become a possible therapeutic target.

Keywords Excitotoxicity • Glutamate • Retina • Retinal ganglion cell

1.1 Introduction

Glutamate is an excitatory neurotransmitter in the central nervous system (CNS). Neurotoxicity in the retina caused by elevated glutamate levels was first reported by Lucas and Newhouse in 1957 [1]. This phenomenon is referred to as excitotoxicity

Y. Munemasa (✉)
Department of Ophthalmology, St. Marianna University School of Medicine,
2-16-1 Sugao, Miyamae, Kawasaki 216-8511, Japan
e-mail: munemasa@marianna-u.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_1,

and it induces neuronal degeneration. Glutamate stored within cells is not harmful. Currently, excitotoxicity is known to play a role in various neurological disorders, including acute insult and chronic stress [2]. After the original report of Lucas and Newhouse, numerous studies focusing on excitotoxicity have been performed in neurodegenerative diseases including glaucomatous optic neuropathy (GON) [3, 4]. One contested study found higher levels of glutamate in the vitreous of glaucomatous patients than in controls [5, 6]. Therefore, it appears reasonable to suggest that if ganglion cell death in glaucoma is caused by excessive activation of *N*-methyl-D-aspartate (NMDA) receptors, then the NMDA receptor antagonist memantine would be worth exploring as a potential neuroprotective agent. Based on this reasoning, a clinical trial was conducted to determine the efficacy of memantine as a neuroprotectant against glaucoma. However, that trial was unsuccessful. Unfortunately, one of the authors associated with the reports by Dreyer et al. and Brooks et al. was later discredited, and attempts by others to reproduce some of those findings were not successful [5–8]. As a result, reliance on these data as a basis for undertaking further trials is difficult to justify. However, after those reports, elevated glutamate levels in animal experimental glaucoma models were reported [9, 10]. Furthermore, ectopic vesicular glutamate release at the optic nerve head was observed 2 days after intraocular pressure (IOP) elevation and caused subsequent axonal degeneration and RGC loss [11]. Although the memantine trial failed, understanding RGC degeneration with elevated glutamate levels may be important. This chapter discusses mechanistic insights into excitotoxicity and how excitotoxicity can contribute to GON.

1.1.1 Physiology

Glutamate is the most abundant excitatory neurotransmitter in the CNS. Nerve impulses trigger the release of glutamate from presynaptic cells [12]. In the opposing postsynaptic cells, glutamate receptors, such as the NMDA receptor, bind glutamate and are activated [13]. Because of its role in synaptic plasticity, glutamate is involved in cognitive functions in the CNS. The form of plasticity known as long-term potentiation occurs at glutamatergic synapses in the brain and retina [14, 15]. Glutamate functions not only as a point-to-point transmitter but also through spillover synaptic cross talk between synapses in which glutamate released from a neighboring synapse creates extrasynaptic signaling/volume transmission. In addition, glutamate plays important roles in the regulation of growth cones and synaptogenesis during development [16, 17].

1.1.2 Glutamate Transporters

In the retina, glutamate transporters that rapidly remove excess glutamate from the extracellular space are found in neuronal and glial membranes. In retinal injury or disease, they can work in reverse, and excess glutamate can accumulate outside cells. It was reported that five distinct excitatory amino acid transporters (EAATs) that transport glutamate have been cloned: the glutamate/aspartate transporter (GLAST) [18], glial glutamate transporter (GLT)-1 [19], excitatory amino acid carrier (EAAC)-1 [20], excitatory amino acid transporter (EAAT)-4 [21], and excitatory amino acid transporter (EAAT)-5 [22]. GLAST and GLT-1, which are exclusively located in glial cells in the brain [23–25], are also present in the retina. In the retina, GLAST is considered to be the major glutamate transporter and is expressed in Müller cells [26–28], while GLT-1, which is known to be responsible for up to 90 % of all glutamate transport in the forebrain region [29], is found in retinal neurons, such as cone photoreceptors and certain types of cone bipolar cell [30].

Little is known concerning the functional significance of GLT-1 expression in these retinal neurons. The elimination of excessive glutamate in the retina and vitreous is also important in slowing retinal neurodegenerative disease. The glutamate transporter is the only mechanism for the removal of glutamate from the extracellular fluid in the retina [31]. Increased glutamate levels may result from a failure of glutamate transporters adjacent to RGCs. Mice deficient in the glutamate transporters GLAST and EAAC-1, which are glutamate transporters, demonstrate spontaneous RGC death and optic nerve degeneration without IOP elevation, suggesting that glutamate transporters are necessary to prevent excitotoxic retinal damage [32, 33]. GLAST immunoreactivity is present throughout the retina and can be double-labeled with glutamine synthetase, a specific marker of Müller glial cells. These observations indicate that the intracellular glutamate concentration is dependent on glutamate uptake via GLAST in Müller glial cells.

1.1.3 Glutamate Receptors

Glutamate receptors are categorized into two main classes: ionotropic and metabotropic. The ionotropic glutamate receptors, which are all nonselective cation channels, are described as either NMDA or non-NMDA subtypes. NMDA receptors are characterized by a high permeability to Ca^{2+}, voltage-dependent blockage by Mg^{2+}, and slow gating kinetics. These receptors are known to be involved in a variety of physiological processes in the CNS [34–36]. Cloning experiments have demonstrated that there are at least five NMDA receptor subunits: NR1 and NR2A through NR2D [37].

Most recently, the novel subunits NR3A and NR3B have been cloned [38–40]. NR3A and NR3B assemble as heterotetramers in the endoplasmic reticulum to form functional channels through distinct combinations, producing various

postsynaptic responses. In the mature nervous system, NMDA receptors are composed primarily of NR1 and NR2A through NR2C [41]. The receptor in some neurons may contain only NR1 combined with either NR2A or NR2B. The NR1 subunit in rats has at least eight splice variant forms [42, 43], and splice variants are also found in the NR2B-D and NR3A subunits [44]. On the other hand, unique genes code for each NR2 subunit [45] and for NR3A [46, 47]. NR1 serves a fundamental subunit of the NMDA receptor, without which the receptor cannot function, whereas NR2A through NR2D can be regarded as modulatory subunits [48, 49]. The NMDA receptor channel is highly permeable to Ca^{2+} [50, 51]. The increase in intracellular calcium levels ($[Ca^{2+}]i$) in neuronal cells resulting from the activation of the NMDA receptor channel has been shown to be responsible for modulating neuronal activity and producing neurotoxicity [52, 53]. There is recent evidence that the inclusion of an NR3A subunit attenuates the calcium current [54]. A unique feature of the NMDA receptor channel is the voltage dependence of the receptor-mediated inward ionic currents. This is because the channel becomes clogged by Mg^{2+} at negative membrane potentials and Mg^{2+} is driven out of the channel pore when the membrane is depolarized. The NMDA receptor-mediated inward current is maximal between −20 and −30 mV in external medium containing physiological concentrations of Mg^{2+} (approximately 1 mM), is reduced at more hyperpolarized potentials, and becomes negligible at −80 mV [55]. Therefore, at the resting membrane potentials of most spiking neurons (−70 to −90 mV), NMDA receptors undergo significant channel blockage by Mg^{2+}, and the blockage is relieved in a voltage-dependent manner when the neurons are depolarized by the activation of co-localized postsynaptic non-NMDA receptors. In other words, the NMDA receptor could serve as a molecular apparatus for detecting presynaptic signals occurring along postsynaptic depolarization at the synapse. This voltage dependence is important for synaptic integration in the CNS.

1.1.4 Downstream Signaling Cascade After Stimulation of NMDA Receptors

Stimulation of NMDA receptors is observed in the retina in various animal models of neurodegeneration, such as experimental glaucoma, axotomy, ischemia-reperfusion, and NMDA injection [56–59]. Intravitreal injection of NMDA causes relatively acute neuronal death, especially in the inner retina, through several molecular pathways (Fig. 1.1). The increase in Ca^{2+}[i] influx is the initial key molecular event in NMDA receptor-mediated cell death [60]. Ca^{2+}[i] overload activates Ca^{2+}-dependent enzyme systems such as calpain- and calcium-/calmodulin-dependent kinase 2 (CaMK2) [61, 62]. Calpain is localized in TUNEL-positive apoptotic cells in the inner retina after NMDA injection, and the inhibition of calpain results in less NMDA-induced neuronal cell death, suggesting a proapoptotic role of calpain in NMDA-induced neurotoxicity. In contrast, phosphorylation of CaMK2

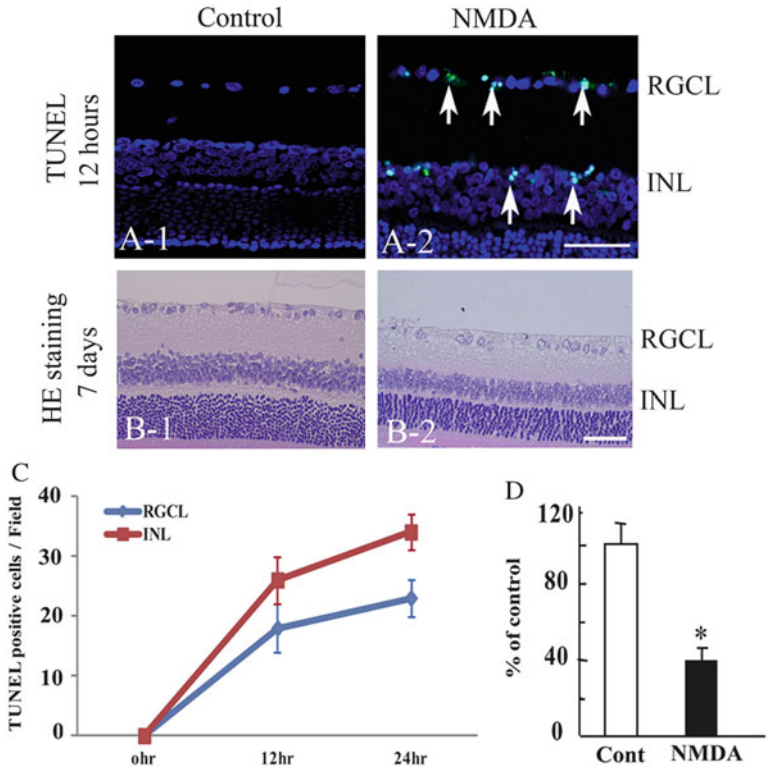


Fig. 1.1 TUNEL staining after NMDA injection (**A**-1, 2). Hematoxylin and eosin staining (**B**-1, 2). TUNEL-positive cells were observed in the inner retina after NMDA injection. There are no TUNEL-positive cells in the control retina. *Arrows* are TUNEL-positive apoptotic cells. Time course of TUNEL-positive cells in the inner retina (**C**). Intravitreal injection of 20 nmol NMDA induced neurotoxicity in the inner retina. Approximately 60 % neuronal cell loss was observed in the RGCL after NMDA injection (**D**). Scale bar = 50 μm

is observed in the retina relatively early after NMDA injection, and inhibition of CaMK2 synthesis accelerates NMDA-induced RGC loss, indicating an antiapoptotic role of CaMK2 [62]. Ca^{2+}[i] influx also affects mitochondrial activity, such as the release of cytochrome C and reactive oxygen species, and results in the activation of several apoptotic pathways. Apoptosis-inducing factor is one key factor released from mitochondria and also contributes to DNA damage with overactivation of NMDA receptors [63]. PARP-1 activation occurs in the retina after NMDA injection in the vitreous and is accompanied by a decrease in ATP levels [64]. Conversely, a PARP inhibitor protects RGCs from NMDA-induced excitotoxicity. Our previous studies on the downstream Ca^{2+}[i] influx led us to propose that the activation of proapoptotic molecules, such as nuclear factor-κB p65 and p38, c-Jun *N*-terminal kinase, and c-Jun, plays a role in NMDA-induced neurotoxicity [65–68] (Fig. 1.2). An inflammatory response, i.e., the upregulation of interleukin-1β, is also observed in the glia and RGCs after NMDA administration,

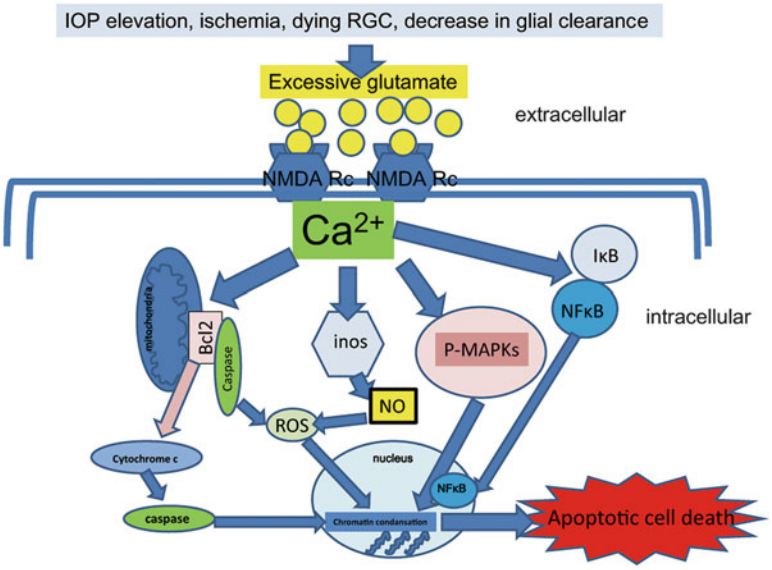


Fig. 1.2 Schema for molecular pathway for glutamate neurotoxicity

suggesting the involvement of inflammation in response to excitotoxicity [69]. Our previous study showed that axonal degeneration with neurofilament loss is evident 3 days after intravitreal injection of NMDA [70]. Although NMDA penetrates into the inner retina after intravitreal injection and induces various molecular changes in the glia and RGCs which lead to RGC apoptotic degeneration and inner retinal thinning, those changes occur after TUNEL-positive DNA fragmentation in RGCs, indicating anterograde degenerative change in NMDA-induced neurotoxicity. Furthermore, apoptotic cell body death affects axonal transport to axons through the disruption of kinesin-1 activity, an anterograde axonal motor protein related to microtubules and neurofilament. These findings indicate anterograde neurodegeneration in NMDA-induced neurotoxicity [70].

1.2 Future Perspectives

Glutamate levels in the vitreous have been measured under the premise that the vitreous represents the extracellular space of the retina. The true extracellular space is the intracellular space between retinal cells. Because of technical difficulties in measuring such glutamate concentrations in the human retina, it has not been confirmed whether glutamate levels are elevated in human patients with glaucoma. The epicenter of GON is proposed to be the optic nerve head or lamina cribrosa, where soft tissues, including RGC axons and blood vessels circulating the optic nerve head, are likely to be compressed as a result of deformation of the laminar

structure [71, 72]. Excitotoxicity may play a role in the pathophysiology of glaucoma by causing secondary RGC death because of glutamate leaking from injured cells, thereby triggering the activation of the apoptotic pathway. Harada and coworkers found that mice deficient in the glutamate transporters GLAST or EAAC-1 demonstrate spontaneous RGC and optic nerve degeneration without elevated IOP [33]. The retinas of glaucoma patients showed lower immunoreactivity of the EAAT-1 [73]. Furthermore, other studies showed that the optic nerve head is ischemic in glaucoma [74, 75]. Excitotoxicity can also contribute to the development of glaucomatous axonal degeneration because glutamate clearance by the glia decreases under ischemic conditions [76]. Although excitotoxicity is not a primary event in GON, glutamate leaking from dying/dead RGCs or compromised glia may contribute to the secondary death of neighboring RGCs via excessive activation of NMDA receptors in the development of glaucoma (Fig. 1.2). Therefore, the more appropriate therapeutic modulation of NMDA receptor-mediated retinal excitotoxicity remains to be elucidated and may become a potential therapeutic target.

References

1. Lucas DR, Newhouse JP (1957) The toxic effect of sodium L-glutamate on the inner layers of the retina. AMA Arch Ophthalmol 58:193–201
2. Tapia R (1996) Release and uptake of glutamate as related to excitotoxicity. Rev Bras Biol 56:165–174
3. Kalloniatis M (1995) Amino acids in neurotransmission and disease. J Am Optom Assoc 66:750–757
4. Haefliger IO, Fleischhauer JC, Flammer J (2000) In glaucoma, should enthusiasm about neuroprotection be tempered by the experience obtained in other neurodegenerative disorders? Eye 14:464–472
5. Dreyer EB, Zurakowski D, Schumer RA et al (1996) Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch Ophthalmol 114:299–305
6. Brooks DE, Garcia GA, Dreyer EB et al (1997) Vitreous body glutamate concentration in dogs with glaucoma. Am J Vet Res 58:864–867
7. Levkovitch-Verbin H, Martin KR, Quigley HA et al (2002) Measurement of amino acid levels in the vitreous humor of rats after chronic intraocular pressure elevation or optic nerve transection. J Glaucoma 11:396–405
8. Wamsley S, Gabelt BT, Dahl DB et al (2005) Vitreous glutamate concentration and axon loss in monkeys with experimental glaucoma. Arch Ophthalmol 123:64–70
9. Kim JH, Lee NY, Jung SW et al (2007) Expression of *N*-methyl-D-aspartate receptor 1 in rats with chronic ocular hypertension. Neuroscience 149:908–916
10. Luo XG, Chiu K, Lau FH et al (2009) The selective vulnerability of retinal ganglion cells in rat chronic ocular hypertension model at early phase. Cell Mol Neurobiol 29:1143–1151
11. Fu CT, Sretavan DW (2012) Ectopic vesicular glutamate release at the optic nerve head and axon loss in mouse experimental glaucoma. J Neurosci 32:15859–15876
12. Hamberger A, Nyström B, Sellström A et al (1976) Amino acid transport in isolated neurons and glia. Adv Exp Med Biol 69:221–236
13. Garcia-Munoz M, Young SJ, Groves PM (1991) Terminal excitability of the corticostriatal pathway. II. Regulation by glutamate receptor stimulation. Brain Res 551:207–215

14. Sugioka M, Fukuda Y, Yamashita M (1998) Development of glutamate-induced intracellular Ca^{2+} rise in the embryonic chick retina. J Neurobiol 34:113–125
15. King AE, Chung RS, Vickers JC et al (2006) Localization of glutamate receptors in developing cortical neurons in culture and relationship to susceptibility to excitotoxicity. J Comp Neurol 498:277–294
16. Herkert M, Röttger S, Becker CM (1998) The NMDA receptor subunit NR2B of neonatal rat brain: complex formation and enrichment in axonal growth cones. Eur J Neurosci 10:1553–1562
17. Haberecht MF, Redburn DA (1996) High levels of extracellular glutamate are present in retina during neonatal development. Neurochem Res 21:285–291
18. Storck T, Schulte S, Hofmann K (1992) Structure, expression, and functional analysis of a Na (+)-dependent glutamate/aspartate transporter from rat brain. Proc Natl Acad Sci USA 89:10955–10959
19. Pines G, Danbolt NC, Bjørås M et al (1992) Cloning and expression of a rat brain L-glutamate transporter. Nature 360:464–467
20. Kanai Y, Hediger MA (1992) Primary structure and functional characterization of a high-affinity glutamate transporter. Nature 360:467–471
21. Fairman WA, Vandenberg RJ, Arriza JL et al (1995) An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. Nature 375:599–603
22. Arriza JL, Eliasof S, Kavanaugh MP et al (1997) Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. Proc Natl Acad Sci USA 94:4155–4160
23. Danbolt NC, Storm-Mathisen J, Kanner BI (1992) An [Na++ K+]-coupled L-glutamate transporter purified from rat brain is located in glial cell processes. Neuroscience 2:295–310
24. Lehre KP, Levy LM, Ottersen OP et al (1995) Differential expression of two glial glutamate transporters in the rat brain: quantitative and immunocytochemical observations. J Neurosci 15:1835–1853
25. Levy LM, Lehre KP, Rolstad B et al (1993) A monoclonal antibody raised against an [Na(+) +K+]coupled L-glutamate transporter purified from rat brain confirms glial cell localization. FEBS Lett 317:79–84
26. Izumi Y, Shimamoto K, Benz AM et al (2002) Glutamate transporters and retinal excitotoxicity. Glia 39:58–68
27. Pow DV (2001) Visualising the activity of the cystine-glutamate antiporter in glial cells using antibodies to aminoadipic acid, a selectively transported substrate. Glia 34:27–38
28. Rauen T, Wiessner M (2000) Fine tuning of glutamate uptake and degradation in glial cells: common transcriptional regulation of GLAST1 and GS. Neurochem Int 37:179–189
29. Robinson MB (1998) The family of sodium-dependent glutamate transporters: a focus on the GLT-1/EAAT2 subtype. Neurochem Int 33:479–491
30. Rauen T, Rothstein JD, Wässle H (1996) Differential expression of three glutamate transporter subtypes in the rat retina. Cell Tissue Res 286:325–336
31. Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1–105
32. Harada T, Harada C, Watanabe M et al (1998) Functions of the two glutamate transporters GLAST and GLT-1 in the retina. Proc Natl Acad Sci USA 95:4663–4666
33. Harada T, Harada C, Nakamura K et al (2007) The potential role of glutamate transporters in the pathogenesis of normal tension glaucoma. J Clin Invest 117:1763–1770
34. Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39
35. Carroll RC, Zukin RS (2002) NMDA receptor trafficking and targeting: implications for synaptic transmission and plasticity. Trends Neurosci 25:571–577
36. Li B, Chen N, Luo T, Otsu Y et al (2002) Differential regulation of synaptic and extra-synaptic NMDA receptors. Nat Neurosci 5:833–834
37. Ozawa S, Kamiya H, Tsuzuki K (1998) Glutamate receptors in the mammalian central nervous system. Prog Neurobiol 54:581–618

38. Cull-Candy S, Brickley S, Farrant M (2001) NMDA receptor subunits: diversity, development and disease. Curr Opin Neurobiol 11:327–335
39. Sucher NJ, Kohler K, Tenneti L et al (2003) *N*-methyl-D-aspartate receptor subunit NR3A in the retina: developmental expression, cellular localization, and functional aspects. Invest Ophthalmol Vis Sci 44:4451–4456
40. Prybylowski K, Wenthold RJ (2004) *N*-Methyl-D-aspartate receptors: subunit assembly and trafficking to the synapse. J Biol Chem 279:9673–9676
41. Dingledine R, Borges K, Bowie D et al (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7–61
42. Durand GM, Gregor P, Zheng X et al (1992) Cloning of an apparent splice variant of the rat *N*-methyl-D-aspartate receptor NMDAR1 with altered sensitivity to polyamines and activators of protein kinase C. Proc Natl Acad Sci USA 89:9359–9363
43. Sugihara H, Moriyoshi K, Ishii T et al (1992) Structures and properties of seven isoforms of the NMDA receptor generated by alternative splicing. Biochem Biophys Res Commun 185:826–832
44. Chatterton JE, Awobuluyi M, Premkumar LS et al (2002) Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. Nature 415:793–798
45. Monyer H, Burnashev N, Laurie DJ et al (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529–540
46. Yao Y, Harrison CB, Freddolino PL et al (2008) Molecular mechanism of ligand recognition by NR3 subtype glutamate receptors. EMBO J 27:2158–2170
47. Das S, Sasaki YF, Rothe T et al (1998) Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. Nature 393:377–381
48. Chazot PL (2004) The NMDA receptor NR2B subunit: a valid therapeutic target for multiple CNS pathologies. Curr Med Chem 11:389–396
49. Luo J, Wang Y, Yasuda RP et al (1997) The majority of *N*-methyl-D-aspartate receptor complexes in adult rat cerebral cortex contain at least three different subunits (NR1/NR2A/NR2B). Mol Pharmacol 51:79–86
50. Cline HT, Tsien RW (1991) Glutamate-induced increases in intracellular Ca^{2+} in cultured frog tectal cells mediated by direct activation of NMDA receptor channels. Neuron 6:259–267
51. MacDermott AB, Mayer ML, Westbrook GL et al (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature 321:519–522
52. Siliprandi R, Canella R, Carmignoto G et al (1992) *N*-methyl-D-aspartate-induced neurotoxicity in the adult rat retina. Vis Neurosci 8:567–573
53. Sucher NJ, Wong LA, Lipton SA (1990) Redox modulation of NMDA receptor-mediated Ca^{2+} flux in mammalian central neurons. Neuroreport 1:29–32
54. Sucher NJ, Aizenman E, Lipton SA (1991) *N*-methyl-D-aspartate antagonists prevent kainate neurotoxicity in rat retinal ganglion cells in vitro. J Neurosci 11:966–971
55. Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature 309:261–263
56. Dong CJ, Guo Y, Agey P et al (2008) Alpha2 adrenergic modulation of NMDA receptor function as a major mechanism of RGC protection in experimental glaucoma and retinal excitotoxicity. Invest Ophthalmol Vis Sci 49:4515–4522
57. Kikuchi M, Tenneti L, Lipton SA (2000) Role of p38 mitogen-activated protein kinase in axotomy-induced apoptosis of rat retinal ganglion cells. J Neurosci 20:5037–5044
58. Lam TT, Abler AS, Kwong JM et al (1999) *N*-methyl-D-aspartate (NMDA)-induced apoptosis in rat retina. Invest Ophthalmol Vis Sci 40:2391–2397
59. Lam TT, Siew E, Chu R et al (1997) Ameliorative effect of MK-801 on retinal ischemia. J Ocul Pharmacol Ther 13:129–137
60. Laabich A, Li G, Cooper NG (2001) Characterization of apoptosis-genes associated with NMDA mediated cell death in the adult rat retina. Brain Res Mol Brain Res 91:34–42
61. Chiu K, Lam TT, Ying Li WW et al (2005) Calpain and *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity in rat retinas. Brain Res 1046:207–215

62. Takeda H, Kitaoka Y, Hayashi Y et al (2007) Calcium/calmodulin-dependent protein kinase II regulates the phosphorylation of CREB in NMDA-induced retinal neurotoxicity. Brain Res 1184:306–315
63. Yu SW, Wang H, Dawson TM et al (2003) Poly(ADP-ribose) polymerase-1 and apoptosis inducing factor in neurotoxicity. Neurobiol Dis 14:303–317
64. Goebel DJ, Winkler BS (2006) Blockade of PARP activity attenuates poly(ADP-ribosyl)ation but offers only partial neuroprotection against NMDA-induced cell death in the rat retina. J Neurochem 98:1732–1745
65. Munemasa Y, Ohtani-Kaneko R, Kitaoka Y et al (2005) Contribution of mitogen-activated protein kinases to NMDA-induced neurotoxicity in the rat retina. Brain Res 1044:227–240
66. Kitaoka Y, Kumai T, Kitaoka Y et al (2004) Nuclear factor-kappa B p65 in NMDA-induced retinal neurotoxicity. Brain Res Mol Brain Res 131:8–16
67. Munemasa Y, Ohtani-Kaneko R, Kitaoka Y (2006) Pro-apoptotic role of c-Jun in NMDA-induced neurotoxicity in the rat retina. J Neurosci Res 83:907–918
68. Takada K, Munemasa Y, Kuribayashi J et al (2011) Protective effect of thalidomide against *N*-methyl-D-aspartate-induced retinal neurotoxicity. J Neurosci Res 89:1596–1604
69. Kitaoka Y, Munemasa Y, Nakazawa T et al (2007) NMDA-induced interleukin-1beta expression is mediated by nuclear factor-kappa B p65 in the retina. Brain Res 1142:247–255
70. Kuribayashi J, Kitaoka Y, Munemasa Y et al (2010) Kinesin-1 and degenerative changes in optic nerve axons in NMDA-induced neurotoxicity. Brain Res 1362:133–140
71. Quigley HA, Nickells RW, Kerrigan LA et al (1995) Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest Ophthalmol Vis Sci 36:774–786
72. Quigley HA (1999) Neuronal death in glaucoma. Prog Retin Eye Res 18:39–57
73. Naskar R, Vorwerk CK, Dreyer EB (2000) Concurrent downregulation of a glutamate transporter and receptor in glaucoma. Invest Ophthalmol Vis Sci 41:1940–1944
74. Flammer J, Orgül S (1998) Optic nerve blood-flow abnormalities in glaucoma. Prog Retin Eye Res 17:267–289
75. Osborne NN, Melena J, Chidlow G et al (2001) A hypothesis to explain ganglion cell death caused by vascular insults at the optic nerve head: possible implication for the treatment of glaucoma. Br J Ophthalmol 85:1252–1259
76. Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 330:613–622

Chapter 2
Calcium and Calpain Activation

Morin Ryu and Toru Nakazawa

Abstract Even with clinical treatment to reduce intraocular pressure (IOP), visual field defects still progress in many patients. Recent research has shown that the optic nerve head (ONH) is a key location in this process, where mechanical, ischemic, and inflammatory stresses are associated with axonal transport defects and retinal ganglion cell (RGC) apoptosis.

Glutamate, an excitatory neurotransmitter, causes excitotoxic damage in RGCs via *N*-methyl-D-aspartate receptors (NMDARs) in certain pathologic conditions, particularly ischemia. Activated NMDARs cause Ca^{2+} dysregulation, which activates the Ca^{2+}-dependent cascade and results in RGC degeneration, calpain activation, the degeneration of neuronal processes, and, finally, apoptosis.

Axonal transport damage induced by optic nerve crush (ONC) is commonly used as a model in glaucoma research. This model is known to induce deficits in neurotrophic factors in the central nervous system and ATP, to impair the mitochondria, to create oxidative stress and Ca^{2+} dysregulation, and to cause calpain activation.

Additionally, hyperglycemia-induced dysfunction and pathology occurs earlier in the RGCs than in the retinal vessels. Previous reports also identified Ca^{2+} dysregulation and calpain activation in this process.

Although various triggers induce RGC degeneration and apoptosis via various mechanisms, it is believed that they all result in Ca^{2+} dysregulation and calpain activation. Targeting this point of convergence thus holds promise as a neuroprotective therapy, especially for patients who do not respond to IOP-lowering treatment.

Keywords Calcium channel blocker • Calcium dysregulation • Calpain inhibitor • Neuroprotection • Oxidative stress

M. Ryu • T. Nakazawa (✉)
Department of Ophthalmology, Tohoku Graduate University School of Medicine,
1-1 Seiryocho, Aobaku, Sendai city, Miyagi 980-8574, Japan
e-mail: ntoru@oph.med.tohoku.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_2,

2.1 Ca^{2+} and RGC Degeneration

Intracellular Ca^{2+} homeostasis plays a pivotal role in many biological processes, including fertilization, gene expression, and apoptosis [1, 2]. The products of cell metabolism accumulate with age and together with oxidative stress gradually impair Ca^{2+} homeostasis, making neurons more vulnerable to stresses that can lead to neurodegeneration [3, 4]. Ca^{2+} dyshomeostasis in these chronic pathologies comprises mitochondrial and endoplasmic reticulum (ER) dysfunction, Ca^{2+} buffering impairment, and glutamate excitotoxicity.

RGC degeneration in glaucoma has similarities with processes in other neurological diseases [5, 6] such as Parkinson's disease and Alzheimer's disease. These similarities include the accumulation of amyloid precursor protein [7], the dependence on neurotrophic factors [8], and the involvement of various Ca^{2+}-mediated cascades [6].

Intracellular Ca^{2+} dysregulation triggers many pathogenic mechanisms in the retina that affect RGC survival, including glutamatergic excitotoxicity via *N*-methyl-D-aspartate (NMDA) receptors [9], optic nerve blockade-induced RGC axonal damage (as occurs in the optic nerve crush (ONC) model) [10], and hyperglycemia-induced RGC degeneration [11]. All these mechanisms begin with a Ca^{2+} influx from extra- and intracellular storage in the ER and mitochondria into the cytoplasm, followed by the activation of Ca^{2+}-dependent cascades, ultimately resulting in RGC degeneration and apoptosis. These Ca^{2+}-dependent cascades include calpain, the caspase pathway, and oxidative stress.

Despite the varying etiology of glaucoma and other ocular neural degenerative diseases, there are many important common elements, including compartmentalized programs of degeneration targeting axons, dendrites, and cell bodies [12–14]. These programs, which are linked to several specific neuronal cascades, all converge in the dysregulation of Ca^{2+} [14] (Fig. 2.1).

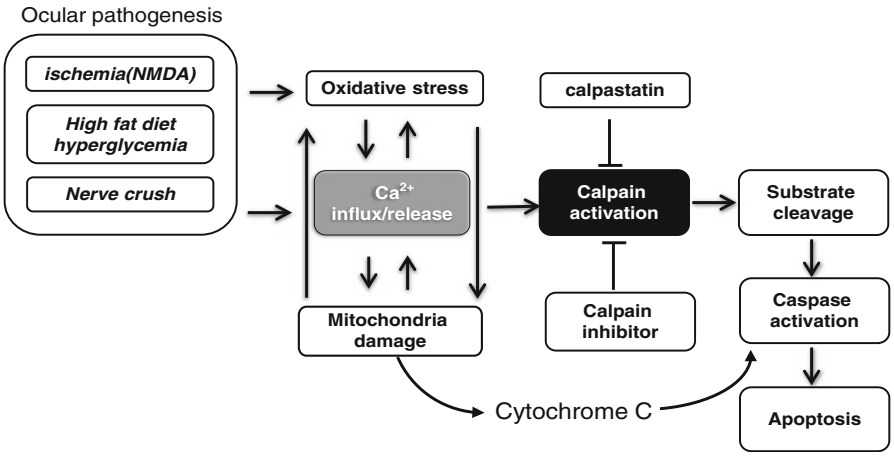


Fig. 2.1 Calcium dysregulation and calpain activation in RGCs

2.2 Calpain Activation

Calpains are a family of cytoplasmic, Ca^{2+}-activated, cysteine proteases. They are ubiquitous in the cells of all living creatures, from human beings to microorganisms. The enzymatic activity of calpains is regulated by Ca^{2+} and the endogenous inhibitory protein calpastatin [15].

In normal cells, Ca^{2+} concentration is lower than 0.05 mM. Under these conditions, calpains act as a biomodulator for Ca^{2+}-dependent events, such as signal transduction, cell proliferation, cell cycle progression, differentiation, and apoptosis [16]. The most abundant members of the calpain family are calpains 1 and 2. They differ in their sensitivity to Ca^{2+}, with their half maximal Ca^{2+} requirements in vitro being approximately 3–50 mM and 400–800 mM, respectively [16]. The level of Ca^{2+} required to activate calpains in vivo, however, has not been determined.

Uncontrolled and prolonged calpain-mediated proteolysis has been associated with a number of neurodegenerative conditions, including Parkinson's disease, Alzheimer's disease [17, 18], stroke [19], and trauma [20].

In the RGCs, calpain activation has been demonstrated in many pathologies, such as in the optic nerve crush model [10], the hyperglycemia model [11], and the NMDA-induced excitotoxic injury [21]. Calpains have various substrates including their endogenous inhibitor calpastatin [22], caspase 3, poly (ADP-ribose) polymerase [23], fodrin [24], p53 [25], p35 [26], and calcineurin [27]. Cleavage of calpastatin, fodrin, p35, or calcineurin is associated with the induction of apoptosis.

2.3 Pathologies Causing Ca^{2+} Dysregulation and Calpain Activation

2.3.1 Oxidative Stress and Ca^{2+} Dysregulation

The central nervous system (CNS) becomes increasingly susceptible to oxidative stress with age. DNA, proteins, and lipids are all affected [28, 29]. Markers of oxidative stress, such as lipid peroxidation [30], protein carbonyl [31], and advanced glycation end products (AGEs) [32], are also detectable in glaucoma models and in the tissues of glaucoma patients [33].

Furthermore, NF-E2-related factor 2 (Nrf2), a transcription factor that plays a pivotal role in endogenous protection against oxidative stress, is implicated in glaucoma models [34]. Nrf2, which is activated by oxidative stress and electrophiles, controls various detoxifying enzymes and antioxidants. In normal conditions, the cytosolic regulatory protein Kelch-like ECH associated protein 1 (Keap1) represses Nrf2 and anchors it in the cytoplasm. When cells are exposed to oxidative or electrophilic stress, Nrf2 is released from Keap1 and translocates to the nucleus,

where it enhances the transcription of antioxidant genes [35]. The authors identified the expression of Nrf2 and its translocation in the RGCs with an ONC model. Furthermore, CDDO-Im, a potent activator of Nrf2, has a neuroprotective effect.

Oxidative stress impairs intracellular Ca^{2+} homeostasis, and the resulting Ca^{2+} overload causes additional oxidative stress, creating a positive feedback loop. Ca^{2+} dysregulation induces oxidative stress through several mechanisms, including an increased metabolic rate [36] and the activation of reactive oxygen species (ROS)-producing enzymes, such as nitric oxide synthase and nicotinamide adenine dinucleotide phosphate oxidase [37, 38]. ROS can directly damage proteins, lipids, and nucleic acids, thereby impairing mitochondrial respiration and depolarizing the mitochondrial membrane. This decreases the ability to buffer Ca^{2+} [39, 40]. Furthermore, it is likely that ROS not only increase the release of Ca^{2+} from the ER [41], but also directly damage the plasma membrane proteins (Ca^{2+} ATPase) responsible for maintaining Ca^{2+} homeostasis [42, 43].

2.3.2 Glutamate Toxicity

Elevated intracellular Ca^{2+} can cause the influx of Ca^{2+} from the extracellular environment or its release from the ER, where the Ca^{2+} concentration is 1,000 times higher than in the cytoplasm. Essentially, Ca^{2+} enters cells through two Ca^{2+} channels in the plasma membrane: receptor-operated channels (ROCs) and voltage-operated Ca^{2+} channels (VOCCs) [44]. These ROCs include *N*-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid receptors (AMPARs). Ca^{2+} influx through the ROCs is activated by the direct binding of a specific agonist (e.g., glutamate).

RGC apoptosis in glaucoma has been attributed to glutamate-mediated toxicity [45]. Under hypoxic conditions, retinal cells release glutamate [46], exposure to which is toxic at high levels. Low but chronic levels of elevated glutamate have also been found to be toxic to ganglion cells [47].

Glutamate is the principal excitatory neurotransmitter in the CNS, which includes the retina. In response to a variety of insults, the balance of glutamate shifts to a net accumulation of extracellular glutamate, which induces the overactivation of glutamatergic ion channels in the neurons. Due to their high permeability to Ca^{2+}, neurons are injured by the activation of NMDARs, and treatment with NMDAR antagonists can reduce the damage associated with glutamatergic excitotoxic injury [48].

Nakazawa et al. showed that after NMDAR activation induced an influx of Ca^{2+}, the protein phosphatase 2A (PP2A) was activated to dephosphorylate Akt. Phosphorylated Akt is apoptosis suppressive and thus prevents RGC death. On the other hand, brain-derived neurotrophic factor (BDNF) stimulates the TrkB/ShcC/PI3K signaling pathway and results in an increase in phosphorylated Akt [9].

2.3.3 Hyperglycemia-Induced RGC Degeneration

Diabetic retinopathy (DR) remains one of the major causes of blindness [49]. DR is diagnosed based on vascular pathology findings in the retina, but in animal models, hyperglycemia has been observed to lead to neurological effects after 2 weeks [50]. In patients without retinopathy, the thickness of the circumpapillary retinal fiber layer has been observed to decrease with the progress of DR [51].

Hyperglycemia-related calcium dysregulation may be caused by a decrease in releasable Ca^{2+} stored in the ER [52], Ca^{2+} influx via impaired Ca^{2+} channels in the plasma membrane, and Ca^{2+} release from the ER and mitochondria [53]. The disturbance of Ca^{2+} homeostasis is a significant cause of RGC degeneration, as it leads to changes in synaptic plasticity, the dysfunction of neurons, and, eventually, apoptosis [54]. Oxidative stress also plays a key role in the development and progression of diabetes and its complications, by increasing the production of free radicals and impairing antioxidant defenses [55]. Hyperglycemia-induced overproduction of superoxide in the mitochondrial electron-transport chain seems to be the key event in the pathogenesis of all diabetic complications [56]. Diabetes mellitus can thus be considered to be among major metabolic disorders causing neuropathy via various mechanisms, including Ca^{2+} dysregulation.

Hyperglycemia combined with a high-fat diet as a model of metabolic stress has been observed to induce RGC axonal degeneration and apoptosis within 4 weeks [11]. In this model, Ca^{2+} dysregulation induced by hyperglycemia causes calpain activation and oxidative stress, leading to RGC apoptosis. Calpastatin-knockout mice, animals in which endogenous calpain inhibitors are defective, undergo enhanced RGC death in hyperglycemic conditions. Furthermore, RGC apoptosis and axonal degeneration are significantly prevented by SNJ-1945, a potent exogenous calpain inhibitor that can cross the blood–retinal barrier [57]. Together, these results indicate that calpain activation is a critical part of RGC degeneration in animal models.

2.3.4 Axonal Flow Damage and Optic Nerve Crush-Induced RGC Degeneration

Recent evidence indicates that the causes of RGC degeneration are compartmentalized into processes such as deficits in axonal transport, remodeling of dendrites and synapses, and physiological dysfunction. These new findings are changing the way we think about glaucoma [6].

It has been known for some time that glaucoma involves deficits in axonal transport. Since the 1970s [58, 59], research on this subject had focused on events in the optic nerve head (ONH) because that is the site of the mechanical blockade that causes axonal impairment [60, 61]. Blockade of the ONH causes retrograde

transport impairment, and the resulting deficit of neuronal survival factors (e.g., BDNF), which normally come from the brain, results in apoptosis [8].

Axonal transport impairment precedes and eventually causes axonal and somatic degeneration in glaucoma [62]. The disorders involved in this axonal transport deficit include changes in molecular motors [63] and cytoskeletal structures [64]. ONC is a common animal model of axonal impairment and is often used to research the pathologies involved in glaucoma and axonal degeneration [65–67]. In addition to ONC, the administration of vinblastine to the optic nerve also prevents axoplasmic flow [10]. The authors successfully established a new glaucoma model not involving mechanical damage to the axons. Vinblastine (VB) is a microtubule disassembly chemical that inhibits mitosis and stops axonal transport in RGCs by binding microtubular protein [68, 69]. Results obtained with this model indicated that RGC apoptosis involves BDNF deficits, impairment of the mitochondria, and calpain activation, a finding that agrees with a number of other reports [8, 70, 71].

2.4 Neuroprotective Therapy

All clinical treatments for glaucoma, including eyedrops and surgery, aim at lowering IOP. However, IOP-lowering drugs do not prevent the progress of visual field defects in all glaucoma patients, especially patients with normal IOP. It is therefore imperative to establish new neuroprotective treatments for these patients.

Neuroprotective therapy is directed specifically at the neurons. In glaucoma, neuroprotection is directed at the neurons in the central visual pathway, particularly at the RGCs, and does not target IOP or any other risk factors.

2.4.1 Calpain Inhibitors (Fig. 2.2)

Calpain activation is implicated in numerous mechanisms underlying glaucomatous RGC degeneration, suggesting that suppression of overactivated calpain pathways may be a good candidate for neuroprotection in glaucoma. This alternative treatment would be especially useful for patients with normal IOP.

The calpain inhibitor ((1S)-1-((((1S)-1-benzyl-3-cyclopropylamino-2, 3-di-oxopropyl)amino)carbonyl)-3-methylbutyl)carbamic acid 5-methoxy-3-oxapentyl ester (SNJ-1945) is an agent with high cellular permeability and high metabolic stability [72], which has the potential to reduce retinal cell death in vivo and in vitro [10, 11, 73, 74] (Fig. 2.2). Compared to other calpain inhibitors, such as 4-fluorophenylsulfonyl-Val-Leu-CHO (SJA6017) and *N*-acetyl-Leu-Leu-Nle-CHO (ALLN), SNJ-1945 is readily administered orally [72]. In one report, SNJ-1945 reached a maximum concentration in the retina 15 min after oral administration of 10 mg/kg

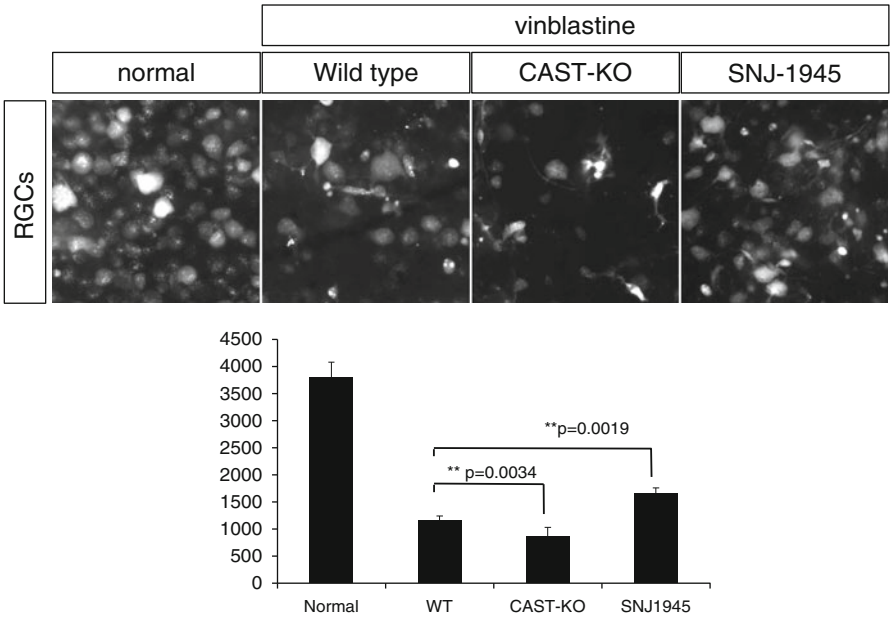


Fig. 2.2 Calpain inhibitor suppressed RGC death from axonal damage induced by vinblastine (CAST-KO, calpastatin-knockout mice; SNJ-1945, a novel calpain inhibitor)

and then gradually decreased over 8 h. The concentration of SNJ-1945 in the retina still maintained IC50 against calpains 1 and 2 even 4 h after oral administration (Y. Shirasaki, Senju Pharmaceuticals, unpublished data).

2.4.2 Calcium Channel Antagonist

Adrenoceptor antagonists for lowering IOP also suppress the influx of Na^+ and Ca^{2+} into the RGCs [75, 76]. This effect may explain the ability of beta-blockers to protect RGCs in DBA/2J mice, which are animals with inherited glaucoma [77]. The selective beta-blockers betaxolol and levobetaxolol have a greater suppressive effect on Ca^{2+} influx [78] than timolol, a nonselective beta-blocker. Furthermore, research in animals on the topical application of betaxolol and levobetaxolol has shown the necessary quantity at the back of the eye to effectively protect RGCs from various insults [79].

A number of independent clinical trials have compared the effect of betaxolol with timolol on both IOP and the progression of visual field (VF) loss in glaucoma patients [80, 81]. Some have reported that betaxolol provides greater suppression of the progression of VF loss than timolol, despite the greater reduction of IOP by timolol. The association between IOP reduction and VF preservation remains

unclear, but may involve neuroprotective mechanisms. In addition to reducing ischemic injury by acting as a vasodilator or Ca^{2+} influx suppressor, betaxolol can also increase the expression of BDNF mRNA in the retina [82].

The primary effect of calcium channel blockers (CCBs) is the inhibition of Ca^{2+} influx, resulting in the relaxation of vascular smooth muscle and consequent increased blood flow [83]. In an ischemic model, CCBs can reduce vascular constriction in the retina and optic nerve head [84].

Although CCBs primarily protect ischemic RGCs by restoring impaired blood flow through vasodilation, they also directly suppress Ca^{2+} influx-related cell death pathways, including ischemia- and excitotoxicity-induced apoptosis and necrosis [85].

An investigation exploring the effect of nimodipine (a Ca^{2+} channel antagonist) on ocular blood flow in NTG [86] found that while the drug did not affect blood flow or IOP, contrast sensitivity was dramatically improved in the treatment group. The researchers concluded that nimodipine's efficacy was a direct result of decreasing Ca^{2+} influx to the RGCs and their axons, a finding that agreed with other studies of Ca^{2+} channel antagonists.

References

1. Wojda U, Salinska E, Kuznicki J (2008) Calcium ions in neuronal degeneration. IUBMB Life 60(9):575–590. doi:10.1002/iub.91
2. Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1(1):11–21. doi:10.1038/35036035
3. Squier TC, Bigelow DJ (2000) Protein oxidation and age-dependent alterations in calcium homeostasis. Front Biosci 5:D504–D526
4. Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443(7113):787–795
5. Tatton W, Chen D, Chalmers-Redman R, Wheeler L, Nixon R, Tatton N (2003) Hypothesis for a common basis for neuroprotection in glaucoma and Alzheimer's disease: anti-apoptosis by alpha-2-adrenergic receptor activation. Surv Ophthalmol 48(Suppl 1):S25–S37
6. Whitmore AV, Libby RT, John SW (2005) Glaucoma: thinking in new ways-a role for autonomous axonal self-destruction and other compartmentalised processes? Prog Retin Eye Res 24(6):639–662. doi:10.1016/j.preteyeres.2005.04.004
7. McKinnon SJ (2003) Glaucoma: ocular Alzheimer's disease? Front Biosci 8:s1140–s1156
8. Quigley HA, McKinnon SJ, Zack DJ, Pease ME, Kerrigan-Baumrind LA, Kerrigan DF, Mitchell RS (2000) Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest Ophthalmol Vis Sci 41(11):3460–3466
9. Nakazawa T, Shimura M, Endo S, Takahashi H, Mori N, Tamai M (2005) *N*-Methyl-D-Aspartic acid suppresses Akt activity through protein phosphatase in retinal ganglion cells. Mol Vis 11:1173–1182
10. Ryu M, Yasuda M, Shi D, Shanab AY, Watanabe R, Himori N, Omodaka K, Yokoyama Y, Takano J, Saido T, Nakazawa T (2012) Critical role of calpain in axonal damage-induced retinal ganglion cell death. J Neurosci Res 90(4):802–815. doi:10.1002/jnr.22800
11. Shanab AY, Nakazawa T, Ryu M, Tanaka Y, Himori N, Taguchi K, Yasuda M, Watanabe R, Takano J, Saido T, Minegishi N, Miyata T, Abe T, Yamamoto M (2012) Metabolic stress

response implicated in diabetic retinopathy: the role of calpain, and the therapeutic impact of calpain inhibitor. Neurobiol Dis 48(3):556–567. doi:10.1016/j.nbd.2012.07.025
12. Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, Raman R, Davies P, Masliah E, Williams DS, Goldstein LS (2005) Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science 307(5713):1282–1288. doi:10.1126/science.1105681
13. Fischer LR, Culver DG, Tennant P, Davis AA, Wang M, Castellano-Sanchez A, Khan J, Polak MA, Glass JD (2004) Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. Exp Neurol 185(2):232–240
14. Coleman M (2005) Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci 6(11):889–898. doi:10.1038/nrn1788
15. Saido TC, Nagao S, Shiramine M, Tsukaguchi M, Sorimachi H, Murofushi H, Tsuchiya T, Ito H, Suzuki K (1992) Autolytic transition of mu-calpain upon activation as resolved by antibodies distinguishing between the pre- and post-autolysis forms. J Biochem 111(1):81–86
16. Goll DE, Thompson VF, Li H, Wei W, Cong J (2003) The calpain system. Physiol Rev 83(3):731–801. doi:10.1152/physrev.00029.2002
17. Samantaray S, Ray SK, Banik NL (2008) Calpain as a potential therapeutic target in Parkinson's disease. CNS Neurol Disord Drug Targets 7(3):305–312
18. Nixon RA (2003) The calpains in aging and aging-related diseases. Ageing Res Rev 2(4):407–418
19. Markgraf CG, Velayo NL, Johnson MP, McCarty DR, Medhi S, Koehl JR, Chmielewski PA, Linnik MD (1998) Six-hour window of opportunity for calpain inhibition in focal cerebral ischemia in rats. Stroke 29(1):152–158
20. Kampfl A, Posmantur RM, Zhao X, Schmutzhard E, Clifton GL, Hayes RL (1997) Mechanisms of calpain proteolysis following traumatic brain injury: implications for pathology and therapy: implications for pathology and therapy: a review and update. J Neurotrauma 14(3):121–134
21. Nakazawa T, Shimura M, Mourin R, Kondo M, Yokokura S, Saido TC, Nishida K, Endo S (2009) Calpain-mediated degradation of G-substrate plays a critical role in retinal excitotoxicity for amacrine cells. J Neurosci Res 87(6):1412–1423. doi:10.1002/jnr.21953
22. Porn-Ares MI, Samali A, Orrenius S (1998) Cleavage of the calpain inhibitor, calpastatin, during apoptosis. Cell Death Differ 5(12):1028–1033. doi:10.1038/sj.cdd.4400424
23. McGinnis KM, Gnegy ME, Park YH, Mukerjee N, Wang KK (1999) Procaspase-3 and poly(ADP)ribose polymerase (PARP) are calpain substrates. Biochem Biophys Res Commun 263(1):94–99. doi:10.1006/bbrc.1999.1315
24. Siman R, Baudry M, Lynch G (1984) Brain fodrin: substrate for calpain I, an endogenous calcium-activated protease. Proc Natl Acad Sci U S A 81(11):3572–3576
25. Pariat M, Carillo S, Molinari M, Salvat C, Debussche L, Bracco L, Milner J, Piechaczyk M (1997) Proteolysis by calpains: a possible contribution to degradation of p53. Mol Cell Biol 17(5):2806–2815
26. Kusakawa G, Saito T, Onuki R, Ishiguro K, Kishimoto T, Hisanaga S (2000) Calpain-dependent proteolytic cleavage of the p35 cyclin-dependent kinase 5 activator to p25. J Biol Chem 275(22):17166–17172. doi:10.1074/jbc.M907757199
27. Qu J, Wang D, Grosskreutz CL (2010) Mechanisms of retinal ganglion cell injury and defense in glaucoma. Exp Eye Res 91(1):48–53. doi:10.1016/j.exer.2010.04.002
28. Cakatay U, Telci A, Kayali R, Tekeli F, Akcay T, Sivas A (2001) Relation of oxidative protein damage and nitrotyrosine levels in the aging rat brain. Exp Gerontol 36(2):221–229
29. Floyd RA, Hensley K (2002) Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. Neurobiol Aging 23(5):795–807
30. Moreno MC, Campanelli J, Sande P, Sanez DA, Keller Sarmiento MI, Rosenstein RE (2004) Retinal oxidative stress induced by high intraocular pressure. Free Radic Biol Med 37(6):803–812

31. Tezel G, Yang X, Cai J (2005) Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma. Invest Ophthalmol Vis Sci 46 (9):3177–3187. doi:10.1167/iovs.05-0208
32. Tezel G, Luo C, Yang X (2007) Accelerated aging in glaucoma: immunohistochemical assessment of advanced glycation end products in the human retina and optic nerve head. Invest Ophthalmol Vis Sci 48(3):1201–1211. doi:10.1167/iovs.06-0737
33. Tezel G (2006) Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. Prog Retin Eye Res 25(5):490–513. doi:10.1016/j.preteyeres.2006.07.003
34. Himori N, Yamamoto K, Maruyama K, Ryu M, Taguchi K, Yamamoto M, Nakazawa T (2013) Critical role of Nrf2 in oxidative stress-induced retinal ganglion cell death. J Neurochem. doi:10.1111/jnc.12325
35. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 236(2):313–322
36. Zundorf G, Kahlert S, Bunik VI, Reiser G (2009) alpha-Ketoglutarate dehydrogenase contributes to production of reactive oxygen species in glutamate-stimulated hippocampal neurons in situ. Neuroscience 158(2):610–616. doi:10.1016/j.neuroscience.2008.10.015
37. Feissner RF, Skalska J, Gaum WE, Sheu SS (2009) Crosstalk signaling between mitochondrial Ca^{2+} and ROS. Front Biosci 14:1197–1218
38. Abramov AY, Scorziello A, Duchen MR (2007) Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. J Neurosci 27(5):1129–1138. doi:10.1523/JNEUROSCI.4468-06.2007
39. Ward MW, Rego AC, Frenguelli BG, Nicholls DG (2000) Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells. J Neurosci 20(19):7208–7219
40. Murchison D, Griffith WH (2007) Calcium buffering systems and calcium signaling in aged rat basal forebrain neurons. Aging Cell 6(3):297–305. doi:10.1111/j.1474-9726.2007.00293.x
41. Feng W, Liu G, Allen PD, Pessah IN (2000) Transmembrane redox sensor of ryanodine receptor complex. J Biol Chem 275(46):35902–35907. doi:10.1074/jbc.C000523200
42. Zaidi A, Michaelis ML (1999) Effects of reactive oxygen species on brain synaptic plasma membrane Ca(2+)-ATPase. Free Radic Biol Med 27(7–8):810–821
43. Huschenbett J, Zaidi A, Michaelis ML (1998) Sensitivity of the synaptic membrane Na^{+}/Ca^{2+} exchanger and the expressed NCX1 isoform to reactive oxygen species. Biochim Biophys Acta 1374(1–2):34–46
44. Berridge MJ (1997) Elementary and global aspects of calcium signalling. J Exp Biol 200 (Pt 2):315–319
45. Dreyer EB, Zurakowski D, Schumer RA, Podos SM, Lipton SA (1996) Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch Ophthalmol 114 (3):299–305
46. Neal MJ, Cunningham JR, Hutson PH, Hogg J (1994) Effects of ischaemia on neurotransmitter release from the isolated retina. J Neurochem 62(3):1025–1033
47. Vorwerk CK, Lipton SA, Zurakowski D, Hyman BT, Sabel BA, Dreyer EB (1996) Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. Invest Ophthalmol Vis Sci 37(8):1618–1624
48. Osborne NN, Ugarte M, Chao M, Chidlow G, Bae JH, Wood JP, Nash MS (1999) Neuroprotection in relation to retinal ischemia and relevance to glaucoma. Surv Ophthalmol 43(Suppl 1):S102–S128
49. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, Mariotti SP (2004) Global data on visual impairment in the year 2002. Bull World Health Organ 82 (11):844–851
50. Hancock HA, Kraft TW (2004) Oscillatory potential analysis and ERGs of normal and diabetic rats. Invest Ophthalmol Vis Sci 45(3):1002–1008

51. Lopes de Faria JM, Russ H, Costa VP (2002) Retinal nerve fibre layer loss in patients with type 1 diabetes mellitus without retinopathy. Br J Ophthalmol 86(7):725–728
52. Kruglikov I, Gryshchenko O, Shutov L, Kostyuk E, Kostyuk P, Voitenko N (2004) Diabetes-induced abnormalities in ER calcium mobilization in primary and secondary nociceptive neurons. Pflugers Arch 448(4):395–401. doi:10.1007/s00424-004-1263-8
53. Berridge MJ (1998) Neuronal calcium signaling. Neuron 21(1):13–26
54. Biessels GJ, ter Laak MP, Hamers FP, Gispen WH (2002) Neuronal Ca^{2+} disregulation in diabetes mellitus. Eur J Pharmacol 447(2–3):201–209
55. Bonnefont-Rousselot D (2002) Glucose and reactive oxygen species. Curr Opin Clin Nutr Metab Care 5(5):561–568
56. Ceriello A (2003) New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care 26(5):1589–1596
57. Shirasaki Y, Yamaguchi M, Miyashita H (2006) Retinal penetration of calpain inhibitors in rats after oral administration. J Ocul Pharmacol Ther 22(6):417–424. doi:10.1089/jop.2006.22.417
58. Anderson DR, Hendrickson A (1974) Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve. Invest Ophthalmol 13(10):771–783
59. Minckler DS, Tso MO, Zimmerman LE (1976) A light microscopic, autoradiographic study of axoplasmic transport in the optic nerve head during ocular hypotony, increased intraocular pressure, and papilledema. Am J Ophthalmol 82(5):741–757
60. Quigley HA, Addicks EM, Green WR, Maumenee AE (1981) Optic nerve damage in human glaucoma. II. The site of injury and susceptibility to damage. Arch Ophthalmol 99(4):635–649
61. Burgoyne CF, Downs JC, Bellezza AJ, Suh JK, Hart RT (2005) The optic nerve head as a biomechanical structure: a new paradigm for understanding the role of IOP-related stress and strain in the pathophysiology of glaucomatous optic nerve head damage. Prog Retin Eye Res 24(1):39–73. doi:10.1016/j.preteyeres.2004.06.001
62. Band LR, Hall CL, Richardson G, Jensen OE, Siggers JH, Foss AJ (2009) Intracellular flow in optic nerve axons: a mechanism for cell death in glaucoma. Invest Ophthalmol Vis Sci 50 (8):3750–3758. doi:10.1167/iovs.08-2396
63. Martin KR, Quigley HA, Valenta D, Kielczewski J, Pease ME (2006) Optic nerve dynein motor protein distribution changes with intraocular pressure elevation in a rat model of glaucoma. Exp Eye Res 83(2):255–262. doi:10.1016/j.exer.2005.11.025
64. Kashiwagi K, Ou B, Nakamura S, Tanaka Y, Suzuki M, Tsukahara S (2003) Increase in dephosphorylation of the heavy neurofilament subunit in the monkey chronic glaucoma model. Invest Ophthalmol Vis Sci 44(1):154–159
65. Bridge PM, Ball DJ, Mackinnon SE, Nakao Y, Brandt K, Hunter DA, Hertl C (1994) Nerve crush injuries–a model for axonotmesis. Exp Neurol 127(2):284–290. doi:10.1006/exnr.1994.1104
66. Nakazawa T, Takahashi H, Shimura M (2006) Estrogen has a neuroprotective effect on axotomized RGCs through ERK signal transduction pathway. Brain Res 1093(1):141–149. doi:10.1016/j.brainres.2006.03.084
67. Schwartz M (2004) Optic nerve crush: protection and regeneration. Brain Res Bull 62 (6):467–471. doi:10.1016/S0361-9230(03)00076-5
68. Hansson HA (1972) Retinal changes induced by treatment with vincristine and vinblastine. Doc Ophthalmol 31(1):65–88
69. Bunt AH (1973) Effects of vinblastine on microtubule structure and axonal transport in ganglion cells of the rabbit retina. Invest Ophthalmol 12(8):579–590
70. Tatton WG, Chalmers-Redman RM, Sud A, Podos SM, Mittag TW (2001) Maintaining mitochondrial membrane impermeability. an opportunity for new therapy in glaucoma? Surv Ophthalmol 45(Suppl 3):S277–S283, discussion S295-276
71. Huang W, Fileta J, Rawe I, Qu J, Grosskreutz CL (2010) Calpain activation in experimental glaucoma. Invest Ophthalmol Vis Sci 51(6):3049–3054. doi:10.1167/iovs.09-4364

72. Shirasaki Y, Miyashita H, Yamaguchi M, Inoue J, Nakamura M (2005) Exploration of orally available calpain inhibitors: peptidyl alpha-ketoamides containing an amphiphile at P3 site. Bioorg Med Chem 13(14):4473–4484. doi:10.1016/j.bmc.2005.04.059
73. Shimazawa M, Suemori S, Inokuchi Y, Matsunaga N, Nakajima Y, Oka T, Yamamoto T, Hara H (2010) A novel calpain inhibitor, ((1S)-1-((((1S)-1-Benzyl-3-cyclopropylamino-2,3-dioxopropyl)amino)carbonyl)-3-me thylbutyl)carbamic acid 5-methoxy-3-oxapentyl ester (SNJ-1945), reduces murine retinal cell death in vitro and in vivo. J Pharmacol Exp Ther 332(2):380–387. doi:10.1124/jpet.109.156612
74. Oka T, Walkup RD, Tamada Y, Nakajima E, Tochigi A, Shearer TR, Azuma M (2006) Amelioration of retinal degeneration and proteolysis in acute ocular hypertensive rats by calpain inhibitor ((1S)-1-((((1S)-1-benzyl-3-cyclopropylamino-2,3-di-oxopropyl)amino)carbonyl)-3-me thylbutyl)carbamic acid 5-methoxy-3-oxapentyl ester. Neuroscience 141 (4):2139–2145. doi:10.1016/j.neuroscience.2006.05.060
75. Osborne NN, Wood JP, Chidlow G, Casson R, DeSantis L, Schmidt KG (2004) Effectiveness of levobetaxolol and timolol at blunting retinal ischaemia is related to their calcium and sodium blocking activities: relevance to glaucoma. Brain Res Bull 62(6):525–528. doi:10.1016/S0361-9230(03)00070-4
76. Hirooka K, Kelly ME, Baldridge WH, Barnes S (2000) Suppressive actions of betaxolol on ionic currents in retinal ganglion cells may explain its neuroprotective effects. Exp Eye Res 70 (5):611–621. doi:10.1006/exer.2000.0822
77. Schuettauf F, Quinto K, Naskar R, Zurakowski D (2002) Effects of anti-glaucoma medications on ganglion cell survival: the DBA/2 J mouse model. Vision Res 42(20):2333–2337
78. Melena J, Wood JP, Osborne NN (1999) Betaxolol, a beta1-adrenoceptor antagonist, has an affinity for L-type Ca^{2+} channels. Eur J Pharmacol 378(3):317–322
79. Osborne NN, DeSantis L, Bae JH, Ugarte M, Wood JP, Nash MS, Chidlow G (1999) Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischaemia to the retina. Exp Eye Res 69(3):331–342. doi:10.1006/exer.1999.0706
80. Drance SM (1998) A comparison of the effects of betaxolol, timolol, and pilocarpine on visual function in patients with open-angle glaucoma. J Glaucoma 7(4):247–252
81. Kaiser HJ, Flammer J, Stumpfig D, Hendrickson P (1994) Longterm visual field follow-up of glaucoma patients treated with beta-blockers. Surv Ophthalmol 38(Suppl):S156–S159, discussion S160
82. Wood JP, DeSantis L, Chao HM, Osborne NN (2001) Topically applied betaxolol attenuates ischaemia-induced effects to the rat retina and stimulates BDNF mRNA. Exp Eye Res 72 (1):79–86. doi:10.1006/exer.2000.0929
83. Abernethy DR, Schwartz JB (1999) Calcium-antagonist drugs. N Engl J Med 341 (19):1447–1457. doi:10.1056/NEJM199911043411907
84. Ishii K, Matsuo H, Fukaya Y, Tanaka S, Sakaki H, Waki M, Araie M (2003) Iganidipine, a new water-soluble Ca^{2+} antagonist: ocular and periocular penetration after instillation. Invest Ophthalmol Vis Sci 44(3):1169–1177. doi:10.1167/iovs.02-0482
85. Yamada H, Chen YN, Aihara M, Araie M (2006) Neuroprotective effect of calcium channel blocker against retinal ganglion cell damage under hypoxia. Brain Res 1071(1):75–80. doi:10.1016/j.brainres.2005.11.072
86. Boehm AG, Breidenbach KA, Pillunat LE, Bernd AS, Mueller MF, Koeller AU (2003) Visual function and perfusion of the optic nerve head after application of centrally acting calcium-channel blockers. Graefes Arch Clin Exp Ophthalmol 241(1):34–38. doi:10.1007/s00417-002-0592-6

Chapter 3
Classical Signaling Pathways

Hideki Hayashi

Abstract In this chapter, I introduce a number of important aspects of intracellular signaling pathways related to the protection and degeneration in cells, especially neurons, under physiological and pathological conditions. Extracellular stimuli activate intracellular signaling pathways by receptor- and/or channel-mediated manner or simple diffusion across the plasma membrane. A variety of intracellular signaling molecules responses to each extracellular stimulus and reflects the coordinated actions of the cells. While there are many signaling pathways contributing to cellular functions and survival, I will focus on the MAPK/ERK and the Akt pathways in this chapter. The roles of these pathways have been extensively studied in neurons and other cells. However, the contribution and/or the cross talk of these intracellular signaling pathways in the degeneration and protection of the retina remains unclear because each signaling molecule in these pathways has diverse roles under physiological and pathological conditions. I would be happy if this chapter would help the readers to understand the complex and the ingenious regulating mechanisms of the intracellular signaling pathways for the degeneration and protection in neurons of the retina.

Keywords MAPK/ERK • Neuroprotection • PI3K-Akt

H. Hayashi (✉)
Department of Ophthalmology, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan

Department of Applied Biochemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 191-0392, Japan
e-mail: hhayashi@toyaku.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_3, © Springer Japan 2014

3.1 MAPK/ERK Pathways

Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases conserved in eukaryotes from yeasts to vertebrates and one of the most important enzyme groups, which regulate a large variety of events including development, cell migration, differentiation, metabolism, survival, and degeneration. In mammals, four distinct MAPK subfamilies have been identified (Fig. 3.1): extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-jun N-terminal kinases 1, 2, and 3 (JNK1/2/3), p38 MAPKα, β, γ, and δ, and ERK5. Although MAPKs are not directly phosphorylated by receptor-type tyrosine kinases or receptor-associated protein kinases, a signal transduction cascade composed of three modules in each MAPK subfamily activates MAPKs in series by phosphorylation. The phosphorylation of a specific MAPK is induced by a specific MAP kinase kinase (MAPKK or MAP2K) and a specific MAP kinase kinase kinase (MAPKKK or MAP3K), which phosphorylates MAPKK.

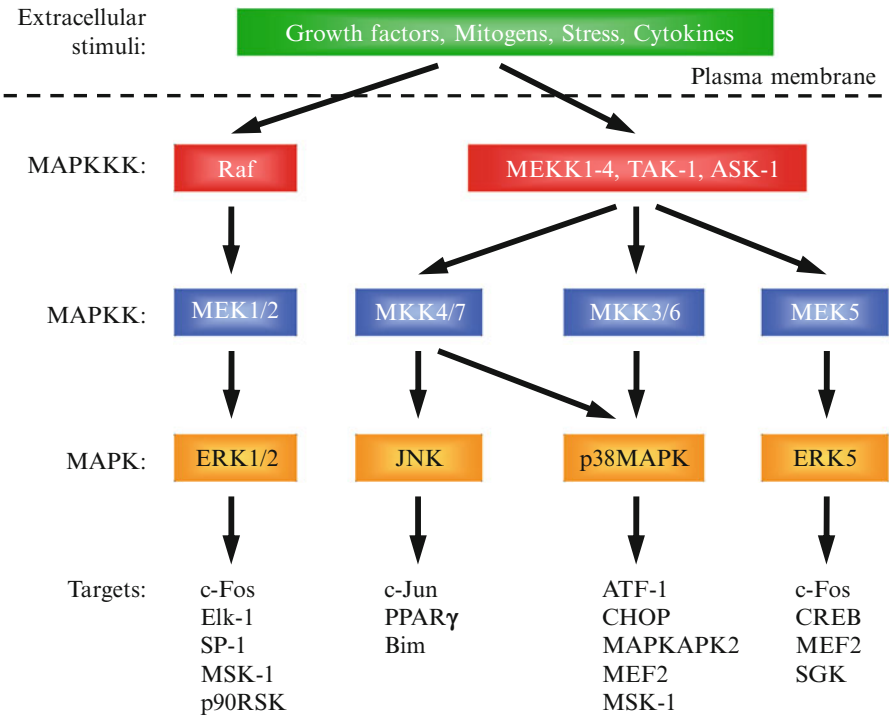


Fig. 3.1 The simplified scheme of the MAPK cascade and its regulations and targets (see text for details)

3.1.1 ERK1/2

ERK1 (MAPK3) and ERK2 (MAPK1) are 90 % identical in primary sequence [1] and are expressed in all tissues with various extents. ERK1/2 are phosphorylated by MAPKK1/2 (MEK1/2), which are activated by Raf-1, B-Raf, or other MAPKKK proteins in the Ras-Raf-MEK-ERK cascade. A double phosphorylation at threonine and tyrosine residues of ERK1/2 by MEK1/2 is required for maximum activation. In most cases, the activation of the ERK1/2 cascade is initiated by receptor-type tyrosine kinases, G-protein-coupled receptors, and ion channels at the plasma membrane. An inactive Ras-GDP is converted into an active Ras-GTP by guanine nucleotide exchange factors. The conversion of Ras-GDP to Ras-GTP is stimulated by growth factor receptors such as vascular endothelial growth factor (VEGF) receptor, epidermal growth factor receptor, and insulin-like growth factor receptor [2]. In addition to these growth factor receptors, tropomyosin-sensitive receptor kinases (Trks), which are the high-affinity receptors for neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 and neurotrophin 4, also activate the Ras-Raf-MEK-ERK pathway [3]. The ERK1/2 pathway has a large number of downstream targets including transcription factors such as Elk-1 and c-Fos and protein kinases such as p90 ribosomal S6 kinase and mitogen- and stress-activated protein kinase-1 (MSK-1). Thus, changes of ERK1/2 activity will cause intricate and profound effects on the cell function and survival.

BDNF is a well-known neurotrophin as a neuroprotective molecule against optic nerve injury and optic neuropathy. Nakazawa et al. [4] reported that an intravitreal injection of BDNF, but not NGF and neurotrophin 3, protected rat retinal ganglion cells (RGCs) from optic nerve transection-induced cell death via signaling pathways involving ERK as well as PI3K. It has also been reported that tears in normal tension glaucoma patients contain less BDNF than those in normal subjects [5]. Thus, the administration of BDNF and/or stimulation of the TrkB, a receptor of BDNF, -ERK pathway has been considered as one of the neuroprotective strategies in potential glaucoma therapies.

7,8-Dihydroxyflavone, a member of the flavonoid family, is a selective and a bioactive high-affinity TrkB agonist, which inhibits kainic acid-induced apoptosis in the hippocampus, decreases infarct volumes induced by the transient middle cerebral artery occlusion, and reduces the neurotoxicity of the dopaminergic toxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice [6]. It has been recently reported that the 7,8-dihydroxyflavone promoted the survival signaling pathways of ERK as well as Akt and protected RGCs and the RGC-5 cell line, a retinal neuronal precursor cell line, from oxidative stress and excitotoxicity [7].

Zhou et al. [8] performed an intravitreal injection of recombinant adeno-associated virus to transduce RGCs with genes encoding a constitutively active form of MEK1, an upstream activator of ERK1/2. This treatment induced in vivo activation of ERK1/2 in the retina at 4 weeks after injection of adeno-associated

virus and significantly increased RGC soma and axon survival at 5 and 7 weeks after ocular hypertension surgery in a rat glaucoma model. This study demonstrated that the gene therapy by the adeno-associated virus to transduce RGCs efficiently improved the RGC survival in ocular hypertensive insult, and the ERK1/2 pathway performed an important role in the protection of RGCs from neurodegeneration induced by glaucoma.

If the ERK signaling pathway plays an important role in the neuronal survival, an inhibition of this pathway would cause a survival defect in neurons. Methylprednisolone, a synthetic glucocorticoid, has been used in the therapy of neurological autoimmune central nervous system diseases. Diem et al. [9] determined the effect of high-dosage methylprednisolone therapy, which is the standard approach for acute inflammation of the optic nerve, on the survival of RGCs in an animal model of severe optic neuritis. This study demonstrated that the administration of methylprednisolone significantly exacerbated RGC apoptosis caused by myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis, and the mechanism of the aggravated RGC apoptosis was an inhibition of ERK1/2 phosphorylation by the steroid treatment.

Perron and Bixby [10] investigated that N-cadherin, laminin, and basic fibroblast growth factor (bFGF) promoted neurite outgrowth in embryonic chick retinal neurons and then found that the neurite outgrowth induced by these proteins required the activation of ERK1/2, but not p38 MAPK. It has also been reported that bFGF modulates axon extension and growth cone guidance of RGC in *Xenopus laevis* via the ERK as well as the phospholipase C pathways, but not the phosphatidylinositol 3-kinase (PI3K) pathway [11]. Furthermore, activated leukocyte cell adhesion molecule (ALCAM) expresses only in extending axons of RGCs and plays important roles of extension and guidance of RGC axons in the early stage of developing retina. The translation of ALCAM occurs in axonal growth cones of chick RGCs and requires the activity of the ERK1/2 pathway [12]. Thus, the ERK1/2 signaling pathway not only plays key roles in neuron survival but also regulates the axon growth of RGCs.

3.1.2 JNK

JNK1/2/3 (MAPK8/9/10), also known as stress-activated protein kinase (SAPK) γ/α/β, respectively, are activated by MAPKK4 and MAPKK7, also known as MKK4 and MKK7, respectively (Fig. 3.1). MKK4 and MKK7 lead to the activation of JNKs by simultaneous phosphorylation at a threonine and a tyrosine residue in protein kinase subdomain of JNK. Although MKK7 specifically activates JNKs, MKK4 can also activate p38 MAPKs. Downstream targets of JNKs include transcription factors such as c-jun and FOXO4, nuclear hormone receptors such as peroxisome proliferator-activated receptor-γ and the retinoic acid receptor,

cytosolic proteins such as insulin receptor substrate-1 and the E3 ligase Itch, and mitochondrial proteins of the Bcl-2 family such as Bad and Bim [13]. Therefore, it is considered that JNKs mediate many nuclear, cytosolic, and mitochondrial responses in developmental, physiological, and pathological conditions.

JNK1 and JNK2 are present in a wide variety of tissues, but JNK3 is primarily expressed in neurons of the central and peripheral nervous system [14]. Knockout mice in *Jnk1*, *Jnk2*, or *Jnk3* survive without apparent structural abnormalities, and *Jnk1*/*Jnk3* or *Jnk2*/*Jnk3* double-deficient mice also survive normally. However, *Jnk1*/*Jnk2* double deficiency leads the dysregulation of apoptosis in brain development and then causes embryonic lethality, indicating that JNK1 and JNK2 play a crucial role in the regulation of apoptosis during the CNS development [15]. In further details, although $Jnk1^{-/-}$ mice survive normally, they show a considerable reduction of the phosphorylation of microtubule-associated protein 2, the cytoskeletal protein, and exhibit axonal and dendritic degeneration in the brain [16]. JNK2 deficiency in mice did not show significant morphological changes but, nevertheless, caused impairment of long-term potentiation in the hippocampus during development [17]. JNK3 knockout attenuates the activation of the proapoptotic caspase cascade and leads to a strong resistance to cerebral hypoxic-ischemic injury compared to wild-type mice [18].

In retinal explants, JNK activation caused by calcium overload can induce a caspase-dependent apoptosis of photoreceptors by an increase of the BH3-only protein Bim_{EL}, which is a proapoptotic Bcl-2 family member [19]. In addition, Fernandes et al. [20] demonstrated that JNK signaling was activated in RGCs following optic nerve crush in mice, and double deficiency of *Jnk2* and *Jnk3* strongly protected RGCs from axon injury-induced death. Dual leucine zipper kinase identified by a high-throughput RNA interference screen has been shown as a critical kinase for JNK activation and cell death in primary RGCs and a rodent model of optic neuropathy. Furthermore, the small-molecule kinase inhibitor tozasertib decreases the phosphorylation of MKK7 and JNK, which are the downstream targets of dual leucine zipper kinase, and then protects RGCs from experimental glaucoma and traumatic optic neuropathy [21]. The D-form of JNK inhibitor 1, a synthetic cell-permeable JNK pathway inhibitor, protected RGCs from chronic ocular hypertension-induced [22] and *N*-methyl-D-aspartate (NMDA)-induced neurodegeneration [23]. Agmatine, an endogenous polyamine synthesized by the decarboxylation of L-arginine, suppresses the phosphorylation of JNK and nuclear factor-kappa B, but not ERK1/2 and p38 MAPK, caused by hypoxic damage and then protects RGC-5 cells from apoptotic death [24].

Increasing evidence supports that the JNK isoforms play important roles as capable mediators of proapoptotic signaling events under developmental and pathological conditions [25, 13]. Therefore, it has been considered that isoform-specific inhibitors for JNKs are beneficial for therapeutic use in neurodegenerative diseases including glaucoma.

3.1.3 p38 MAPK

The p38 MAPKα/β/γ/δ (MAPK14/11/12/13) are activated by MKK3 and MKK6 (Fig. 3.1). p38 MAPKγ is known as ERK6 and SAPK3, and p38 MAPKδ is also termed as SAPK4. In addition to the MKK3 and MKK6, p38 MAPKs can also be phosphorylated by MKK4, an upstream kinase of JNK, as shown in Fig. 3.1 depicting the cross talk between the p38 MAPK and the JNK pathways, and they share a substantial proportion of substrates. Therefore, p38 MAPKs and JNKs are often co-activated by the same upstream regulators and/or stresses and then demonstrate the synergic regulation for the downstream targets [26, 27]. Many transcription factors have been shown as downstream substrates of p38 MAPKs including CCAAT/enhancer binding protein β (C/EBPβ), C/EBP homologous protein (CHOP), myocyte enhancer factors 2A and 2C, activating transcription factors 1, 2, and 6 (ATF1/2/6), and p53 [28]. Other substrates of p38 MAPKs are MAP kinase-activated protein kinase 2 (MAPKAPK2 or MK2) and MSK-1. MAPKAPK2 collaborates with p38 MAPKs for activating various substrates such as heat shock protein 27 [29], tyrosine hydroxylase [30], cAMP response element-binding protein (CREB), and ATF1 [31]. MSK-1 is activated by p38 MAPKs and ERK1/2 and contributes to the activation of CREB [32].

Although p38 MAPKα is expressed ubiquitously in human tissues, p38 MAPKβ is abundantly expressed in the brain. p38 MAPKγ and p38 MAPKδ are expressed at high level in skeletal muscle and gland tissues, respectively [33]. Deficiency of *p38 Mapkα* results in embryonic lethality at E10.5–12.5 because of defective placental development by abnormal angiogenesis in the embryo itself and the visceral yolk sac [34]. Xing et al. [35] have reported that cortical neurons in coculture with microglia isolated from p38 MAPKα conditional knockout mice were protected from lipopolysaccharide-induced neurodegeneration. p38 MAPKα conditional knockout microglia secreted much less tumor necrosis factor-α (TNF-α), the pro-inflammatory cytokine, in response to lipopolysaccharide compared to wild-type microglia. Beardmore et al. [36] demonstrated that *p38 Mapkβ*$^{-/-}$ mice were viable and exhibit no obvious phenotype and concluded, with additional experiments, that p38 MAPKα was the main isoform of p38 MAPKs contributing to the immune response. Knockout mice in *p38 Mapkγ*, *p38 Mapkδ*, or *p38 Mapkγ/δ* were also viable and fertile without apparent health problem [37]. In addition, the knockout of p38 MAPKγ or p38 MAPKδ or both kinases did not alter the expression or the activity of the other isoforms.

p38 MAPKα in the nuclei of RGCs was activated after optic nerve transection [38]. The maximum phosphorylation of p38 MAPKα was detected 1 day after axotomy and then gradually decreased. Intravitreal administration of SB203580, an inhibitor of p38 MAPKα and p38 MAPKβ, protected RGCs from axotomy-induced cell death. Furthermore, the administration of MK801, an inhibitor of NMDA receptor, increased the number of survived RGC and also decreased p38 MAPK activation in a dose-dependent manner. This study indicated that RGC death induced after optic nerve damage was mediated through NMDA receptor and

then the p38 MAPK signaling pathway. Manabe et al. [39] reported that retinal injury caused by intravitreal injection of NMDA increased the phosphorylation of p38 MAPK and Akt in the ganglion cells and inner nuclear layers. Then, SB203580 rescued RGCs, whereas an inhibitor of the PI3K-Akt pathway, LY294002, exacerbated RGC death after NMDA injection. The authors concluded that the p38 MAPK pathway is proapoptotic and the PI3K-Akt pathway is antiapoptotic in RGCs against NMDA injury. Intravitreal injection of NMDA also upregulates thioredoxin interacting protein, an endogenous inhibitor of thioredoxin, and decreases thioredoxin activity [40]. Elevated thioredoxin interacting protein leads to the increase of oxidative stress, release of inflammatory cytokines such as TNF-α and IL-1β, and activation of the p38 MAPK and JNK pathways. In response to this neurotoxicity, treatment with verapamil, a calcium channel blocker and an inhibitor of thioredoxin interacting protein, attenuated these effects caused by the upregulation of thioredoxin interacting protein. It has been reported that phosphorylated p38 MAPK and JNK were detected in the nuclei of RGCs and amacrine cells and phosphorylated ERK1/2 were found in Müller cells after retinal ischemia induced by an elevation of intraocular pressure [41]. Inhibiting activation of p38 MAPK or ERK1/2, but not JNK, markedly protected retinal cells and restored retinal function from ischemic damage in the retina.

As described above, p38 MAPKs are considered as proapoptotic kinases of neurons and some other cell types in many cases. However, p38 MAPK is activated by the induction of ischemic tolerance, which is a phenomenon that a brief ischemia reduces the lethal damage of subsequent prolonged ischemia, in the gerbil hippocampus, and SB203580 attenuates the ischemic tolerance effect [42]. Intravitreal injection of bFGF shows the neuroprotective effect by elevating phosphorylated p38 MAPK, ERK1/2, and CREB in Müller glia against an NMDA-induced retinal damage [43]. Thus, the roles of p38 MAPKs in neurodegeneration or protection are highly dependent on stimuli, cell types, and other conditions.

3.1.4 ERK5

ERK5 (MAPK7) is activated in response to growth factors including epidermal growth factor, NGF, and BDNF, oxidative stress, and cytokines via the dual phosphorylation at threonine and tyrosine of its TEY sequence by MEK5 (Fig. 3.1). MEK5 is the only identified upstream kinase of ERK5 at present [44]. ERK5 is also known as a big MAPK (BMK) named due to a high molecular weight (a predicted molecular mass of 98 kDa) compared to ERK1/2 [45]. Substrates of ERK5 are serum- and glucocorticoid-inducible kinase (SGK) and the transcription factors such as c-Myc, c-Fos, NF-κB, Sap1a, CREB, and the family of the myocyte enhancer factor 2 (MEF2A, MEF2C, and MEF2D) [46]. Since inhibitors of MEK1/2 such as U0126 and PD98056, which have been used as specific inhibitors for MEK1/2, can also inhibit MEK5, data using these inhibitors should be reconsidered with a role of the MEK5-ERK5 pathway [47].

Deficiency of *Erk5* results in embryonic lethality at days 9.5–10.5 because of severe defects of cardiovascular development and angiogenesis [48], whereas neuron-specific knockout of *Erk5* shows normal development in two different transgenic lines of mice [49]. On the other hand, the expression of a dominant-negative form of ERK5 blocked the protective effect of BDNF from serum withdrawal-induced apoptosis in primary rat embryonic cortical neurons [50]. Thus, it has been considered that the ERK5 signaling pathway contributes to at least the neurotrophin-mediated neuroprotective effect.

The phosphorylation of ERK5 is increased in dorsal root ganglion neurons in response to peripheral inflammation, suggesting that ERK5 may contribute to the development of inflammatory pain [51]. Watson et al. [52] demonstrated that a retrogradely transported TrkA signal initiated by NGF at distal axons activated ERK5 at the cell bodies of dorsal root ganglion neurons, induced nuclear translocation of ERK5 and the phosphorylation of CREB, and promoted neuron survival.

Wu et al. [53] demonstrated that the knockdown of *Erk5* by siRNA increased VEGF expression and angiogenesis and constitutively active MEK5 adenovirus causing sustained ERK5 activation decreased VEGF expression in glucose-exposed human microvascular endothelial cells. In addition, the reduction of ERK5 protein and increase of VEGF mRNA were found in the retinas of streptozotocin-induced diabetic rats. It has also been reported that increased fibronectin production, one of the characteristic features of diabetic retinopathy, decreased ERK5 expression in the retinae of diabetic rats [54]. In experimental glaucoma with chronic intraocular pressure elevation, only Brn3a among three Brn3 proteins, Brn3a, b, and c, showed a significant reduction with severe nerve damage, and the expression of *Erk5* mRNA, but not *Erk1/2* mRNA, demonstrated a positive correlation with a severity of optic nerve damage [55]. These studies indicate that ERK5 may perform important roles in the retina under pathological conditions such as diabetic retinopathy and glaucoma. However, further investigations have been required to define the roles of ERK5 in the retina and RGCs under physiological and pathological conditions.

3.2 PI3K-Akt Pathway

Akt is a 57 kDa serine/threonine kinase and activated by PI3K. Akt1, 2, and 3 are also known as protein kinase B (PKB) α, β, and γ, respectively. A catalytic domain of Akt/PKB is considerably similar to protein kinases A and C. Akt1/PKBα and Akt2/PKBβ are ubiquitously expressed with various extents, whereas Akt3/PKBγ is more restricted to the brain and testis [56]. Akt is rapidly activated by various growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor, bFGF, insulin, and NGF. Then, Akt activation stimulated by the growth factor is inhibited by a PI3K inhibitor such as wortmannin and by a dominant-negative form of PI3K [57]. However, PI3K does not directly activate Akt. PI3K phosphorylates membrane phospholipids such as phosphatidylinositol 4-phosphate

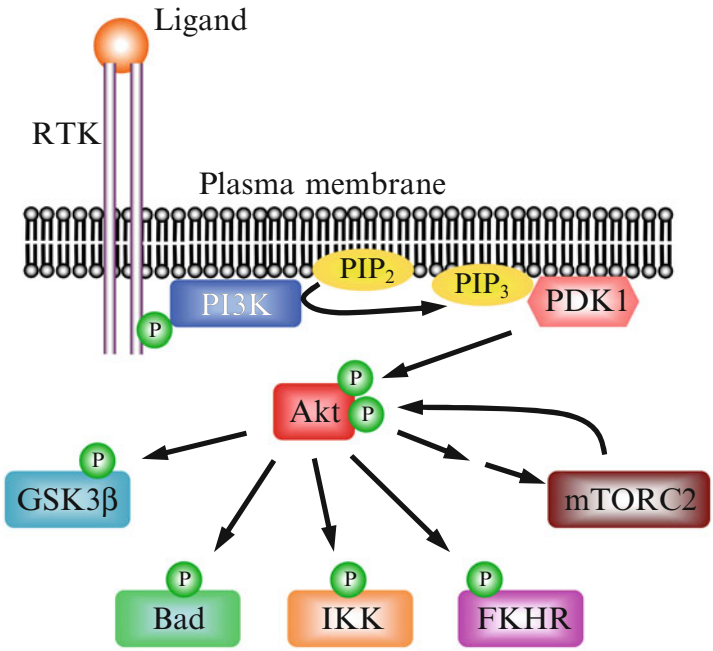


Fig. 3.2 A schematic model for the classic Akt pathway and its substrates (see text for details). *RTK* receptor tyrosine kinase, *PI3K* phosphatidylinositol 3-kinase, *PIP2* PtdIns(4,5)P2, *PIP3* PtdIns(3,4,5)P3, *PDK1* phosphoinositide-dependent protein kinase 1, *GSK3β* glycogen synthase kinase 3β, *IKK* inhibitor of κB kinase, *FKHR* forkhead transcription factor, *mTORC2* mammalian target of rapamycin complex 2

(PtdIns(4)P) and phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2), thereby producing phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P_2) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3). The binding of the pleckstrin homology (PH) domain of Akt to PtdIns(3,4)P_2 and/or PtdIns(3,4,5)P_3 is the driving force to recruit Akt to the plasma membrane [58]. Akt has two major phosphorylation sites at Thr308 in the kinase domain and Ser473 in the carboxy-terminal tail. These phosphorylation sites are phosphorylated by distinct kinases. Phosphoinositide-dependent protein kinase 1 (PDK1) is responsible for the phosphorylation of Thr308 [59], and Akt at Ser473 is phosphorylated by the mammalian target of rapamycin (mTOR) complex 2 [60, 61], referred to as PDK2 (Fig. 3.2). On the one hand, the phosphatase and tensin homologue deleted on chromosome ten (PTEN) negatively regulates the PI3K-Akt pathway by dephosphorylating PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2 [62, 63].

It has been reported that elevated intracellular cAMP by pharmacological reagent like forskolin can activate Akt, whereas this effect was not blocked by the PI3K inhibitor wortmannin [64]. Wortmannin also did not prevent heat-shock-induced Akt activation [65]. Thus, while the activation of PI3K is still considered as

the main factor for Akt activation, several alternative pathways stimulating Akt may exist.

Studies in Akt knockout mice have shown the various important roles of Akt in mammals. Deficiency of Akt2 induced severe insulin resistance and diabetes [66]. Insulin action, which stimulates glucose uptake in muscle, fat, and several other tissues, was markedly decreased, resulting in the attenuation of whole-body glucose disposal. In contrast to Akt2 knockout mice, Akt1 knockout mice were normal with insulin-dependent glucose metabolism. However, these mice demonstrated embryonic and postnatal defects in growth, and then this defect was sustained in adulthood [67]. In addition, deficiency of Akt1 increased neonatal mortality and shortened life span [68]. Akt1/Akt2 double-knockout neonates were severely growth deficient with about a half of a birth weight of their wild-type or double-heterozygous littermates. These mice died shortly after birth with impaired skin development, skeletal muscle atrophy, and delayed bone development [69]. On the other hand, Akt3-deficient mice exhibited normal growth and glucose metabolism. However, brain size and weight of adult $Akt3^{-/-}$ mice were markedly decreased by approximately 25 % because of cell size and cell number, suggesting a critical role of Akt3 in postnatal brain development of mammals [70]. It has been reported that Akt1/Akt3 double-knockout mice were embryonic lethal and the lethality could be attributed to placental insufficiency, cardiovascular anomalies, and impaired nervous system development [71].

Akt regulates a variety of biological functions [56] such as glucose and glycogen metabolisms [72], angiogenesis [73], cell cycle [74], and also cell survival. Since this chapter focuses on intracellular signaling pathways of degeneration and protection, the following section describes about the roles of Akt in cell survival. Akt has numerous downstream substrates related to apoptosis and survival, for instance, glycogen synthase kinase 3β (GSK3β), inhibitor of κB kinase (IKK), forkhead transcription factor (FKHR), and the Bcl-2 family protein Bad.

Akt inactivates GSK3β by phosphorylating it at Ser9. GSK3β contributes to the regulation of glycogen synthesis [75] and the canonical Wnt signaling pathway in phosphorylating β-catenin [76]. β-Catenin phosphorylated by GSK3β is degraded by ubiquitin-proteasome system, but non-phosphorylated β-catenin acts as a transcription factor and plays key roles in cell survival [77]. Endo et al. [78] demonstrated that activated Akt markedly phosphorylated/inactivated GSK3β in the hippocampus after transient global cerebral ischemia and the inhibition of the Akt/GSK3β signaling pathway by a PI3K inhibitor facilitated ischemic injury. Then, the authors conclude that the activation of the Akt/GSK3β pathway may regulate the survival of vulnerable neurons after ischemia. It has also been reported that PDGF-CC, one of the PDGF family members, protects cortical neurons from middle cerebral artery occlusion-induced ischemic injury and rescues RGCs from optic nerve crush or intravitreal injection of NMDA-induced neuronal death [79]. This study mechanistically showed that PDGF-CC induced the phosphorylation of GSK3β at Ser9 with significant Akt activation.

In response to stress signals such as oxidative stress and endoplasmic reticulum stress, apoptosis signal-regulating kinase 1 (ASK1) can initiate the JNK-dependent

proapoptotic signaling. Puckett et al. [80] have shown that insulin-like growth factor 1 (IGF-1) induced ASK1 inactivation by phosphorylating it at Ser967 with Akt activation. This process was mediated by Akt-activated IKK forming a complex with and phosphorylating ASK1 at Ser967, suggesting that the Akt/IKK pathway can counteract stress signal initiated by the ASK1/JNK proapoptotic pathway.

It has been reported that advanced glycation end product or TNF-α induced apoptosis in human retinal pericytes through the activation of the proapoptotic transcription factor FKHR via p38 MAPK and JNK [81]. In this study, Akt inhibitor facilitated the FKHR-mediated apoptosis in the retina. In addition, the Akt survival signal inactivating FKHR was inhibited by PTEN activation during the photoreceptor cell death in the *rd* mouse strain, the well-characterized animal model of retinitis pigmentosa [82]. These studies indicate the importance of the Akt/FKHR pathway in retinal cell survival.

Akt inhibits the mitochondrion-mediated proapoptotic function of Bad by phosphorylating it [83]. RGCs of goldfish can regenerate after optic nerve injury. Koriyama et al. [84] have demonstrated that IGF-1 was upregulated 2–3 days after optic nerve injury and then Akt activity and phosphorylation of Bad were increased. Subsequently, an increased level of Bcl-2 and reduced activity of caspase 3 were observed. In addition, IGF-1 induced Akt phosphorylation and neurite growth in cultured goldfish retina. In contrast to goldfish, rat RGCs die by apoptosis after optic nerve injury. The level of IGF-1 in the retina was decreased 1–2 days prior to the parallel decrease of phosphorylations of Akt and Bad by optic nerve injury. The administration of IGF-1 induced Akt phosphorylation and improved RGC survival in vitro and in vivo [85]. In the rat glaucoma model with chronic elevation of intraocular pressure, the phosphorylation levels of Akt, Bad, and CREB in the retina were simultaneously increased 1 week after the injury and decreased thereafter compared to normal intraocular pressure control, suggesting important roles of Akt at the early stage of injury in control of cell death or survival [86]. Furthermore, advanced glycation products caused cell death in rat retinal tissue cultures, primary neuron cultures, and retinal neuron cell lines (R28). Erythropoietin protected these retinal cells by enhancing Bad phosphorylation and Bcl-xl expression and reducing Bax expression via ERK- and Akt-dependent signaling pathways [87]. In addition, the Ser473 kinase for Akt mTOR complex 2 is also indirectly regulated by Akt [60, 61] (Fig. 3.2) and contributes to cell survival. Nitric oxide can protect neurons from apoptosis through the PI3K-Akt pathway [88]. In cultured retinal neurons, Akt phosphorylation at Thr308 and Ser473 induced by the nitric oxide donor *S*-nitroso-*N*-acetylpenicillamine was blocked by the mTOR inhibitor KU0063794, indicating the participation of the mTOR complex 2 in the antiapoptotic PI3K-Akt process [89].

As described above, Akt mediates antiapoptotic processes via various molecules and performs crucial roles in not only neuroprotection but also other biological functions such as glucose metabolism, development, and cell fate in neurons and other types of cells.

Acknowledgments This work was supported by Grant-in-Aid for Scientific Research C 24500441 from Japan Society for the Promotion of Science.

References

1. Ring AY, Sours KM, Lee T, Ahn NG (2011) Distinct patterns of activation-dependent changes in conformational mobility between ERK1 and ERK2. Int J Mass Spectrom 302 (1–3):101–109. doi:10.1016/j.ijms.2010.08.020
2. Steelman LS, Chappell WH, Abrams SL, Kempf RC, Long J, Laidler P, Mijatovic S, Maksimovic-Ivanic D, Stivala F, Mazzarino MC, Donia M, Fagone P, Malaponte G, Nicoletti F, Libra M, Milella M, Tafuri A, Bonati A, Basecke J, Cocco L, Evangelisti C, Martelli AM, Montalto G, Cervello M, McCubrey JA (2011) Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. Aging 3(3):192–222
3. Yoshii A, Constantine-Paton M (2010) Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Dev Neurobiol 70(5):304–322. doi:10.1002/dneu.20765
4. Nakazawa T, Tamai M, Mori N (2002) Brain-derived neurotrophic factor prevents axotomized retinal ganglion cell death through MAPK and PI3K signaling pathways. Invest Ophthalmol Vis Sci 43(10):3319–3326
5. Ghaffariyeh A, Honarpisheh N, Shakiba Y, Puyan S, Chamacham T, Zahedi F, Zarrineghbal M (2009) Brain-derived neurotrophic factor in patients with normal-tension glaucoma. Optometry 80(11):635–638. doi:10.1016/j.optm.2008.09.014
6. Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, Liu Y, Wilson WD, Xiao G, Blanchi B, Sun YE, Ye K (2010) A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. Proc Natl Acad Sci U S A 107(6):2687–2692. doi:10.1073/pnas.0913572107
7. Gupta VK, You Y, Li JC, Klistorner A, Graham SL (2013) Protective effects of 7,8-dihydroxyflavone on retinal ganglion and RGC-5 cells against excitotoxic and oxidative stress. J Mol Neurosci 49(1):96–104. doi:10.1007/s12031-012-9899-x
8. Zhou Y, Pernet V, Hauswirth WW, Di Polo A (2005) Activation of the extracellular signal-regulated kinase 1/2 pathway by AAV gene transfer protects retinal ganglion cells in glaucoma. Mol Ther 12(3):402–412. doi:10.1016/j.ymthe.2005.04.004
9. Diem R, Hobom M, Maier K, Weissert R, Storch MK, Meyer R, Bahr M (2003) Methylprednisolone increases neuronal apoptosis during autoimmune CNS inflammation by inhibition of an endogenous neuroprotective pathway. J Neurosci 23(18):6993–7000
10. Perron JC, Bixby JL (1999) Distinct neurite outgrowth signaling pathways converge on ERK activation. Mol Cell Neurosci 13(5):362–378. doi:10.1006/mcne.1999.0753
11. Webber CA, Chen YY, Hehr CL, Johnston J, McFarlane S (2005) Multiple signaling pathways regulate FGF-2-induced retinal ganglion cell neurite extension and growth cone guidance. Mol Cell Neurosci 30(1):37–47. doi:10.1016/j.mcn.2005.05.005
12. Thelen K, Maier B, Faber M, Albrecht C, Fischer P, Pollerberg GE (2012) Translation of the cell adhesion molecule ALCAM in axonal growth cones: regulation and functional importance. J Cell Sci 125(Pt 4):1003–1014. doi:10.1242/jcs.096149
13. Bogoyevitch MA (2006) The isoform-specific functions of the c-Jun N-terminal Kinases (JNKs): differences revealed by gene targeting. BioEssays 28(9):923–934. doi:10.1002/bies.20458
14. Martin JH, Mohit AA, Miller CA (1996) Developmental expression in the mouse nervous system of the p493F12 SAP kinase. Brain Res Mol Brain Res 35(1–2):47–57

15. Kuan CY, Yang DD, Samanta Roy DR, Davis RJ, Rakic P, Flavell RA (1999) The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. Neuron 22(4):667–676
16. Chang L, Jones Y, Ellisman MH, Goldstein LS, Karin M (2003) JNK1 is required for maintenance of neuronal microtubules and controls phosphorylation of microtubule-associated proteins. Dev Cell 4(4):521–533
17. Chen JT, Lu DH, Chia CP, Ruan DY, Sabapathy K, Xiao ZC (2005) Impaired long-term potentiation in c-Jun N-terminal kinase 2-deficient mice. J Neurochem 93(2):463–473. doi:10.1111/j.1471-4159.2005.03037.x
18. Pirianov G, Brywe KG, Mallard C, Edwards AD, Flavell RA, Hagberg H, Mehmet H (2007) Deletion of the c-Jun N-terminal kinase 3 gene protects neonatal mice against cerebral hypoxic-ischaemic injury. J Cereb Blood Flow Metab 27(5):1022–1032. doi:10.1038/sj.jcbfm.9600413
19. Donovan M, Doonan F, Cotter TG (2011) Differential roles of ERK1/2 and JNK in retinal development and degeneration. J Neurochem 116(1):33–42. doi:10.1111/j.1471-4159.2010.07056.x
20. Fernandes KA, Harder JM, Fornarola LB, Freeman RS, Clark AF, Pang IH, John SW, Libby RT (2012) JNK2 and JNK3 are major regulators of axonal injury-induced retinal ganglion cell death. Neurobiol Dis 46(2):393–401. doi:10.1016/j.nbd.2012.02.003
21. Welsbie DS, Yang Z, Ge Y, Mitchell KL, Zhou X, Martin SE, Berlinicke CA, Hackler L Jr, Fuller J, Fu J, Cao LH, Han B, Auld D, Xue T, Hirai S, Germain L, Simard-Bisson C, Blouin R, Nguyen JV, Davis CH, Enke RA, Boye SL, Merbs SL, Marsh-Armstrong N, Hauswirth WW, DiAntonio A, Nickells RW, Inglese J, Hanes J, Yau KW, Quigley HA, Zack DJ (2013) Functional genomic screening identifies dual leucine zipper kinase as a key mediator of retinal ganglion cell death. Proc Natl Acad Sci U S A 110(10):4045–4050. doi:10.1073/pnas.1211284110
22. Yang X, Luo C, Cai J, Pierce WM, Tezel G (2008) Phosphorylation-dependent interaction with 14-3-3 in the regulation of bad trafficking in retinal ganglion cells. Invest Ophthalmol Vis Sci 49(6):2483–2494. doi:10.1167/iovs.07-1344
23. Bessero AC, Chiodini F, Rungger-Brandle E, Bonny C, Clarke PG (2010) Role of the c-Jun N-terminal kinase pathway in retinal excitotoxicity, and neuroprotection by its inhibition. J Neurochem 113(5):1307–1318. doi:10.1111/j.1471-4159.2010.06705.x
24. Hong S, Lee JE, Kim CY, Seong GJ (2007) Agmatine protects retinal ganglion cells from hypoxia-induced apoptosis in transformed rat retinal ganglion cell line. BMC Neurosci 8:81. doi:10.1186/1471-2202-8-81
25. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270(5240):1326–1331
26. Llopis A, Salvador N, Ercilla A, Guaita-Esteruelas S, Barrantes Idel B, Gupta J, Gaestel M, Davis RJ, Nebreda AR, Agell N (2012) The stress-activated protein kinases p38alpha/beta and JNK1/2 cooperate with Chk1 to inhibit mitotic entry upon DNA replication arrest. Cell Cycle 11(19):3627–3637. doi:10.4161/cc.21917
27. Al-Gayyar MM, Mysona BA, Matragoon S, Abdelsaid MA, El-Azab MF, Shanab AY, Ha Y, Smith SB, Bollinger KE, El-Remessy AB (2013) Diabetes and overexpression of proNGF cause retinal neurodegeneration via activation of RhoA pathway. PLoS One 8(1):e54692. doi:10.1371/journal.pone.0054692
28. Zarubin T, Han J (2005) Activation and signaling of the p38 MAP kinase pathway. Cell Res 15(1):11–18. doi:10.1038/sj.cr.7290257
29. Stokoe D, Engel K, Campbell DG, Cohen P, Gaestel M (1992) Identification of MAPKAP kinase 2 as a major enzyme responsible for the phosphorylation of the small mammalian heat shock proteins. FEBS Lett 313(3):307–313
30. Thomas G, Haavik J, Cohen P (1997) Participation of a stress-activated protein kinase cascade in the activation of tyrosine hydroxylase in chromaffin cells. Eur J Biochem 247(3):1180–1189

31. Tan Y, Rouse J, Zhang A, Cariati S, Cohen P, Comb MJ (1996) FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. EMBO J 15 (17):4629–4642
32. Deak M, Clifton AD, Lucocq LM, Alessi DR (1998) Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. EMBO J 17(15):4426–4441. doi:10.1093/emboj/17.15.4426
33. Wang XS, Diener K, Manthey CL, Wang S, Rosenzweig B, Bray J, Delaney J, Cole CN, Chan-Hui PY, Mantlo N, Lichenstein HS, Zukowski M, Yao Z (1997) Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. J Biol Chem 272 (38):23668–23674
34. Mudgett JS, Ding J, Guh-Siesel L, Chartrain NA, Yang L, Gopal S, Shen MM (2000) Essential role for p38alpha mitogen-activated protein kinase in placental angiogenesis. Proc Natl Acad Sci U S A 97(19):10454–10459. doi:10.1073/pnas.180316397
35. Xing B, Bachstetter AD, Van Eldik LJ (2011) Microglial p38alpha MAPK is critical for LPS-induced neuron degeneration, through a mechanism involving TNFalpha. Mol Neurodegener 6:84. doi:10.1186/1750-1326-6-84
36. Beardmore VA, Hinton HJ, Eftychi C, Apostolaki M, Armaka M, Darragh J, McIlrath J, Carr JM, Armit LJ, Clacher C, Malone L, Kollias G, Arthur JS (2005) Generation and characterization of p38beta (MAPK11) gene-targeted mice. Mol Cell Biol 25(23):10454–10464. doi:10.1128/MCB.25.23.10454-10464.2005
37. Sabio G, Arthur JS, Kuma Y, Peggie M, Carr J, Murray-Tait V, Centeno F, Goedert M, Morrice NA, Cuenda A (2005) p38gamma regulates the localisation of SAP97 in the cytoskeleton by modulating its interaction with GKAP. EMBO J 24(6):1134–1145. doi:10.1038/sj.emboj.7600578
38. Kikuchi M, Tenneti L, Lipton SA (2000) Role of p38 mitogen-activated protein kinase in axotomy-induced apoptosis of rat retinal ganglion cells. J Neurosci 20(13):5037–5044
39. Manabe S, Lipton SA (2003) Divergent NMDA signals leading to proapoptotic and antiapoptotic pathways in the rat retina. Invest Ophthalmol Vis Sci 44(1):385–392
40. Al-Gayyar MM, Abdelsaid MA, Matragoon S, Pillai BA, El-Remessy AB (2011) Thioredoxin interacting protein is a novel mediator of retinal inflammation and neurotoxicity. Br J Pharmacol 164(1):170–180. doi:10.1111/j.1476-5381.2011.01336.x
41. Roth S, Shaikh AR, Hennelly MM, Li Q, Bindokas V, Graham CE (2003) Mitogen-activated protein kinases and retinal ischemia. Invest Ophthalmol Vis Sci 44(12):5383–5395
42. Nishimura M, Sugino T, Nozaki K, Takagi Y, Hattori I, Hayashi J, Hashimoto N, Moriguchi T, Nishida E (2003) Activation of p38 kinase in the gerbil hippocampus showing ischemic tolerance. J Cereb Blood Flow Metab 23(9):1052–1059. doi:10.1097/01.WCB.0000084251.20114.65
43. Fischer AJ, Scott MA, Ritchey ER, Sherwood P (2009) Mitogen-activated protein kinase-signaling regulates the ability of Muller glia to proliferate and protect retinal neurons against excitotoxicity. Glia 57(14):1538–1552. doi:10.1002/glia.20868
44. Drew BA, Burow ME, Beckman BS (2012) MEK5/ERK5 pathway: the first fifteen years. Biochim Biophys Acta 1825(1):37–48. doi:10.1016/j.bbcan.2011.10.002
45. Lee JD, Ulevitch RJ, Han J (1995) Primary structure of BMK1: a new mammalian map kinase. Biochem Biophys Res Commun 213(2):715–724
46. Plotnikov A, Zehorai E, Procaccia S, Seger R (2011) The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. Biochim Biophys Acta 1813 (9):1619–1633. doi:10.1016/j.bbamcr.2010.12.012
47. Kamakura S, Moriguchi T, Nishida E (1999) Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. J Biol Chem 274(37):26563–26571
48. Regan CP, Li W, Boucher DM, Spatz S, Su MS, Kuida K (2002) Erk5 null mice display multiple extraembryonic vascular and embryonic cardiovascular defects. Proc Natl Acad Sci U S A 99(14):9248–9253. doi:10.1073/pnas.142293999

49. Hayashi M, Lee JD (2004) Role of the BMK1/ERK5 signaling pathway: lessons from knockout mice. J Mol Med 82(12):800–808. doi:10.1007/s00109-004-0602-8
50. Liu L, Cavanaugh JE, Wang Y, Sakagami H, Mao Z, Xia Z (2003) ERK5 activation of MEF2-mediated gene expression plays a critical role in BDNF-promoted survival of developing but not mature cortical neurons. Proc Natl Acad Sci U S A 100(14):8532–8537. doi:10.1073/pnas.1332804100
51. Katsura H, Obata K, Mizushima T, Sakurai J, Kobayashi K, Yamanaka H, Dai Y, Fukuoka T, Sakagami M, Noguchi K (2007) Activation of extracellular signal-regulated protein kinases 5 in primary afferent neurons contributes to heat and cold hyperalgesia after inflammation. J Neurochem 102(5):1614–1624. doi:10.1111/j.1471-4159.2007.04698.x
52. Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat Neurosci 4(10):981–988. doi:10.1038/nn720
53. Wu Y, Zuo Y, Chakrabarti R, Feng B, Chen S, Chakrabarti S (2010) ERK5 contributes to VEGF alteration in diabetic retinopathy. J Ophthalmol 2010:465824. doi:10.1155/2010/465824
54. Wu Y, Feng B, Chen S, Chakrabarti S (2012) ERK5 regulates glucose-induced increased fibronectin production in the endothelial cells and in the retina in diabetes. Invest Ophthalmol Vis Sci 53(13):8405–8413. doi:10.1167/iovs.12-10553
55. Guo Y, Johnson E, Cepurna W, Jia L, Dyck J, Morrison JC (2009) Does elevated intraocular pressure reduce retinal TRKB-mediated survival signaling in experimental glaucoma? Exp Eye Res 89(6):921–933. doi:10.1016/j.exer.2009.08.003
56. Coffer PJ, Jin J, Woodgett JR (1998) Protein kinase B (c-Akt): a multifunctional mediator of phosphatidylinositol 3-kinase activation. Biochem J 335(Pt 1):1–13
57. Marte BM, Downward J (1997) PKB/Akt: connecting phosphoinositide 3-kinase to cell survival and beyond. Trends Biochem Sci 22(9):355–358
58. Franke TF, Kaplan DR, Cantley LC, Toker A (1997) Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science 275(5300):665–668
59. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB, Cohen P (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Current Biol 7(4):261–269
60. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307(5712):1098–1101. doi:10.1126/science.1106148
61. Hresko RC, Mueckler M (2005) mTOR.RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. J Biol Chem 280(49):40406–40416. doi:10.1074/jbc.M508361200
62. Cantley LC, Neel BG (1999) New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci U S A 96(8):4240–4245
63. McCubrey JA, Steelman LS, Kempf CR, Chappell WH, Abrams SL, Stivala F, Malaponte G, Nicoletti F, Libra M, Basecke J, Maksimovic-Ivanic D, Mijatovic S, Montalto G, Cervello M, Cocco L, Martelli AM (2011) Therapeutic resistance resulting from mutations in Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR signaling pathways. J Cell Physiol 226(11):2762–2781. doi:10.1002/jcp.22647
64. Sable CL, Filippa N, Hemmings B, Van Obberghen E (1997) cAMP stimulates protein kinase B in a Wortmannin-insensitive manner. FEBS Lett 409(2):253–257
65. Konishi H, Matsuzaki H, Tanaka M, Ono Y, Tokunaga C, Kuroda S, Kikkawa U (1996) Activation of RAC-protein kinase by heat shock and hyperosmolarity stress through a pathway independent of phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A 93(15):7639–7643
66. Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ (2001) Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292(5522):1728–1731. doi:10.1126/science.292.5522.1728

67. Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ (2001) Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276(42):38349–38352. doi:10.1074/jbc.C100462200
68. Chen WS, Xu PZ, Gottlob K, Chen ML, Sokol K, Shiyanova T, Roninson I, Weng W, Suzuki R, Tobe K, Kadowaki T, Hay N (2001) Growth retardation and increased apoptosis in mice with homozygous disruption of the Akt1 gene. Genes Dev 15(17):2203–2208. doi:10.1101/gad.913901
69. Peng XD, Xu PZ, Chen ML, Hahn-Windgassen A, Skeen J, Jacobs J, Sundararajan D, Chen WS, Crawford SE, Coleman KG, Hay N (2003) Dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development, and impeded adipogenesis in mice lacking Akt1 and Akt2. Genes Dev 17(11):1352–1365. doi:10.1101/gad.1089403
70. Tschopp O, Yang ZZ, Brodbeck D, Dummler BA, Hemmings-Mieszczak M, Watanabe T, Michaelis T, Frahm J, Hemmings BA (2005) Essential role of protein kinase B gamma (PKB gamma/Akt3) in postnatal brain development but not in glucose homeostasis. Development 132(13):2943–2954. doi:10.1242/dev.01864
71. Yang ZZ, Tschopp O, Di-Poi N, Bruder E, Baudry A, Dummler B, Wahli W, Hemmings BA (2005) Dosage-dependent effects of Akt1/protein kinase Balpha (PKBalpha) and Akt3/PKBgamma on thymus, skin, and cardiovascular and nervous system development in mice. Mol Cell Biol 25(23):10407–10418. doi:10.1128/MCB.25.23.10407-10418.2005
72. Whiteman EL, Cho H, Birnbaum MJ (2002) Role of Akt/protein kinase B in metabolism. Trends Endocrinol Metab 13(10):444–451
73. Radisavljevic Z (2013) AKT as locus of cancer angiogenic robustness and fragility. J Cell Physiol 228(1):21–24. doi:10.1002/jcp.24115
74. Liang J, Slingerland JM (2003) Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. Cell Cycle 2(4):339–345
75. Plyte SE, Hughes K, Nikolakaki E, Pulverer BJ, Woodgett JR (1992) Glycogen synthase kinase-3: functions in oncogenesis and development. Biochim Biophys Acta 1114 (2–3):147–162
76. Wu D, Pan W (2010) GSK3: a multifaceted kinase in Wnt signaling. Trends Biochem Sci 35 (3):161–168. doi:10.1016/j.tibs.2009.10.002
77. Wada A (2009) Lithium and neuropsychiatric therapeutics: neuroplasticity via glycogen synthase kinase-3beta, beta-catenin, and neurotrophin cascades. J Pharmacol Sci 110(1):14–28
78. Endo H, Nito C, Kamada H, Nishi T, Chan PH (2006) Activation of the Akt/GSK3beta signaling pathway mediates survival of vulnerable hippocampal neurons after transient global cerebral ischemia in rats. J Cereb Blood Flow Metab 26(12):1479–1489. doi:10.1038/sj.jcbfm.9600303
79. Tang Z, Arjunan P, Lee C, Li Y, Kumar A, Hou X, Wang B, Wardega P, Zhang F, Dong L, Zhang Y, Zhang SZ, Ding H, Fariss RN, Becker KG, Lennartsson J, Nagai N, Cao Y, Li X (2010) Survival effect of PDGF-CC rescues neurons from apoptosis in both brain and retina by regulating GSK3beta phosphorylation. J Exp Med 207(4):867–880. doi:10.1084/jem.20091704
80. Puckett MC, Goldman EH, Cockrell LM, Huang B, Kasinski AL, Du Y, Wang CY, Lin A, Ichijo H, Khuri F, Fu H (2013) Integration of apoptosis signal-regulating kinase 1-mediated stress signaling with the Akt/protein kinase B-IkappaB kinase cascade. Mol Cell Biol 33 (11):2252–2259. doi:10.1128/MCB.00047-13
81. Alikhani M, Roy S, Graves DT (2010) FOXO1 plays an essential role in apoptosis of retinal pericytes. Mol Vis 16:408–415
82. Jomary C, Cullen J, Jones SE (2006) Inactivation of the Akt survival pathway during photoreceptor apoptosis in the retinal degeneration mouse. Invest Ophthalmol Vis Sci 47 (4):1620–1629. doi:10.1167/iovs.05-1176
83. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 91 (2):231–241

84. Koriyama Y, Homma K, Sugitani K, Higuchi Y, Matsukawa T, Murayama D, Kato S (2007) Upregulation of IGF-I in the goldfish retinal ganglion cells during the early stage of optic nerve regeneration. Neurochem Int 50(5):749–756. doi:10.1016/j.neuint.2007.01.012
85. Homma K, Koriyama Y, Mawatari K, Higuchi Y, Kosaka J, Kato S (2007) Early downregulation of IGF-I decides the fate of rat retinal ganglion cells after optic nerve injury. Neurochem Int 50(5):741–748. doi:10.1016/j.neuint.2007.01.011
86. Kim HS, Park CK (2005) Retinal ganglion cell death is delayed by activation of retinal intrinsic cell survival program. Brain Res 1057(1–2):17–28. doi:10.1016/j.brainres.2005.07.005
87. Shen J, Wu Y, Xu JY, Zhang J, Sinclair SH, Yanoff M, Xu G, Li W, Xu GT (2010) ERK- and Akt-dependent neuroprotection by erythropoietin (EPO) against glyoxal-AGEs via modulation of Bcl-xL, Bax, and BAD. Invest Ophthalmol Vis Sci 51(1):35–46. doi:10.1167/iovs.09-3544
88. Brown GC (2010) Nitric oxide and neuronal death. Nitric Oxide 23(3):153–165. doi:10.1016/j.niox.2010.06.001
89. Mejia-Garcia TA, Portugal CC, Encarnacao TG, Prado MA, Paes-de-Carvalho R (2013) Nitric oxide regulates AKT phosphorylation and nuclear translocation in cultured retinal cells. Cell Signal 25(12):2424–2439. doi:10.1016/j.cellsig.2013.08.001

Chapter 4
Antioxidative Treatment for Neuroprotection in Glaucoma

Kenya Yuki

Abstract Glaucoma is a neurodegenerative disease with progressive retinal ganglion cell apoptosis. IOP elevation is a modifiable proven risk factor for glaucoma. Although a lot of new medicines and surgeries for lowering IOP have come in, glaucoma is still a leading cause of blindness in the world, motivating us to search IOP-independent treatment. Accumulating evidences point to an association between oxidative stress and glaucoma. Increased level of oxidative DNA damage, lipid peroxidation, and protein oxidation and reduced antioxidative status were observed in serum, trabecular meshwork, and aqueous humor of human glaucoma subjects. Antioxidants protect retinal ganglion cell apoptosis from IOP elevation, optic nerve injury, inflammation, and impaired ocular blood flow in vivo and in vitro. Some population-based epidemiological studies showed that higher antioxidant intake reduced risk of glaucoma. This review focused on the present evidence that support the possibility of antioxidant neuroprotective treatment for glaucoma.

Keywords Antioxidant • Glaucoma • Mitochondria • Oxidative stress • Reactive oxygen species

4.1 Introduction

Glaucoma is the second leading cause of blindness in the world, affecting about five million people [1]. Glaucomatous optic neuropathy is a disease with progressive retinal ganglion cell (RGC) loss and nerve fiber layer defect accompanied by typical visual field defects. Glaucoma is significantly associated with reduced

K. Yuki (✉)
Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjyuku-ku, Tokyo 160-8582, Japan
e-mail: yuuki@a3.keio.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_4, © Springer Japan 2014

patients' quality of life and affects public health [2, 3]; thus finding a new treatment of glaucoma is a critical issue in a society. Although elevated IOP is a proven risk factor for glaucoma, the etiology of glaucoma is still unknown and it is thought to be multifactorial [4]. Proposed mechanisms of glaucomatous optic neuropathy are IOP elevation, optic nerve axonal injury, neurotrophic factor depletion, impaired ocular blood flow, TNF-α neurotoxicity, glutamate neurotoxicity, inflammation, and oxidative stress [5].

Oxidative stress is a condition that reactive oxygen species (ROS) exist in cell or extracellular space without being metabolized by antioxidative mechanism [6]. ROS is usually generated in electron transport chain in mitochondria. Superoxide anion (O_2^-) and hydroxyl peroxide (H_2O_2) are a major type of ROS. Under normal condition, superoxide anion is generated as a by-product of mitochondrial respiration mainly by complexes I and III [7, 8].

Superoxide anion is reduced by superoxide dismutase (SOD) to less reactive hydrogen peroxide. Generated hydrogen peroxide is reduced by catalase or glutathione peroxidase (Gpx) to nonreactive water. ROS has an important role in cell signaling and cell homeostasis [9]. However, excessive ROS which was not metabolized by antioxidative enzyme or substances results in oxidative insult to protein, lipid, and DNA. DNA oxidation [10], protein oxidation [11], and lipid oxidation [12] are associated with neurodegenerative diseases such as Alzheimer's disease [13] and Parkinson's disease [14, 15]. Oxidative DNA damage causes DNA single- and double-strand breaks, base modifications, and abasic sites. Guanine is the most easily oxidized purine in DNA. After oxidation, guanine becomes 8-hydroxy-deoxyguanosine (8-OHdG) [16]. Oxidized guanine can pair with cytosine and with adenine. The latter yields transversion, which is mutagenic and apoptotic for cell [17]. Oxidative modification of protein induces protein carbonylation, structural changes in a protein, protein misfolding, and protein aggregation that might lead to its functional impairment. Increase of protein carbonyl content in creatinine kinase was observed in brain with Alzheimer's disease [18]. Lipid oxidation is also associated with neuronal cell apoptosis [12]. Cell membrane is composed of lipid. Lipid peroxidation in neuronal cell membrane increases cell rigidity, decreased membrane binding enzyme activity, and altered permeability, resulting in neuronal apoptosis [19].

4.2 Evidences of the Association Between Oxidative Stress and Human Glaucoma

Accumulating evidences point out to the association between oxidative stress and primary open-angle glaucoma (POAG) (Table 4.1). In association with oxidative DNA damage and glaucoma, increased 8-OHdG level was observed in serum [20], aqueous humor [20], and trabecular meshwork [21, 22]. 8-OHdG is one of the most reliable DNA oxidation markers due to its stability [23]. Studies found that levels of 8-OHdG in trabecular meshwork cells were significantly higher in glaucoma

Table 4.1 Current evidence implicating systemic and ocular oxidative stress involvement in the pathophysiology of POAG and NTG

	Serum	Urine	Erythrocyte	Aqueous humor	Trabecular meshwork
POAG					
Oxidative damage	TOS↑ [26]				
Lipid peroxidation	MDA↑ [26, 28]			MDA↑ [28] MDA↑ [27]	
DNA oxidation	8-OHdG↑ [20]			8-OHdG↑ [20]	8-OHdG↑ [21, 22]
Antioxidant capacity and enzyme	TAS↓ [20] BAP↓ [31] TAC↓ [26, 28] SOD ↑ [26]		SOD↓ [24] Catalase↓ [24] Gpx↓ [24]	TRAP↓ [30] TAS↓ [20, 27] TAC↓ [28] SOD↓ [33] SOD↑ [30] GST↓ [33] Gpx↑ [30]	
NTG					
DNA damage		8-OHdG↓ [41]			
Antioxidant capacity and enzyme	SOD1↓ [135] TAS↑ [41]				

POAG primary open-angle glaucoma, *NTG* normal-tension glaucoma, *TOS* total oxidant status, *TAC* total antioxidant capacity, *SOD* superoxide dismutase, *MDA* malondialdehyde, *TAS* total antioxidant status, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *BAP* biological antioxidant potential, *TRAP* total reactive antioxidant potential, *Gpx* glutathione peroxidase, *GST* glutathione S-transferase, *TAS* total antioxidant status

patients than in controls [21]. Oxidative DNA damage in patients with glaucoma correlated significantly with IOP and visual field defects in subjects with glaucoma [21, 22]. Another study found that aqueous and serum levels of 8-OHdG were higher in glaucoma patients than in cataract group [20]. In another study, higher oxidative DNA damage after lymphocyte incubation with hydrogen peroxide was observed in glaucoma patients than in that of healthy controls [24]. In lipid peroxidation, malondialdehyde (MDA), a lipid peroxidation marker [25], level was significantly higher in serum [26] and aqueous humor [27, 26] of glaucoma patients than that of controls. Nucci et al. compared MDA level in blood and aqueous humor between that of 40 patients with POAG scheduled for cataract surgery and 26 patients of age-matched subjects scheduled for cataract surgery and found that blood and aqueous humor MDA levels in POAG patients were significantly increased over those of the control group [28]. Serum levels of total oxidant status (TOS) [29] of patients with POAG were reported to be significantly higher in POAG compared with controls [26].

In association with antioxidant status with POAG, total antioxidant status (TAS) is reported to be less in serum [20, 26, 28, 31] and aqueous humor [20, 27, 28, 30] in glaucoma patients than that of controls. Tanito et al. examined serum level of

antioxidant capacity by biological antioxidant potential (BAP) test [32] in subjects with POAG and controls and found that BAP level was significantly lower in the POAG group than in the control group [31]. Bagnis et al. compared cytosolic SOD (SOD1), mitochondrial SOD (SOD2), and glutathione S-transferase 1 (GST1) level in the aqueous humor of POAG subjects and controls. GST1 is an enzyme which activates thiol group of glutathione, enabling antioxidative function on the substrate. Levels of SOD1, SOD2, and GST1 were significantly lower in POAG patients than in the control group [33]. A significant decrease of antioxidant enzymes, catalase, SOD, and Gpx level in erythrocyte is observed in glaucoma patients compared with controls [24]. On the other hand, an increase of SOD activity and glutathione activity was observed in glaucoma patients compared with cataract subjects in another study [30].

These studies suggest that subjects with POAG were topically and systemically exposed to higher ROS compared with controls and may have susceptibility to ROS due to low level of antioxidant status. Systemic accumulation of oxidative DNA damage, lipid peroxidation, and reduced systemic antioxidant status were observed in Alzheimer's disease and Parkinson's disease [34–39]. Alzheimer's disease, Parkinson's disease, and glaucoma are thought to share a common mechanism of neuronal loss [40]. Human studies in POAG suggest us that reduced systemic and ocular antioxidant status increased ROS level in the retina, cause oxidative DNA damage, and lipid peroxidation in RGC, may result in glaucoma.

There is still not enough study evaluating the association of oxidative stress in normal-tension glaucoma (NTG). Serum levels of TAS and urinary 8-OHdG level in patients with NTG were reported. In this study, serum level of TAS is significantly higher in NTG patients compared with controls. Furthermore, lower levels of urinary 8-OHdG were observed in NTG patients [41]. Although this result is incompatible with the result of POAG studies and the reason for discrepancy is unknown, this study suggests us that systemic oxidative stress including systemic DNA damage is associated with NTG pathomechanism. On the other hand, higher systemic oxidative DNA damage is reported to be associated with progression of NTG [42]. The authors examined urinary 8-OHdG level at baseline and evaluated visual field progression of 40 consecutive NTG subjects for 5 years in a retrospective manner. Seventeen subjects showed progression and twenty-three subjects showed no progression [42]. No significant difference was observed in untreated IOP, treated IOP, systemic disease status, and age between the two groups. Urinary 8-OHdG level was significantly higher in the progressive group than in the nonprogressive group (Fig. 4.1). This result suggests that higher level of systemic oxidative DNA damage is associated with IOP-independent glaucomatous visual field progression.

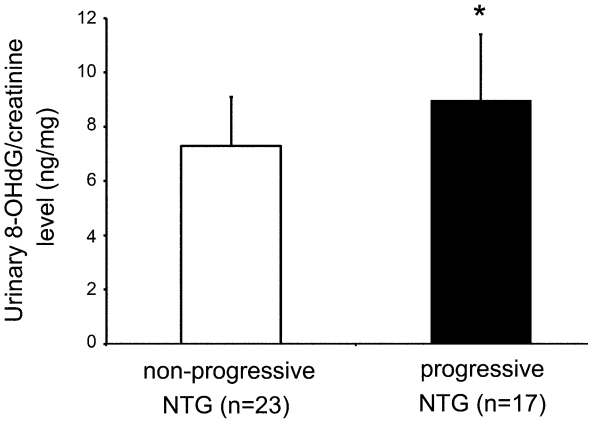


Fig. 4.1 Urinary 8-OHdG/creatinine level was significantly higher in subjects with progressive NTG group compared with nonprogressive NTG group. No significant difference was observed in age, male/female ratio, systolic and diastolic blood pressure, central corneal thickness, mean deviation, untreated IOP, and treated IOP between the two groups. *$p = 0.02$ unpaired *t*-test

4.3 Antioxidative Neuroprotective Treatment Effect in Experimental Glaucoma

4.3.1 Antioxidative Treatment for IOP Elevation-Induced RGC Apoptosis

The mechanism of IOP elevation-induced RGC apoptosis is still unclear. However, oxidative insult is thought to be involved in an apoptotic pathway of IOP-induced RGC loss.

Episcleral vein cauterization increased IOP to 25 mmHg after cauterization [43]. After 30 days of IOP elevation, increased chemiluminescence, which indicates generation of ROS [44], was observed in the retina. The level of total antioxidant capacity in aqueous humor and vitreous humor significantly reduces, and thiobarbituric acid reactive substances (TBARS) [45], level of lipid peroxidation, significantly increased after IOP elevation. This study showed that IOP elevation to 25 mmHg causes antioxidant enzyme reduction in aqueous humor and vitreous humor and increases lipid peroxidation and ROS generations in the retina. In another study using episcleral vein cauterization model, lucigenin-dependent chemiluminescence [46] and nitroblue tetrazolium (NBT) [47] were used as a probe for superoxide anion. A number of NBT-positive and lucigenin-dependent chemiluminescence-positive RGCs, MDA level, SOD1, SOD2, and catalase activity in the retina increased after IOP elevation [48]. These results

showed that IOP elevation causes superoxide anion production in RGC and lipid peroxidation in the retina. In human studies, Ferreira et al. reported higher level of SOD and Gpx in aqueous humor of POAG patients compared with controls [30]. Increase of antioxidant enzyme due to IOP elevation may be due to compensatory antioxidant mechanism against oxidative stress.

Another experimental glaucoma model showed compatible results. In a glaucoma model by anterior chamber injection of hyaluronic acid, IOP elevated to 25 mmHg 1 week after injection. In this model, level of MDA significantly increased and level of glutathione, SOD, and catalase significantly decreased in the retina [49, 50]. In a study using micro-needle-induced acute IOP elevation to 30 mmHg or 60 mmHg for 2 h, level of 4-hydroxy-2-nonenal (4-HNE) [51], a lipid oxidation marker, and hemoxygenase-1 (HO-1), an inducible antioxidative enzyme against oxidative stress, in the retina significantly increased [52]. Another study showed protein oxidation in RGCs of rat with ocular hypertension [53]. Multiple models of IOP elevation in mice and rat showed increased level of DNA damage, lipid oxidation, and protein oxidation and reduced level of antioxidant status in the retina. These results strongly support the results of human study that subjects with POAG were topically exposed to excessive ROS due to IOP elevation.

However, we cannot conclude oxidative insult as a signal of apoptosis or just a by-product of RGC apoptosis from these studies. Inman et al. answered this question using DBA2/J mice. DBA2/J mice are a glaucoma model with chronic IOP elevation and progressive RGC loss [54]. The authors investigated oxidative insult in DBA2/J mice [55]. In DBA2/J mice with IOP elevation, level of MDA, glial fibrillary acidic protein, HO-1, and receptor of advanced glycation end product in the retina was significantly higher in DBA2/J mice compared with C57 mice. Level of 8-OHdG and 8-hydroxyguanosine, DNA oxidized marker, is observed significantly higher in ganglion cell layer of DBA2/J mice compared with C57. Authors delivered α-lipoic acid (ALA), an antioxidant substance [56], and evaluated whether it alters glaucoma. ALA treatment did not reduce IOP elevation in DBA2/J mice. However, the retina from ALA-treated mice showed significantly lower level of MDA and 4-hydroxyalkenal. The DBA2/J retina without ALA had half the number of RGC cells compared with the ALA-treated DBA2/J mice retina. These results suggest that antioxidant treatment, ALA intake, protected RGC, not by reduction of IOP, but by enhancing intrinsic antioxidant pathway. ROS generated by IOP elevation in DBA2/J mice is not a by-product of RGC death, but a key player of IOP-induced RGC apoptosis.

High IOP increases lucigenin-dependent chemiluminescence and NBT-positive cell in the retina [48], suggesting that superoxide anion was produced in the retina by IOP elevation. How does IOP generate superoxide anion? Mitochondria are thought to be the main source of superoxide anion. The major role of mitochondria is to produce ATP using electron transport chain. Superoxide anions were naturally produced in mitochondria while electron was transported. RGC has a lot of mitochondria for anterograde and retrograde axonal transport. IOP elevation reduces the number of mitochondria with normal membrane potential in the optic nerve head [57]. In DBA2/J mice with IOP elevation, mitochondrial fission, matrix swelling,

substantially reduced cristae volume, and abnormal cristae depletion were observed by electron microscopy in glaucomatous optic nerve head axons [58]. Superoxide anion is basically non-membrane permeable [59, 60]. While the mitochondrial membrane is intact, it exits in mitochondria and may not harm cells. However, mitochondrial membrane was once damaged by IOP, superoxide anion goes out to cytoplasm, and damage DNA, may result in RGC apoptosis. Superoxide anion leaking from mitochondria also damages mitochondrial membrane and mitochondrial DNA. Molecules in mitochondria are thought to be susceptible to ROS due to its closeness to the ROS production cite and lack of DNA protecting histone in mitochondrial DNA. ROS leaking from mitochondria attacks mitochondrial membrane and mitochondrial DNA, which results in more mitochondrial dysfunction, causing a vicious cycle.

4.3.2 Antioxidant Treatment Effect for Optic Nerve Crush-Induced RGC Apoptosis

Recent studies using optical coherence tomography showed that IOP elevation dynamically deforms lamina cribrosa in the human eye [61]. Lamina cribrosa deformation plays an important role in glaucoma pathogenesis by inducing loss of retrograde transport of neurotrophic factors such as brain-derived neurotrophic factors at the optic nerve head [62]. Optic nerve crush model and optic nerve transaction model are is thought to be a glaucoma model of loss of retrograde transport of neurotrophic factors.

Superoxide anion is thought to be an early signal of apoptosis of RGC after optic nerve crush. Optic nerve transaction increases superoxide production measured by dihydroethidium. Superoxide anion level in RGC increased within 24 h after optic nerve transaction, peaking at 4 days. Intravitreal injection of polyethylene glycol–SOD after optic nerve transaction significantly increases level of SOD and reduces TUNEL-positive cells in RGC [63]. Higher MitoSox™, red superoxide anion indicator, level was observed in RGCs after optic nerve transaction, which means that superoxide anion production occurs at mitochondria [64]. Another study found that overexpression of thioredoxin-1 or thioredoxin-2, a 12 kD oxidoreductase enzyme which facilitates reduction of other proteins by cysteine thiol–disulfide exchange, by plasmid intravitreal injection protects RGC from optic nerve transaction [65]. These results showed that superoxide anion leaking from mitochondria is the early events of apoptotic pathway after optic nerve transaction and suggests possible antioxidative treatment for glaucoma by reducing superoxide anion.

Himori et al. evaluated role of NF-E2-related factor 2 (NRF2) in optic nerve crush using Nrf2-deficient mice [66]. Nrf2 is a key transcriptional factor and an antioxidant pathway that plays a major role in endogenous protection against the cytotoxic effect of oxidative stress [67]. Nrf2 is kept in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and Cullin 3 which degrade Nrf2 by

ubiquitination. Under normal condition, Nrf2 is ubiquitinated and degraded quickly. Exposed to oxidative stress, Nrf2 is not ubiquitinated, builds up in the cytoplasm, translocates into nucleus, binds to the antioxidant response element in the upstream promoter region of many antioxidative genes, and initiates their transcription. Nrf2 levels in nucleus significantly increased and mRNA level of antioxidant enzymes like HO-1 also significantly increased in wild-type mice 1 day after optic nerve crush. In Nrf2-deficient mice, significant increase of 8-OHdG-positive cells, 4-HNE-positive cells, and level of TBARS was observed in RGC compared with wild type after optic nerve crush. The number of RGC had significantly decreased in Nrf2-deficient mice compared to wild-type mice. 1-(2-cyano-3,12-dioxooleana-1,9 (11)-dien-28-oyl)imidazole (CDDO-Im) is an Nrf2 activator. CDDO-Im treatment induced expression of antioxidative genes such as *Nqo1*, *Ho-1*, *Gclm*, *Gclc*, *Gsta4*, and *Txnrd* in WT mice, but not in Nrf2-deficient mice, indicating that these responses are Nrf2 dependent. CDDO-Im treatment significantly inhibited RGC loss compared with vehicle treatment 7 days after optic nerve crush in WT mice. This protective effect is not observed in Nrf2-deficient mice. Nrf2 is a possible target for antioxidative neuroprotective glaucoma treatment. Further analysis is warranted.

4.3.3 Antioxidant Treatment for Impaired Ocular Blood Flow-Induced RGC Apoptosis

A number of evidence suggest the association between impaired ocular blood flow and glaucoma [68, 69]. In the Barbados Eye Study, 10 mmHg lower systolic blood pressure increased 9 % higher risk of incident POAG [70]. In the Proyecto VER study, subjects with diastolic perfusion pressure less than 45 mmHg had three times more likely to have POAG compared with subjects with more than 65 mmHg [71]. In Early Manifest Glaucoma Trial, lower systolic perfusion pressure less than 125 mmHg had 40 % higher risk of glaucoma progression [72]. Impaired ocular blood flow and its correlation with visual field defect were observed in subjects with glaucoma [73, 74] and low diastolic blood pressure is associated with progression of NTG under IOP-lowering treatment [75]. The exact mechanism that impaired blood flow causes glaucomatous optic neuropathy is still unknown, but tissue hypoxia is thought to be an associated mechanism with glaucoma and impaired ocular blood flow. Hypoxia-inducible factor 1 alpha (HIF1-α) was observed in human glaucomatous optic nerve head and retina which suggests that glaucomatous optic nerve head and retina were exposed to hypoxic insult [76].

Ischemia/reperfusion injury to the retina is thought to be a mice model of hypoxic retinal insult. Transient increase of IOP causes retinal ischemia, caspase activation, increased number of TUNEL-positive cells in the inner nuclear layer and RGC layer, and inner retinal degeneration including RGC loss [77]. Superoxide

anion generation was observed in the retina after ischemia/reperfusion injury [78] and was prevented by mitochondrial uncoupler in hippocampal neuron [79], which means superoxide anion was produced in mitochondria after ischemia/reperfusion injury. Successful antioxidant therapy for ischemia/reperfusion injury was reported [80–83]. Intravitreal injection of AAV-SOD2 significantly reduces superoxide anion level, MDA level, and 8-OHdG level in the retina after ischemia/reperfusion injury and protected RGC loss from apoptosis [84]. AAV-catalase also protected inner neuron from ischemia/reperfusion injury [85].

Oharazawa et al. conducted clinically applicable important examination. The authors used H_2-loaded eye drops. H_2-loaded eye drops were prepared by dissolving H_2 gas into a saline to saturated level and administered to ocular surface. After H_2-loaded eye drops were administered, H_2 concentration in vitreous immediately increased and superoxide anion level decreased after ischemia/reperfusion injury. H_2-loaded eye drops reduced the number of 4-HNE-positive cells, 8-OHdG-positive cells, and TUNEL-positive cells in the retina and prevented retinal thinning [86]. H_2 gas was reported to protect brain injury by ischemia/reperfusion injury and by traumatic brain injury via suppressing hydroxyl peroxide, which is most cytotoxic ROS, in vivo and protect neuronal cells against oxidative injury in vitro [87–89]. Inhalation of H_2 gas selectively reduced hydroxyl peroxide, but did not affect level of H_2O_2, oxidized form of NAD^+, oxidized form of FAD, or oxidized form of cytochrome c, which suggests that H_2 gas does not affect the normal metabolism involved in oxidation–reduction reaction's essential role in signal transduction. Irrigation of the cornea by H_2-enriched solution significantly reduced angiogenesis after alkali burn injury by reduction of ROS [90]. H_2 gas protects from cataract [91] and blue light-induced retinal damage [92] in rat. Inhalation of 3 % H_2 gas delivers enough H_2 in blood in human. Preliminary study for treatment of brain stem infarction [93], acute erythematous skin disease [94], and acute cerebral ischemia [95] showed that H_2 gas inhalation suppressed brain damage and erythema symptoms without compromising safety. Hydrogen is the lightest element; therefore, H_2 gas is highly permeable to cell membrane. H_2 gas would reach RGC by topical application of H_2-enriched water to ocular surface. H_2 gas effect should be evaluated in another animal model of glaucoma in the future.

Another clinically applicable interesting study is also reported. Caloric restriction is a dietary regimen recognized to delay aging and extend life span in various kind of organisms including *C. elegans*, *F. pyramitela*, and *D. melanogaster*, mice, and monkey [96]. Caloric restriction reduces lipid peroxidation, protein carbonylation, and nitration in neuronal cells in vivo and in vitro [97]. Caloric restriction also slowed the age-dependent protein insolubilization and increased antioxidant substances, like glutathione and ascorbic acid levels in neural retina [98]. Outer nuclear layer cell densities and retinal thickness were significantly greater in the caloric restricted mice versus ad libitum diet group in 12, 24, and 30 months of age [99]. After IOP elevation-induced ischemic injury, caloric restriction for 24 h every other day for 6 months significantly reduced HO-1 level, HNE level, and age-dependent decline in mitochondrial oxidative phosphorylation enzyme activity and prevented RGC functional loss [100]. These results suggest that caloric

restriction improves age-related mitochondrial function, reduces lipid peroxidation, and improves RGC function. Caloric restriction may be an alternative antioxidative treatment for glaucoma.

4.3.4 Antioxidant Treatment for Glutamate Neurotoxicity-Induced RGC Apoptosis

Glutamate is an excitatory neurotransmitter in the retina. Excessive glutamate exposure to the retina results in neuronal apoptosis [101] and is called excitotoxicity. Glutamate-induced excitotoxicity is thought to be associated with the pathomechanism of Alzheimer's disease [102], Parkinson's disease [103], Huntington disease [104], and glaucoma [105, 106]. N-methyl-D-aspartate receptor is a glutamate receptor, which plays a predominant role in excitotoxicity in the retina. NMDA is a selective agonist which binds to NMDA receptor. Excessive glutamate overactivates NMDA receptor, and a nonspecific cation channel opens that allows the passage of Ca^{2+} into neuron. Excessive Ca^{2+} influx into neuron destabilizes mitochondrial membrane, release of cytochrome c, release of superoxide anion, and apoptotic neuronal death. IOP elevation in rat causes reduction of glutamate transporter 1 (GLT1) and glutamate aspartate transporter (GLAST) in the retina [107, 108]. GLAST-deficient mice showed RGC loss without IOP elevation, which is suppressed by an NMDA receptor antagonist, memantine, suggesting the association of glutamate neurotoxicity and pathogenesis of NTG [106].

ROS is involved in excitotoxicity. The application of NMDA to cerebellar granular cell in vitro produces superoxide anion, resulting in cell death [109] and SOD attenuates NMDA-induced rat cortical neuronal death in vitro [110]. NMDA induced superoxide anion generation [111]. SOD may play an important role in protecting the retina against glutamate neurotoxicity. In cultured cortical neuron, overexpression of SOD1 prevents neuronal cell loss from NMDA-induced neurotoxicity [112, 113]. NMDA intravitreally injected SOD1-deficient mice showed significantly higher number of TUNEL-positive cells in RGC layer and inner nuclear layer compared with NMDA-injected wild-type mice [114]. The amplitude of a and b wave of dark-adapted full-field single-flash electroretinogram (ERG) was significantly lower in NMDA-injected SOD1-deficient mice compared with NMDA-injected wild-type mice. The number of 8-OHdG-positive cells and cells with DNA double-strand break was significantly higher in RGC layer in NMDA-injected SOD1-deficient mice. Level of ROS measured by dihydroethidium in the retina is significantly higher in NMDA-injected SOD1-deficient mice. SOD1 is an enzyme which reduces superoxide anion to hydroxyl peroxide. This study showed that NMDA-induced superoxide anion production is a cause of RGC apoptosis via DNA oxidation damage, and lack of SOD1 ameliorates NMDA neurotoxicity.

Numerous reports showed that antioxidative substance protects RGC from NMDA-induced neurotoxicity in vivo and in vitro [115–123]. Edaravone is a

novel free radical scavenger, which is approved for clinical use in management of acute ischemic stroke. In the Cochrane Database of Systematic Reviews, edaravone treatment increases twofold the number of subjects who improved neurological impairment compared with controls after acute ischemic stroke [124]. Intravitreal injection of edaravone treatment reduced number of TUNEL-positive cells in RGC layer after NMDA intravitreal injection. It also reduced the number of 8-OHdG-positive cells and 4-HNE-positive cells [119]. Intravitreal injection of submicron-sized liposomes containing edaravone also significantly reduced the number of TUNEL-positive RGC cell after NMDA injection [115]. Edaravone-loaded liposome eye drops protect the retina against light-induced damage, suggesting its possibility of clinical usage [125]. Edaravone has been already widely clinically used for acute stroke. It may be one of the promising candidates for glaucoma antioxidant treatment.

4.3.5 Antioxidant Treatment for TNF-α Neurotoxicity-Induced RGC Apoptosis

TNF-α is a pro-inflammatory cytokine that stimulates acute phase of inflammation with multiple functions [126]. TNF-α is produced by activated microglia in the retina after IOP elevation and is thought to be associated with RGC apoptosis by binding to the TNF receptor [127]. TNF-α and TNFR1 in the retina and optic nerve head are upregulated in RGCs and their axons in human glaucoma [128]. TNF-α level also increased in aqueous humor of POAG subjects [129]. TNF-α-deficient mice and TNF-receptor 2-deficient mice showed significantly less RGC loss after IOP elevation [130]. Etanercept, a widely used TNF-α inhibitor, prevents high IOP-induced RGC loss and optic nerve degeneration [131]. These results suggest that TNF-α neurotoxicity is highly involved in glaucoma pathophysiology.

TNF-α-induced RGC apoptosis is reported to be associated with oxidative stress. TNF-α administration to primary culture of rat RGC increases number of Annexin V + /PI + cells with increase of ROS [132]. TNF-α administration induces loss of mitochondrial membrane potential and release of AIF. Although caspase inhibitor treatment temporally decreases rate of apoptosis of RGC, the effect is not enough when mitochondrial membrane has already lost their potential. Combination with caspase inhibitor and tempol, a ROS scavenger, provides additional 20 % survival in RGC [132]. These results suggest that ROS produced in mitochondria is a cause of TNF-α-induced RGC apoptosis. Peroxiredoxin 6 (Prdx6) is a ubiquitous antioxidant enzyme. Peroxiredoxin metabolizes hydrogen peroxide using reduced thioredoxin. TNF-α decreased the level of Prdx6 and increased the level of ROS in RGC-5, resulting in cell death. Prdx6 delivery protected RGC loss by TNF-α by limiting ROS level [133]. Thioredoxin 1 was significantly decreased in the optic nerve and RGC after TNF-α injection. 17β-E2 significantly prevented TNF-α-induced axonal loss, and this axonal protective effect was inhibited by

intravitreal injection of thioredoxin 1 siRNA [134]. These results suggest that TNF-α impairs mitochondrial membrane potential, which results in superoxide anion release, thus causing axonal loss and RGC apoptosis.

4.4 Superoxide Anion and Glaucomatous Optic Neuropathy

IOP elevation, optic nerve crush, TNF-α neurotoxicity, NMDA neurotoxicity, and impaired ocular blood flow cause ROS generation, mainly superoxide anion via mitochondrial membrane dysfunction (Fig. 4.2). SOD is an antioxidative enzyme which metabolizes superoxide anion to hydroxyl peroxide. SOD1 exists in cytosol, SOD2 in mitochondria, and SOD 3 in extracellular space. SOD1 is ubiquitously expressed in the retina but mainly in RGC, inner plexiform layer, and inner nuclear layer in the mice retina which suggest that SOD1 plays an important role for reducing superoxide anion in the inner retina [135]. The number of RGC showed no significant change between SOD1-deficient mice and wild-type mice in young age. However, significant decrease was observed in SOD1-deficient mice in the number of RGC compared with wild-type mice in 24 weeks of age. RGC function measured by pattern ERG was also decreased. The amplitude of pattern ERG is correlated with RGC function [136, 137]. Significant increase of ROS measured by dihydroethidium was

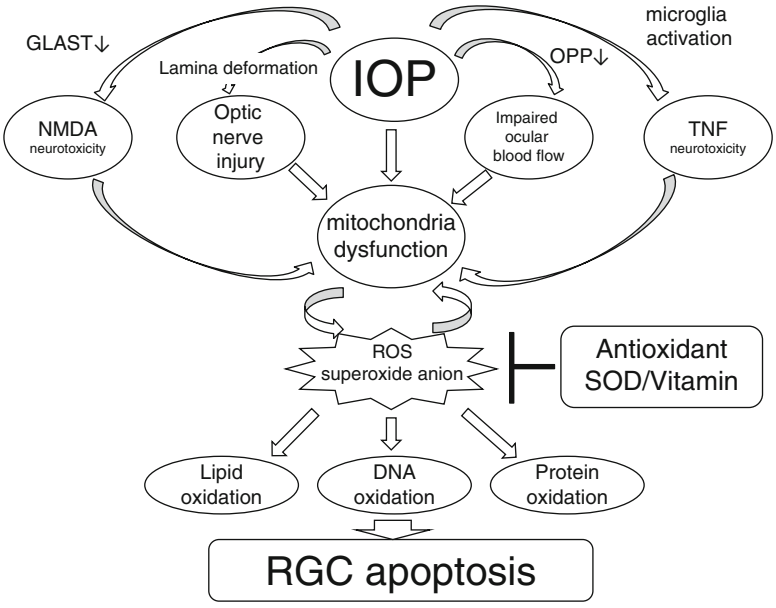


Fig. 4.2 Multiple pathogenic mechanisms cause increased level of ROS in glaucoma. Mitochondrial dysfunction is a primary source of ROS including superoxide anion

observed in SOD1-deficient mice compared with controls. IOP does not show any changes between the two groups [135]. This study suggests that superoxide anion during normal respiration in mitochondria can cause enough oxidative insult for RGC loss, when it is not metabolized by SOD1. Reduced level of SOD1 in serum of NTG subjects was observed [135]. Impairment of SOD1 and mitochondrial dysfunction may be associated with IOP-independent RGC apoptosis.

4.5 Evidence of Possible Neuroprotective Role of Antioxidant Intake for Glaucomatous Optic Neuropathy

Epidemiological studies evaluated the association between antioxidant intake and glaucoma (Table 4.2).

In the Nurses' Health Study ($n = 76{,}200$) and the Health Professionals Follow-Up Study ($n = 40{,}284$). Four hundred seventy-four self-reported glaucoma cases confirmed by medical chart review to have POAG with visual field loss were analyzed in this study. Dietary intakes were measured using validated food frequency questionnaires. No association was observed in intake level of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, vitamin C, vitamin E, and vitamin A [138].

In an osteoporotic fracture study with a sample of 1,155 women in a cross-sectional manner, 95 were diagnosed to have glaucoma by glaucoma specialist. Odds ratio for glaucoma was decreased by 69 % (OR, 0.31; 95 % CI 0.11–0.91) who consumed at least one serving/month of collard greens and kale, by 64 % (OR, 0.36; 95 % CI 0.17–0.77) who consumed more than two servings/week of carrots, and by 47 % (OR, 0.53; 95 % CI 0.29–0.97) who consumed at least one serving/week of canned or dried peaches compared with those who consumed fewest group [139]. Later, prospective analysis was performed in this study. Among 584 African-American women, 77 subjects had new onset POAG. Women who ate three or more servings/day of fruits/fruit juices were 79 % (OR, 0.21; 95 % CI 0.08–0.60) less likely to have glaucoma than women who ate less than one serving/day. Women who consumed more than two servings/week of fresh oranges (OR, 0.18; 95 % CI 0.06–0.51) and peaches (OR, 0.30; 95 % CI 0.13–0.67) had a decreased odds of glaucoma compared to those consuming less than one serving/week. For vegetables, >1 serving/week compared to ≤ 1 serving/month of collard greens/kale decreased the odds of glaucoma by 57 % (OR, 0.43; 95 % CI 0.21–0.85). Individual nutrient intake from food sources found protective trends with higher intakes of vitamin A ($p = 0.011$), vitamin C ($p = 0.018$), and α-carotene ($p = 0.021$) [140]. The effect of peaches and collard/kale for glaucoma is confirmed in both cross-sectional and prospective study.

In the Rotterdam Eye Study, 3,502 participants without glaucoma at baseline were followed and 91 subjects had new onset OAG. Hazard ratio for onset of OAG for vitamin A equivalents (highest versus lowest tertile) was 0.45 (95 %

Table 4.2 Dietary intake of antioxidant and incidence or prevalence of open-angle glaucoma

Study	*n* (glaucoma)	Glaucoma diagnosis	Factors which are not associated with glaucoma	Factors which reduced the risk of glaucoma
The Nurses' Health Study and the Health Professionals Follow-Up Study [138]	116,484 (474)	Self-reported	α-Carotene β-Carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, vitamin E Vitamin C Vitamin A	N/A
Osteoporotic fracture study [139]	1,155 (95)	Optic disc evaluation and visual field testing	All the fruits and fruit juices All vegetables All vitamins Fresh apple Fresh banana Fresh orange Orange juice Fresh peach Spinach Green salad	Canned/dried peach Collard greens and kale Carrot
Osteoporotic fracture study [140]	584 (77)	Disc photograph and visual field testing	All vegetables Fresh apple Fresh banana Orange juice Canned/dried peach Fresh carrot Spinach Green salad Folic acid Vitamin Bs Vitamin D Vitamin E β-Carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin	All fruits/fruit juices Fresh orange Fresh peach Collard greens/kale Vitamin A Vitamin C α-Carotene
Rotterdam Eye Study [141]	3,502 (91)	Ophthalmic examination with visual field testing	α-Carotene β-Carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, vitamin E Vitamin Bs Vitamin C	Vitamin A Vitamin B1
National Health and Nutrition Examination Survey [142]	2,912 (203)	Self-reported	Vitamin A Vitamin E	Vitamin C

CI 0.23–0.90) and for vitamin B1 0.50 (0.25–0.98) [141]. No association was observed in α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, vitamin E, and vitamin B12.

In 2005–2006 National Health and Nutrition Examination Survey, among 2,912 participants, 203 subjects have self-reported glaucoma. Odds ratio for glaucoma, comparing the highest quartile of consumption to no consumption, was 0.47 (95 % CI 0.23–0.97) for vitamin C. However, no significant association was observed in vitamins A and E with glaucoma [142].

Higher collard greens/kale intake, vitamin C intake, and vitamin A intake showed protective effect against glaucoma. Collard greens/kale is a vitamin A- and C-rich nutrient, which means that subjects with higher collard greens/kale intake have higher vitamin C and A intake as a nutrient. Vitamin A is a well-known antioxidant vitamin. Vitamin A is necessary by the retina in the form of retinal to combine with opsin to form rhodopsin, the light-absorbing molecule. Lack of vitamin A causes night blindness due to insufficient production of rhodopsin. Protective effect of vitamin A for neurodegenerative disease has been already reported [143, 144]. Retinoic acid is a metabolite of vitamin A that mediates the functions of vitamin A. Retinoic acid protected hippocampal neuron from staurosporine-induced oxidative stress in mitochondria by enhancing protein levels of SOD1 and SOD2 [145]. Retinoic acid also inhibits staurosporine-induced glutathione depletion in neuron and reduced the number of apoptotic neuronal cell in vivo [145].

The association between glaucoma and vitamin C has long been evaluated and the association is more obvious than vitamin A. Higher level of vitamin C exists in aqueous humor, which is thought to have an antioxidative protection on lens and trabecular meshwork cells [146]. Reduced vitamin C level is associated with human NTG [147] and POAG [148]. The rs1279386 (A > G) SNP in SLC23A2, which encodes Na-dependent vitamin C transporter, was significantly associated with lower plasma concentrations of vitamin C and with higher risk of POAG [149]. Lack of vitamin C may be involved in glaucoma pathogenesis.

4.6 Adverse Effect of Antioxidant Therapy

Antioxidant treatment protects RGC from IOP elevation, optic nerve injury, TNF-α neurotoxicity, glutamate neurotoxicity, and impaired ocular blood flow. These results suggest that antioxidant treatment is a promising neuroprotective treatment for glaucomatous optic neuropathy. However, we should take care of side effects of antioxidant treatment. Some antioxidative treatment may increase mortality by affecting human essential defense mechanism [150].

Carcinogenesis is supposed to be an adverse event of systemic antioxidant treatment. Cancer cells demand high ATP level for proliferation and higher activity for mitochondria, which results in higher generation of ROS in cancer cells. Cancer cells have strong antioxidative mechanism to metabolize ROS. Pharmacologically depletion of antioxidant enzyme in cancer stem cell decreases clonogenicity and

results in radiosensitization [151]. A lot of inhibitors of antioxidant enzyme are approved by the FDA [152]. In a large, double-blind, randomized, placebo-controlled study, systemic administration of beta-carotene increased 1.2-fold higher risk of lung cancer in men. Total mortality was also 8 % higher in subjects who received beta-carotene [153]. In another multicenter, double-blind, placebo-controlled, randomized study, systemic administration of vitamin A and beta-carotene increased 28 % in risk of incident lung cancer and 17 % in the risk of death [154]. Furthermore, another study showed that vitamin E supplementation increased 17 % in the risk of prostate cancer [155]. In mice, median life span of $Gpx4^{+/-}$ mice was significantly longer than that of wild-type mice because of a delayed occurrence of fatal lymphoma. The authors concluded that lifelong reduction in Gpx4 reduced cancer incidence most likely through alterations in sensitivity of tissues to apoptosis and increased life span [156]. Glaucoma is a chronic neurodegenerative disease. For the treatment of glaucoma, lifelong antioxidative treatment would be necessary. Safety of systemic administration of antioxidant for glaucoma patients should be carefully evaluated in the future study. Topical administration of antioxidant seems to be safer, more acceptable method for glaucoma patients. Drug delivery system using eye drops, implants, liposomes, nanosphere, and contact lens would be better way for antioxidant treatment for glaucoma. H_2-loaded water is commercially available and used as a supplement. H_2-loaded eye drops may be a good candidate for topical antioxidant delivery for glaucoma treatment.

4.7 Conclusion

Numerous reports suggest the association between oxidative stress and glaucomatous optic neuropathy in human. A lot of in vivo and in vitro studies show the promising neuroprotective effect of antioxidant treatment for glaucomatous optic neuropathy. In a population-based epidemiological study, some antioxidant vitamins such as vitamin C have neuroprotective effect for glaucoma. However, lifelong systemic administration of antioxidant may cause unexpected complications including carcinogenesis. Topical administration of adequate ROS scavengers may be a safe and an effective antioxidant treatment of glaucoma.

References

1. Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90:262–267. doi:10.1136/bjo.2005.081224
2. Tanabe S, Yuki K, Ozeki N et al (2011) The association between primary open-angle glaucoma and motor vehicle collisions. Invest Ophthalmol Vis Sci 52:4177–4181. doi:10.1167/iovs.10-6264
3. Yuki K, Tanabe S, Kouyama K et al (2013) The association between visual field defect severity and fear of falling in primary open-angle glaucoma. Invest Ophthalmol Vis Sci 54:7739–7745. doi:10.1167/iovs.13-12079

4. Coleman AL, Miglior S (2008) Risk factors for glaucoma onset and progression. Surv Ophthalmol 53(Suppl 1):S3–S10. doi:10.1016/j.survophthal.2008.08.006
5. Weinreb RN, Khaw PT (2004) Primary open-angle glaucoma. Lancet 363:1711–1720. doi:10.1016/S0140-6736(04)16257-0
6. Finkel T, Holbrook NJ (2000) Oxidants, oxidative stress and the biology of ageing. Nature 408:239–247. doi:10.1038/35041687
7. Brand MD (2010) The sites and topology of mitochondrial superoxide production. Exp Gerontol 45:466–472. doi:10.1016/j.exger.2010.01.003
8. Barja G (1999) Mitochondrial oxygen radical generation and leak: sites of production in states 4 and 3, organ specificity, and relation to aging and longevity. J Bioenerg Biomembr 31:347–366
9. Janssen-Heininger YM, Mossman BT, Heintz NH et al (2008) Redox-based regulation of signal transduction: principles, pitfalls, and promises. Free Radic Biol Med 45:1–17. doi:10.1016/j.freeradbiomed.2008.03.011
10. Radak Z, Zhao Z, Goto S et al (2011) Age-associated neurodegeneration and oxidative damage to lipids, proteins and DNA. Mol Aspects Med 32:305–315. doi:10.1016/j.mam.2011.10.010 11
11. Sultana R, Perluigi M, Butterfield DA (2009) Oxidatively modified proteins in Alzheimer's disease (AD), mild cognitive impairment and animal models of AD: role of Abeta in pathogenesis. Acta Neuropathol 118:131–150. doi:10.1007/s00401-009-0517-0
12. Sultana R, Perluigi M, Allan Butterfield D (2012) Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. Free Radic Biol Med 62:157–169. doi:10.1016/j.freeradbiomed.2012.09.027
13. Nunomura A, Castellani RJ, Zhu X et al (2006) Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol 65:631–641. doi:10.1097/01.jnen.0000228136.58062.bf
14. Varcin M, Bentea E, Michotte Y et al (2012) Oxidative stress in genetic mouse models of Parkinson's disease. Oxid Med Cell Longev 2012:624925. doi:10.1155/2012/624925
15. Yao Z, Wood NW (2009) Cell death pathways in Parkinson's disease: role of mitochondria. Antioxid Redox Signal 11:2135–2149. doi:10.1089/ARS.2009.2624
16. Valavanidis A, Vlachogianni T, Fiotakis C (2009) 8-hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27:120–139. doi:10.1080/10590500902885684
17. Moriya M, Grollman AP (1993) Mutations in the mutY gene of *Escherichia coli* enhance the frequency of targeted G:C–>T:a transversions induced by a single 8-oxoguanine residue in single-stranded DNA. Mol Gen Genet 239:72–76
18. Aksenova MV, Aksenov MY, Payne RM et al (1999) Oxidation of cytosolic proteins and expression of creatine kinase BB in frontal lobe in different neurodegenerative disorders. Dement Geriatr Cogn Disord 10:158–165
19. Anzai K, Ogawa K, Goto Y et al (1999) Oxidation-dependent changes in the stability and permeability of lipid bilayers. Antioxid Redox Signal 1:339–347
20. Sorkhabi R, Ghorbanihaghjo A, Javadzadeh A et al (2011) Oxidative DNA damage and total antioxidant status in glaucoma patients. Mol Vis 17:41–46
21. Izzotti A, Sacca SC, Cartiglia C et al (2003) Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. Am J Med 114:638–646
22. Sacca SC, Pascotto A, Camicione P et al (2005) Oxidative DNA damage in the human trabecular meshwork: clinical correlation in patients with primary open-angle glaucoma. Arch Ophthalmol 123(4):458–463. doi:10.1001/archopht.123.4.458
23. Wu LL, Chiou CC, Chang PY et al (2004) Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 339:1–9
24. Majsterek I, Malinowska K, Stanczyk M et al (2011) Evaluation of oxidative stress markers in pathogenesis of primary open-angle glaucoma. Exp Mol Pathol 90:231–237. doi:10.1016/j.yexmp.2011.01.001

25. Del Rio D, Stewart AJ, Pellegrini N (2005) A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 15:316–328. doi:10.1016/j.numecd.2005.05.003
26. Erdurmus M, Yagci R, Atis O et al (2011) Antioxidant status and oxidative stress in primary open angle glaucoma and pseudoexfoliative glaucoma. Curr Eye Res 36:713–718. doi:10.3109/02713683.2011.584370
27. Zanon-Moreno V, Marco-Ventura P, Lleo-Perez A et al (2008) Oxidative stress in primary open-angle glaucoma. J Glaucoma 17:263–268. doi:10.1097/IJG.0b013e31815c3a7f
28. Nucci C, Di Pierro D, Varesi C et al (2013) Increased malondialdehyde concentration and reduced total antioxidant capacity in aqueous humor and blood samples from patients with glaucoma. Mol Vis 19:1841–1846
29. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–1111. doi:10.1016/j.clinbiochem.2005.08.008
30. Ferreira SM, Lerner SF, Brunzini R et al (2004) Oxidative stress markers in aqueous humor of glaucoma patients. Am J Ophthalmol 137:62–69
31. Tanito M, Kaidzu S, Takai Y et al (2012) Status of systemic oxidative stresses in patients with primary open-angle glaucoma and pseudoexfoliation syndrome. PLoS One 7:e49680. doi:10.1371/journal.pone.0049680
32. Pasquini A, Luchetti E, Marchetti V et al (2008) Analytical performances of d-ROMs test and BAP test in canine plasma. Definition of the normal range in healthy Labrador dogs. Vet Res Commun 32:137–143. doi:10.1007/s11259-007-9014-x
33. Bagnis A, Izzotti A, Centofanti M et al (2012) Aqueous humor oxidative stress proteomic levels in primary open angle glaucoma. Exp Eye Res 103:55–62. doi:10.1016/j.exer.2012.07.011
34. Zengi O, Karakas A, Ergun U et al (2011) Urinary 8-hydroxy-2′-deoxyguanosine level and plasma paraoxonase 1 activity with Alzheimer's disease. Clin Chem Lab Med 50:529–534. doi:10.1515/CCLM.2011.792
35. McGrath LT, McGleenon BM, Brennan S et al (2001) Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. QJM 94:485–490
36. Seet RC, Lee CY, Lim EC et al (2009) Oxidative damage in Parkinson disease: measurement using accurate biomarkers. Free Radic Biol Med 48:560–566. doi:10.1016/j.freeradbiomed.2009.11.026
37. Chen CM, Liu JL, Wu YR et al (2009) Increased oxidative damage in peripheral blood correlates with severity of Parkinson's disease. Neurobiol Dis 33:429–435. doi:10.1016/j.nbd.2008.11.011
38. van Rensburg SJ, van Zyl JM, Potocnik FC et al (2006) The effect of stress on the antioxidative potential of serum: implications for Alzheimer's disease. Metab Brain Dis 21:171–179. doi:10.1007/s11011-006-9020-7
39. Sharma A, Kaur P, Kumar B et al (2008) Plasma lipid peroxidation and antioxidant status of Parkinson's disease patients in the Indian population. Parkinsonism Relat Disord 14:52–57. doi:10.1016/j.parkreldis.2007.06.009
40. Bayer AU, Keller ON, Ferrari F et al (2002) Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. Am J Ophthalmol 133:135–137
41. Yuki K, Murat D, Kimura I et al (2010) Increased serum total antioxidant status and decreased urinary 8-hydroxy-2′-deoxyguanosine levels in patients with normal-tension glaucoma. Acta Ophthalmol 88:e259–e264. doi:10.1111/j.1755-3768.2010.01997.x
42. Yuki K, Tsubota K (2013) Increased urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG)/creatinine level is associated with the progression of normal-tension glaucoma. Curr Eye Res 38:983–988. doi:10.3109/02713683.2013.800889

43. Ferreira SM, Lerner SF, Brunzini R et al (2010) Time course changes of oxidative stress markers in a rat experimental glaucoma model. Invest Ophthalmol Vis Sci 51:4635–4640. doi:10.1167/iovs.09-5044
44. Boveris A, Cadenas E, Reiter R et al (1980) Organ chemiluminescence: noninvasive assay for oxidative radical reactions. Proc Natl Acad Sci U S A 77:347–351
45. Bird RP, Draper HH (1984) Comparative studies on different methods of malonaldehyde determination. Methods Enzymol 105:299–305
46. Faulkner K, Fridovich I (1993) Luminol and lucigenin as detectors for O_2. Free Radic Biol Med 15:447–451
47. Vasquez-Vivar J, Martasek P, Hogg N et al (1999) Electron spin resonance spin-trapping detection of superoxide generated by neuronal nitric oxide synthase. Methods Enzymol 301:169–177
48. Ko ML, Peng PH, Ma MC et al (2005) Dynamic changes in reactive oxygen species and antioxidant levels in retinas in experimental glaucoma. Free Radic Biol Med 39:365–373. doi:10.1016/j.freeradbiomed.2005.03.025
49. Ozdemir G, Tolun FI, Gul M et al (2009) Retinal oxidative stress induced by intraocular hypertension in rats may be ameliorated by brimonidine treatment and N-acetyl cysteine supplementation. J Glaucoma 18:662–665. doi:10.1097/IJG.0b013e31819c46b1
50. Moreno MC, Campanelli J, Sande P et al (2004) Retinal oxidative stress induced by high intraocular pressure. Free Radic Biol Med 37:803–812
51. Spickett CM (2013) The lipid peroxidation product 4-hydroxy-2-nonenal: advances in chemistry and analysis. Redox Biol 1:145–152. doi:10.1016/j.redox.2013.01.007
52. Liu Q, Ju WK, Crowston JG et al (2007) Oxidative stress is an early event in hydrostatic pressure induced retinal ganglion cell damage. Invest Ophthalmol Vis Sci 48:4580–4589. doi:10.1167/iovs.07-0170
53. Tezel G, Yang X, Cai J (2005) Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma. Invest Ophthalmol Vis Sci 46:3177–3187. doi:10.1167/iovs.05-0208
54. Chang B, Smith RS, Hawes NL et al (1999) Interacting loci cause severe iris atrophy and glaucoma in DBA/2J mice. Nat Genet 21:405–409. doi:10.1038/7741
55. Inman DM, Lambert WS, Calkins DJ et al (2013) Alpha-lipoic acid antioxidant treatment limits glaucoma-related retinal ganglion cell death and dysfunction. PLoS One 8:e65389. doi:10.1371/journal.pone.0065389
56. Rochette L, Ghibu S, Richard C et al (2013) Direct and indirect antioxidant properties of alpha-lipoic acid and therapeutic potential. Mol Nutr Food Res 57:114–125. doi:10.1002/mnfr.201200608
57. Munemasa Y, Kitaoka Y, Kuribayashi J et al (2010) Modulation of mitochondria in the axon and soma of retinal ganglion cells in a rat glaucoma model. J Neurochem 115:1508–1519. doi:10.1111/j.1471-4159.2010.07057.x
58. Ju WK, Kim KY, Lindsey JD et al (2008) Intraocular pressure elevation induces mitochondrial fission and triggers OPA1 release in glaucomatous optic nerve. Invest Ophthalmol Vis Sci 49:4903–4911. doi:10.1167/iovs.07-1661
59. Bleier L, Drose S (2013) Superoxide generation by complex III: from mechanistic rationales to functional consequences. Biochim Biophys Acta 1827:1320–1331. doi:10.1016/j.bbabio.2012.12.002
60. Gus'kova RA, Ivanov II, Kol'tover VK et al (1984) Permeability of bilayer lipid membranes for superoxide (O2-.) radicals. Biochim Biophys Acta 778:579–585
61. Lee EJ, Kim TW, Weinreb RN et al (2013) Reversal of lamina cribrosa displacement after intraocular pressure reduction in open-angle glaucoma. Ophthalmology 120:553–559. doi:10.1016/j.ophtha.2012.08.047
62. Quigley HA, McKinnon SJ, Zack DJ et al (2000) Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest Ophthalmol Vis Sci 41:3460–3466

63. Kanamori A, Catrinescu MM, Kanamori N et al (2010) Superoxide is an associated signal for apoptosis in axonal injury. Brain 133:2612–2625. doi:10.1093/brain/awq105
64. Lieven CJ, Hoegger MJ, Schlieve CR et al (2006) Retinal ganglion cell axotomy induces an increase in intracellular superoxide anion. Invest Ophthalmol Vis Sci 47:1477–1485. doi:10.1167/iovs.05-0921
65. Munemasa Y, Kim SH, Ahn JH et al (2008) Protective effect of thioredoxins 1 and 2 in retinal ganglion cells after optic nerve transection and oxidative stress. Invest Ophthalmol Vis Sci 49:3535–3543. doi:10.1167/iovs.08-1716
66. Himori N, Yamamoto K, Maruyama K et al (2013) Critical role of Nrf2 in oxidative stress-induced retinal ganglion cell death. J Neurochem. doi:10.1111/jnc.12325
67. Gan L, Johnson JA (2013) Oxidative damage and the Nrf2-ARE pathway in neurodegenerative diseases. Biochim Biophys Acta. doi:10.1016/j.bbadis.2013.12.011
68. Cherecheanu AP, Garhofer G, Schmidl D et al (2012) Ocular perfusion pressure and ocular blood flow in glaucoma. Curr Opin Pharmacol 13:36–42. doi:10.1016/j.coph.2012.09.003
69. Caprioli J, Coleman AL (2010) Blood pressure, perfusion pressure, and glaucoma. Am J Ophthalmol 149:704–712. doi:10.1016/j.ajo.2010.01.018
70. Leske MC, Wu SY, Hennis A et al (2008) Risk factors for incident open-angle glaucoma: the Barbados eye studies. Ophthalmology 115:85–93. doi:10.1016/j.ophtha.2007.03.017
71. Quigley HA, West SK, Rodriguez J et al (2001) The prevalence of glaucoma in a population-based study of Hispanic subjects: Proyecto VER. Arch Ophthalmol 119:1819–1826
72. Leske MC, Heijl A, Hyman L et al (2007) Predictors of long-term progression in the early manifest glaucoma trial. Ophthalmology 114:1965–1972. doi:10.1016/j.ophtha.2007.03.016
73. Chiba N, Omodaka K, Yokoyama Y et al (2013) Association between optic nerve blood flow and objective examinations in glaucoma patients with generalized enlargement disc type. Clin Ophthalmol 5:1549–1556. doi:10.2147/OPTH.S22097
74. Yokoyama Y, Aizawa N, Chiba N et al (2011) Significant correlations between optic nerve head microcirculation and visual field defects and nerve fiber layer loss in glaucoma patients with myopic glaucomatous disk. Clin Ophthalmol 5:1721–1727. doi:10.2147/OPTH.S23204
75. Okumura Y, Yuki K, Tsubota K (2012) Low diastolic blood pressure is associated with the progression of normal-tension glaucoma. Ophthalmologica 228:36–41. doi:10.1159/000335978
76. Tezel G, Wax MB (2004) Hypoxia-inducible factor 1alpha in the glaucomatous retina and optic nerve head. Arch Ophthalmol 122:1348–1356. doi:10.1001/archopht.122.9.1348
77. Lam TT, Abler AS, Tso MO (1999) Apoptosis and caspases after ischemia-reperfusion injury in rat retina. Invest Ophthalmol Vis Sci 40:967–975
78. Szabo ME, Droy-Lefaix MT, Doly M (1997) Direct measurement of free radicals in ischemic/reperfused diabetic rat retina. Clin Neurosci 4:240–245
79. Abramov AY, Scorziello A, Duchen MR (2007) Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. J Neurosci 27:1129–1138. doi:10.1523/JNEUROSCI.4468-06.2007
80. Song Y, Gong YY, Xie ZG et al (2008) Edaravone (MCI-186), a free radical scavenger, attenuates retinal ischemia/reperfusion injury in rats. Acta Pharmacol Sin 29:823–828. doi:10.1111/j.1745-7254.2008.00822.x
81. Nayak MS, Kita M, Marmor MF (1993) Protection of rabbit retina from ischemic injury by superoxide dismutase and catalase. Invest Ophthalmol Vis Sci 34:2018–2022
82. Dilsiz N, Sahaboglu A, Yildiz MZ et al (2006) Protective effects of various antioxidants during ischemia-reperfusion in the rat retina. Graefes Arch Clin Exp Ophthalmol 244:627–633. doi:10.1007/s00417-005-0084-6
83. Zhang Z, Qin X, Zhao X et al (2011) Valproic acid regulates antioxidant enzymes and prevents ischemia/reperfusion injury in the rat retina. Curr Eye Res 37:429–437. doi:10.3109/02713683.2011.653616

84. Liu Y, Tang L, Chen B (2012) Effects of antioxidant gene therapy on retinal neurons and oxidative stress in a model of retinal ischemia/reperfusion. Free Radic Biol Med 52:909–915. doi:10.1016/j.freeradbiomed.2011.12.013
85. Chen B, Tang L (2011) Protective effects of catalase on retinal ischemia/reperfusion injury in rats. Exp Eye Res 93:599–606. doi:10.1016/j.exer.2011.07.007
86. Oharazawa H, Igarashi T, Yokota T et al (2010) Protection of the retina by rapid diffusion of hydrogen: administration of hydrogen-loaded eye drops in retinal ischemia-reperfusion injury. Invest Ophthalmol Vis Sci 51:487–492. doi:10.1167/iovs.09-4089
87. Ohsawa I, Ishikawa M, Takahashi K et al (2007) Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med 13:688–694. doi:10.1038/nm1577
88. Ji X, Tian Y, Xie K et al (2012) Protective effects of hydrogen-rich saline in a rat model of traumatic brain injury via reducing oxidative stress. J Surg Res 178:e9–e16. doi:10.1016/j.jss.2011.12.038
89. Ji X, Liu W, Xie K et al (2010) Beneficial effects of hydrogen gas in a rat model of traumatic brain injury via reducing oxidative stress. Brain Res 1354:196–205. doi:10.1016/j.brainres.2010.07.038
90. Kubota M, Shimmura S, Kubota S et al (2011) Hydrogen and N-acetyl-L-cysteine rescue oxidative stress-induced angiogenesis in a mouse corneal alkali-burn model. Invest Ophthalmol Vis Sci 52:427–433. doi:10.1167/iovs.10-6167
91. Yang CX, Yan H, Ding TB (2013) Hydrogen saline prevents selenite-induced cataract in rats. Mol Vis 19:1684–1693
92. Feng M, Wang XH, Yang XB et al (2012) Protective effect of saturated hydrogen saline against blue light-induced retinal damage in rats. Int J Ophthalmol 5:151–157. doi:10.3980/j.issn.2222-3959.2012.02.07
93. Ono H, Nishijima Y, Adachi N et al (2011) Improved brain MRI indices in the acute brain stem infarct sites treated with hydroxyl radical scavengers, Edaravone and hydrogen, as compared to Edaravone alone. A non-controlled study. Med Gas Res 1:12. doi:10.1186/2045-9912-1-12
94. Ono H, Nishijima Y, Adachi N et al (2012) Hydrogen(H2) treatment for acute erythymatous skin diseases. A report of 4 patients with safety data and a non-controlled feasibility study with H2 concentration measurement on two volunteers. Med Gas Res 2:14. doi:10.1186/2045-9912-2-14
95. Ono H, Nishijima Y, Adachi N et al (2012) A basic study on molecular hydrogen (H2) inhalation in acute cerebral ischemia patients for safety check with physiological parameters and measurement of blood H2 level. Med Gas Res 2:21. doi:10.1186/2045-9912-2-21
96. Fontana L, Partridge L, Longo VD (2010) Extending healthy life span–from yeast to humans. Science 328:321–326. doi:10.1126/science.1172539
97. Hyun DH, Emerson SS, Jo DG et al (2006) Calorie restriction up-regulates the plasma membrane redox system in brain cells and suppresses oxidative stress during aging. Proc Natl Acad Sci U S A 103:19908–19912. doi:10.1073/pnas.0608008103
98. Li D, Sun F, Wang K (2003) Caloric restriction retards age-related changes in rat retina. Biochem Biophys Res Commun 309:457–463
99. Obin M, Pike A, Halbleib M et al (2000) Calorie restriction modulates age-dependent changes in the retinas of Brown Norway rats. Mech Ageing Dev 114:133–147
100. Kong YX, van Bergen N, Bui BV et al (2012) Impact of aging and diet restriction on retinal function during and after acute intraocular pressure injury. Neurobiol Aging 33:1126.e15–e25. doi:10.1016/j.neurobiolaging.2011.11.026
101. Lucas DR, Newhouse JP (1957) The toxic effect of sodium L-glutamate on the inner layers of the retina. AMA Arch Ophthalmol 58:193–201

102. Hynd MR, Scott HL, Dodd PR (2004) Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. Neurochem Int 45:583–595. doi:10.1016/j.neuint.2004.03.007
103. Hallett PJ, Standaert DG (2004) Rationale for and use of NMDA receptor antagonists in Parkinson's disease. Pharmacol Ther 102:155–174. doi:10.1016/j.pharmthera.2004.04.001
104. Mittal SK, Eddy C (2013) The role of dopamine and glutamate modulation in Huntington disease. Behav Neurol 26:255–263. doi:10.3233/BEN-2012-120268
105. Seki M, Lipton SA (2008) Targeting excitotoxic/free radical signaling pathways for therapeutic intervention in glaucoma. Prog Brain Res 173:495–510. doi:10.1016/S0079-6123(08)01134-5
106. Harada T, Harada C, Nakamura K et al (2007) The potential role of glutamate transporters in the pathogenesis of normal tension glaucoma. J Clin Invest 117:1763–1770. doi:10.1172/JCI30178
107. Martin KR, Levkovitch-Verbin H, Valenta D et al (2002) Retinal glutamate transporter changes in experimental glaucoma and after optic nerve transection in the rat. Invest Ophthalmol Vis Sci 43:2236–2243
108. Ishikawa M, Yoshitomi T, Zorumski CF et al (2011) Downregulation of glutamine synthetase via GLAST suppression induces retinal axonal swelling in a rat ex vivo hydrostatic pressure model. Invest Ophthalmol Vis Sci 52:6604–6616. doi:10.1167/iovs.11-7375
109. Lafon-Cazal M, Pietri S, Culcasi M et al (1993) NMDA-dependent superoxide production and neurotoxicity. Nature 364:535–537. doi:10.1038/364535a0
110. Patel M, Day BJ, Crapo JD et al (1996) Requirement for superoxide in excitotoxic cell death. Neuron 16:345–355
111. Dugan LL, Sensi SL, Canzoniero LM et al (1995) Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate. J Neurosci 15:6377–6388
112. Borg J, London J (2002) Copper/zinc superoxide dismutase overexpression promotes survival of cortical neurons exposed to neurotoxins in vitro. J Neurosci Res 70:180–189. doi:10.1002/jnr.10404
113. Boulos S, Meloni BP, Arthur PG et al (2007) Peroxiredoxin 2 overexpression protects cortical neuronal cultures from ischemic and oxidative injury but not glutamate excitotoxicity, whereas Cu/Zn superoxide dismutase 1 overexpression protects only against oxidative injury. J Neurosci Res 85:3089–3097. doi:10.1002/jnr.21429
114. Yuki K, Yoshida T, Miyake S et al (2013) Neuroprotective role of superoxide dismutase 1 in retinal ganglion cells and inner nuclear layer cells against N-methyl-D-aspartate-induced cytotoxicity. Exp Eye Res 115:230–238. doi:10.1016/j.exer.2013.07.002
115. Hironaka K, Inokuchi Y, Fujisawa T et al (2011) Edaravone-loaded liposomes for retinal protection against oxidative stress-induced retinal damage. Eur J Pharm Biopharm 79:119–125. doi:10.1016/j.ejpb.2011.01.019
116. Sakamoto K, Suzuki Y, Kurauchi Y et al (2014) Hydrogen sulfide attenuates NMDA-induced neuronal injury via its anti-oxidative activity in the rat retina. Exp Eye Res. doi:10.1016/j.exer.2014.01.008
117. Kim KA, Kang KD, Lee EH et al (2011) Edible wild vegetable, *Gymnaster koraiensis* protects retinal ganglion cells against oxidative stress. Food Chem Toxicol 49:2131–2143. doi:10.1016/j.fct.2011.05.028
118. Akane M, Shimazawa M, Inokuchi Y et al (2011) SUN N8075, a novel radical scavenger, protects against retinal cell death in mice. Neurosci Lett 488:87–91. doi:10.1016/j.neulet.2010.11.008
119. Inokuchi Y, Imai S, Nakajima Y et al (2009) Edaravone, a free radical scavenger, protects against retinal damage in vitro and in vivo. J Pharmacol Exp Ther 329:687–698. doi:10.1124/jpet.108.148676

120. Nakajima Y, Inokuchi Y, Shimazawa M et al (2008) Astaxanthin, a dietary carotenoid, protects retinal cells against oxidative stress in-vitro and in mice in-vivo. J Pharm Pharmacol 60:1365–1374. doi:10.1211/jpp/60.10.0013
121. Nakajima Y, Inokuchi Y, Nishi M et al (2008) Coenzyme Q10 protects retinal cells against oxidative stress in vitro and in vivo. Brain Res 1226:226–233. doi:10.1016/j.brainres.2008.06.026
122. Inomata Y, Nakamura H, Tanito M et al (2006) Thioredoxin inhibits NMDA-induced neurotoxicity in the rat retina. J Neurochem 98:372–385. doi:10.1111/j.1471-4159.2006.03871.x
123. Aruoma OI, Moncaster JA, Walsh DT et al (2003) The antioxidant cocktail, effective microorganism X (EM-X), protects retinal neurons in rats against N-methyl-D-aspartate excitotoxicity in vivo. Free Radic Res 37:91–97
124. Feng S, Yang Q, Liu M et al (2011) Edaravone for acute ischaemic stroke. Cochrane Database Syst Rev 12, CD007230. doi:10.1002/14651858.CD007230.pub2
125. Shimazaki H, Hironaka K, Fujisawa T et al (2011) Edaravone-loaded liposome eyedrops protect against light-induced retinal damage in mice. Invest Ophthalmol Vis Sci 52:7289–7297. doi:10.1167/iovs.11-7983
126. Tezel G (2008) TNF-alpha signaling in glaucomatous neurodegeneration. Prog Brain Res 173:409–421. doi:10.1016/S0079-6123(08)01128-X
127. Tezel G, Wax MB (2000) Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. J Neurosci 20:8693–8700
128. Tezel G, Li LY, Patil RV et al (2001) TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. Invest Ophthalmol Vis Sci 42:1787–1794
129. Sawada H, Fukuchi T, Tanaka T et al (2010) Tumor necrosis factor-alpha concentrations in the aqueous humor of patients with glaucoma. Invest Ophthalmol Vis Sci 51:903–906. doi:10.1167/iovs.09-4247
130. Nakazawa T, Nakazawa C, Matsubara A et al (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J Neurosci 26:12633–12641. doi:10.1523/JNEUROSCI.2801-06.2006
131. Roh M, Zhang Y, Murakami Y et al (2012) Etanercept, a widely used inhibitor of tumor necrosis factor-alpha (TNF-alpha), prevents retinal ganglion cell loss in a rat model of glaucoma. PLoS One 7:e40065. doi:10.1371/journal.pone.0040065
132. Tezel G, Yang X (2004) Caspase-independent component of retinal ganglion cell death, in vitro. Invest Ophthalmol Vis Sci 45:4049–4059. doi:10.1167/iovs.04-0490
133. Fatma N, Kubo E, Sen M et al (2008) Peroxiredoxin 6 delivery attenuates TNF-alpha-and glutamate-induced retinal ganglion cell death by limiting ROS levels and maintaining Ca^{2+} homeostasis. Brain Res 1233:63–78. doi:10.1016/j.brainres.2008.07.076
134. Kitaoka Y, Munemasa Y, Hayashi Y et al (2011) Axonal protection by 17beta-estradiol through thioredoxin-1 in tumor necrosis factor-induced optic neuropathy. Endocrinology 152:2775–2785. doi:10.1210/en.2011-0046
135. Yuki K, Ozawa Y, Yoshida T et al (2011) Retinal ganglion cell loss in superoxide dismutase 1 deficiency. Invest Ophthalmol Vis Sci 52:4143–4150. doi:10.1167/iovs.10-6294
136. Bach M, Unsoeld AS, Philippin H et al (2006) Pattern ERG as an early glaucoma indicator in ocular hypertension: a long-term, prospective study. Invest Ophthalmol Vis Sci 47:4881–4887. doi:10.1167/iovs.05-0875
137. Nagaraju M, Saleh M, Porciatti V (2007) IOP-dependent retinal ganglion cell dysfunction in glaucomatous DBA/2J mice. Invest Ophthalmol Vis Sci 48:4573–4579. doi:10.1167/iovs.07-0582
138. Kang JH, Pasquale LR, Willett W et al (2003) Antioxidant intake and primary open-angle glaucoma: a prospective study. Am J Epidemiol 158:337–346

139. Coleman AL, Stone KL, Kodjebacheva G et al (2008) Glaucoma risk and the consumption of fruits and vegetables among older women in the study of osteoporotic fractures. Am J Ophthalmol 145:1081–1089. doi:10.1016/j.ajo.2008.01.022
140. Giaconi JA, Yu F, Stone KL et al (2012) The association of consumption of fruits/vegetables with decreased risk of glaucoma among older African–American women in the study of osteoporotic fractures. Am J Ophthalmol 154:635–644. doi:10.1016/j.ajo.2012.03.048
141. Ramdas WD, Wolfs RC, Kiefte-de Jong JC et al (2012) Nutrient intake and risk of open-angle glaucoma: the Rotterdam study. Eur J Epidemiol 27:385–393. doi:10.1007/s10654-012-9672-z
142. Wang SY, Singh K, Lin SC (2013) Glaucoma and vitamins A, C, and E supplement intake and serum levels in a population-based sample of the United States. Eye (Lond) 27:487–494. doi:10.1038/eye.2013.10
143. Obulesu M, Dowlathabad MR, Bramhachari PV (2011) Carotenoids and Alzheimer's disease: an insight into therapeutic role of retinoids in animal models. Neurochem Int 59:535–541. doi:10.1016/j.neuint.2011.04.004
144. Tafti M, Ghyselinck NB (2007) Functional implication of the vitamin A signaling pathway in the brain. Arch Neurol 64:1706–1711. doi:10.1001/archneur.64.12.1706
145. Ahlemeyer B, Bauerbach E, Plath M et al (2001) Retinoic acid reduces apoptosis and oxidative stress by preservation of SOD protein level. Free Radic Biol Med 30:1067–1077
146. Purcell EF, Lerner LH, Kinsey VE (1954) Ascorbic acid in aqueous humor and serum of patients with and without cataract; physiologic significance of relative concentrations. AMA Arch Ophthalmol 51:1–6
147. Yuki K, Murat D, Kimura I et al (2010) Reduced-serum vitamin C and increased uric acid levels in normal-tension glaucoma. Graefes Arch Clin Exp Ophthalmol 248:243–248. doi:10.1007/s00417-009-1183-6
148. Zanon-Moreno V, Asensio-Marquez EM, Ciancotti-Oliver L et al (2013) Effects of polymorphisms in vitamin E-, vitamin C-, and glutathione peroxidase-related genes on serum biomarkers and associations with glaucoma. Mol Vis 19:231–242
149. Zanon-Moreno V, Ciancotti-Olivares L, Asencio J et al (2011) Association between a SLC23A2 gene variation, plasma vitamin C levels, and risk of glaucoma in a Mediterranean population. Mol Vis 17:2997–3004
150. Bjelakovic G, Nikolova D, Gluud LL et al (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297:842–857. doi:10.1001/jama.297.8.842
151. Diehn M, Cho RW, Lobo NA et al (2009) Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 458:780–783. doi:10.1038/nature07733
152. Gorrini C, Harris IS, Mak TW (2013) Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12:931–947. doi:10.1038/nrd4002
153. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 330:1029–1035. doi:10.1056/NEJM199404143301501
154. Omenn GS, Goodman GE, Thornquist MD et al (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 334:1150–1155. doi:10.1056/NEJM199605023341802
155. Klein EA, Thompson IM Jr, Tangen CM et al (2011) Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E cancer prevention trial (SELECT). JAMA 306:1549–1556. doi:10.1001/jama.2011.1437
156. Ran Q, Liang H, Ikeno Y et al (2007) Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. J Gerontol A Biol Sci Med Sci 62:932–942

Chapter 5
ER Stress

Masamitsu Shimazawa and Hideaki Hara

Abstract Glaucoma, an optic neuropathy resulting from retinal ganglion cell (RGC) death, is one of the leading causes of blindness worldwide. The causes of RGC death in glaucoma have been reported to arise from intraocular pressure, dysregulation of ocular circulation, autoimmune diseases, and genetic predisposition and so on. However, its pathological mechanisms remain unclear. Recently, it is focused on the involvement of endoplasmic reticulum (ER) stress in glaucoma. The authors demonstrated, for the first time, that various types of cellular stress induce ER stress before proceeding to RGC death. ER stress is caused by the accumulation of misfolded or unfolded proteins within the ER lumen. The excess ER stress leads to ER-stress-induced cell death, highlighting the possible mechanisms of neurodegenerative diseases, such as Alzheimer disease, amyotrophic lateral sclerosis, and Parkinson disease. This chapter introduces the involvement of ER stress in retinal cell death causing glaucoma and its therapeutic strategy.

Keywords Endoplasmic reticulum stress • Glucose-regulated protein 78 • Unfolded protein response

5.1 ER Stress and Neurodegeneration

In chronic neurodegenerative disorders such as Alzheimer disease, Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis (ALS), abnormally unfolded proteins are known to aggregate and accumulate in neurons, and they are thought to be closely related to the initiation and development of these neurodegenerative diseases [1–3]. Recently, endoplasmic reticulum (ER) stress has been

M. Shimazawa (✉) • H. Hara
Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, 1-5-25 Daigaku-nishi, Gifu 501-1196, Japan
e-mail: shimazawa@gifu-pu.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_5, © Springer Japan 2014

reported to induce neuronal cell death and, moreover, to play roles in neurodegenerative diseases [3]. ER stress is caused by a number of biochemical and physiological stimuli that result in the accumulation of unfolded proteins in the ER lumen, and it is closely associated with the neuronal cell injury caused by vascular and neurodegenerative diseases such as stroke, Alzheimer disease, and Parkinson disease [1, 4, 5].

The ER is the cellular organelle in which secreted and transmembrane proteins are newly synthesized, posttranslationally modified, and properly folded to function. Agents or conditions that adversely affect ER protein folding lead to an accumulation of unfolded or misfolded proteins in the ER, a condition defined as ER stress. ER stress activates signaling pathways, including the unfolded protein response (UPR) that counteracts the effects of the original stress. The accumulation of unfolded or misfolded proteins in the ER causes ER stress; a complex signal-transduction cascade, known as UPR, is activated to cope with ER stress [2]. UPR is mediated by three types of ER transmembrane proteins: inositol-requiring enzyme 1 (IRE1), RNA-dependent protein kinase-like ER eukaryotic translation initiation factor 2α kinase (PERK), and activating transcription factor 6 (ATF6) [3], and the expression of both glucose-regulated protein 78 (GRP78)/BiP and C/EBP-homologous protein (CHOP) mRNAs is upregulated by the activation of these pathways (Fig. 5.1). UPR activates at least four pathways. One major component of the UPR is the elevated expression of molecular chaperones, such as GRP78/BiP, GRP94, and calreticulin, to increase protein folding activity and prevent protein aggregation [6]. Another component of UPR is the suppression of the protein burden through the global inhibition of translation. PERK is the sensor protein of UPR. The third component, ER-associated degradation, is extensive degradation of unfolded proteins. The final pathway induced by ER stress, CHOP, triggers cell cycle arrest and apoptosis [7]. ER stress can be induced by agents or conditions that interfere with (a) protein glycosylation (e.g., glucose starvation, tunicamycin, glucosamine), (b) disulfide-bond formation (e.g., DTT, homocysteine), (c) Ca^{2+} balance (A23187, thapsigargin, EGTA), and/or (d) a general overloading of the ER with proteins (e.g., viral or nonviral oncogenesis) [3, 6, 8].

5.1.1 ER Stress and Retinal Ganglion Cell Death

Retinal ganglion cell (RGC) death is a common feature of many ophthalmic disorders such as glaucoma, optic neuropathies, and retinovascular diseases, such as diabetic retinopathy and retinal vein occlusions. RGC death has been reported to occur via a variety of mechanisms involving, for example, oxidative stress [9], excitatory amino acids [10], nitric oxide (NO) [11], and apoptosis [12]. Glutamate, one of the excitatory amino acids, is the main neurotransmitter in the retinal signaling pathway. Excessive glutamate increases both intracellular Ca^{2+} and NO production through activation of the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor, resulting in retinal cell death [13, 14]. However, little is known about the

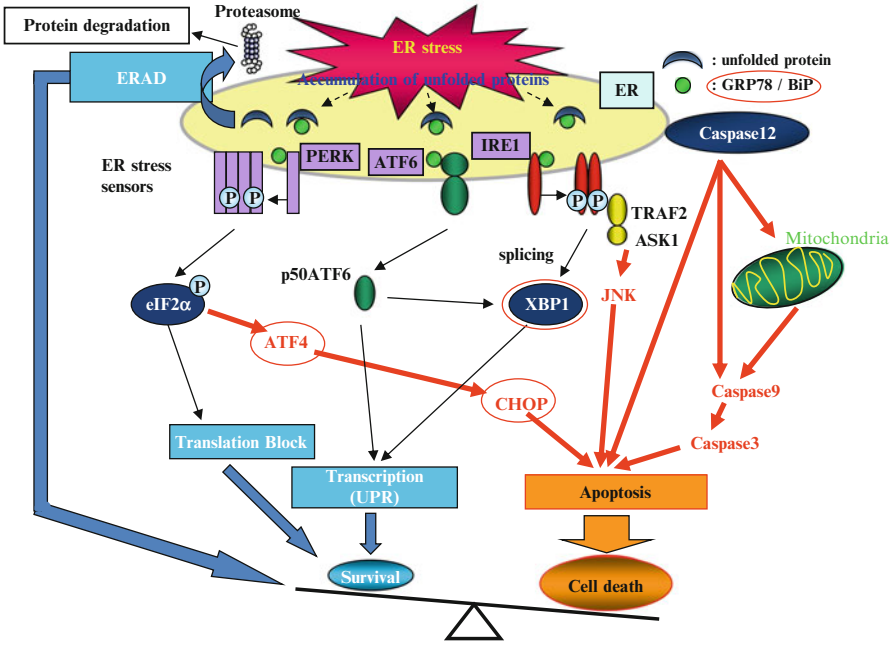


Fig. 5.1 ER-stress-signal pathways. *BiP*, glucose-regulated protein (GRP)78/BiP; *eIF2*α, eukaryotic initiation factor 2α; *CHOP*, C/EBP-homologous protein; *XBP-1*, X-box-binding protein 1; *ATF4*, activating transcription factor 4; *TRAF2*, TNF receptor-associated factor 2; *ASK1*, apoptosis signal-regulating kinase 1; *JNK*, c-Jun NH(2)-terminal kinase; *IRE1*, inositol-requiring enzyme 1; *PERK*, PKR-like ER kinase

role, if any, of ER stress in retinal damage. Recently, Uehara et al. [15] reported that in primary cortical culture, even mild exposure of NMDA induces apoptotic cell death. They demonstrated to be caused by an accumulation of polyubiquitinated proteins and increases in X-box-binding protein (XBP-1) mRNA splicing and CHOP mRNA, representing activation of the UPR signaling pathway. They also found that protein-disulfide isomerase (PDI), which assists in the maturation and transport of unfolded secretory proteins, prevented the neurotoxicity associated with ER stress. They suggest that neurodegenerative disorders might be mediated by S-nitrosylation of PDI, which would reduce its enzymatic activity. Their results strongly suggest that the activation of ER stress may participate in the retinal cell death occurring after NMDA-receptor activation and/or ischemic insult.

NMDA receptors may participate in the processes of excitotoxicity and neuronal death in the retina [16, 17]. Previous studies have found that TUNEL-positive cells can be observed in the retinal ganglion cell layer (GCL) and inner nuclear layer (INL) of the mouse retina at an early stage (within 24 h) after an intravitreal injection of NMDA [18, 19]. The hallmark of NMDA-induced neuronal death is a sustained increase in the intracellular Ca^{2+} concentration accompanied by overactivation of vital Ca^{2+}-dependent cellular enzymes [20]. Thus, the signal-transduction

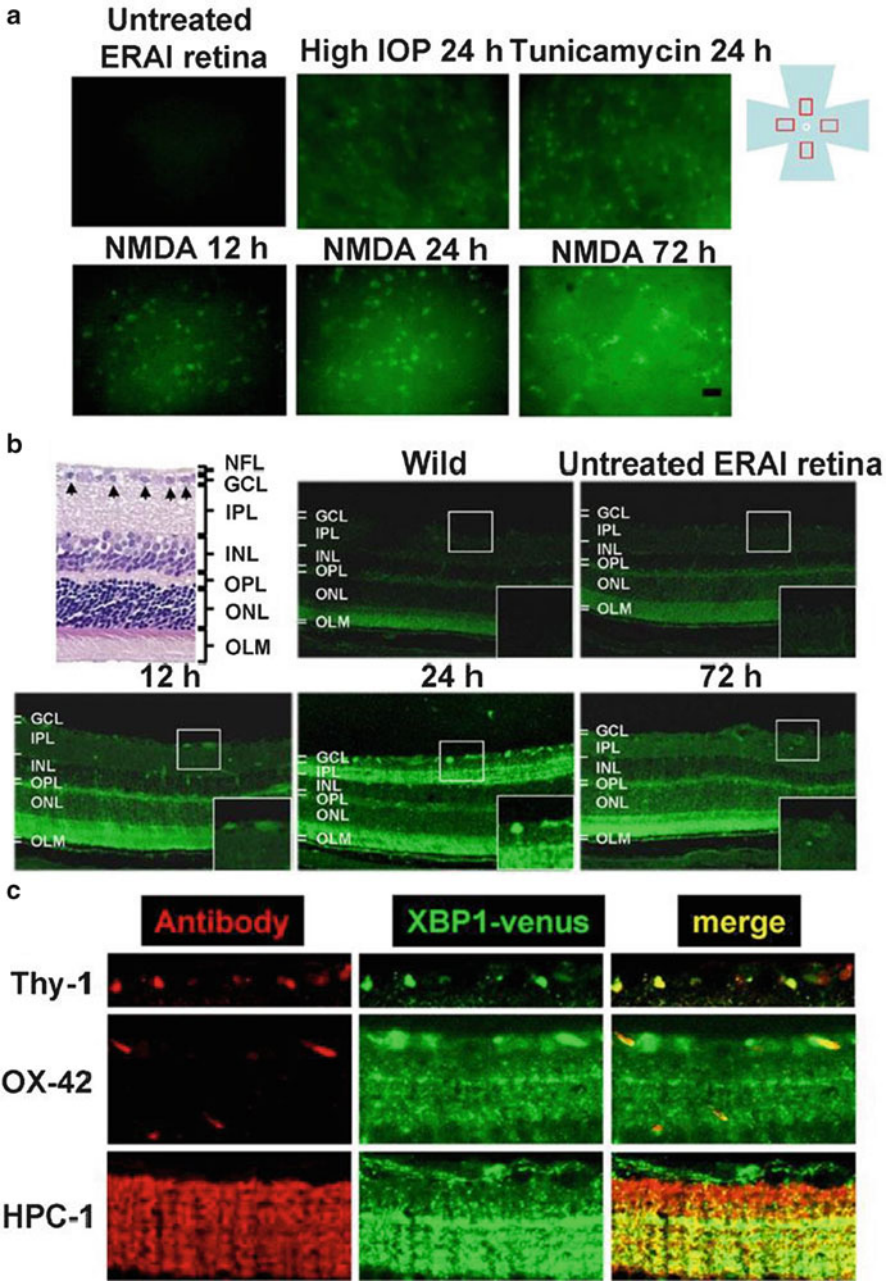


Fig. 5.2 Expression and localization of XBP-1–venus fusion protein in ERAI mouse retinas after various types of retinal damage. (**a**) Representative fluorescence photographs of increased XBP-1–venus fusion protein in ERAI mouse flat-mounted retina after *N*-methyl-D-aspartate (NMDA), intraocular pressure (IOP) elevation, or tunicamycin insult. The fluorescence (*green*) arising from

pathways for NMDA-mediated cell death in the retina are well studied, but not yet fully understood. To illuminate the role and distribution of ER stress in vivo, we focused on the retina of ER-stress-activated indicator (ERAI) transgenic mice carrying a human XBP-1 and venus, a variant of green fluorescent protein (GFP) fusion gene, in which effective identification of cells under ER-stress conditions is possible in vivo, as described in our previous report [21]. In flat-mounted retinas, fluorescence arising from the XBP-1–venus fusion protein was detected following various stimulations [tunicamycin, NMDA, and intraocular pressure (IOP) elevation] (Fig. 5.2). To our knowledge, this is the first report demonstrating that NMDA and ischemic insult (elevating IOP), in addition to tunicamycin, can activate the ER-stress signal (measured as the splicing of the XBP-1 and venus fusion gene in ERAI transgenic mice) in the retina in vivo. Interestingly, ER stress was also induced in the retina after a transient IOP elevation, defined as an ischemia–reperfusion model. It has been reported that this model exhibits retinal cell damage similar to that induced by NMDA and that both of these examples of damage are protected against by dizocilpine, an NMDA-receptor antagonist, and by NO synthetase-inhibitor treatment [13, 22]. Although little is known about the precise mechanisms responsible for activation of ER stress after NMDA or IOP elevation (ischemia–reperfusion), both stimuli cause intracellular Ca^{2+} overload and increased NO production, resulting in apoptotic cell death. Several lines of study suggest that intracellular Ca^{2+} overload and excessive production of NO deplete Ca^{2+} in the ER, thereby resulting in ER stress [23, 24]. Uehara et al. [15] reported that NO induces S-nitrosylation of PDI, an enzyme that assists in the maturation and transport of unfolded secretory proteins and thereby helps to prevent the neurotoxicity associated with ER stress. S-nitrosylated PDI exhibits reduced enzymatic activity and induces cell death through the ER-stress pathway. These mechanisms may contribute to the activation of ER stress in the retina after NMDA stimulation or IOP elevation. Accordingly, our findings may provide important new insights into the mechanisms underlying the retinal cell damage induced by NMDA and by ischemia–reperfusion. In transverse retinal sections, we observed an increase in fluorescence intensity within the cells of the GCL and IPL at 12 and 24 h, respectively, after NMDA injection. The cells displaying increased fluorescence were ganglion cells (at 12 h after the injection), amacrine cells in IPL (at 24 h), and

Fig. 5.2 (continued) XBP-1–venus fusion protein was observed under an epifluorescence microscope. The scale bar represents 25 μm. (**b**) Distribution of increased XBP-1–venus fusion protein in retinal cross sections from ERAI mice after NMDA injection at 40 nmol/eye. The distribution of fluorescence (*green*) arising from XBP-1–venus fusion protein was observed under a laser confocal microscope. Each large box shows an enlargement of the area within the corresponding small box. (**c**) Localization of XBP-1–venus fusion protein in ERAI mouse retina after NMDA injection. In the retinal nerve fiber layer (*upper panels*), Thy-1-positive cells (*red*) can be seen to merge with XBP-1–venus fusion protein (*green*). In the *middle panels*, OX-42 (a microglia marker)-positive cells (*red*) are partly merged with XBP-1–venus fusion protein (*green*). In the inner plexiform layer (*lower panels*), HPC-1 (an amacrine marker)-positive cells (*red*) are merged with XBP-1–venus fusion protein (*green*). Modified from Shimazawa et al. [95]

microglia in GCL (at 72 h). These data indicate that ganglion cells may be more sensitive to ER stress than the other retinal cells examined. To further clarify the participation of ER stress, we examined the changes in GRP78/BiP and CHOP in the retina after NMDA-induced injury. We found (a) that NMDA induced GRP78/BiP proteins in the retina at 12 h after its injection (on the basis of immunoblots) and (b) that NMDA induced both GRP78/BiP and CHOP in the retina (especially within retinal ganglion cells and INL) at 12 h after its injection (on the basis of our immunostaining results). The expression of the CHOP gene reportedly increases in the rat retina after intravitreal injection of NMDA [25]. Furthermore, Awai et al. [26] found that treatment with MK-801, an NMDA-receptor antagonist, inhibited the increases in CHOP mRNA and protein in the mouse retina that are observed after intravitreal injection of NMDA and moreover that CHOP-deficient mice were resistant to NMDA-induced retinal damage. However, CHOP-deficient mice partially suppressed the NMDA-induced cell death, and therefore other pathways, such as mitochondrial dysfunction, may be engaged in the retinal cell death. Collectively, the above results indicate that NMDA can cause ER stress in the retina and that the neurotoxicity induced by NMDA is due in part to a mechanism dependent on CHOP protein induction through excessive ER stress. In conclusion, we have identified a close association between ER stress and retinal damage, and these results suggest that the ER-stress-signal pathway might be a good target in the treatment of retinal diseases.

5.1.2 ER Stress in Glaucoma

Glaucoma is a multifactorial optic neuropathy characterized by RGC death [27]. This irreversible RGC death results in progressive visual field loss along with decreased color sensitivity and contrast [28]. Although RGC death can be observed in patients with normal ocular tension [29], if genetic, environmental, and other factors are involved [30], elevated IOP is a recognized risk factor for RGC degeneration in glaucoma. At present, the only well-established treatment of glaucoma involves lowering the IOP; however, visual field loss continues to progress in a subset of glaucoma patients even if medical and surgical treatments successfully lower the IOP [31]. Thus, new approaches to treating glaucoma, such as directly preventing RGC death, have been required in addition to regulating IOP, but the associated pathological mechanisms remain unclear. Therefore, further studies will be needed to clarify the precise mechanisms in glaucoma pathogenesis.

Previous studies suggest that there is a significantly higher rate of glaucoma occurrence among patients with Alzheimer disease (AD), the most common form of dementia, than control subjects, suggesting a possible relationship between these two diseases [32]. Briefly, Bayer et al. [32] noted that Alzheimer's patients had a greater rate of glaucoma occurrence (25.9 %) than a control group (5.2 %). Subsequently, we reported (a) that the concentrations of $A\beta_{1-42}$ and tau were decreased and increased, respectively, in vitreous fluid from patients with glaucoma and

diabetic retinopathy (vs. macular hole controls) [33], (b) that in AD transgenic mice (Tg2576/PS1 mutant), there was a significant decrease in the visual function [34], and (c) that β-secretase inhibitors reduced glutamate-induced cell death in rat primary cultured retinal ganglion cells [35]. On the other hand, a chronic elevation of IOP induces Aβ in RGCs in experimental rat glaucoma [30]. This result is consistent with some reports on experimental glaucoma models of rats [36] and mice [37]. Furthermore, Guo et al. [36] reported that neutralizing antibody to Aβ significantly delays and attenuates RGC apoptosis in experimental glaucoma. These findings indicate that $A\beta_{1-42}$ neurotoxicity as AD brain may be involved in RGC death in glaucoma; however, the study by Guo and colleagues [36] did not use primate, but rodents (mouse and rat) for the study of experimental glaucoma. Recently, we found that the expression of $A\beta_{1-42}$ was increased in the retina and the optic nerve head (ONH) of monkeys with experimental glaucoma [38]. Thus, accumulations of Aβ in the retina and ONH may be involved in glaucoma pathogenesis. So far, many researchers [39–42] reported that the accumulation of Aβ may be associated with ER stress in the brains of AD model mice and AD patients; therefore, ER stress may be closely related to RGC death in glaucoma. Doh et al. [43] investigated whether ER stress induced RGC death in chronic ocular hypertension, one of the RGC death mechanisms, using an experimental glaucoma rat model and demonstrated that GRP78/BiP, p-PERK, and CHOP were significantly expressed in the retina with chronic IOP elevation. This finding strongly suggests that ER stress is involved in RGC death in glaucoma, and the PERK–p-eIF2α–CHOP pathway plays a role in the RGC death associated with ER stress. Recently, Yang et al. [44] reported that the expression of various ER-resident proteins, including GRP78/BiP and PDI, and ER sensor proteins detecting UPR including ATF6 and IRE1 was increased in the retina of glaucoma patients. These findings strongly supported the involvement of ER stress in glaucoma pathogenesis, but further studies will be needed to demonstrate it.

5.1.3 ER Stress in Lateral Geniculate Nucleus of Glaucoma

Recent evidence indicates that glaucomatous damage extends from the retina to the visual center of the brain, including the lateral geniculate nucleus (LGN) and the primary visual cortex [45–47]. In particular, neuronal damage in the LGN in monkey glaucoma models can be detected in the early phase (the first few weeks) after IOP elevation [48–50]. Furthermore, relay neurons, a type of LGN neuron that proceed to synapse in the visual cortex, were more vulnerable than the other types of LGN neurons after IOP elevation [51, 52]. In addition, most of the degenerative and compensatory changes in the LGN occur in the relay neurons after total deafferentation [53, 54]. Therefore, protecting relay and retinal neurons may be effective in preventing blindness in glaucoma cases because visual information entering the eye is processed in the retina and then transmitted to the LGN, from where signals are relayed to the visual cortex.

In the LGN, the relay neurons predominately express the NMDA receptor [55–58]. In glaucoma models, excessive activation of this receptor leads to an overload of intracellular Ca^{2+} [59, 60]. Such elevations in Ca^{2+} elicit the activation of NO formation through NO-synthase activation [15, 61–63]. NO induces S-nitrosylation of PDI and inhibits its enzymatic activity as described in Sect. 5.1.1 [15]. As a consequence, this leads to excess accumulation of misfolded or unfolded proteins within the ER that may lead to ER-stress-induced cell death via the p-eIF2α–CHOP signal pathway [15, 64–66]. The involvement of ER stress in relay neuronal atrophy and death within the LGN via the NMDA–Ca^{2+}–NO synthase/NO pathway after IOP elevation supports our results: memantine, an NMDA antagonist, and lomerizine, a Ca^{2+} channel blocker, reduced neuronal atrophy in the LGN after retinal damage [60, 67]. Furthermore, we demonstrated that TUNEL-positive apoptotic cells and the production of ER-stress-related proteins were increased in LGN neurons of glaucoma monkeys [68]. Regarding the localization of ER-stress-related proteins, parvalbumin-positive relay neurons in the LGN layers connected to the eye with elevated IOP were found to express p-eIF2a and CHOP at 11–24 weeks after chronic IOP elevation. Delayed neuronal death in these regions progressed gradually following RGC axon loss by IOP elevation. As expression of the ER-stress-related proteins measured here may be detected before dying cells at each sampling point (i.e., at 4, 11, 15, and 24 weeks after IOP elevation), the number of cells positive for ER-stress markers may be relatively low in comparison with the number (and extent) of parvalbumin-positive relay neuron loss in the LGN. The total number of detectable neuron loss in the LGN, but not cells positive for ER-stress markers, increased during the study period. Therefore, the number of ER-stress-positive cells in the LGN at each sampling point may be smaller than those undergoing neuronal cell death. In this context, it is noteworthy that the timing of the upregulation of p-eIF2α and CHOP proteins coincided with the timing of the decrease in neuronal cells during the study period and these ER-stress-related proteins were co-localized with parvalbumin-positive relay neurons. Furthermore, the increase in polyubiquitinated proteins was preceded by an increase in TUNEL-positive cells in the LGN after the laser photocoagulation treatment during the study period. These findings indicate that excessive ER stress (caused by the accumulation of misfolded or unfolded proteins) induces LGN neuronal death via the activation of the ER-dependent apoptotic pathway, suggesting that the ER-stress pathway may play an important role in LGN neuronal death after IOP elevation. On the other hand, an increase in GRP78/BiP was not detected in the LGN after IOP elevation during the study period as assessed by immunostaining (data not shown). A possible explanation for this observation is a direct cytotoxic effect of p-eIF2α [69] and activation of the caspase-3 pathway [70–72], whereby p-eIF2α directly mediates apoptosis in response to activation of the double-stranded RNA-dependent protein kinase (PKR). In fact, we have previously shown that inhibition of PKR activation was neuroprotective against ER-stress-induced RGC death [73]. Although GRP78/BiP expression requires further studies, the present data suggest that impaired induction of antiapoptotic GRP78/BiP is accompanied by a strong induction of proapoptotic signal in the ER, indicating a signal imbalance

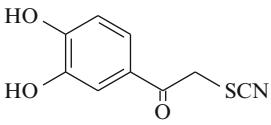


Fig. 5.3 Chemical structure of 1-(3,4-dihydroxyphenyl)-2-thiocyanate-ethanone (BIX)

leaning toward cell death. We have previously shown that a preferential inducer of GRP78/BiP exhibited the potential to be a therapeutic agent for ER-stress-induced retinal diseases [74–76]. In conclusion, the present study indicates that ER stress may be involved in LGN neuronal death after IOP elevation and the upregulation of p-eIF2α and CHOP protein levels in the parvalbumin-positive relay neurons may play roles in the cell death process induced by high IOP in monkey. These findings also indicate that ER stress induced by retinal damage may play a pivotal role in the pathogenesis of the blindness caused by retinal diseases such as glaucoma.

5.1.4 ER Stress Targeting Agents for Neuroprotection in Glaucoma

GRP78/BiP, a highly conserved member of the 70 kDa heat-shock protein family, is one of the chaperones localized to the ER membrane [23, 77], and it is a major ER-luminal Ca^{2+}-storage protein [78, 79]. GRP78/BiP works to restore folding in misfolded or incompletely assembled proteins [80–82], the interaction between BiP and misfolded proteins being dependent on its hydrophobic motifs [83–85]. Proteins stably bound to BiP are subsequently translocated from the ER into the cytosol, where they are degraded by proteasomes [86, 87]. Previous reports have show that induction of BiP prevents the neuronal death induced by ER stress [88–91]. Hence, a selective inducer of BiP might attenuate ER stress and be a new, useful therapeutic agent for the treatment of ER-stress-associated diseases. This seemed an interesting idea, and we recently identified BiP inducer X (BIX, Fig. 5.3) while screening for low-molecular-mass compounds that might induce BiP using high-throughput screening with a BiP reporter assay system (Dual-Luciferase Reporter Assay; Promega Corporation, Madison, WI) [74]. We found that BIX preferentially induced BiP mRNA and protein in SK-N-SH cells and reduced tunicamycin-induced cell death. Intracerebroventricular pretreatment with BIX reduced the infarction size after focal cerebral ischemia in mice. In view of the retinal research described above, we wondered whether BIX might reduce the retinal ganglion cell loss and CHOP expression induced by tunicamycin or NMDA treatment.

BIX preferentially induced GRP78/BiP mRNA in RGC-5 (a retinal precursor cell line) [76]. Although it also induced GRP94, calreticulin, p58IPK, and ASNS, these inductions were lower than that of GRP78/BiP. This is consistent with our previous study that BIX preferentially induced BiP with slight inductions of GRP94, calreticulin, and CHOP mediated by the ATF6 pathway accompanied by

activation of ERSEs and that BIX does not affect the pathway downstream of IRE1 or the translational control branch downstream of PERK in SK-N-SH cells [74]. Therefore, BIX is not just an ER stressor such as tunicamycin or thapsigargin, and we consider that the induction of GRP78/BiP by BIX is mediated by the ATF6 pathway in RGC-5 similar to that in SK-N-SH cells. Next, we evaluated the effects of BIX, as a preferential inducer of GRP78/BiP, on ER-stress-induced in vitro cell death in RGC-5 and in vivo retinal damage in mice. BIX reduced tunicamycin-induced cell death in RGC-5 and also reduced both tunicamycin-induced and NMDA-induced retinal damage in mice [76]. Our previous study revealed that BIX (a) reduced tunicamycin-induced cell death in SK-N-SH cells, (b) contributed to the induction of GRP78/BiP expression via the ATF-6 pathway (but not via the PERK or IRE1 pathways), and (c), on intracerebroventricular injection, prevented the neuronal damage induced by focal ischemia in mice [74]. Furthermore, immunostaining revealed that intravitreal injection of BIX significantly induced GRP78/BiP protein in mouse retina. On the other hand, there was little protective effect of BIX against RGC-5 damages after staurosporine treatment. Staurosporine is well known as a nonspecific inhibitor of protein kinases and initiates caspase-dependent apoptosis in many cell types [92, 93]. Our previous studies revealed that staurosporine induced cell death without any changes in the expression of BiP or CHOP protein [73, 94]. Furthermore, a preliminary study showed that treatment with BIX (1 and 5 μM) did not inhibit RGC-5 cell death 48 h after serum deprivation, which does not induce any UPR responses such as BiP or CHOP (unpublished data). These results strongly support that BIX selectively protects cell damage induced by ER stress. Recently, we reported that in mice, increased expressions of XBP-1 splicing, BiP, and CHOP could be detected after the induction of retinal damage by tunicamycin, NMDA, or an elevation of IOP [95]. That report was the first to demonstrate an involvement of ER stress and BiP in retinal cell death in mice. Hence, we asked whether BIX can prevent such retinal damage. By histologic analysis and TUNEL staining, we estimated that BIX reduced tunicamycin-induced retinal damage. Furthermore, we used Thy-1–CFP transgenic mice to examine the effect of BIX in a large retinal area [96]. This transgene contains a CFP gene under the direction of regulatory elements derived from the mouse Thy-1 gene, and the transgenic mice express CFP protein in RGC and in the inner part of the IPL of the retina [96]. BIX exerted protective effects against tunicamycin-induced retinal damage in the Thy-1–CFP transgenic mice (Fig. 5.4) and NMDA-induced retinal damage in ddY mice. NMDA is well known to induce RGC death and optic nerve loss (effects mediated by excitatory glutamate receptor), and such neuronal death is believed to play a role in many neurologic and neurodegenerative diseases [14, 97]. Uehara et al. [15] noted that mild exposure to NMDA induced apoptotic cell death in primary cortical culture, and they demonstrated this effect to be caused by an accumulation of polyubiquitinated proteins and increases in XBP-1 mRNA splicing and CHOP mRNA (reflecting activation of the UPR signaling pathway). They also found that PDI, which assists in the maturation and transport of unfolded secretory proteins, prevented the neurotoxicity associated with ER stress. These findings suggested that activation of ER stress

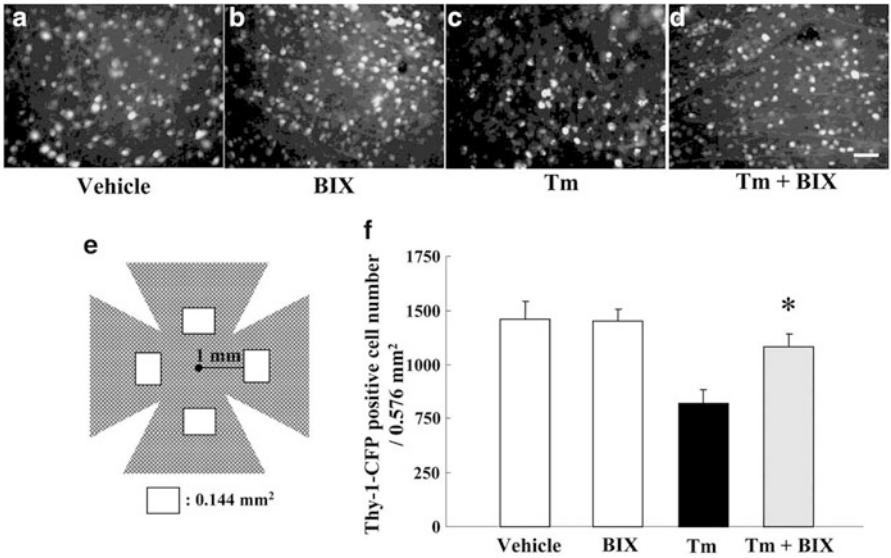


Fig. 5.4 Effects of BIX on retinal damage induced by intravitreal injection of tunicamycin (Tm) in Thy-1–CFP transgenic mice. Mouse retinas (flat mounts) at 7 days after intravitreal injection of (**a**) vehicle, (**b**) BIX (5 nmol), (**c**) Tm (1 μg), or (**d**) Tm (1 μg) plus BIX (5 nmol). Damage was evaluated by counting Thy-1–CFP-positive cell numbers in the four *white areas* shown in (**e**) (each area 0.144 $mm^2 \times 4$ areas; total 0.576 mm^2) at 7 days after the above intravitreal injections. (**f**) Effect of BIX against Tm-induced damage (indicated by decreased number of Thy-1–CFP-positive cells) at 7 days after intravitreal injection. Data are shown as mean ± SE ($n = 9$ or 10). $^*P < 0.05$ versus tunicamycin alone. Scale bar represents 25 μm. Modified from Inokuchi et al. [76]

may participate in the retinal cell death occurring after NMDA-receptor activation and/or an ischemic insult [15]. BIX also attenuated the CHOP protein expression induced by either tunicamycin or NMDA in the mouse retina in vivo. As mentioned above, BIX may affect CHOP protein expression through ATF6 pathway, but no change was observed in BIX-treated RGC-5. In SK-N-SH cells, BIX slightly increased CHOP mRNA only at 2 h after the treatment. Expression of CHOP is mainly regulated by three transcription factors—ATF4, cleaved ATF6, and XBP-1—which are downstream effectors during ER stress in similar to other ER chaperones. These differences between BiP and CHOP expression by BIX may be due to the difference of their promoters. CHOP promoter contains at least two ERSE motifs (CHOP ERSE-1 and CHOP ERSE-2) located in opposite directions with a 9 bp overlap, and one of ERSEs is inactive [98]. On the other hand, BiP promoter has three functional ERSE motifs of the rat GRP78/BiP promoter (ERSE-163, ERSE-131, and ERSE-98) [99]. These variations in each promoter may contribute to the differences among the expressions of ER chaperons induced by BIX and the lack of CHOP expression.

In conclusion, we have demonstrated that BIX, a preferential inducer of BiP, inhibits both the neuronal cell death induced by ER stress in vitro in RGC-5 cells and in vivo in the mouse retina. Hence, an increase in BiP might be one of the targets of mechanisms bestowing neuroprotection in retinal diseases.

References

1. Aridor M, Balch WE (1999) Integration of endoplasmic reticulum signaling in health and disease. Nat Med 5(7):745–751
2. Harding HP, Calfon M, Urano F, Novoa I, Ron D (2002) Transcriptional and translational control in the Mammalian unfolded protein response. Annu Rev Cell Dev Biol 18:575–599
3. Kaufman RJ (1999) Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev 13(10):1211–1233
4. Bando Y, Onuki R, Katayama T, Manabe T, Kudo T, Taira K, Tohyama M (2005) Double-strand RNA dependent protein kinase (PKR) is involved in the extrastriatal degeneration in Parkinson's disease and Huntington's disease. Neurochem Int 46(1):11–18
5. Gale M Jr, Katze MG (1998) Molecular mechanisms of interferon resistance mediated by viral-directed inhibition of PKR, the interferon-induced protein kinase. Pharmacol Ther 78 (1):29–46
6. Lee AS (2001) The glucose-regulated proteins: stress induction and clinical applications. Trends Biochem Sci 26(8):504–510
7. Oyadomari S, Mori M (2004) Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 11(4):381–389
8. Pahl HL (1999) Signal transduction from the endoplasmic reticulum to the cell nucleus. Physiol Rev 79(3):683–701
9. Bonne C, Muller A, Villain M (1998) Free radicals in retinal ischemia. Gen Pharmacol 30 (3):275–280
10. Dreyer EB (1998) A proposed role for excitotoxicity in glaucoma. J Glaucoma 7(1):62–67
11. Neufeld AH (1999) Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. Surv Ophthalmol 43(Suppl 1):S129–S135
12. McKinnon SJ (1997) Glaucoma, apoptosis, and neuroprotection. Curr Opin Ophthalmol 8 (2):28–37
13. Adachi K, Kashii S, Masai H, Ueda M, Morizane C, Kaneda K, Kume T, Akaike A, Honda Y (1998) Mechanism of the pathogenesis of glutamate neurotoxicity in retinal ischemia. Graefes Arch Clin Exp Ophthalmol 236(10):766–774
14. Sucher NJ, Lipton SA, Dreyer EB (1997) Molecular basis of glutamate toxicity in retinal ganglion cells. Vision Res 37(24):3483–3493. doi:10.1016/S0042-6989(97)00047-3
15. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, Lipton SA (2006) S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. Nature 441(7092):513–517
16. Sabel BA, Sautter J, Stoehr T, Siliprandi R (1995) A behavioral model of excitotoxicity: retinal degeneration, loss of vision, and subsequent recovery after intraocular NMDA administration in adult rats. Exp Brain Res 106(1):93–105
17. Siliprandi R, Canella R, Carmignoto G, Schiavo N, Zanellato A, Zanoni R, Vantini G (1992) N-methyl-D-aspartate-induced neurotoxicity in the adult rat retina. Vis Neurosci 8(6):567–573
18. Hara A, Niwa M, Kumada M, Kitaori N, Yamamoto T, Kozawa O, Mori H (2004) Fragmented DNA transport in dendrites of retinal neurons during apoptotic cell death. Brain Res 1007(1–2):183–187
19. Li Y, Schlamp CL, Nickells RW (1999) Experimental induction of retinal ganglion cell death in adult mice. Invest Ophthalmol Vis Sci 40(5):1004–1008

20. Fukunaga K, Soderling TR, Miyamoto E (1992) Activation of Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. J Biol Chem 267(31):22527–22533
21. Iwawaki T, Akai R, Kohno K, Miura M (2004) A transgenic mouse model for monitoring endoplasmic reticulum stress. Nat Med 10(1):98–102
22. Lam TT, Siew E, Chu R, Tso MO (1997) Ameliorative effect of MK-801 on retinal ischemia. J Ocul Pharmacol Ther 13(2):129–137
23. Li WW, Alexandre S, Cao X, Lee AS (1993) Transactivation of the grp78 promoter by Ca^{2+} depletion. A comparative analysis with A23187 and the endoplasmic reticulum Ca(2+)-ATPase inhibitor thapsigargin. J Biol Chem 268(16):12003–12009
24. Oyadomari S, Araki E, Mori M (2002) Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells. Apoptosis 7(4):335–345
25. Laabich A, Li G, Cooper NG (2001) Characterization of apoptosis-genes associated with NMDA mediated cell death in the adult rat retina. Brain Res Mol Brain Res 91(1–2):34–42
26. Awai M, Koga T, Inomata Y, Oyadomari S, Gotoh T, Mori M, Tanihara H (2006) NMDA-induced retinal injury is mediated by an endoplasmic reticulum stress-related protein, CHOP/GADD153. J Neurochem 96(1):43–52
27. Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90(3):262–267. doi:10.1136/bjo.2005.081224
28. Mozaffarieh M, Grieshaber MC, Flammer J (2008) Oxygen and blood flow: players in the pathogenesis of glaucoma. Mol Vis 14:224–233
29. Tomita G (2000) The optic nerve head in normal-tension glaucoma. Curr Opin Ophthalmol 11 (2):116–120
30. McKinnon SJ, Lehman DM, Kerrigan-Baumrind LA, Merges CA, Pease ME, Kerrigan DF, Ransom NL, Tahzib NG, Reitsamer HA, Levkovitch-Verbin H, Quigley HA, Zack DJ (2002) Caspase activation and amyloid precursor protein cleavage in rat ocular hypertension. Invest Ophthalmol Vis Sci 43(4):1077–1087
31. The effectiveness of intraocular pressure reduction in the treatment of normal-tension glaucoma. Collaborative normal-tension glaucoma study group (1998) Am J Ophthalmol 126 (4):498–505. doi:S0002939498002724
32. Bayer AU, Ferrari F, Erb C (2002) High occurrence rate of glaucoma among patients with Alzheimer's disease. Eur Neurol 47(3):165–168
33. Yoneda S, Hara H, Hirata A, Fukushima M, Inomata Y, Tanihara H (2005) Vitreous fluid levels of beta-amyloid((1-42)) and tau in patients with retinal diseases. Jpn J Ophthalmol 49 (2):106–108
34. Shimazawa M, Inokuchi Y, Okuno T, Nakajima Y, Sakaguchi G, Kato A, Oku H, Sugiyama T, Kudo T, Ikeda T, Takeda M, Hara H (2008) Reduced retinal function in amyloid precursor protein-over-expressing transgenic mice via attenuating glutamate-N-methyl-d-aspartate receptor signaling. J Neurochem 107(1):279–290. doi:10.1111/j.1471-4159.2008.05606.x, JNC5606 [pii]
35. Yamamoto R, Yoneda S, Hara H (2004) Neuroprotective effects of beta-secretase inhibitors against rat retinal ganglion cell death. Neurosci Lett 370(1):61–64
36. Guo L, Salt TE, Luong V, Wood N, Cheung W, Maass A, Ferrari G, Russo-Marie F, Sillito AM, Cheetham ME, Moss SE, Fitzke FW, Cordeiro MF (2007) Targeting amyloid-beta in glaucoma treatment. Proc Natl Acad Sci U S A 104(33):13444–13449. doi:10.1073/pnas.0703707104, 0703707104 [pii]
37. Goldblum D, Kipfer-Kauer A, Sarra GM, Wolf S, Frueh BE (2007) Distribution of amyloid precursor protein and amyloid-beta immunoreactivity in DBA/2J glaucomatous mouse retinas. Invest Ophthalmol Vis Sci 48(11):5085–5090. doi:10.1167/iovs.06-1249
38. Ito Y, Shimazawa M, Tsuruma K, Mayama C, Ishii K, Onoe H, Aihara M, Araie M, Hara H (2012) Induction of amyloid-beta(1-42) in the retina and optic nerve head of chronic ocular hypertensive monkeys. Mol Vis 18:2647–2657

39. Soejima N, Ohyagi Y, Nakamura N, Himeno E, Iinuma KM, Sakae N, Yamasaki R, Tabira T, Murakami K, Irie K, Kinoshita N, LaFerla FM, Kiyohara Y, Iwaki T, Kira J (2013) Intracellular accumulation of toxic turn amyloid-beta is associated with endoplasmic reticulum stress in Alzheimer's disease. Curr Alzheimer Res 10(1):11–20
40. Lee JH, Won SM, Suh J, Son SJ, Moon GJ, Park UJ, Gwag BJ (2010) Induction of the unfolded protein response and cell death pathway in Alzheimer's disease, but not in aged Tg2576 mice. Exp Mol Med 42(5):386–394. doi:10.3858/emm.2010.42.5.040
41. Hosoi T, Ozawa K (2012) Molecular approaches to the treatment, prophylaxis, and diagnosis of Alzheimer's disease: endoplasmic reticulum stress and immunological stress in pathogenesis of Alzheimer's disease. J Pharmacol Sci 118(3):319–324
42. Scheper W, Nijholt DA, Hoozemans JJ (2011) The unfolded protein response and proteostasis in Alzheimer disease: preferential activation of autophagy by endoplasmic reticulum stress. Autophagy 7(8):910–911
43. Doh SH, Kim JH, Lee KM, Park HY, Park CK (2010) Retinal ganglion cell death induced by endoplasmic reticulum stress in a chronic glaucoma model. Brain Res 1308:158–166. doi:10.1016/j.brainres.2009.10.025
44. Yang X, Luo C, Cai J, Powell DW, Yu D, Kuehn MH, Tezel G (2011) Neurodegenerative and inflammatory pathway components linked to TNF-alpha/TNFR1 signaling in the glaucomatous human retina. Invest Ophthalmol Vis Sci 52(11):8442–8454. doi:10.1167/iovs.11-8152
45. Gupta N, Yucel YH (2003) Brain changes in glaucoma. Eur J Ophthalmol 13(Suppl 3): S32–S35
46. Vrabec JP, Levin LA (2007) The neurobiology of cell death in glaucoma. Eye (Lond) 21(Suppl 1):S11–S14. doi:10.1038/sj.eye.6702880
47. Yucel Y, Gupta N (2008) Glaucoma of the brain: a disease model for the study of transsynaptic neural degeneration. Prog Brain Res 173:465–478. doi:10.1016/S0079-6123(08)01132-1
48. Weber AJ, Chen H, Hubbard WC, Kaufman PL (2000) Experimental glaucoma and cell size, density, and number in the primate lateral geniculate nucleus. Invest Ophthalmol Vis Sci 41 (6):1370–1379
49. Ito Y, Shimazawa M, Chen YN, Tsuruma K, Yamashima T, Araie M, Hara H (2009) Morphological changes in the visual pathway induced by experimental glaucoma in Japanese monkeys. Exp Eye Res 89(2):246–255. doi:10.1016/j.exer.2009.03.013
50. Shimazawa M, Ito Y, Inokuchi Y, Yamanaka H, Nakanishi T, Hayashi T, Ji B, Higuchi M, Suhara T, Imamura K, Araie M, Watanabe Y, Onoe H, Hara H (2012) An alteration in the lateral geniculate nucleus of experimental glaucoma monkeys: in vivo positron emission tomography imaging of glial activation. PLoS One 7(1):e30526
51. Jones EG, Hendry SH (1989) Differential calcium binding protein immunoreactivity distinguishes classes of relay neurons in monkey thalamic nuclei. Eur J Neurosci 1(3):222–246
52. Johnson JK, Casagrande VA (1995) Distribution of calcium-binding proteins within the parallel visual pathways of a primate (*Galago crassicaudatus*). J Comp Neurol 356(2):238–260. doi:10.1002/cne.903560208
53. Le Vay S (1971) On the neurons and synapses of the lateral geniculate nucleus of the monkey, and the effects of eye enucleation. Z Zellforsch Mikrosk Anat 113(3):396–419
54. Somogyi J, Eysel U, Hamori J (1987) A quantitative study of morphological reorganization following chronic optic deafferentation in the adult cat dorsal lateral geniculate nucleus. J Comp Neurol 255(3):341–350. doi:10.1002/cne.902550303
55. Salt TE (1986) Mediation of thalamic sensory input by both NMDA receptors and non-NMDA receptors. Nature 322(6076):263–265. doi:10.1038/322263a0
56. Salt TE (1987) Excitatory amino acid receptors and synaptic transmission in the rat ventrobasal thalamus. J Physiol 391:499–510
57. Scharfman HE, Lu SM, Guido W, Adams PR, Sherman SM (1990) N-methyl-D-aspartate receptors contribute to excitatory postsynaptic potentials of cat lateral geniculate neurons recorded in thalamic slices. Proc Natl Acad Sci U S A 87(12):4548–4552

58. Jones EG, Tighilet B, Tran BV, Huntsman MM (1998) Nucleus- and cell-specific expression of NMDA and non-NMDA receptor subunits in monkey thalamus. J Comp Neurol 397(3):371–393
59. Yucel YH, Gupta N, Zhang Q, Mizisin AP, Kalichman MW, Weinreb RN (2006) Memantine protects neurons from shrinkage in the lateral geniculate nucleus in experimental glaucoma. Arch Ophthalmol 124(2):217–225. doi:10.1001/archopht.124.2.217
60. Ito Y, Nakamura S, Tanaka H, Shimazawa M, Araie M, Hara H (2008) Memantine protects against secondary neuronal degeneration in lateral geniculate nucleus and superior colliculus after retinal damage in mice. CNS Neurosci Ther 14(3):192–202. doi:10.1111/j.1755-5949.2008.00050.x
61. Lipton SA, Singel DJ, Stamler JS (1994) Nitric oxide in the central nervous system. Prog Brain Res 103:359–364
62. Wang X, Sam-Wah Tay S, Ng YK (2000) Nitric oxide, microglial activities and neuronal cell death in the lateral geniculate nucleus of glaucomatous rats. Brain Res 878(1–2):136–147
63. Nucci C, Morrone L, Rombola L, Nistico R, Piccirilli S, Cerulli L (2003) Multifaceted roles of nitric oxide in the lateral geniculate nucleus: from visual signal transduction to neuronal apoptosis. Toxicol Lett 139(2–3):163–173
64. Ilieva EV, Ayala V, Jove M, Dalfo E, Cacabelos D, Povedano M, Bellmunt MJ, Ferrer I, Pamplona R, Portero-Otin M (2007) Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain 130(Pt 12):3111–3123
65. Okada K, Minamino T, Tsukamoto Y, Liao Y, Tsukamoto O, Takashima S, Hirata A, Fujita M, Nagamachi Y, Nakatani T, Yutani C, Ozawa K, Ogawa S, Tomoike H, Hori M, Kitakaze M (2004) Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. Circulation 110(6):705–712
66. Kim I, Xu W, Reed JC (2008) Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 7(12):1013–1030
67. Ito Y, Nakamura S, Tanaka H, Tsuruma K, Shimazawa M, Araie M, Hara H (2010) Lomerizine, a Ca^{2+} channel blocker, protects against neuronal degeneration within the visual center of the brain after retinal damage in mice. CNS Neurosci Ther 16(2):103–114. doi:10.1111/j.1755-5949.2009.00081.x
68. Ito Y, Shimazawa M, Inokuchi Y, Yamanaka H, Tsuruma K, Imamura K, Onoe H, Watanabe Y, Aihara M, Araie M, Hara H (2011) Involvement of endoplasmic reticulum stress on neuronal cell death in the lateral geniculate nucleus in the monkey glaucoma model. Eur J Neurosci 33(5):843–855. doi:10.1111/j.1460-9568.2010.07578.x
69. Srivastava SP, Kumar KU, Kaufman RJ (1998) Phosphorylation of eukaryotic translation initiation factor 2 mediates apoptosis in response to activation of the double-stranded RNA-dependent protein kinase. J Biol Chem 273(4):2416–2423
70. Martin LJ, Kaiser A, Yu JW, Natale JE, Al-Abdulla NA (2001) Injury-induced apoptosis of neurons in adult brain is mediated by p53-dependent and p53-independent pathways and requires Bax. J Comp Neurol 433(3):299–311
71. Martin LJ, Price AC, McClendon KB, Al-Abdulla NA, Subramaniam JR, Wong PC, Liu Z (2003) Early events of target deprivation/axotomy-induced neuronal apoptosis in vivo: oxidative stress, DNA damage, p53 phosphorylation and subcellular redistribution of death proteins. J Neurochem 85(1):234–247
72. Repici M, Atzori C, Migheli A, Vercelli A (2003) Molecular mechanisms of neuronal death in the dorsal lateral geniculate nucleus following visual cortical lesions. Neuroscience 117(4):859–867
73. Shimazawa M, Ito Y, Inokuchi Y, Hara H (2007) Involvement of double-stranded RNA-dependent protein kinase in ER stress-induced retinal neuron damage. Invest Ophthalmol Vis Sci 48(8):3729–3736. doi:10.1167/iovs.06-1122

74. Kudo T, Kanemoto S, Hara H, Morimoto N, Morihara T, Kimura R, Tabira T, Imaizumi K, Takeda M (2008) A molecular chaperone inducer protects neurons from ER stress. Cell Death Differ 15(2):364–375. doi:10.1038/sj.cdd.4402276
75. Oida Y, Izuta H, Oyagi A, Shimazawa M, Kudo T, Imaizumi K, Hara H (2008) Induction of BiP, an ER-resident protein, prevents the neuronal death induced by transient forebrain ischemia in gerbil. Brain Res 1208:217–224. doi:10.1016/j.brainres.2008.02.068
76. Inokuchi Y, Nakajima Y, Shimazawa M, Kurita T, Kubo M, Saito A, Sajiki H, Kudo T, Aihara M, Imaizumi K, Araie M, Hara H (2009) Effect of an inducer of BiP, a molecular chaperone, on endoplasmic reticulum (ER) stress-induced retinal cell death. Invest Ophthalmol Vis Sci 50(1):334–344. doi:10.1167/iovs.08-2123
77. Lee YK, Brewer JW, Hellman R, Hendershot LM (1999) BiP and immunoglobulin light chain cooperate to control the folding of heavy chain and ensure the fidelity of immunoglobulin assembly. Mol Biol Cell 10(7):2209–2219
78. van de Put FH, Elliott AC (1997) The endoplasmic reticulum can act as a functional Ca^{2+} store in all subcellular regions of the pancreatic acinar cell. J Biol Chem 272(44):27764–27770
79. Lievremont JP, Rizzuto R, Hendershot L, Meldolesi J (1997) BiP, a major chaperone protein of the endoplasmic reticulum lumen, plays a direct and important role in the storage of the rapidly exchanging pool of Ca^{2+}. J Biol Chem 272(49):30873–30879
80. Helenius A (1994) How N-linked oligosaccharides affect glycoprotein folding in the endoplasmic reticulum. Mol Biol Cell 5(3):253–265
81. Kuznetsov G, Chen LB, Nigam SK (1997) Multiple molecular chaperones complex with misfolded large oligomeric glycoproteins in the endoplasmic reticulum. J Biol Chem 272 (5):3057–3063
82. Klausner RD, Sitia R (1990) Protein degradation in the endoplasmic reticulum. Cell 62 (4):611–614
83. Blond-Elguindi S, Cwirla SE, Dower WJ, Lipshutz RJ, Sprang SR, Sambrook JF, Gething MJ (1993) Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP. Cell 75(4):717–728
84. Knarr G, Gething MJ, Modrow S, Buchner J (1995) BiP binding sequences in antibodies. J Biol Chem 270(46):27589–27594
85. Knarr G, Modrow S, Todd A, Gething MJ, Buchner J (1999) BiP-binding sequences in HIV gp160. Implications for the binding specificity of bip. J Biol Chem 274(42):29850–29857
86. Brodsky JL, Werner ED, Dubas ME, Goeckeler JL, Kruse KB, McCracken AA (1999) The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. J Biol Chem 274(6):3453–3460
87. Meerovitch K, Wing S, Goltzman D (1998) Proparathyroid hormone-related protein is associated with the chaperone protein BiP and undergoes proteasome-mediated degradation. J Biol Chem 273(33):21025–21030
88. Katayama T, Imaizumi K, Sato N, Miyoshi K, Kudo T, Hitomi J, Morihara T, Yoneda T, Gomi F, Mori Y, Nakano Y, Takeda J, Tsuda T, Itoyama Y, Murayama O, Takashima A, St George-Hyslop P, Takeda M, Tohyama M (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. Nat Cell Biol 1(8):479–485. doi:10.1038/70265
89. Yu Z, Luo H, Fu W, Mattson MP (1999) The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. Exp Neurol 155(2):302–314. doi:10.1006/exnr.1998.7002, S0014-4886(98)97002-9 [pii]
90. Rao RV, Peel A, Logvinova A, del Rio G, Hermel E, Yokota T, Goldsmith PC, Ellerby LM, Ellerby HM, Bredesen DE (2002) Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. FEBS Lett 514(2–3):122–128, S0014579302022895 [pii]

91. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, Lee AS (2003) Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. J Biol Chem 278 (23):20915–20924
92. Weil M, Jacobson MD, Coles HS, Davies TJ, Gardner RL, Raff KD, Raff MC (1996) Constitutive expression of the machinery for programmed cell death. J Cell Biol 133 (5):1053–1059
93. Taylor J, Gatchalian CL, Keen G, Rubin LL (1997) Apoptosis in cerebellar granule neurones: involvement of interleukin-1 beta converting enzyme-like proteases. J Neurochem 68 (4):1598–1605
94. Inokuchi Y, Shimazawa M, Nakajima Y, Suemori S, Mishima S, Hara H (2006) Brazilian green propolis protects against retinal damage in vitro and in vivo. Evid Based Complement Alternat Med 3(1):71–77. doi:10.1093/ecam/nek005
95. Shimazawa M, Inokuchi Y, Ito Y, Murata H, Aihara M, Miura M, Araie M, Hara H (2007) Involvement of ER stress in retinal cell death. Mol Vis 13:578–587
96. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR (2000) Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron 28(1):41–51
97. Henneberry RC, Novelli A, Cox JA, Lysko PG (1989) Neurotoxicity at the N-methyl-D-aspartate receptor in energy-compromised neurons. An hypothesis for cell death in aging and disease. Ann N Y Acad Sci 568:225–233
98. Ubeda M, Habener JF (2000) CHOP gene expression in response to endoplasmic-reticular stress requires NFY interaction with different domains of a conserved DNA-binding element. Nucleic Acids Res 28(24):4987–4997
99. Foti DM, Welihinda A, Kaufman RJ, Lee AS (1999) Conservation and divergence of the yeast and mammalian unfolded protein response. Activation of specific mammalian endoplasmic reticulum stress element of the grp78/BiP promoter by yeast Hac1. J Biol Chem 274 (43):30402–30409

Chapter 6
Nitric Oxide Contributes to Retinal Ganglion Cell Survival Through Protein S-Nitrosylation After Optic Nerve Injury

Yoshiki Koriyama and Satoru Kato

Abstract Neuroprotective strategies to attenuate retinal ganglion cell (RGC) death could lead to novel therapies for chronic optic neuropathies such as glaucoma. Nitric oxide (NO) signaling results in both neurotoxic and neuroprotective effects in CNS neurons after nerve lesion. However, the functional mechanisms of NO in the nervous system are not fully understood. Protein S-nitrosylation by NO is a posttranslational modification that regulates protein function through the reaction of NO with a cysteine thiol group on target proteins. NO/S-nitrosylation is now thought to be important in regulating cell death, survival, and gene expression. However, there are few reports on the role of protein S-nitrosylation in glaucoma. Therefore, we investigated the role of protein S-nitrosylation signaling in RGC survival after optic nerve injury.

Keywords Glaucoma • Keap1 • Nitric oxide • Nrf2 • Retinal ganglion cell • S-nitrosylation

6.1 NO Signaling in Glaucoma

6.1.1 Nerve Injury Model of Glaucoma in Rodents

Glaucoma is a neurodegenerative disorder characterized by the progressive loss of retinal ganglion cells (RGCs) and by degeneration of optic axons. Elevated intraocular pressure is considered to be one of the major risk factors associated with this

Y. Koriyama (✉) • S. Kato
Department of Molecular Neurobiology, Graduate School of Medicine, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-8640, Japan

Graduate School and Faculty of Pharmaceutical Sciences, Suzuka University of Medical Sciences, 3500-3 Minamitamagaki, Suzuka 513-8670, Japan
e-mail: koriyama@suzuka-u.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_6, © Springer Japan 2014

neuropathy [1]. However, in some glaucomatous patients, loss of RGCs and a subsequent loss of vision can occur even with normal intraocular pressure. Although the causes of glaucoma are unclear, the various pathogenetic mechanisms of glaucoma result in the common end stage of RGC apoptosis. For example, almost 90 % of RGCs die within 2 weeks after optic nerve injury [2]. In particular, oxidative stress and nitrative stress appear to play important roles in this progressive neuronal death. Eye tissue uses four times more oxygen than brain tissue and thus is highly exposed to various reactive oxygen species (ROS) including hydrogen peroxide, hydroxyl radicals, and superoxide anions. Consequently, eye tissue contains very high amounts of antioxidants, such as superoxide dismutase, catalase, ascorbate, and vitamins [3]. In the pathogenetic stages of RGC death, increasing intracellular ROS generation has been reported to accompany glutathione depletion [4]. Evidence also indicates that there are reductions in endogenous antioxidants in aging and induction of lipid peroxidation [5]. On the other hand, mechanical injury of axons, and thereby a lack of neurotrophins being supplied to RGC bodies, is one of the mechanisms proposed for the retrograde degeneration of RGCs following optic nerve injury [2]. RGC death after nerve injury is mostly caused by apoptosis associated with upregulation of proapoptotic Bax and downregulation of antiapoptotic Bcl-2 and Bcl-xL [6]. As RGC cell loss and optic nerve degeneration in the crush injury model mimics many of the features of glaucoma, we use this model to study the mechanisms of RGC survival and/or axonal regeneration [7].

Nitric oxide (NO) levels also increase in many retinal cells within a few days of optic nerve damage [8]. Many reports suggest that excess NO plays a crucial role in neuronal cell death. However, NO can also prevent neuronal cell death. In general, NO mediates neuroprotection through two main signaling pathways: the NO/cyclic guanosine monophosphate (cGMP) pathway and the protein S-nitrosylation pathway. However, whether S-nitrosylation of target protein promotes RGC survival after injury is unknown. This is the focus of this review.

6.1.2 Role of Three Isoforms of Nitric Oxide Synthase in Glaucomatous Retina

NO is an important signaling molecule that regulates a range of physiological processes, including vasodilatation, neuronal function, inflammation, and immune function [9]. NO is an organic gas ubiquitously synthesized by NO synthase (NOS). In mammalian cells, NOS is subclassified into three types: brain or neuronal NOS (nNOS or NOSI), inducible NOS (iNOS or NOSII), and endothelial NOS (eNOS or NOSIII). The function of NO is different depending on the cell type and enzyme isotype.

nNOS may be involved in neurotransmission by creating retrograde signaling between synapses. At synapses, nNOS is coupled to *N*-methyl-D-aspartate receptors (NMDA-R) via postsynaptic density-95 protein complexes [10]. Upon glutamate stimulation of NMDA-R, calcium ions enter the cytoplasm through the ion channel.

In conjunction with calmodulin, calcium ion influx triggers nNOS activation and NO generation [11]. Low levels of NO (picomole order) that are produced under physiological conditions stimulate many normal intracellular signaling pathways. In contrast, overstimulation of NMDA-R and subsequent calcium ion influx promote pathological signaling, resulting in neural damage and death through production of toxic amounts of NO [12]. Increased expression of nNOS in the retina and optic nerve head was reported in rats with elevated intraocular pressure. Most axotomized RGCs express nNOS protein, and these cells degenerate within 2 weeks after optic nerve injury [13]. Excessive NO generated from injured RGCs might be one risk factor for RGC cell death in glaucoma.

iNOS can be upregulated by acute inflammatory stimuli. For example, neurotoxic levels of NO (nanomole order [14]) via iNOS induction in activated glial cells give arise various neurodegenerative diseases. NO will further oxidize to nitrite, peroxynitrite, and free radicals to highly interact with thiols and iron–sulfur centers of various enzymes [14] to alter the biological function of cells and result in apoptosis, neurotoxicity, optic nerve degeneration, and numerous eye diseases. It has been reported that glaucoma could be due to neurotoxic effects of NO at the optic nerve head and in the RGCs which results in optic nerve head degeneration and visual field loss [15]. Inducible NOS was triggered by ocular inflammation, LPS, endotoxin, or cytokines including tumor necrosis factor α (TNFα) and interleukin-1 or interleukin-6 [16]. Therefore, one possibility for the treatment of glaucoma could be the use of inhibitors of iNOS induction and/or its activity. In the retina, Müller glial cells can express the iNOS isoform after endotoxin and cytokine exposure [17]. Retinal pigment epithelium (RPE) cells also contain iNOS in various species [18]. Goureau et al. [19] reported that fibroblast growth factors (FGFs) and transforming growth factor β (TGFβ) have opposite effects on the regulation of the production of NO in RPE cells. FGFs inhibit the induction of iNOS at the transcriptional level. Conversely, TGFβ frequently acts as an immunosuppressor. TGFβ attenuates NO production in human and rat RPE and Müller glial cells [20]. These molecules might be candidates to reverse or treat NO-related glaucoma. On the other hand, upregulation of TNFα and TNFα receptor-1 expression was accompanied by progressive optic nerve degeneration in the glaucomatous optic nerve head [21]. It has been reported that TNFα contributes to the progression of optic nerve degeneration by inducing iNOS expression in glial cells. Thus, the TNFα inhibitor etanercept or other antagonists of TNFα or suppressors of inflammation could be considered as therapeutic tools against glaucoma [22].

The major function of eNOS is vasodilation, by regulating vascular smooth muscle relaxation. Immunoreactivity of eNOS is seen in the retinal vascular endothelial cells, choroid and retina [23]. In glaucomatous eyes, overexpression of nNOS and iNOS is linked to glaucomatous RGC apoptosis through increased levels of NO, while enhanced staining for eNOS is assumed to be a compensatory neuroprotective reaction [24]. Furthermore, no significant changes in eNOS expression have been observed in the chronic glaucoma model.

The reagents that can regulate NO levels in the retina could become a reasonable neuroprotective agent for treating glaucoma.

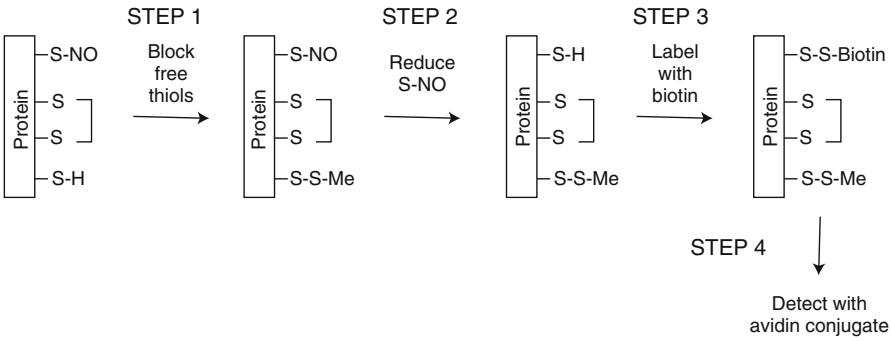


Fig. 6.1 Schematic diagram for detecting S-nitrosylated proteins (biotin-switch assay). Step 1: free thiols are blocked by methylthiolation reagent. Step 2: the reduction of SNO bonds to thiols with reductant. Step 3: newly reformed cysteines react with biotin-conjugated thiol-modifying reagent. Step 4: target biotinylated proteins are collected by avidin-coupled reagents

6.1.3 Anti- and Proapoptotic Mechanisms by NO and Protein S-Nitrosylation

Because NO is a free gas, it can easily penetrate the biological membrane. Superoxide radical shows a high affinity toward NO. The reaction between NO and superoxide anion produces peroxynitrite [25], a highly reactive molecule that can cause extensive damage to proteins, lipids, and DNA molecules. Furthermore, NO can react with thiols, organosulfur compounds containing a carbon-bonded sulfhydryl (–C–SH or R–SH) group. Some actions of NO, including neurotransmission and vasodilation, are mediated via the activation of soluble guanylate cyclase and subsequent elevation of cGMP levels [26]. Other actions of NO are mediated via S-nitrosylation of the free cysteine SH group of proteins and regulate their activities when cysteines are present at their active site [26].

Since Jafferey and Snyder discovered the "biotin-switch assay" for protein S-nitrosylation [27], nearly 1,000 proteins have been identified as *S*-nitrosoproteins. The biotin-switch assay consists of four steps (Fig. 6.1):

- Step 1—the methylthiolation of free cysteine thiols with methyl methanethiosulfonate.
- Step 2—the reduction of SNO bounded to thiols with reductant.
- Step 3—newly reformed cysteines are reacted with biotin-conjugated thiol-modifying reagent.
- Step 4—target biotinylated proteins are collected by avidin-coupled reagents.

S-nitrosylation can mediate either neuroprotective or neurotoxic effects, depending on the action of the target protein [28]. For the neuroprotective effect, S-nitrosylation of caspases inhibits their activation. Most caspases contain a single cysteine at their catalytic site, which is susceptible to redox modification and can be effectively modified by S-nitrosylation in the presence of NO with subsequent

Table 6.1 S-nitrosylated proteins targeting for cell survival/death

Functions	Name	References
Cell survival/death	14-3-3	[41]
	Apoptosis signal-regulating kinase 1	[42]
	Bcl-2	[43]
	Caspases	[30]
	Cyclin-dependent kinase 5	[44]
	Cyclooxygenase-2	[45]
	Dynamin-related protein 1	[46]
	Erk	[47]
	Fas	[48]
	GAPDH	[34]
	GOSPEL	[49]
	Keap1	[50, 51]
	Matrix metalloproteinase 9	[52]
	Parkin	[37]
	Peroxiredoxin2	[53]
	PTEN	[39, 40]
	STAT3	[54]
	X-linked inhibitor of apoptosis protein	[55]

inhibition of enzyme activity [29]. S-nitrosylation has been shown to reduce the activity of caspases such as caspase-3, caspase-8, and caspase-9 in various types of neurons [30]. These results indicate that endogenous NO generated by NOS exerts an antiapoptotic function by S-nitrosylation-dependent inactivation of caspases. In contrast, another target protein of S-nitrosylation promotes activation of caspases and induces cell death. Caspase activation during NO stimulation also occurs as a result of downregulation of X-linked inhibitor of apoptosis protein (XIAP) [31]. Under normal conditions, XIAP efficiently binds to the catalytic sites of caspases and inhibits them [32]. However, NO inactivates the E3 ligase activity of XIAP through S-nitrosylation, thus stabilizing caspases [33]. On the other hand, various S-nitrosylated target proteins are also involved in cell death signaling. For example, NO S-nitrosylates glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and binds to Siah, an E3 ubiquitin ligase [34]. Inside the nucleus, S-nitrosylated GAPDH stabilizes Siah, and the complex facilitates ubiquitination and degradation of the nuclear coreceptor [35]. This mechanism is thought to regulate gene expression associated with cellular dysfunction and death [36]. Recent studies have shown unregulated S-nitrosylation of many proteins involved in neuronal death and neurodegenerative disorders, such as Parkin [37], protein disulfide-isomerase (PDI) [38], and phosphatase and tensin homologue deleted on chromosome 10 (PTEN) [39, 40]. The target S-nitrosylated proteins for cell death or cell survival are listed in Table 6.1. In the future, there might be new targets that could be used for the treatment of glaucoma.

6.2 Antioxidative Effects of NO Through Keap1 S-Nitrosylation Signaling in RGCs

6.2.1 Protective Effects of Nip Against Oxidative Stress

Although a growing number of S-nitrosylated proteins are reported to contribute to pathogenetic brain disease, there are few such reports for retinal diseases. Therefore, we studied the role of protein S-nitrosylation by NO in RGC survival and axonal regeneration after optic nerve injury [50, 51, 56–58]. Many chemicals such as glyceryl trinitrate, sodium nitroprusside, and S-nitrosothiols have been reported as NO donors [59]. Recently, novel hybrid NO donor drugs have been designed as NO-releasing compounds such as NO–NSAIDS and nipradilol (Nip), which already has been registered as an antiglaucoma agent in Japan. Nip acts as a vasodilator by releasing NO from the nitroxy moiety [60]. Nip lowers intraocular pressure via both selective α1-adrenoceptor and nonselective β-adrenoceptor antagonists. The protective effects of Nip have been shown in various neuronal cells [61] including RGCs [62]. Mizuno et al. demonstrated that the protective effects of Nip were NO dependent because both selective α1- and nonselective β-adrenoceptor antagonists had no effect on RGCs [63]. However, the detailed mechanism of NO-dependent protection is not clear. Several lines of evidence indicate that NO can suppress RGC cell death [64] through an NO/cGMP-dependent pathway [65]. Furthermore, Tomita et al. [66] reported that the beneficial effect of Nip on RGC cell death was partially cancelled by inhibiting protein kinase G. Moreover, Naito [67] reported that Nip attenuates hydrogen peroxide-induced lipid peroxidation. Thus, we wanted to know whether the neuroprotective action of Nip is mediated by antioxidative processes via a NO/cGMP- or a NO/S-nitrosylation-dependent mechanism. In addition, as Nip did not S-nitrosylate caspase-3 under our experimental conditions, we focused on the possibility of Kelch-like ECH-associated protein 1 (Keap1) S-nitrosylation to understand its antioxidative action.

The Keap1 and NF-E2-related factor 2 (Nrf2) pathways regulate the expression of cytoprotective genes in response to oxidative stress or electrophilic stress [68]. Keap1 is the redox-sensor protein that allows the activation of Nrf2 by modification including oxidation or S-nitrosylation [69]. Once activated, Nrf2 translocates from the cytosol to the nucleus, binds to the antioxidant responsible element (ARE) of target genes, and drives their expression of antioxidative heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1 (NQO-1), and glutamate cysteine ligase C (GCLC). Keap1 sensor protein contains 27 cysteine residues. Several analyses have identified multiple Keap1 cysteine residues to be involved in the reaction with oxygen or electrophiles. The most frequently reported targets are Cys151, Cys257, Cys273, Cys288, Cys297, and Cys613 [70, 71]. However, there are notable differences between laboratories, electrophilic probes, and species. Interestingly, NO activates the Keap1/Nrf2 pathway by S-nitrosylation of Keap1 protein in colon carcinoma cells [72]. Two reactive cysteines, Csy273 and Cys288, of Keap1 have been identified as key sites of translocational activity of Nrf2 [73].

Thus, we focused on the neuroprotective mechanism of Nip thorough Keap1 S-nitrosylation.

We first tested whether Nip could induce NO generation in a retinal ganglion cell line: RGC-5 [51]. Nip (20 μM) significantly increased (1.6-fold) fluorescence intensity of NO indicator compared to no treatment (control) within 1 h of treatment. Denitro-nipradilol (DeNip) has weak selective α1-adrenoceptor and nonselective β-adrenoceptor antagonist properties, but no NO-donating action. DeNip did not increase NO production in RGC-5 cells.

To evaluate the protective action of Nip against oxidative stress in RGC-5 cells, we used oxidative stress models in culture with hydrogen peroxide, tBOOH, and serum withdrawal. Charles et al. [74] studied the signaling cascades involved in RGC-5 cell death under serum deprivation. They demonstrated that serum deprivation increases malondialdehyde and decreases reduced glutathione. Furthermore, several groups have already reported that serum deprivation produced ROS in RGC-5 cells [75]. While pretreatment with Nip 4–6 h before oxidative stress exposure showed the maximum protective effect, no protective effect was observed when Nip was applied to RGC-5 0–2 h before oxidative stress stimulation. Another NO donor, NOR1, also showed protective effects against oxidative stress [50]. These protective effects were reversed by a NO scavenger. The protective effects of Nip were suppressed by the protein synthesis inhibitor cycloheximide. In contrast, DeNip did not show any neuroprotective effect against oxidative stress.

6.2.2 NO/S-Nitrosylation-Dependent Antioxidative Protein Induction by Nip

As the protective effect of Nip was dependent upon newly synthesized proteins, we tested the inducibility of antioxidative enzymes in RGC-5 cells. After 4 h of Nip treatment, we found that heme oxygenase-1 (HO-1) mRNA and protein had increased in RGC-5 cells. HO-1 [76] is an enzyme that degrades intracellular heme to free iron, carbon monoxide, and biliverdin. Bilirubin, converted from biliverdin, acts as a strong endogenous ROS scavenger and attenuates lipid peroxidation related to 4-hydroxy-2-nonenal (4HNE). Other antioxidative proteins, such as NQO-1 and GCLC, did not increase for up to 12 h after treatment of Nip. DeNip did not alter HO-1 expression. These results indicate that the protective effect of Nip is dependent on NO. An HO-1 inhibitor prevented the neuroprotective effect of Nip against oxidative stress. The data indicate that the neuroprotective action of Nip is caused by NO generation following antioxidative HO-1 expression.

To test the involvement of the Keap1/Nrf2 system in the Nip-induced increase in HO-1 expression, we analyzed the translocation of Nrf2 to the nucleus. Nip facilitated the translocation of Nrf2 into the nucleus. Both a NO scavenger and an S-nitrosylation blocker (dithiothreitol) inhibited nuclear translocation of Nrf2 by Nip. Furthermore, translocated Nrf2 was bound to the E1 enhancer of HO-1 promoter as an ARE site [50]. These results suggest that Nip-mediated translocation

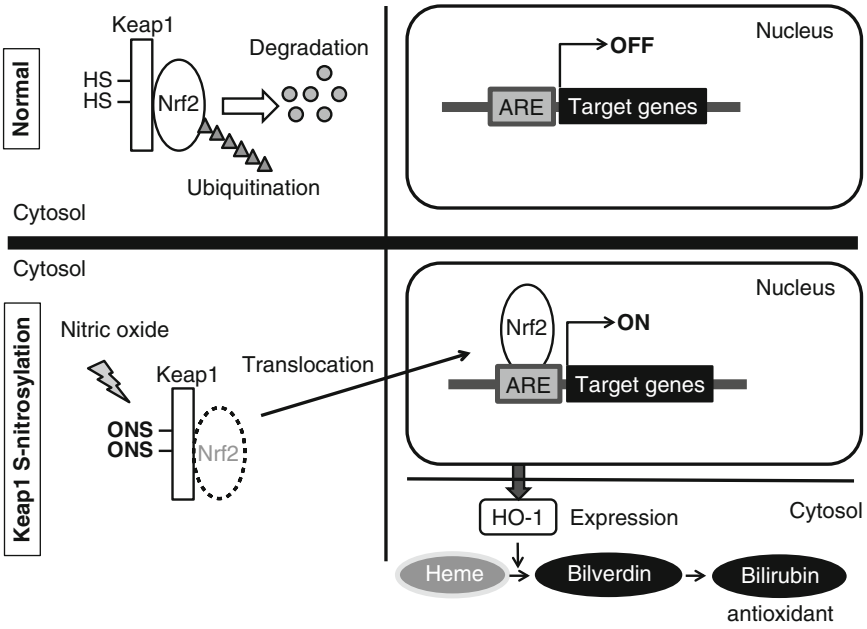


Fig. 6.2 Schematic diagram of Keap1 S-nitrosylation-dependent antioxidative signaling pathway. NO donors induce S-nitrosylation of Keap1 and thereafter HO-1 expression through Nrf2/ARE signaling

of Nrf2 to the nucleus is dependent on NO/S-nitrosylation pathways. We further investigated the effects of the NO scavenger and S-nitrosylation blocker on HO-1 induction by Nip. As expected, the increase in HO-1 was blocked by both reagents. By using the biotin-switch assay, we determined that Nip increased S-nitrosylated Keap1 but not caspase-3.

To extend the protective effect of Nip in vivo, we used an optic nerve injury model in mice. Nip suppressed the final products of lipid peroxidation: 4HNE accumulation mediated by inducing HO-1 expression in RGCs after nerve injury. Finally, RGC death after nerve injury was reduced by Nip. These results demonstrate for the first time that Nip protects RGC death against oxidative stress both in vitro and in vivo through the induction of HO-1 by S-nitrosylation of Keap1 (Fig. 6.2). This novel neuroprotective action of Nip in RGCs may shed additional light on possible antiglaucomatous agents.

6.3 PTEN S-Nitrosylation-Induced Optic Nerve Regeneration by Nip

Nip has also been reported as having neuritogenic action in cat RGCs [77]. However, the mechanism of Nip-induced optic nerve regeneration has not been fully elucidated. It has been reported that PTEN deletion strongly showed optic nerve

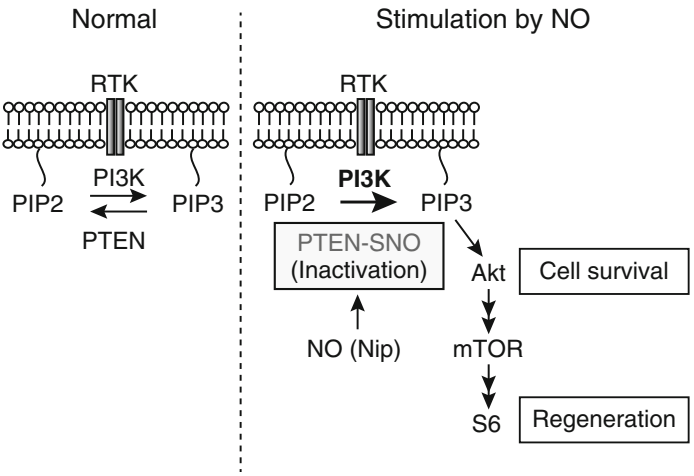


Fig. 6.3 Schematic diagram of PTEN S-nitrosylation-dependent axonal regeneration. In the case of NO stimulation, PTEN was inactivated through S-nitrosylation. Subsequently, Akt phosphorylates a wide range of substrates involved in the regulation of cellular functions, including cell growth and survival. Akt activation also induced mTOR/S6 signaling, which is known as a novel pathway of axonal regeneration. *RTK*, tyrosine kinase receptor; *PIP2*, phosphatidylinositol (4, 5) biphosphate; *PIP3*, phosphatidylinositol (3, 4, 5) triphosphate

regeneration after injury in part by increasing protein translation through the mammalian target of rapamycin (mTOR) pathway [78–81]. PTEN and mTOR are critical factors for controlling the regenerative capacity of mouse RGCs and corticospinal neurons [81]. It has been known that PTEN is inactivated by S-nitrosylation and then activates phosphoinositide 3-kinase (PI3K) and its downstream pathway [39]. We showed a correlation between Akt/mTOR activities and optic nerve regeneration through S-nitrosylation of PTEN in RGCs [80] (Fig. 6.3).

6.4 Future Studies

For treatment of glaucomatous degeneration, neuroprotective and neuritogenic actions in RGCs play a central role. As there are a few reports on protein S-nitrosylation in glaucoma, elucidating specific targets of S-nitrosylation and understanding their regulatory mechanism could assist the development of therapeutic intervention and be a next-era target for the treatment of injured RGCs in glaucomatous retina.

References

1. García-Campos J, Villena A, Díaz F et al (2007) Morphological and functional changes in experimental ocular hypertension and role of neuroprotective drugs. Histol Histopathol 22:1399–1411
2. Berkelaar M, Clarke DB, Wang YC et al (1994) Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. J Neurosci 14:4368–4374
3. Anderson B Jr, Saltzman HA (1964) Retinal oxygen utilization measured by hyperbaric blackout. Arch Ophthalmol 72:792–795
4. Maher P, Hanneken A (2005) Flavonoids protect retinal ganglion cells from oxidative stress-induced death. Invest Ophthalmol Vis Sci 46:4796–4803
5. Wang AL, Lukas TJ, Yuan M et al (2010) Age-related increase in mitochondrial DNA damage and loss of DNA repair capacity in the neural retina. Neurobiol Aging 31:2002–2010
6. Chierzi S, Strettoi E, Cenni MC et al (1999) Optic nerve crush: axonal responses in wild-type and bcl-2 transgenic mice. J Neurosci 19:8367–8376
7. Levkovitch-Verbin H, Harris-Cerruti C, Groner Y et al (2000) RGC death in mice after optic nerve crush injury: oxidative stress and neuroprotection. Invest Ophthalmol Vis Sci 41:4169–4174
8. Groppe M, Thanos S, Schuhmann W et al (2003) Measurement of nitric oxide production by the lesioned rat retina with a sensitive nitric oxide electrode. Anal Bioanal Chem 376:797–807
9. Calabrese V, Cornelius C, Rizzarelli E et al (2009) Nitric oxide in cell survival: a janus molecule. Antioxid Redox Signal 11:2717–2739
10. Sattler R, Xiong Z, Lu WY et al (1999) Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. Science 284:1845–1848
11. Bredt DS, Hwang PM, Glatt CE et al (1991) Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 351:714–718
12. Lipton SA, Choi YB, Pan ZH et al (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364:626–632
13. Lee EJ, Kim KY, Gu TH et al (2003) Neuronal nitric oxide synthase is expressed in the axotomized ganglion cells of the rat retina. Brain Res 986:174–180
14. Chiou GC (2001) Review: effects of nitric oxide on eye diseases and their treatment. J Ocul Pharmacol Ther 17:189–198
15. Neufeld AH, Sawada A, Becker B (1999) Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. Proc Natl Acad Sci USA 96:9944–9948
16. Dawson VL, Dawson TM (1995) Physiological and toxicological actions of nitric oxide in the central nervous system. Adv Pharmacol 34:323–342
17. Goureau O, Hicks D, Courtois Y et al (1994) Induction and regulation of nitric oxide synthase in retinal Müller glial cells. J Neurochem 63:310–317
18. Liversidge J, Grabowski P, Ralston S et al (1994) Rat retinal pigment epithelial cells express an inducible form of nitric oxide synthase and produce nitric oxide in response to inflammatory cytokines and activated T cells. Immunology 83:404–409
19. Goureau O, Lepoivre M, Becquet F et al (1993) Differential regulation of inducible nitric oxide synthase by fibroblast growth factors and transforming growth factor beta in bovine retinal pigmented epithelial cells: inverse correlation with cellular proliferation. Proc Natl Acad Sci USA 90:4276–4280
20. Becquet F, Courtois Y, Goureau O (1997) Nitric oxide in the eye: multifaceted roles and diverse outcomes. Surv Ophthalmol 42:71–82
21. Yuan L, Neufeld AH (2000) Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. Glia 32:42–50

22. Roh M, Zhang Y, Murakami Y et al (2012) Etanercept, a widely used inhibitor of tumor necrosis factor-α (TNF-α), prevents retinal ganglion cell loss in a rat model of glaucoma. PLoS One 7:e40065
23. Chakravarthy U, Stitt AW, McNally J et al (1995) Nitric oxide synthase activity and expression in retinal capillary endothelial cells and pericytes. Curr Eye Res 14:285–294
24. Neufeld AH (1999) Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. Surv Ophthalmol 43:129–135
25. Beckman JS, Beckman TW, Chen J et al (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87:1620–1624
26. Schmidt HH, Lohmann SM, Walter U (1993) The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biochim Biophys Acta 1178:153–175
27. Jaffrey SR, Erdjument-Bromage H, Ferris CD et al (2001) Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat Cell Biol 3:193–197
28. Nakamura T, Lipton SA (2011) Redox modulation by S-nitrosylation contributes to protein misfolding, mitochondrial dynamics, and neuronal synaptic damage in neurodegenerative diseases. Cell Death Differ 18:1478–1486
29. Melino G, Bernassola F, Knight RA et al (1997) S-nitrosylation regulates apoptosis. Nature 388:432–433
30. Tenneti L, D'Emilia DM, Lipton SA (1997) Suppression of neuronal apoptosis by S-nitrosylation of caspases. Neurosci Lett 236:139–142
31. Manderscheid M, Messmer UK, Franzen R et al (2001) Regulation of inhibitor of apoptosis expression by nitric oxide and cytokines: relation to apoptosis induction in rat mesangial cells and raw 264.7 macrophages. J Am Soc Nephrol 12:1151–1163
32. Yang YL, Li XM (2000) The IAP family: endogenous caspase inhibitors with multiple biological activities. Cell Res 10:169–177
33. Nakamura T, Wang L, Wong CC et al (2010) Transnitrosylation of XIAP regulates caspase-dependent neuronal cell death. Mol Cell 39:184–195
34. Hara MR, Agrawal N, Kim SF et al (2005) S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. Nat Cell Biol 7:665–674
35. Zhang J, Guenther MG, Carthew RW et al (1998) Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 12:1775–1780
36. Sen N, Hara MR, Kornberg MD et al (2008) Nitric oxide-induced nuclear GAPDH activates p300/CBP and mediates apoptosis. Nat Cell Biol 10:866–873
37. Sunico CR, Nakamura T, Rockenstein E et al (2013) S-nitrosylation of parkin as a novel regulator of p53-mediated neuronal cell death in sporadic Parkinson's disease. Mol Neurodegener 8:29
38. Walker AK, Farg MA, Bye CR et al (2010) Protein disulphide isomerase protects against protein aggregation and is S-nitrosylated in amyotrophic lateral sclerosis. Brain 133:105–116
39. Kwak YD, Ma T, Diao S et al (2010) NO signaling and S-nitrosylation regulate PTEN inhibition in neurodegeneration. Mol Neurodegener 5:49
40. Numajiri N, Takasawa K, Nishiya T et al (2011) On-off system for PI3-kinase-Akt signaling through S-nitrosylation of phosphatase with sequence homology to tensin (PTEN). Proc Natl Acad Sci USA 108:10349–10354
41. Kuncewicz T, Sheta EA, Goldknopf IL et al (2003) Proteomic analysis of S-nitrosylated proteins in mesangial cells. Mol Cell Proteomics 2:156–163
42. Sumbayev VV (2003) S-nitrosylation of thioredoxin mediates activation of apoptosis signal-regulating kinase 1. Arch Biochem Biophys 415:133–136
43. Azad N, Vallyathan V, Wang L et al (2006) S-nitrosylation of Bcl-2 inhibits its ubiquitin-proteasomal degradation. A novel antiapoptotic mechanism that suppresses apoptosis. J Biol Chem 281:34124–34134

44. Qu J, Nakamura T, Cao G et al (2011) S-nitrosylation activates Cdk5 and contributes to synaptic spine loss induced by beta-amyloid peptide. Proc Natl Acad Sci USA 108:14330–14335
45. Tian J, Kim SF, Hester L et al (2008) S-nitrosylation/activation of COX-2 mediates NMDA neurotoxicity. Proc Natl Acad Sci USA 105:10537–10540
46. Westermann B (2009) Nitric oxide links mitochondrial fission to Alzheimer's disease. Sci Signal 2:pe29
47. Feng X, Sun T, Bei Y et al (2013) S-nitrosylation of ERK inhibits ERK phosphorylation and induces apoptosis. Sci Rep 3:1814
48. Yin XH, Yan JZ, Hou XY et al (2013) Neuroprotection of S-nitrosoglutathione against ischemic injury by down-regulating Fas S-nitrosylation and downstream signaling. Neuroscience 248C:290–298
49. Sen N, Hara MR, Ahmad AS et al (2009) GOSPEL: a neuroprotective protein that binds to GAPDH upon S-nitrosylation. Neuron 63:81–91
50. Koriyama Y, Chiba K, Yamazaki M et al (2010) Long-acting genipin derivative protects retinal ganglion cells from oxidative stress models in vitro and in vivo through the Nrf2/antioxidant response element signaling pathway. J Neurochem 115:79–91
51. Koriyama Y, Kamiya M, Takadera T et al (2012) Protective action of nipradilol mediated through S-nitrosylation of Keap1 and HO-1 induction in retinal ganglion cells. Neurochem Int 61:1242–1253
52. Gu Z, Kaul M, Yan B et al (2002) S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. Science 297:1186–1190
53. Fang J, Nakamura T, Cho DH et al (2007) S-nitrosylation of peroxiredoxin 2 promotes oxidative stress-induced neuronal cell death in Parkinson's disease. Proc Natl Acad Sci USA 104(47):18742–18747
54. Kim J, Won JS, Singh AK et al (2014) STAT3 regulation by S-nitrosylation: implication for inflammatory disease. Antioxid Redox Signal 20:2514–2527
55. Dawson TM, Chung KK (2009) S-nitrosylation of XIAP compromises neuronal survival in Parkinson's disease. Proc Natl Acad Sci USA 106:4900–4905
56. Koriyama Y (2013) Role of protein S-nitrosylation in central nervous system survival and regeneration. Yakugaku Zasshi 133:843–848
57. Koriyama Y, Takagi Y, Chiba K (2011) Neuritogenic activity of a genipin derivative in retinal ganglion cells is mediated by retinoic acid receptor β expression through nitric oxide/S-nitrosylation signaling. J Neurochem 119:1232–1242
58. Koriyama Y, Takagi Y, Chiba K (2013) Requirement of retinoic acid receptor β for genipin derivative-induced optic nerve regeneration in adult rat retina. PLoS One 8:e71252
59. Miller MR, Megson IL (2007) Recent developments in nitric oxide donor drugs. Br J Pharmacol 151:305–321
60. Adachi T, Hori S, Miyazaki K et al (1995) Rapid increase in plasma nitrite concentration following intravenous administration of nipradilol. Eur J Pharmacol 286:201–204
61. Taguchi R, Shirakawa H, Yamaguchi T et al (2006) Nitric oxide-mediated effect of nipradilol, an alpha- and beta-adrenergic blocker, on glutamate neurotoxicity in rat cortical cultures. Eur J Pharmacol 535:86–94
62. Nakazawa T, Tomita H, Yamaguchi K et al (2002) Neuroprotective effect of nipradilol on axotomized rat retinal ganglion cells. Curr Eye Res 24:114–122
63. Mizuno K, Koide T, Yoshimura M et al (2001) Neuroprotective effect and intraocular penetration of nipradilol, a beta-blocker with nitric oxide donative action. Invest Ophthalmol Vis Sci 42:688–694
64. Imai N, Tsuyama Y, Murayama K et al (1997) Protective effect of nitric oxide on ischemic retina. Nihon Ganka Gakkai Zasshi 101:639–643
65. Ando A, Yamazaki Y, Kaneko S et al (2005) Cytoprotection by nipradilol, an anti-glaucomatous agent, via down-regulation of apoptosis related gene expression and activation of NF-kappaB. Exp Eye Res 80:501–507

66. Tomita H, Nakazawa T, Sugano E et al (2002) Nipradilol inhibits apoptosis by preventing the activation of caspase-3 via S-nitrosylation and the cGMP-dependent pathway. Eur J Pharmacol 452:263–268
67. Naito A, Aniya Y, Sakanashi M (1994) Antioxidative action of the nitrovasodilator nicorandil: inhibition of oxidative activation of liver microsomal glutathione S-transferase and lipid peroxidation. Jpn J Pharmacol 65:209–213
68. Baird L, Dinkova-Kostova AT (2011) The cytoprotective role of the Keap1-Nrf2 pathway. Arch Toxicol 85:241–272
69. Himori N, Yamamoto K, Maruyama K et al (2013) Critical role of Nrf2 in oxidative stress-induced retinal ganglion cell death. J Neurochem 127:669–680
70. Eggler AL, Liu G, Pezzuto JM et al (2005) Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. Proc Natl Acad Sci USA 102:10070–10075
71. Kansanen E, Bonacci G, Schopfer FJ et al (2011) Electrophilic nitro-fatty acids activate NRF2 by a KEAP1 cysteine 151-independent mechanism. J Biol Chem 286:14019–14027
72. Li CQ, Kim MY, Godoy LC et al (2009) Nitric oxide activation of Keap1/Nrf2 signaling in human colon carcinoma cells. Proc Natl Acad Sci USA 106:14547–14551
73. Buckley BJ, Li S, Whorton AR (2008) Keap1 modification and nuclear accumulation in response to S-nitrosocysteine. Free Radic Biol Med 44:692–698
74. Charles I, Khalyfa A, Kumar DM et al (2005) Serum deprivation induces apoptotic cell death of transformed rat retinal ganglion cells via mitochondrial signaling pathways. Invest Ophthalmol Vis Sci 46:1330–1338
75. Nakajima Y, Shimazawa M, Otsubo K et al (2009) Zeaxanthin, a retinal carotenoid, protects retinal cells against oxidative stress. Curr Eye Res 34:311–318
76. Maines MD (1988) Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J 2:2557–2568
77. Watanabe M, Tokita Y, Yata T (2006) Axonal regeneration of cat retinal ganglion cells is promoted by nipradilol, an anti-glaucoma drug. Neuroscience 140:517–528
78. Park KK, Liu K, Hu Y et al (2008) Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 322:963–966
79. de Lima S, Koriyama Y, Kurimoto T et al (2012) Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. Proc Natl Acad Sci USA 109:9149–9154
80. Koriyama Y, Kamiya M, Arai K et al (2014) Nipradilol promotes axon regeneration through S-nitrosylation of PTEN in retinal ganglion cells Adv Exp Med Biol 801:751–757
81. Liu K, Lu Y, Lee JK et al (2010) PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat Neurosci 13:1075–1081

Chapter 7
Neurotrophic Factors

Chikako Harada and Takayuki Harada

Abstract Neurotrophic factors regulate neural cell survival and differentiation and control the number of retinal ganglion cells (RGCs) during retinal development. Several studies have reported a possibility that blockade of axonal transport in glaucoma leads to deficits in the neurotrophic factors and subsequent RGC death in adult eyes. Interestingly, not only mature neurotrophins but also the uncleaved neurotrophin precursors, the pro-neurotrophins, may play a critical role in survival or death of retinal neurons. Neurotrophic factors act on their receptors expressed on RGCs, but they also act on surrounding cells including Müller glia and microglia and indirectly affect the state of RGCs. Although ligand–receptor systems of neurotrophins are complex and their effects are still controversial, clinical trials using neurotrophins are underway for several retinal diseases. The current therapy for glaucoma is to lower intraocular pressure (IOP), but neurotrophic factors may be available for preventing IOP-independent RGC loss and future treatment of glaucoma.

Keywords Glaucoma • Glia–neuron interaction • Neuroprotection • Neurotrophins • Retinal ganglion cell

7.1 Introduction

Neurotrophic factors including those belonging to the neurotrophin family [nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5)] play important roles in the development of the retina and the visual system. Neurotrophins induce neural cell survival and

C. Harada • T. Harada (✉)
Visual Research Project, Tokyo Metropolitan Institute of Medical Science,
2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan
e-mail: harada-tk@igakuken.or.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_7,

differentiation through the high-affinity tyrosine kinase (Trk) receptors (TrkA, TrkB, and TrkC). On the other hand, they also bind to the low-affinity neurotrophin receptor p75 ($p75^{NTR}$) and induce programmed cell death (PCD). Neurotrophins, as well as many transcription factors and cell death-regulating factors, have been implicated in the regulation of developmental retinal ganglion cell (RGC) death. For example, competition for limited amounts of neurotrophins in the superior colliculus (SC), the target area of RGC axons, determines the apoptosis of RGCs during the early postnatal period. Furthermore, unprocessed precursor forms of neurotrophin family members (pro-neurotrophins) also play important roles in the retina. This chapter reviews the roles of neurotrophic factors during retinal development and degeneration and their possible use for supplementation therapies in glaucoma.

7.2 Roles of Neurotrophins and Pro-neurotrophins During Retinal Development

Neurotrophins play important roles during development in the central nervous system and regulate the number of RGCs in the early phase of retinal development [1–3]. There are two periods of cell death in the developing murine retina. The first peak occurs during embryonic days 15–17 (E15–E17) and coincides with the main onset of neurogenesis, neural migration, and initial axon growth. Post-mitotic RGCs at the optic nerve exit undergo $p75^{NTR}$-induced PCD; however, retinal morphology, RGC number, and BrdU-positive cell number in $p75^{NTR}$ knockout (KO) mice are normal after E15 [4, 5], suggesting the involvement of multiple mechanisms. The second peak of retinal PCD coincides with the phase of tectal and thalamic innervation and synapse formation. In the rat, ~50 % of the total RGC population dies in the first postnatal week soon after their axons reach their targets, the SC and the dorsal lateral geniculate nucleus (dLGN) [6]. While BDNF or NT-4/5 injected into the SC promotes the survival of neonatal RGCs [7, 8], BDNF or NT-4/5 mutant mice have normal RGC numbers and double-mutant mice show delay in the inner retinal development [9, 10]. Thus, neurotrophins and their receptors seem to be differentially involved in RGC apoptosis according to the developmental stages.

Recent studies have shown that the unprocessed precursor form of neurotrophin family members (pro-neurotrophins), such as proNGF and proBDNF, can act through a co-receptor system of $p75^{NTR}$ and sortilin to mediate cell apoptosis [11–14]. In the E15 retina, sortilin is mainly detected in the plasma membrane fraction but shifted to the non-plasma membrane fraction by the postnatal day 6 (P6) retina. On the other hand, $p75^{NTR}$ was mainly detected in the plasma membrane fraction in both E15 and P6 retinas. These results suggest that the intracellular sortilin expression pattern may regulate PCD in the developing retina. In addition, proNGF enhanced the death of E15 RGCs approximately twofold

compared with the unstimulated control, but such an increase was inhibited by neurotensin that blocks the interaction between proNGF and sortilin. ProNGF did not cause the death of E15 RGCs in $p75^{NTR}$ KO mice and P6 RGCs in wild-type (WT) mice. Taken together, these results suggest that sortilin and $p75^{NTR}$ receptors form receptor complexes on the cell surface via proNGF, which is required for proNGF-induced RGC death in the developing retina [12]. A recent study has shown that proNGF promotes RGC death in vivo. Importantly, proNGF-induced RGC loss is caused by an indirect effect and requires the $p75^{NTR}$-dependent production of tumor necrosis factor-alpha (TNF-α) by Müller glial cells. Therefore, proNGF-induced neuronal loss in the adult retina occurs through a non-cell-autonomous mechanism [15]. These findings raise the possibility that non-cell-autonomous events may be a general feature of $p75^{NTR}$-dependent cell apoptosis in vivo. An auto/paracrine proapoptotic mechanism based on the interaction of proNGF with the receptor complex $p75^{NTR}$/sortilin participates also in intense light-dependent photoreceptor cell death [16]. Therefore, sortilin may be a putative target for intervention in retinal degenerative disorders. Furthermore, several lines of evidence indicate that the neurotrophin receptor-interacting factor (NRIF) and neurotrophin receptor-interacting MAGE (from Melanoma AntiGEn) homolog (NRAGE) proteins play important roles in death signaling cascades evoked by $p75^{NTR}$ [17, 18], suggesting complex cell death pathways through $p75^{NTR}$. On the other hand, proBDNF controls the branching of retinal axons antagonistically, by inducing the corresponding neurotrophin receptor–ephrinA complexes. Moreover, scavenging pro-neurotrophins, by adding antibodies specific for the pro-domain of proBDNF or a soluble extracellular domain of $p75^{NTR}$, abolish repellent ephrinA reverse signaling. This indicates that retinal cells secrete pro-neurotrophins, inducing the ephrinA–$p75^{NTR}$ interaction and enabling repellent axon guidance. The antagonistic functions of proBDNF raise the possibility that not only cell death but also topographic branching is regulated by local control of processing of pro-neurotrophins [19]. Additional studies to determine the functions of both mature neurotrophins and pro-neurotrophins may uncover the detailed mechanisms of how the RGC number and axon remodeling are regulated.

7.3 Neuroprotection of Retinal Ganglion Cells by Neurotrophic Factors

Recent studies have reported a possibility that blockade of axonal transport in glaucoma leads to deficits in the neurotrophic factors and subsequent RGC death in adult eyes. This hypothesis is supported by the observations that axonal transport in the optic nerve is blocked in experimental glaucoma in primates [20–22], in rodents [23], and in humans [24]. In addition, retrograde transport of radiolabeled BDNF was impaired following intraocular pressure increase in rats, and accumulation of TrkB immunolabeling was found in the optic nerve head in this model [25, 26]. Live-cell imaging revealed the dynamics of the axonal transport of BDNF

in living RGCs, which clearly differed from the movements in dendrites [27]. Consistently, intraocular injection of exogenous BDNF or viral-mediated BDNF gene transfer promotes robust RGC survival after optic nerve injury [28–31] and in experimental glaucoma induced by chronic eye pressure elevation [32, 33]. Therefore, supplementation therapy using neurotrophic factors is an attractive method for the protection of RGCs and axons in glaucoma.

7.3.1 Nerve Growth Factor (NGF)

NGF is an endogenous neurotrophin that exerts trophic and differentiating activity on neurons of the central and peripheral nervous systems. NGF can stimulate both TrkA and $p75^{NTR}$ receptors; thus, it becomes important to establish the precise role of NGF signaling in RGC survival. Recent studies reported that a small molecule peptidomimetic TrkA agonist, but not NGF, promoted RGC protection following optic nerve injury and in experimental glaucoma [34, 35]. In addition, the combination of NGF or TrkA agonists with $p75^{NTR}$ antagonists markedly enhanced RGC survival [36]. These results indicate that NGF can be neuroprotective when acting on TrkA receptors and in the absence of $p75^{NTR}$ activation. As described in Sect. 7.2, proNGF induces RGC death during development and in the adult retina. Mice rendered null for $p75^{NTR}$, sortilin, or NRAGE did not undergo proNGF-induced RGC death [15]. On the other hand, we reported that $p75^{NTR}$/sortilin receptor system has no effect on ischemia-induced RGC death in the adult retina [12]. Further studies determining the detailed functions of NGF/proNGF and their receptor systems may provide important information that lead to the development of new therapeutic methods for glaucoma. A recent study reported that NGF protects degenerating RGCs in a rat model of high-IOP glaucoma [37, 38]. In addition, NGF eyedrops reduced RGC loss in patients with advanced glaucoma and indicated long-lasting improvements in visual field, optic nerve function, contrast sensitivity, and visual acuity [38]. These findings seem to be impressive and promising, but the patient number is limited ($n = 3$). In addition, several studies have failed to observe NGF-mediated RGC survival [34, 35, 39]. Thus, further study is needed before topical NGF can be considered for application in human glaucoma.

7.3.2 Brain-Derived Neurotrophic Factor (BDNF)

BDNF is known to regulate neural cell survival and axonal outgrowth mainly by activating TrkB receptors, which stimulates various signaling cascades, such as the mitogen-activated protein kinase pathway, phosphatidylinositol 3-kinase pathway, and Fyn-mediated actin polymerization pathway [40–42]. Several lines of evidence support a key role for BDNF/TrkB signaling in survival of adult RGCs in acute and

chronic models of optic nerve damage [28, 29, 31, 43–45]. TrkB expression is relatively high in RGCs and Müller glial cells but low in photoreceptors [46–48]. Based on this fact, we previously proposed a model in which exogenously applied or microglia-derived neurotrophins regulate photoreceptor survival indirectly by regulating secondary trophic factor production in Müller cells [49]. However, the effects that such a glia–neuron interaction would have in vivo or whether it would be relevant for the protection of neural cell types that express TrkB remained unclear. Therefore, to explore this point, we prepared two conditional KO mice in which TrkB was deleted from Müller glia ($TrkB^{GFAP}$ KO mice) or from two types of retinal neurons (RGCs and amacrine cells, $TrkB^{c\text{-}kit}$ KO mice) [50]. For this purpose, we utilized *GFAP*-cre and *c-kit*-cre transgenic mice (Fig. 7.1). These mice enabled us to separately examine direct and indirect effects of TrkB on neuroprotection. Surprisingly, the extent of glutamate-induced retinal degeneration was similar in these two mutant mice (Fig. 7.2). Furthermore, BDNF failed to increase the production of BDNF, basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), and glial cell line-derived neurotrophic factor (GDNF) in Müller cells and did not prevent photoreceptor degeneration in $TrkB^{GFAP}$ KO mice. Recent studies have shown that Müller glia dedifferentiate in response to neurotoxic damage or injury, implying that even the adult mammalian retina may have regenerative potential [51]. However, BDNF failed to stimulate Müller cell proliferation and expression of neural markers in the degenerating retina in $TrkB^{GFAP}$ KO mice. These results demonstrate that BDNF signaling in glia is critically involved in the protection and regeneration of surrounding neurons during retinal degeneration [50]. Further studies to stimulate the glia–neuron network as well as direct neuroprotection on RGCs may lead to effective treatment for glaucoma.

7.3.3 Ciliary Neurotrophic Factor (CNTF)

CNTF belongs to a small subfamily of cytokines and plays important roles as a potent neuroprotective molecule in the vertebrate retina [49, 52]. CNTFs mediate their effects through the signal transducing receptors glycoprotein 130 (gp130) and leukemia inhibitory factor (LIF) receptor. Whereas LIF directly interacts with the LIF receptor, which subsequently forms a complex with gp130, CNTF first binds to CNTF receptor-α (CNTFR-α), which then recruits the other signaling receptor subunits [53, 54]. Binding of CNTF to its receptor complex activates the Janus kinases/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), and phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway [55]. These receptor systems and their downstream pathways are functional in RGCs, and CNTF prolongs the survival of RGCs in optic nerve crush or transection

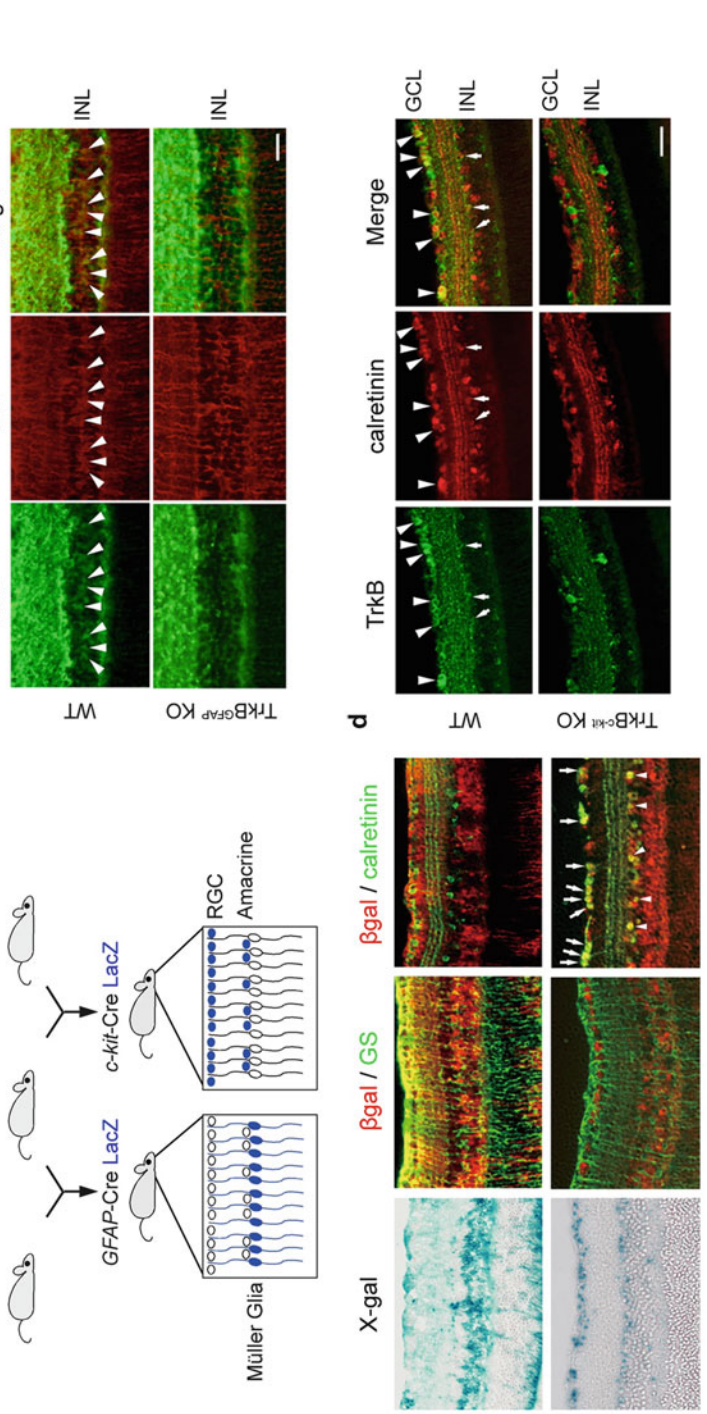


Fig. 7.1 Establishment of glia- and neuron-specific TrkB conditional knockout (KO) mice. (**a**) Schematic diagram of transgenically targeted fate-mapping strategy. (**b**) Overlapping immunoreactivities (*yellow*) of βgal and glutamine synthetase (GS) or calretinin indicate that Cre-mediated recombination occurs in Müller glial cells in GFAP–Cre–LacZ mice and RGCs (*arrows*) and amacrine cells (*arrowheads*) in c-kit–Cre–LacZ mice. (**c**) Immunohistochemical analysis of TrkB (*green*) and GS (*red*) showed the loss of TrkB from Müller cells (*arrowheads*) in $TrkB^{GFAP}$ KO mice. (**d**) Immunohistochemical analysis of TrkB (*green*) and calretinin (*red*) showed the loss of TrkB from RGCs and amacrine cells in $TrkB^{c\text{-}kit}$ KO mice. Scale bars = 100 μm. Modified from Harada et al. [50]

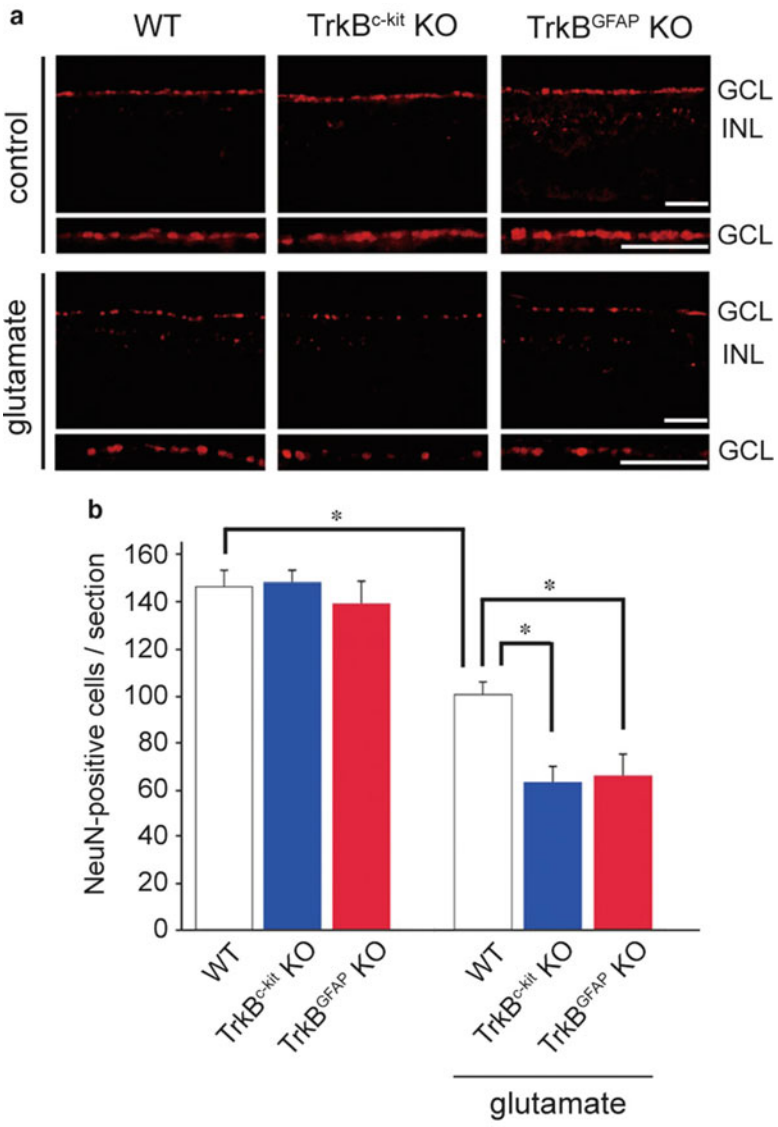


Fig. 7.2 Similar susceptibility to glutamate neurotoxicity in TrkB$^{c\text{-}kit}$ KO and TrkB^{GFAP} KO retinas. (**a**) Immunohistochemical analysis of RGCs with an anti-NeuN antibody. Retinal explants were untreated (control) or treated with 5 mM glutamate for 1 h. Scale bars = 50 μm. (**b**) Quantification of RGCs. *$P < 0.01$. Reproduced from Harada et al. [50]

models [56–58] and experimental glaucoma [59, 60]. Currently, human clinical trials for retinal degenerative diseases involving intravitreal implantation of a device containing encapsulated cells transfected with the human *CNTF* gene are under way [61–63].

One problem is that exogenously delivered CNTF have limited effects on promoting RGC survival and regeneration following optic nerve injury [64, 65]. Among CNTF signaling pathways, the JAK/STAT pathway is negatively regulated by suppressor of cytokine signaling 3 (SOCS3) [66]. Consistently, viral overexpression of SOCS3 in RGCs markedly compromises the axon growth promoting effects of intravitreally applied recombinant CNTF [67]. Accordingly, SOCS3 deletion in RGCs promotes dramatic axon regeneration following optic nerve injury in a gp130-dependent manner. Importantly, exogenously delivered CNTF could further enhance the extent of axon regeneration in SOCS3 deleted mice, suggesting that SOCS3 functions as an intrinsic brake for CNTF-mediated regeneration [68, 69]. Thus, reduced responsiveness to CNTF by SOCS3 may be an important limiting factor for successful neuroprotection and axon regeneration in mature visual system.

7.3.4 Glial Cell Line-Derived Neurotrophic Factor (GDNF)

GDNF and neurturin belong to the GDNF family and are distant members of the transforming growth factor-β (TGF-β) superfamily. GDNF family members mediate their actions through a multicomponent receptor complex composed of a transmembrane tyrosine kinase receptor, Ret, and one of the four glycosylphosphatidylinositol (GPI)-linked GDNF family receptors α, designated GFRα1–GFRα4 [70]. Intraocular injection of GDNF exerts a neuroprotective effect on axotomized RGCs, with less efficacy than BDNF [71–73]. GDNF also protects RGCs in a porcine model of retinal ischemia [74]. In addition, intravitreal injection of GDNF and vitamin E microspheres significantly increased RGC survival compared with GDNF, vitamin E, or blank microspheres in an experimental animal model of glaucoma [75]. Recent study showed that the dipeptide leucine-isoleucine (Leu-Ile) that induce the production of neurotrophic factors in the brain significantly increased the levels of BDNF and GDNF in the retina. Treatment with Leu-Ile significantly increased RGC survival at 14 days after optic nerve injury [76]. In addition to direct effects on RGCs, GDNF increases BDNF, bFGF, and GDNF production in Müller cells [77]. These results suggest that both neurotrophins and GDNF families regulate phenotypic expression in Müller cells and that they affect surrounding neurons through an indirect pathway.

One interesting point is that the upregulation of glutamate/aspartate transporter (GLAST) in Müller cells is required for RGC protection by GDNF and neurturin following optic nerve transection [78]. We recently found that GLAST KO mice show spontaneous RGC death and optic nerve degeneration without elevated IOP [79]. Interestingly, GLAST is essential not only to keep the extracellular glutamate concentration below a neurotoxic level but also to maintain glutathione levels by transporting glutamate, which is a substrate for glutathione synthesis, into Müller cells. As retinal concentration of glutathione, a major cellular antioxidant in the retina, was decreased in GLAST KO mice, both glutamate neurotoxicity and

oxidative stress may be involved in the normal tension glaucoma (NTG)-like pathology. Together with the evidence that downregulation of GLAST (human EAAT1) in the retina and of the glutathione level in the plasma are found in human glaucoma patients [80, 81], it is appropriate to consider GLAST KO mice as a valid and adequate model that offer a powerful system to determine the mechanisms of and evaluate new treatments for NTG. As the receptors for GDNF and neurturin are increased in Müller cells after optic nerve transection, the neuroprotective effects of GDNF may be indirect, at least partly, through the enhancement of glutamate uptake in Müller cells. Furthermore, we have reported that the loss of apoptosis signal-regulating kinase 1 (ASK1), an evolutionarily conserved mitogen-activated protein kinase kinase kinase (MAP3K), prevented the activity of p38 MAPK and TNF-induced production of inducible nitric oxide synthase (iNOS) in Müller cells [82]. TNF and nitric oxide (NO) can induce RGC death and participate in the pathophysiology of glaucoma [83–85]. Taken together, these findings suggest that such a glial–neuronal network may be functional and that ASK1, NO, GLAST, and trophic factors in Müller cells have important roles in this network during glaucoma [82, 86]. Thus, in addition to neurotrophic factors, further efforts to discover new compounds that can enhance glutamate uptake and inhibit ASK1 signaling for a prolonged period may lead to the development of novel strategies for the management of glaucoma, including NTG [87, 88].

7.4 Conclusions and Future Directions

Neurotrophic factors play important roles during retinal development, degeneration and regeneration. There has been considerable progress in the characterization of molecular pathways that regulate RGC survival by neurotrophic factors. In addition, some clinical trials for retinal diseases are underway and therapeutic potentials of several neurotrophic factors in protecting RGCs are emerging [89–91]. Therefore, it is important to discover safe administration methods and determine the long-term effects of these molecules on human retinal function, especially in glaucomatous eyes.

Acknowledgments This work was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (CH) and the Funding Program for Next Generation World-Leading Researchers (NEXT Program) (TH).

References

1. Harada T, Harada C, Parada LF (2007) Molecular regulation of visual system development: more than meets the eye. Genes Dev 21:367–378
2. Isenmann S, Kretz A, Cellerino A (2003) Molecular determinants of retinal ganglion cell development, survival, and regeneration. Prog Retin Eye Res 22:483–543

3. von Bartheld CS (1998) Neurotrophins in the developing and regenerating visual system. Histol Histopathol 13:437–459
4. Frade JM, Barde YA (1999) Genetic evidence for cell death mediated by nerve growth factor and the neurotrophin receptor p75 in the developing mouse retina and spinal cord. Development 126:683–690
5. Harada C, Harada T, Nakamura K et al (2006) Effect of $p75^{NTR}$ on the regulation of naturally occurring cell death and retinal ganglion cell number in the mouse eye. Dev Biol 290:57–65
6. Perry VH, Henderson Z, Linden R (1983) Postnatal changes in retinal ganglion cell and optic axon populations in the pigmented rat. J Comp Neurol 219:356–368
7. Cui Q, Harvey AR (1995) At least two mechanisms are involved in the death of retinal ganglion cells following target ablation in neonatal rats. J Neurosci 15:8143–8155
8. Ma YT, Hsieh T, Forbes ME et al (1998) BDNF injected into the superior colliculus reduces developmental retinal ganglion cell death. J Neurosci 18:2097–2107
9. Cellerino A, Carroll P, Thoenen H et al (1997) Reduced size of retinal ganglion cell axons and hypomyelination in mice lacking brain-derived neurotrophic factor. Mol Cell Neurosci 9:397–408
10. Harada C, Harada T, Quah HM et al (2005) Role of neurotrophin-4/5 in neural cell death during retinal development and ischemic retinal injury in vivo. Invest Ophthalmol Vis Sci 46:669–673
11. Lee R, Kermani P, Teng KK et al (2001) Regulation of cell survival by secreted proneurotrophins. Science 294:1945–1948
12. Nakamura K, Namekata K, Harada C, Harada T (2007) Intracellular sortilin expression pattern regulates proNGF-induced naturally occurring cell death during development. Cell Death Differ 14:1552–1554
13. Nykjaer A, Lee R, Teng KK et al (2004) Sortilin is essential for proNGF-induced neuronal cell death. Nature 427:843–848
14. Teng HK, Teng KK, Lee R et al (2005) ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. J Neurosci 25:5455–5463
15. Lebrun-Julien F, Bertrand MJ, De Backer O et al (2010) ProNGF induces TNFα-dependent death of retinal ganglion cells through a $p75^{NTR}$ non-cell-autonomous signaling pathway. Proc Natl Acad Sci U S A 107:3817–3822. doi:10.1073/pnas.0909276107
16. Santos AM, López-Sánchez N, Martín-Oliva D et al (2012) Sortilin participates in light-dependent photoreceptor degeneration in vivo. PLoS One 7:e36243. doi:10.1371/journal.pone.0036243
17. Bertrand MJ, Kenchappa RS, Andrieu D et al (2008) NRAGE, a p75NTR adaptor protein, is required for developmental apoptosis in vivo. Cell Death Differ 15:1921–1929. doi:10.1038/cdd.2008.127
18. Linggi MS, Burke TL, Williams BB et al (2005) Neurotrophin receptor interacting factor (NRIF) is an essential mediator of apoptotic signaling by the p75 neurotrophin receptor. J Biol Chem 280:13801–13808
19. Marler KJ, Poopalasundaram S, Broom ER et al (2010) Pro-neurotrophins secreted from retinal ganglion cell axons are necessary for ephrinA-$p75^{NTR}$-mediated axon guidance. Neural Dev 5:30. doi:10.1186/1749-8104-5-30
20. Anderson DR, Hendrickson A (1974) Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve. Invest Ophthalmol 13:771–783
21. Hayreh SS, March W, Anderson DR (1979) Pathogenesis of block of rapid orthograde axonal transport by elevated intraocular pressure. Exp Eye Res 28:515–523
22. Quigley H, Anderson DR (1976) The dynamics and location of axonal transport blockade by acute intraocular pressure elevation in primate optic nerve. Invest Ophthalmol 15:606–616
23. Salinas-Navarro M, Alarcón-Martínez L, Valiente-Soriano FJ et al (2010) Ocular hypertension impairs optic nerve axonal transport leading to progressive retinal ganglion cell degeneration. Exp Eye Res 90:168–183. doi:10.1016/j.exer.2009.10.003

24. Knox DL, Eagle RC Jr, Green WR (2007) Optic nerve hydropic axonal degeneration and blocked retrograde axoplasmic transport: histopathologic features in human high-pressure secondary glaucoma. Arch Ophthalmol 125:347–353
25. Pease ME, McKinnon SJ, Quigley HA et al (2000) Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. Invest Ophthalmol Vis Sci 41:764–774
26. Quigley HA, McKinnon SJ, Zack DJ et al (2000) Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest Ophthalmol Vis Sci 41:3460–3466
27. Takihara Y, Inatani M, Hayashi H et al (2011) Dynamic imaging of axonal transport in living retinal ganglion cells in vitro. Invest Ophthalmol Vis Sci 52:3039–3045. doi:10.1167/iovs.10-6435
28. Chen H, Weber AJ (2001) BDNF enhances retinal ganglion cell survival in cats with optic nerve damage. Invest Ophthalmol Vis Sci 42:966–974
29. Di Polo A, Aigner LJ, Dunn RJ et al (1998) Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Müller cells temporarily rescues injured retinal ganglion cells. Proc Natl Acad Sci U S A 95:3978–3983
30. Mansour-Robaey S, Clarke DB, Wang YC et al (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A 91:1632–1636
31. Peinado-Ramón P, Salvador M, Villegas-Pérez MP, Vidal-Sanz M (1996) Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study. Invest Ophthalmol Vis Sci 37:489–500
32. Ko ML, Hu DN, Ritch R et al (2001) Patterns of retinal ganglion cell survival after brain-derived neurotrophic factor administration in hypertensive eyes of rats. Neurosci Lett 305:139–142
33. Martin KR, Quigley HA, Zack DJ et al (2003) Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model. Invest Ophthalmol Vis Sci 44:4357–4365
34. Bai Y, Dergham P, Nedev H et al (2010) Chronic and acute models of retinal neurodegeneration TrkA activity are neuroprotective whereas $p75^{NTR}$ activity is neurotoxic through a paracrine mechanism. J Biol Chem 285:39392–39400. doi:10.1074/jbc.M110.147801
35. Shi Z, Birman E, Saragovi HU (2007) Neurotrophic rationale in glaucoma: a TrkA agonist, but not NGF or a p75 antagonist, protects retinal ganglion cells in vivo. Dev Neurobiol 67:884–894
36. Lebrun-Julien F, Morquette B, Douillette A et al (2009) Inhibition of $p75^{NTR}$ in glia potentiates TrkA-mediated survival of injured retinal ganglion cells. Mol Cell Neurosci 40:410–420. doi:10.1016/j.mcn.2008.12.005
37. Colafrancesco V, Parisi V, Sposato V et al (2011) Ocular application of nerve growth factor protects degenerating retinal ganglion cells in a rat model of glaucoma. J Glaucoma 20:100–108. doi:10.1097/IJG.0b013e3181d787e5
38. Lambiase A, Aloe L, Centofanti M et al (2009) Experimental and clinical evidence of neuroprotection by nerve growth factor eye drops: Implications for glaucoma. Proc Natl Acad Sci U S A 106:13469–13474. doi:10.1073/pnas.0906678106
39. Cohen A, Bray GM, Aguayo AJ (1994) Neurotrophin-4/5 (NT-4/5) increases adult rat retinal ganglion cell survival and neurite outgrowth in vitro. J Neurobiol 25:953–959
40. Blum R, Konnerth A (2005) Neurotrophin-mediated rapid signaling in the central nervous system: mechanisms and functions. Physiology 20:70–78
41. Namekata K, Harada C, Taya C et al (2010) Dock3 induces axonal outgrowth by stimulating membrane recruitment of the WAVE complex. Proc Natl Acad Sci U S A 107:7586–7591. doi:10.1073/pnas.0914514107

42. Nikoletopoulou V, Lickert H, Frade JM et al (2010) Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. Nature 467:59–63. doi:10.1038/nature09336
43. Cheng L, Sapieha P, Kittlerová P et al (2002) TrkB gene transfer protects retinal ganglion cells from axotomy-induced death in vivo. J Neurosci 22:3977–3986
44. Hu Y, Cho S, Goldberg JL (2010) Neurotrophic effect of a novel TrkB agonist on retinal ganglion cells. Invest Ophthalmol Vis Sci 51:1747–1754. doi:10.1167/iovs.09-4450
45. Parrilla-Reverter G, Agudo M, Sobrado-Calvo P et al (2009) Effects of different neurotrophic factors on the survival of retinal ganglion cells after a complete intraorbital nerve crush injury: a quantitative in vivo study. Exp Eye Res 89:32–41. doi:10.1016/j.exer.2009.02.015
46. Grishanin RN, Yang H, Liu X et al (2008) Retinal TrkB receptors regulate neural development in the inner, but not outer, retina. Mol Cell Neurosci 38:431–443. doi:10.1016/j.mcn.2008.04.004
47. Harada T, Harada C, Nakayama N et al (2000) Modification of glial-neuronal cell interactions prevents photoreceptor apoptosis during light-induced retinal degeneration. Neuron 26:533–541
48. Wahlin KJ, Campochiaro PA, Zack DJ, Adler R (2000) Neurotrophic factors cause activation of intracellular signaling pathways in Müller cells and other cells of the inner retina, but not photoreceptors. Invest Ophthalmol Vis Sci 41:927–936
49. Harada T, Harada C, Kohsaka S et al (2002) Microglia-Müller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. J Neurosci 22:9228–9236
50. Harada C, Guo X, Namekata K et al (2011) Glia- and neuron-specific functions of TrkB signalling during retinal degeneration and regeneration. Nat Commun 2:189. doi:10.1038/ncomms1190
51. Lamba D, Karl M, Reh T (2008) Neural regeneration and cell replacement: a view from the eye. Cell Stem Cell 2:538–549. doi:10.1016/j.stem.2008.05.002
52. LaVail MM, Unoki K, Yasumura D et al (1992) Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. Proc Natl Acad Sci U S A 89:11249–11253
53. Fischer D, Leibinger M (2012) Promoting optic nerve regeneration. Prog Retin Eye Res 31:688–701. doi:10.1016/j.preteyeres.2012.06.005
54. Heinrich PC, Behrmann I, Haan S et al (2003) Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374:1–20
55. Ernst M, Jenkins BJ (2004) Acquiring signalling specificity from the cytokine receptor gp130. Trends Genet 20:23–32
56. Leaver SG, Cui Q, Plant GW et al (2006) AAV-mediated expression of CNTF promotes long-term survival and regeneration of adult rat retinal ganglion cells. Gene Ther 13:1328–1341
57. Meyer-Franke A, Kaplan MR, Pfrieger FW, Barres BA (1995) Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. Neuron 15:805–819
58. van Adel BA, Kostic C, Déglon N et al (2003) Delivery of ciliary neurotrophic factor via lentiviral-mediated transfer protects axotomized retinal ganglion cells for an extended period of time. Hum Gene Ther 14:103–115
59. Ji J-T, Elyaman W, Yip HK et al (2004) CNTF promotes survival of retinal ganglion cells after induction of ocular hypertension in rats: the possible involvement of STAT3 pathway. Eur J Neurosci 19:265–272
60. Pease ME, Zack DJ, Berlinicke C et al (2009) Effect of CNTF on retinal ganglion cell survival in experimental glaucoma. Invest Ophthalmol Vis Sci 50:2194–2200. doi:10.1167/iovs.08-3013
61. Sieving PA, Caruso RC, Tao W et al (2006) Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. Proc Natl Acad Sci U S A 103:3896–3901

62. Talcott KE, Ratnam K, Sundquist SM et al (2011) Longitudinal study of cone photoreceptors during retinal degeneration and in response to ciliary neurotrophic factor treatment. Invest Ophthalmol Vis Sci 52:2219–2226. doi:10.1167/iovs.10-6479
63. Tao W (2006) Application of encapsulated cell technology for retinal degenerative diseases. Expert Opin Biol Ther 6:717–726
64. Cui Q, Lu Q, So KF, Yip HK (1999) CNTF, not other trophic factors, promotes axonal regeneration of axotomized retinal ganglion cells in adult hamsters. Invest Ophthalmol Vis Sci 40:760–766
65. Watanabe M, Tokita Y, Kato M, Fukuda Y (2003) Intravitreal injections of neurotrophic factors and forskolin enhance survival and axonal regeneration of axotomized beta ganglion cells in cat retina. Neuroscience 116:733–742
66. Babon JJ, Nicola NA (2012) The biology and mechanism of action of suppressor of cytokine signaling 3. Growth Factors 30:207–219. doi:10.3109/08977194.2012.687375
67. Hellström M, Muhling J, Ehlert EM et al (2011) Negative impact of rAAV2 mediated expression of SOCS3 on the regeneration of adult retinal ganglion cell axons. Mol Cell Neurosci 46:507–515. doi:10.1016/j.mcn.2010.12.003
68. Smith PD, Sun F, Park KK et al (2009) SOCS3 deletion promotes optic nerve regeneration in vivo. Neuron 64:617–623. doi:10.1016/j.neuron.2009.11.021
69. Sun F, Park KK, Belin S et al (2011) Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. Nature 480:372–375. doi:10.1038/nature10594
70. Baloh RH, Enomoto H, Johnson EM Jr, Milbrandt J (2000) The GDNF family ligands and receptors – implications for neural development. Curr Opin Neurobiol 10:103–110
71. Klöcker N, Bräunling F, Isenmann S, Bähr M (1997) In vivo neurotrophic effects of GDNF on axotomized retinal ganglion cells. Neuroreport 8:3439–3442
72. Koeberle PD, Ball AK (1998) Effects of GDNF on retinal ganglion cell survival following axotomy. Vision Res 38:1505–1515
73. Yan Q, Wang J, Matheson CR, Urich JL (1999) Glial cell line-derived neurotrophic factor (GDNF) promotes the survival of axotomized retinal ganglion cells in adult rats: comparison to and combination with brain-derived neurotrophic factor (BDNF). J Neurobiol 38:382–390
74. Kyhn MV, Klassen H, Johansson UE et al (2009) Delayed administration of glial cell line-derived neurotrophic factor (GDNF) protects retinal ganglion cells in a pig model of acute retinal ischemia. Exp Eye Res 89:1012–1020. doi:10.1016/j.exer.2009.08.014
75. Checa-Casalengua P, Jiang C, Bravo-Osuna I et al (2011) Retinal ganglion cells survival in a glaucoma model by GDNF/Vit E PLGA microspheres prepared according to a novel microencapsulation procedure. J Control Release 156:92–100. doi:10.1016/j.jconrel.2011.06.023
76. Nakatani M, Shinohara Y, Takii M et al (2011) Periocular injection of in situ hydrogels containing Leu-Ile, an inducer for neurotrophic factors, promotes retinal ganglion cell survival after optic nerve injury. Exp Eye Res 93:873–879. doi:10.1016/j.exer.2011.09.024
77. Harada C, Harada T, Quah HM et al (2003) Potential role of glial cell line-derived neurotrophic factor receptors in Müller glial cells during light-induced retinal degeneration. Neuroscience 122:229–235
78. Koeberle PD, Bähr M (2008) The upregulation of GLAST-1 is an indirect antiapoptotic mechanism of GDNF and neurturin in the adult CNS. Cell Death Differ 15:471–483
79. Harada T, Harada C, Nakamura K et al (2007) The potential role of glutamate transporters in the pathogenesis of normal tension glaucoma. J Clin Invest 117:1763–1770
80. Gherghel D, Griffiths HR, Hilton EJ et al (2005) Systemic reduction in glutathione levels occurs in patients with primary open-angle glaucoma. Invest Ophthalmol Vis Sci 46:877–883
81. Naskar R, Vorwerk CK, Dreyer EB (2000) Concurrent downregulation of a glutamate transporter and receptor in glaucoma. Invest Ophthalmol Vis Sci 41:1940–1944
82. Harada C, Namekata K, Guo X et al (2010) ASK1 deficiency attenuates neural cell death in GLAST-deficient mice, a model of normal tension glaucoma. Cell Death Differ 17:1751–1759. doi:10.1038/cdd.2010.62

83. Nakazawa T, Nakazawa C, Matsubara A et al (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J Neurosci 26:12633–12641
84. Osborne NN (2008) Pathogenesis of ganglion 'cell death' in glaucoma and neuroprotection: focus on ganglion cell axonal mitochondria. Prog Brain Res 173:339–352. doi:10.1016/S0079-6123(08)01124-2
85. Toda N, Nakanishi-Toda M (2007) Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. Prog Retin Eye Res 26:205–238
86. Bringmann A, Iandiev I, Pannicke T et al (2009) Cellular signaling and factors involved in Müller cell gliosis: neuroprotective and detrimental effects. Prog Retin Eye Res 28:423–451. doi:10.1016/j.preteyeres.2009.07.001
87. Guo X, Harada C, Namekata K et al (2010) Regulation of the severity of neuroinflammation and demyelination by TLR-ASK1-p38 pathway. EMBO Mol Med 2:504–515. doi:10.1002/emmm.201000103
88. Katome T, Namekata K, Guo X et al (2013) Inhibition of ASK1-p38 pathway prevents neural cell death following optic nerve injury. Cell Death Differ 20:270–280. doi:10.1038/cdd.2012.122
89. Kolomeyer AM, Zarbin MA (2014) Trophic factors in the pathogenesis and therapy for retinal degenerative diseases. Surv Ophthalmol 59(2):134–165. doi:10.1016/j.survophthal.2013.09.004
90. Miyazaki M, Ikeda Y, Yonemitsu Y et al (2011) Pigment epithelium-derived factor gene therapy targeting retinal ganglion cell injuries: neuroprotection against loss of function in two animal models. Hum Gene Ther 22:559–565. doi:10.1089/hum.2010.132
91. Tombran-Tink J (2010) PEDF in angiogenic eye diseases. Curr Mol Med 10:267–278

Chapter 8
RIP Kinase-Mediated Programmed Necrosis

Yusuke Murakami, Maki Kayama, Joan W. Miller, and Demetrios Vavvas

Abstract Retinal ganglion cell (RGC) death is the ultimate cause of vision loss in glaucoma. Apoptosis has been thought to be a major form of cell death in various diseases including glaucoma; however, attempts to develop neuroprotective agents that target apoptosis have largely failed. Recent accumulating evidence has shown that non-apoptotic forms of cell death such as necrosis are also regulated by specific molecular machinery, such as those mediated by receptor-interacting protein (RIP) kinases. In this review, we summarize recent advances in our understanding of RIP kinase signaling and its roles in RGC loss. These data suggest that not only apoptosis but also necrosis is involved in RGC death and that combined targeting of these pathways may be an effective strategy for glaucoma.

Keywords Necroptosis • Programmed necrosis • Retinal ganglion cell death • RIP kinase

8.1 Introduction

Glaucoma affects 70 million people worldwide and is characterized by progressive retinal ganglion cell (RGC) death with accompanying optic nerve atrophy [1, 2]. It is often associated with elevated intraocular pressure (IOP) and current

Y. Murakami (✉)
Department of Ophthalmology, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Department of Ophthalmology, Retina Service, Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114, USA
e-mail: ymuraka3@med.kyushu-u.ac.jp

M. Kayama • J.W. Miller • D. Vavvas
Department of Ophthalmology, Retina Service, Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114, USA

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_8, © Springer Japan 2014

management aims at lowering IOP. Although IOP-lowering treatments slow the development and progression of glaucoma, approximately 10 % of people who receive proper treatment still experience severe vision loss. Therefore, investigating the mechanisms of RGC death will be important to better understanding of the disease pathophysiology and development of novel therapeutics.

8.2 Two Distinct Forms of Cell Death: Apoptosis and Necrosis

Apoptosis and necrosis are two major forms of cell death defined by morphological appearance [3]. In 1972, Kerr et al. coined the term apoptosis (Greek for "falling off") to describe a specific form of cell death, which shows condensation of the nucleus and cytoplasm, rounding-up of the cell, reduction of cellular volume, and engulfment by resident phagocyte [4]. They suspected apoptosis as a general mechanism of controlled cell deletion, and indeed, accumulating evidence demonstrates that apoptosis is genetically a regulated process of cell death, where the caspase family of cysteine proteases plays a central role for signal transduction and execution [5]. In contrast, necrosis (Greek for "dead") is associated with swelling of the cytoplasm and organelles, a gain in cell volume, plasma membrane rupture, and connections with the extracellular cavity. Although necrosis was traditionally thought to be an uncontrolled process of cell death, it is now known to also have regulated components, such as those mediated by receptor-interacting protein (RIP) kinases [6].

8.3 RIP Kinase-Mediated Programmed Necrosis

RIP kinase-mediated programmed necrosis was discovered from the extensive studies of death receptor-induced cell death. Death ligands such as TNF-α and Fas-L induce apoptosis, but they also cause necrosis in certain types of cells [7]. Intriguingly, Vercammen and colleagues found that, when apoptosis is blocked by caspase inhibitors, cells undergo an alternative necrotic cell death in response to TNF-α or Fas-L [8, 9]. Twelve years later, Holler and colleagues identified that that this death receptor-induced necrosis is mediated by the activation of RIP1 [10]. Furthermore, three independent studies recently showed that interaction between RIP3 and RIP1 is critical for RIP1 kinase activation and subsequent necrosis [11–13]. These advances in our understanding of the molecular basis of necrosis have revealed previously unrecognized roles of necrosis in health and disease.

8.4 RIP Kinase Signaling

Two members of the RIP kinase family proteins, RIP1 and RIP3, are critical mediators of necrosis [6]. RIP1 was originally identified as a protein that interacts with Fas [14]. RIP1 consists of an N-terminal serine/threonine kinase domain, an intermediate domain, a RIP homotypic interaction motif (RHIM), and a C-terminal DD (Fig. 8.1). RIP1 acts as a multifunctional adaptor protein downstream of death receptors and mediates pro-survival NF-κB activation, caspase-dependent apoptosis, and RIP kinase-dependent necrosis [15]. RIP3 was found as a serine/threonine kinase that shares homology with RIP1 but does not possess a DD [16] (Fig. 8.1). RIP3 contains the RHIM domain in its C-terminus and directly binds to and phosphorylates RIP1 [17]. Although the precise biological function of RIP1-RIP3 interaction was unclear for a long period, recent studies have shown that RIP3-dependent phosphorylation of RIP1 kinase in the RIP1-RIP3 complex is critical for the induction of death receptor-induced necrosis [11–13]. This necrosis-inducing protein complex is termed the "necrosome."

8.4.1 RIP1 Polyubiquitination and Pro-survival NF-κB Activation

In response to TNF-α stimulation, RIP1 is recruited to TNFR and forms a membrane-associated complex with TNF receptor-associated death domain (TRADD), TNF receptor-associated factor 2 or 5 (TRAF2/5), and cIAP1/2, the so-called complex I [18]. cIAP1 and cIAP2 are key ubiquitin ligases that induce RIP1 polyubiquitination in the complex [19]. This ubiquitin chain provides an

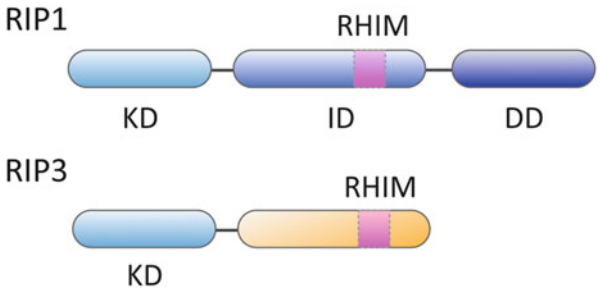


Fig. 8.1 The structure of RIP1 and RIP3. RIP1 consists of an N-terminal serine/threonine kinase domain (KD), an intermediate domain (ID), a RIP homotypic interaction motif (RHIM), and a C-terminal death domain (DD). RIP3 shares homology with RIP1 but does not possess a DD. RIP3 contains the RHIM domain in its C-terminus and directly binds to and phosphorylates RIP1. This phosphorylation in the RIP1-RIP3 complex is critical for the induction of death receptor-induced necrosis. Reproduced with permission from Murakami et al. [40]

assembly site for transforming growth factor-β-activated kinase 1 (TAK1), TAK1-binding protein 2 or 3 (TAB2/3), and inhibitor κB kinase (IKK) complex and mediates NF-κB activation [20]. Activated NF-κB translocates to the nucleus and induces transcription of prosurvival genes such as cIAPs, c-FLIPs, and IL-6 [21]. In addition, it mediates the induction of cylindromatosis (CYLD) or A20, which dephosphorylates RIP1 and acts as a negative feedback loop in NF-κB signaling [22, 23] (Fig. 8.2a).

8.4.2 *RIP1 Deubiquitination and Formation of Cytosolic Pro-death Complex: DISC or Necrosome*

RIP1 switches its function to a regulator of cell death when it is deubiquitinated by CYLD or A20 [24]. Deubiquitination of RIP1 abolishes its ability to activate NF-κB and leads to the formation of cytosolic pro-death complexes, the so-called complex II [18]. These complexes contain TRADD, FADD, RIP1, caspase-8, c-FLIP, and/or RIP3 and mediate either apoptosis or necrosis depending on cellular conditions. Dimerization of caspase-8 in the complex II mediates a conformational change to its active form, thereby inducing apoptosis (Fig. 8.2b). On the other hand, in conditions where caspases are inhibited or cannot be activated efficiently, RIP1 interacts with RIP3 and forms the necrosome (Fig. 8.2c). RIP3-dependent activation of RIP kinase is crucial for necrosis induction in response to TNF-α [11–13]. Other death ligands such as Fas-L are also capable to mediate RIP kinase-dependent necrosis as well as caspase-dependent apoptosis. In contrast to TNF-α, Fas directly recruits RIP1 and FADD to the plasma membrane and forms pro-death complexes with caspase-8 and/or RIP3 [14].

8.4.3 *Regulatory Mechanisms of RIP Kinase Activation*

Because caspase inhibition sensitizes cells to RIP kinase-dependent necrosis, caspases may inhibit RIP kinase activity. Indeed, caspase-8 directly cleaves and inactivates RIP1 and RIP3 [25, 26]. Interestingly, this inactivation does not require proapoptotic caspase-8 activation through its homodimerization, but is mediated by the restricted caspase-8 activity in the heterodimer with c-FLIP, an endogenous inhibitor of caspase-8 [27] (Fig. 8.2). Recent studies have shown that the c-FLIP-caspase-8 heterodimer has a restricted substrate repertoire and mediates pro-survival effect via antagonizing RIP kinase activation [28].

The expression levels of RIP3 are another factor that control RIP kinase activation. Whereas RIP1 is expressed ubiquitously in all cell types, RIP3 expression differs amongst cells and tissue [16]. In addition, the levels of RIP3 correlate with the responsiveness to necrotic cell death induced by TNF-α [12]. The levels of

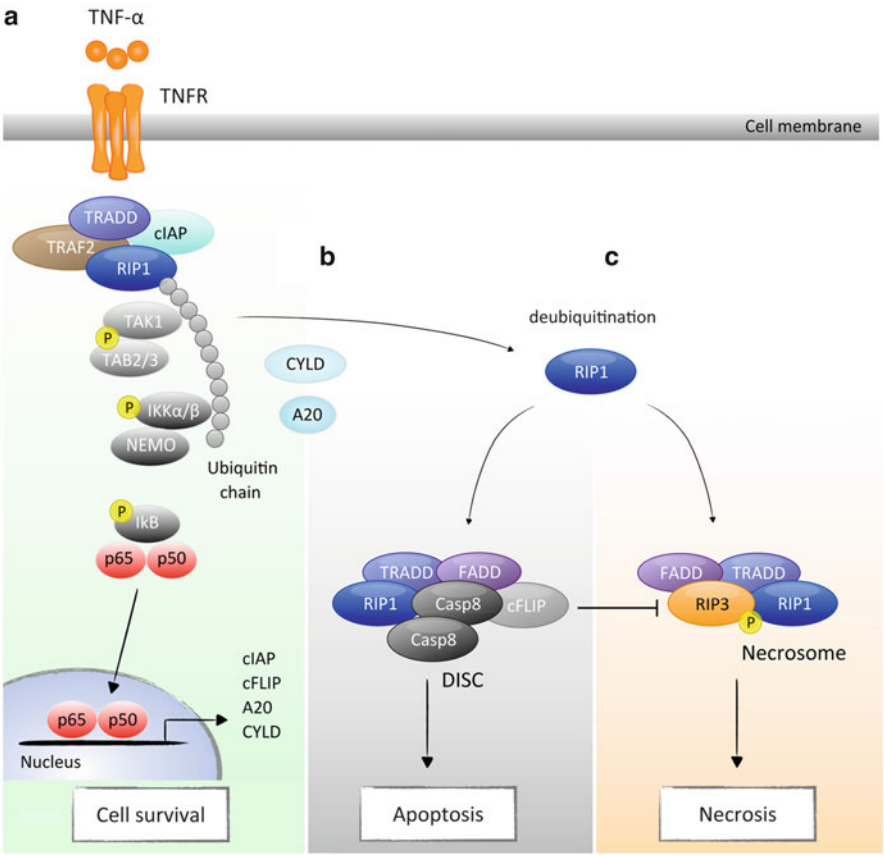


Fig. 8.2 RIP kinase signaling. (**a**) In response to TNF-α stimulation, RIP1 is recruited to TNFR and forms a membrane-associated complex with TRADD, TRAF2 and cIAPs. cIAPs ubiquitinate RIP1, which in turn mediate NF-κB activation. Nuclear translocation of p65/p50 subunits promotes the production of pro-survival genes such as cIAPs and c-FLIPs as well as deubiquitinating enzymes such as CYLD and A20, which act as a negative feedback loop in NF-κB signaling. (**b**) and (**c**). RIP1 switches its function to a regulator of cell death when it is deubiquitinated by CYLD or A20. Deubiquitination of RIP1 abolishes its ability to activate NF-κB and leads to the formation of cytosolic pro-death complexes. These complexes contain TRADD, FADD, RIP1, caspase-8, c-FLIP, and/or RIP3 and mediate either apoptosis or necrosis depending on cellular conditions. Multimerization of caspase-8 in the DISC mediates a conformational change to its active form, thereby inducing apoptosis (**b**). The catalytic activity of caspase-8-c-FLIP heterodimer complex cleaves and inactivates RIP1 and RIP3. In conditions where caspases/c-FLIPs are inhibited or cannot be activated efficiently, RIP1 forms the necrosome with RIP3, thereby promoting necrosis (**c**). Reproduced with permission from Murakami et al. [40]

caspases also change depending on cellular types and conditions. Caspase-dependent apoptosis is downregulated in the mature neurons because of reduced caspase-3 expression after development [29]. Caspase-8 expression is substantially lower in RPE cells compared with other ocular epithelial cells or tumor cells, which

may protect the RPE from apoptosis [30]. Therefore, it is likely that the balance between caspases and RIP3 may be important to decide the cell fate (i.e., apoptosis or necrosis) in response to death receptor stimulation or other signals.

8.5 RIP Kinase Inhibitors

Degterev and colleagues identified small compounds named necrostatin that specifically inhibit death receptor-mediated necrosis in a cell-based screening of ~15,000 chemical compounds [31]. Necrostatin-1 (Nec-1) has been shown to strongly inhibit RIP1 kinase phosphorylation, and structure-activity relationship analysis demonstrated that Nec-1 binds to the adaptive pocket on RIP1 and stabilizes the inactive conformation of RIP1 kinase [32]. Importantly, other two necrostatins, which have different structures than Nec-1, also inhibit RIP1 kinase phosphorylation, suggesting that necrostatins target RIP1 kinase.

However, there are some reports raising concerns about the specificity of necrostatins. For instance, it was shown that Nec-1 partially affects the PAK1 and PKAcα activity on a panel screening of 98 human kinases [33]. More recently, Takahashi and colleagues demonstrated critical issues on the specificity and activity of Nec-1. They report that Nec-1 is identical to methyl-thiohydantoin-tryptophan (MTH-Trp), an inhibitor of indoleamine 2,3-dioxygenase (IDO) [34]. IDO is the rate-limiting enzyme in tryptophan catabolism and modulates immune tolerance. Hence, interpretation of the results obtained by using Nec-1 requires consideration of its nonspecific effect, and additional experiments using RIP3-deficient mice or RNAi knockdown of RIP kinase will help the precise understanding of the role of RIP kinase in diseases.

8.6 Knockout Animals for RIP Kinases

RIP1 is a multifunctional protein that is critical for both cell survival and death, and *Rip1*$^{-/-}$ mice exhibit postnatal lethality with reduced NF-κB activation and extensive cell death in lymphoid and adipose tissues [35]. In contrast, *Rip3*$^{-/-}$ mice are viable and do not show gross abnormality in any of the major organs including the retina [36]. Although *Rip3*$^{-/-}$ mice are indistinguishable from WT mice in physiological conditions, recent studies have revealed that they display marked reduction in death receptor-induced necrosis [12, 11]. Hence, *Rip3*$^{-/-}$ mice have been used as an instrument of investigating death receptor-induced necrosis in physiological and pathological conditions. Using *Rip3*$^{-/-}$ mice, we have investigated the role of RIP kinase in photoreceptor cell death in retinal degenerative diseases such as retinal detachment, retinitis pigmentosa, and age-related macular degeneration [37–40].

8.7 The Role of RIP Kinase in RGC Death

In experimental models of glaucoma, dying RGCs show not only apoptotic but also necrotic features [41]. However, most of studies investigating the mechanisms of RGC death have mainly focused on apoptosis, because of the general concept that necrosis is an uncontrolled process of cell death. Unfortunately, despite more than a decade of work on apoptosis, attempts to prevent or delay RGC degeneration in glaucoma have been unsuccessful, and it would be important to investigate the role of other mechanisms of cell death in glaucoma.

Accumulating evidence indicates that TNF-α is a critical mediator of RGC death in glaucoma [42]. TNF-α is elevated in the aqueous humor of glaucoma patients [43]. Moreover, neutralization of TNF-α prevents RGC death in several models of experimental glaucoma [44, 45]. However, the mechanism by which TNF-α mediates RGC death remains unclear. RIP1 is a key adaptor protein activated downstream of TNF-α [6]. Given the emerging role of RIP kinase in necrosis induction, it can be hypothesized that RIP kinase may be involved in RGC in glaucoma. Rosenbaum and colleagues addressed this question by testing Nec-1 in the retinal ischemia-reperfusion injury model. They showed that intravitreal injection of Nec-1 protects RGC loss and provides functional improvement [46], suggesting the involvement of programmed necrosis in RGC death. We also evaluated the role of RIP kinase-dependent necrosis in other models of RGC death and found that not only caspase pathway but also RIP kinase pathway is important for RGC death (MK, DGV et al. unpublished data). Consistent with these in vivo findings, Lee and colleagues demonstrated that RIP3 mediates rat RGC death through phosphorylation of Daxx after oxygen glucose deprivation in vitro [47]. Taken together, these findings suggest RIP kinase-dependent necrosis as a novel mechanism of RGC death and as a therapeutic target.

8.8 Conclusion

Although apoptosis has been thought to be a major form of cell death in retinal and neurodegenerative diseases, recent studies have shown that non-apoptotic forms of cell death are also important. RIP kinase is a crucial regulator of programmed necrosis and contributes to neuronal cell death in various conditions, including RGC death. Further studies investigating the role of RIP kinase-dependent necrosis in glaucoma will be important for better understanding of the mechanisms of RGC death and development of novel therapeutics to prevent or delay RGC loss.

Competing Interests Statement The Massachusetts Eye and Ear Infirmary has filed patents on the subject of neuroprotection in retinal degenerations. YM, MK, JWM, and DGV are named inventors.

Acknowledgments This work was supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology grant 25861637 (YM), Harvard Ophthalmology Department Support (DGV), and NEI grant EY014104 (MEEI Core Grant).

References

1. Quigley HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90(3):262–267. doi:10.1136/bjo.2005.081224
2. Kwon YH, Fingert JH, Kuehn MH, Alward WL (2009) Primary open-angle glaucoma. N Engl J Med 360(11):1113–1124. doi:10.1056/NEJMra0804630
3. Schweichel JU, Merker HJ (1973) The morphology of various types of cell death in prenatal tissues. Teratology 7(3):253–266. doi:10.1002/tera.1420070306
4. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26(4):239–257
5. Riedl SJ, Shi Y (2004) Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol 5(11):897–907. doi:10.1038/nrm1496
6. Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G (2010) Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol 11(10):700–714. doi:10.1038/nrm2970
7. Laster SM, Wood JG, Gooding LR (1988) Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J Immunol 141(8):2629–2634
8. Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W, Grooten J, Fiers W, Vandenabeele P (1998) Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. J Exp Med 187(9):1477–1485
9. Vercammen D, Brouckaert G, Denecker G, Van de Craen M, Declercq W, Fiers W, Vandenabeele P (1998) Dual signaling of the Fas receptor: initiation of both apoptotic and necrotic cell death pathways. J Exp Med 188(5):919–930
10. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J (2000) Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 1(6):489–495
11. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, Chan FK (2009) Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 137(6):1112–1123
12. He S, Wang L, Miao L, Wang T, Du F, Zhao L, Wang X (2009) Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 137(6):1100–1111
13. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J (2009) RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 325(5938):332–336
14. Stanger BZ, Leder P, Lee TH, Kim E, Seed B (1995) RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 81(4):513–523
15. Festjens N, Vanden Berghe T, Cornelis S, Vandenabeele P (2007) RIP1, a kinase on the crossroads of a cell's decision to live or die. Cell Death Differ 14(3):400–410. doi:10.1038/sj.cdd.4402085
16. Sun X, Lee J, Navas T, Baldwin DT, Stewart TA, Dixit VM (1999) RIP3, a novel apoptosis-inducing kinase. J Biol Chem 274(24):16871–16875
17. Sun X, Yin J, Starovasnik MA, Fairbrother WJ, Dixit VM (2002) Identification of a novel homotypic interaction motif required for the phosphorylation of receptor-interacting protein (RIP) by RIP3. J Biol Chem 277(11):9505–9511

18. Micheau O, Tschopp J (2003) Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 114(2):181–190
19. Mahoney DJ, Cheung HH, Mrad RL, Plenchette S, Simard C, Enwere E, Arora V, Mak TW, Lacasse EC, Waring J, Korneluk RG (2008) Both cIAP1 and cIAP2 regulate TNFalpha-mediated NF-kappaB activation. Proc Natl Acad Sci U S A 105(33):11778–11783
20. Ea CK, Deng L, Xia ZP, Pineda G, Chen ZJ (2006) Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. Mol Cell 22 (2):245–257
21. Micheau O, Lens S, Gaide O, Alevizopoulos K, Tschopp J (2001) NF-kappaB signals induce the expression of c-FLIP. Mol Cell Biol 21(16):5299–5305. doi:10.1128/MCB.21.16.5299-5305.2001
22. Trompouki E, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A, Mosialos G (2003) CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. Nature 424(6950):793–796. doi:10.1038/nature01803
23. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin EV, Dixit VM (2004) De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature 430(7000):694–699. doi:10.1038/nature02794
24. Shembade N, Ma A, Harhaj EW (2010) Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. Science 327(5969):1135–1139
25. Feng S, Yang Y, Mei Y, Ma L, Zhu DE, Hoti N, Castanares M, Wu M (2007) Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. Cell Signal 19(10):2056–2067. doi:10.1016/j.cellsig.2007.05.016
26. Lin Y, Devin A, Rodriguez Y, Liu ZG (1999) Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 13(19):2514–2526
27. Oberst A, Dillon CP, Weinlich R, McCormick LL, Fitzgerald P, Pop C, Hakem R, Salvesen GS, Green DR (2011) Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. Nature 471(7338):363–367. doi:10.1038/nature09852
28. van Raam BJ, Salvesen GS (2012) Proliferative versus apoptotic functions of caspase-8 Hetero or homo: the caspase-8 dimer controls cell fate. Biochim Biophys Acta 1824(1):113–122. doi:10.1016/j.bbapap.2011.06.005
29. Donovan M, Cotter TG (2002) Caspase-independent photoreceptor apoptosis in vivo and differential expression of apoptotic protease activating factor-1 and caspase-3 during retinal development. Cell Death Differ 9(11):1220–1231. doi:10.1038/sj.cdd.4401105
30. Yang P, Peairs JJ, Tano R, Zhang N, Tyrell J, Jaffe GJ (2007) Caspase-8-mediated apoptosis in human RPE cells. Invest Ophthalmol Vis Sci 48(7):3341–3349. doi:10.1167/iovs.06-1340
31. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J (2005) Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 1(2):112–119
32. Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X, Abbott D, Cuny GD, Yuan C, Wagner G, Hedrick SM, Gerber SA, Lugovskoy A, Yuan J (2008) Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol 4(5):313–321
33. Biton S, Ashkenazi A (2011) NEMO and RIP1 control cell fate in response to extensive DNA damage via TNF-alpha feedforward signaling. Cell 145(1):92–103. doi:10.1016/j.cell.2011.02.023
34. Takahashi N, Duprez L, Grootjans S, Cauwels A, Nerinckx W, DuHadaway JB, Goossens V, Roelandt R, Van Hauwermeiren F, Libert C, Declercq W, Callewaert N, Prendergast GC, Degterev A, Yuan J, Vandenabeele P (2012) Necrostatin-1 analogues: critical issues on the specificity, activity and in vivo use in experimental disease models. Cell Death Dis 3:e437. doi:10.1038/cddis.2012.176
35. Kelliher MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P (1998) The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. Immunity 8(3):297–303

36. Newton K, Sun X, Dixit VM (2004) Kinase RIP3 is dispensable for normal NF-kappa Bs, signaling by the B-cell and T-cell receptors, tumor necrosis factor receptor 1, and Toll-like receptors 2 and 4. Mol Cell Biol 24(4):1464–1469
37. Trichonas G, Murakami Y, Thanos A, Morizane Y, Kayama M, Debouck CM, Hisatomi T, Miller JW, Vavvas DG (2010) Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis. Proc Natl Acad Sci U S A. doi:10.1073/pnas.1009179107
38. Murakami Y, Matsumoto H, Roh M, Suzuki J, Hisatomi T, Ikeda Y, Miller JW, Vavvas DG (2012) Receptor interacting protein kinase mediates necrotic cone but not rod cell death in a mouse model of inherited degeneration. Proc Natl Acad Sci U S A 109(36):14598–14603. doi:10.1073/pnas.1206937109
39. Murakami Y, Matsumoto H, Roh M, Giani A, Kataoka K, Morizane Y, Kayama M, Thanos A, Nakatake S, Notomi S, Hisatomi T, Ikeda Y, Ishibashi T, Connor KM, Miller JW, Vavvas DG (2013) Programmed necrosis, not apoptosis, is a key mediator of cell loss and DAMP-mediated inflammation in dsRNA-induced retinal degeneration. Cell Death Differ. doi:10.1038/cdd.2013.109
40. Murakami Y, Notomi S, Hisatomi T, Nakazawa T, Ishibashi T, Miller JW, Vavvas DG (2013) Photoreceptor cell death and rescue in retinal detachment and degenerations. Prog Retin Eye Res 37:114–140. doi:10.1016/j.preteyeres.2013.08.001
41. Whiteman AL, Klauss G, Miller PE, Dubielzig RR (2002) Morphologic features of degeneration and cell death in the neurosensory retina in dogs with primary angle-closure glaucoma. Am J Vet Res 63(2):257–261
42. Tezel G (2008) TNF-alpha signaling in glaucomatous neurodegeneration. Prog Brain Res 173:409–421. doi:10.1016/S0079-6123(08)01128-X
43. Sawada H, Fukuchi T, Tanaka T, Abe H (2010) Tumor necrosis factor-alpha concentrations in the aqueous humor of patients with glaucoma. Invest Ophthalmol Vis Sci 51(2):903–906. doi:10.1167/iovs.09-4247
44. Tezel G, Wax MB (2000) Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. J Neurosci 20(23):8693–8700
45. Nakazawa T, Nakazawa C, Matsubara A, Noda K, Hisatomi T, She H, Michaud N, Hafezi-Moghadam A, Miller JW, Benowitz LI (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J Neurosci 26(49):12633–12641. doi:10.1523/JNEUROSCI.2801-06.2006
46. Rosenbaum DM, Degterev A, David J, Rosenbaum PS, Roth S, Grotta JC, Cuny GD, Yuan J, Savitz SI (2010) Necroptosis, a novel form of caspase-independent cell death, contributes to neuronal damage in a retinal ischemia-reperfusion injury model. J Neurosci Res 88 (7):1569–1576. doi:10.1002/jnr.22314
47. Lee YS, Dayma Y, Park MY, Kim KI, Yoo SE, Kim E (2013) Daxx is a key downstream component of receptor interacting protein kinase 3 mediating retinal ischemic cell death. FEBS Lett 587(3):266–271. doi:10.1016/j.febslet.2012.12.004

Chapter 9
Axonal Degeneration

Yasushi Kitaoka

Abstract Axonal degeneration often leads to the death of retinal ganglion cell (RGC) bodies. The pattern of localized retinal nerve fiber layer defects observed in glaucoma patients suggests that axonal degeneration may occur first, followed by sequential RGC body loss in this pathological condition. The molecular mechanism of axonal degeneration in the optic nerve is still unclear. The tumor necrosis factor injection model and the hypertensive glaucoma model may be useful in understanding the mechanism of axonal degeneration of RGCs because axon loss precedes RGC body loss in both models. There is a growing body of evidence that glaucoma may be correlated with Alzheimer's disease. Autophagy impairment may be involved in neurodegenerative diseases including Alzheimer's disease and glaucoma. Thus, the modulation of these signaling pathways will lead to a new concept of axonal protection.

Keywords Alzheimer's disease • Autophagy • Glaucoma • Tumor necrosis factor

9.1 Amyloidogenic Pathway and Axonal Degeneration

Alzheimer's disease (AD) is a neurodegenerative disorder in which axonal degeneration may precede cell body death [1]. The deposition of β-amyloid (Aβ) in neuronal cells is a hallmark of AD. The unfavorable metabolism of amyloid precursor protein (APP) leads to Aβ production. APP is proteolytically cleaved by β-secretase, generating a short C-terminal fragment (CTFβ) of 99 amino acids. The CTFβ fragment of APP is then cleaved by γ-secretase into an Aβ peptide and a cytosolic APP intracellular domain in the amyloidogenic pathway [2]. Although

Y. Kitaoka (✉)
Department of Ophthalmology, St. Marianna University School of Medicine,
2-16-1 Sugao Miyamae-ku, Kawasaki 216-8511, Japan
e-mail: kitaoka@marianna-u.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_9, © Springer Japan 2014

mutations in the APP or presenilin (PS) genes have been implicated in familial AD [3], the physiopathological events underlying chronic Aβ production/clearance imbalance may be different in sporadic AD and familial AD [4]. Nonetheless, an increase in PS1 and subsequent Aβ accumulation have been found in the hippocampus of senescence-accelerated mice (SAMP8), suggesting the involvement of PS1, one of the γ-secretase complexes, in sporadic AD [5]. It has also been suggested that overexpression of *PS1* in vivo is sufficient to elevate γ-secretase activity and that upregulation of PS1/γ-secretase activity could contribute to increased risk for late-onset sporadic AD [6]. Thus, elucidating the mechanism of the amyloidogenic pathway remains a potential target for discovering a treatment for sporadic AD.

APP has been reported to accumulate in the optic nerve in rat and mouse glaucoma models [7, 8]. Aβ was also found to accumulate in retinal ganglion cells (RGCs) in a rat glaucoma model [9]. Although some studies found no positive association between glaucoma and AD [10, 11], other supported the correlation of these two neurodegenerative diseases [12, 13]. A very recent study has demonstrated that biomarkers of AD, such as apolipoprotein E and transthyretin, were increased in the aqueous humor in glaucoma patients compared with those in a control cataract patients group [14], suggesting that there is a linking pathophysiology in both diseases.

As another linking factor in the pathophysiology of AD and glaucoma, tumor necrosis factor (TNF), a cytokine that is synthesized and released from astrocytes and microglia, has been proposed. For example, TNF has been implicated in the pathogenesis and progression of AD [15, 16]. A meta-analysis demonstrated that AD is accompanied by an inflammatory response, particularly higher peripheral concentrations of TNF, interleukin (IL)-6, IL-1β, transforming growth factor-β, IL-12, and IL-18 [17]. Recent studies have demonstrated that the inhibition of TNF signaling reduces multiple hallmark of AD, including APP, Aβ peptide, and Aβ plaque [18], and prevents pre-plaque amyloid-associated pathology, cognitive deficits, and the loss of neurons in a mouse model of AD [19]. Similar to the findings of its crucial roles in AD, TNF has also been shown to have pivotal roles in the pathogenesis of glaucoma [20]. Glial production of TNF is increased in the glaucomatous optic nerve and TNF-mediated neurotoxicity is a component of neurodegeneration in glaucoma [20]. Increases in TNF have been demonstrated in the retina [21] and optic nerve [22] in hypertensive glaucoma models. A recent study of the aqueous humor has demonstrated that a significantly higher percentage of patients in the glaucoma group were positive for TNF compared with the cataract group [23]. A more recent study of the proteomic data from human glaucoma has shown a prominent upregulation of TNF/TNFR1 signaling in the glaucomatous retina [24]. A meta-analysis demonstrated that patients with open-angle glaucoma may have higher TNF levels in the aqueous humor compared with the control group, and the TNF-308G/A polymorphism is significantly associated with the risk of high-tension glaucoma [25]. Taken together with the finding that APP and Aβ accumulate in the optic nerve and RGCs in glaucoma models, it is possible that

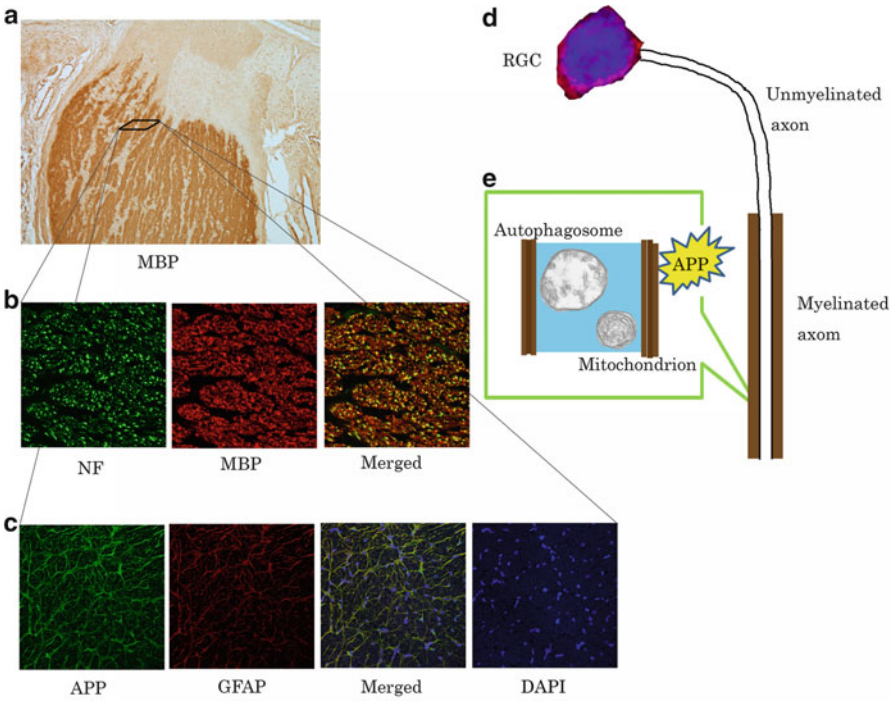


Fig. 9.1 (**a**) Myelin basic protein (MBP) immunostaining in normal rat optic nerve. (**b**) Immunohistochemistry of neurofilament (NF) and MBP in cross sections of the optic nerve. (**c**) Immunohistochemistry of amyloid precursor protein (APP) and glial fibrillary acidic protein (GFAP) in cross sections of the optic nerve. (**d**) Schema of RGC axons in unmyelinated and myelinated areas. (**e**) High-magnification schema of a myelinated axon

TNF signaling and the amyloidogenic pathway are involved in the pathophysiology of both AD and glaucoma.

In longitudinal sections of normal rat optic nerve, myelinated axons can be recognized as myelin basic protein (MBP)-positive staining starting around the laminar portion (Fig. 9.1a, d). In myelinated areas, neurofilament is located inside (green dots) and MBP is located outside rings (red rings) in cross sections (Fig. 9.1b). In TNF-induced optic nerve degeneration, there is an increase in phosphorylated PS1 located in astroglial cells, thereby leading to a subsequent increase in γ-secretase activity in the optic nerve [26]. APP is also located in astroglial cells, because there is substantial colocalization of APP and glial fibrillary acidic protein in cross sections of the optic nerve (Fig. 9.1c, e). The cleavage of APP by the activation of γ-secretase occurs mostly in glial membranes in the optic nerve in this TNF-induced neurodegeneration model [26], which displays primary axonal degeneration with sequential RGC body death [27]. Taken together, the activation of the amyloidogenic pathway in glial cells may be involved in nearby axonal degeneration.

Although several clinical trials of γ-secretase inhibitors (GSIs) for AD treatment have not achieved success so far, some clinical trials are still ongoing [28]. GSIs decreased Aβ production in the human central nervous system [29] and decreased the appearance of new amyloid plaque and the growth of preexisting plaque in APP/PS1 mice [30]. It has been suggested that synapses are the initial target in AD and that learning and memory deficits occur before the formation of plaque [31]. Instead of plaque, soluble Aβ impaired memory function [32], and the GSI LY-411575 reduced soluble Aβ and rescued the neuronal dysfunction in the hippocampus of a mouse model of AD [33]. Therefore, it is likely that GSIs have beneficial effects on neurons including synapses through the inhibition of both amyloid plaque and soluble Aβ. Moreover, the novel GSI ELN594 attenuated the formation and growth of new plaque and led to a normalization of the enhanced dynamics of synaptic structures close to plaque [34]. Furthermore, the GSI N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine *t*-butyl ester (DAPT) induced neural differentiation of human Müller stem cells into RGC precursors and increased the number and length of neurites in cultured cells [35]. Since a recent study has suggested that RGC dendritic atrophy precedes cell loss in a mouse model of AD [36], preventing dendrite atrophy as well as axonal degeneration of RGCs before cell death is important in both AD and glaucoma. In axons, it was shown that DAPT injection immediately after axotomy increased axon regeneration in mature *Caenorhabditis elegans* neurons [37]. In optic nerve axons, treatment with the GSI BMS299897 significantly prevented axonal loss in TNF-induced optic nerve degeneration [26]. These findings suggest that GSIs can protect synapses and axons that are the early manifestation sites in AD and glaucoma. Further studies will be needed to clarify notch conditions after administration of GSIs to examine whether local applications for optic nerve diseases may be feasible.

9.2 Autophagy and Axonal Degeneration

Autophagy is a self-digestion system that is a cellular pathway involved in protein and organelle degradation. Alteration of autophagy is associated with several conditions, including cancer, infectious and immunity disease, liver disease, heart disease, myopathy, and neurodegenerative disease [38]. In the central nervous system (CNS), a previous study suggested that the induction of autophagy serves as an early stress response in axonal dystrophy and may participate in the remodeling of axon structures in Purkinje cells [39]. It was also suggested that autophagy is required for normal axon terminal membrane trafficking and turnover, and an essential role of local autophagy in the maintenance of axonal homeostasis and prevention of axonal degeneration in Purkinje cells was demonstrated [40]. In contrast, in superior cervical ganglion neurons, the autophagy inhibitor 3-methyladenine (3-MA) efficiently suppressed neurite degeneration by protecting neurites from the loss of viability and mitochondrial function [41], suggesting that autophagy may play several distinct roles in axons depending on different types of

neuron or different types of injury. Autophagosomes inside axons can move toward cell bodies, and this movement is dependent on a dynein motor [42]. Therefore, it is possible that axonal transport may affect autophagosome clearance.

In the eyes, the existence of microtubule-associated protein light chain 3 (LC3)-II, an autophagic marker, in RGCs and its transient upregulation after optic nerve transection have been demonstrated [43]. That study showed that the inhibition of autophagy with bafilomycin A1, 3-MA, and wortmannin in RGC-5 cells under serum-deprived conditions decreased cell viability by approximately 40 %, suggesting the activation of autophagy in RGCs after optic nerve transection and its protective role in RGC-5 cells maintained under conditions of serum deprivation [43]. Those findings are consistent with the results of a recent study demonstrating that decreased Brn-3a-immunopositive RGCs in flat-mounted retinas after optic nerve transection were significantly increased by rapamycin, an autophagy inducer [44]. In addition, rapamycin decreased intracellular reactive oxygen species (ROS) production and increased cell viability in RGC-5 cells with the ROS-inducing agent paraquat [44]. That study also showed that the autophagy inhibitor 3-MA increased ROS production and reduced cell viability in RGC-5 cells, implying that autophagy induction protects RGC-5 cells from mitochondrial damage and cell death, whereas autophagy inhibition promotes ROS production and cell death [44]. In contrast, another study demonstrated that decreased 4′,6-diamidino-2-phenylindole (DAPI)-positive cells after intraocular pressure (IOP) elevation in the RGC layer were significantly increased by 3-MA [45]. Thus, the role of autophagy in RGC death is still controversial, although the fact that about 50 % of DAPI-positive cells in the RGC layer are displaced amacrine cells in rats and mice may affect the RGC survival estimation. Nonetheless, all of the above studies and a very recent study in a nonhuman primate chronic hypertensive glaucoma model [46] support the concept that autophagy is activated in RGCs in response to damage such as glaucoma and other optic nerve injuries.

In optic nerve axons, a previous study showed an increase in autophagosomes inside axons until 6 h after optic nerve crush [47]. That electron microscopy finding is consistent with the electron microscopy findings of a recent study demonstrating that abnormal mitochondria and autophagic vacuoles were noted inside axons 3 weeks after glaucoma induction [48]. Thus, autophagosomes and mitochondria move inside axons (Fig. 9.1d, e). Similar to the findings in RGC body death, the role of autophagy in optic nerve axonal degeneration is still controversial. For example, it was shown that the application of 3-MA, an autophagy inhibitor, resulted in a significant delay in axonal degeneration during the acute phase after optic nerve crush [47], while the application of 3-MA exaggerated axonal degeneration induced by IOP elevation [48].

It is interesting to note that the upregulation of autophagy may aid in oligodendrocyte survival in the Long-Evans shaker (les) rat, which has a mutation in MBP that results in severe CNS dysmyelination and subsequent demyelination during development [49]. Because oligodendrocyte loss was observed in the optic nerve after IOP elevation [21], how autophagy in oligodendrocytes can alter axon survival may be particularly interesting. It was shown that the neuroprotective effect of

brain-derived neurotrophic factor (BDNF) was mediated by autophagy through the PI3K/Akt/mTOR pathway, although in cortical neurons rather than oligodendrocytes [50]. Moreover, exogenous BDNF can protect optic nerve axons by recruiting endogenous BDNF located in oligodendrocytes [51]. Oligodendrocytes seem to be sources of BDNF for nearby axons. One hypothesis posits that autophagy induction in oligodendrocytes with the possible involvement of BDNF has beneficial effects on nearby optic nerve axons.

p62, which is also called sequestosome 1, is a multifunctional protein that acts as a critical ubiquitin chain-targeting factor shuttling substrates for proteasomal degradation [52] and interacts with LC3-II, an autophagic marker. p62 is normally degraded by the lysosomal proteases through the interaction with LC3-II [53]. A recent study has shown the accumulation of p62 and LC3-II in the chronically compressed spinal cord, and the forced expression of p62 and the inhibition of autophagy decreased the number of neuronal cells [54]. It has been demonstrated that the inhibition of autophagy is correlated with increased levels of p62 in neuronal cells [39] and that autophagy deficiency leads to the abnormal accumulation of p62 and neurodegenerative changes in the cerebellum [55]. Thus, increased p62 protein levels including autophagic flux impairment may lead to neurodegeneration. An increased p62 protein level was observed in the optic nerve in a hypertensive glaucoma model [48]. Under pathological conditions with impaired autophagy, there is a constitutively high level of p62, thereby leading to the accumulation of damaged mitochondria and subsequent ROS production [56].

Although LC3-II is known as an autophagic marker, it increases not only under autophagy activation but also under autophagy flux impairment [57]. Therefore, increases in both p62 and LC3-II observed in the optic nerve after IOP elevation imply that autophagy flux impairment may be involved in axonal degeneration in glaucoma models [48]. In addition, rapamycin, an autophagy inducer, increased LC3-II further and decreased p62 levels in the optic nerve and exerted axonal protection in a glaucoma model [48]. These findings are in agreement with those of a previous study demonstrating that LiCl, an autophagy inducer, increased the expression of LC3-II under hypoxic stress and decreased the expression of p62 under normoxia and hypoxic stress in a neuronal cell culture [54]. Furthermore, these findings are also supported by a recent study demonstrating that the activation of autophagy increased protein levels of LC3-II and Beclin1 and decreased p62 in neuroblastoma SH-SY5Y cells [58]. Thus, the modulation of autophagy may be a potential strategy against degenerative optic nerve disease.

References

1. Raff MC, Whitmore AV, Finn JT (2002) Axon self-destruction and neurodegeneration. Science 296:868–871
2. Jakob-Roetne R, Jacobsen H (2009) Alzheimer's disease: from pathology to therapeutic approaches. Angew Chem Int Ed Engl 48:3030–3059

3. Bekris LM, Yu CE, Bird TD et al (2010) Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol 23:213–227
4. Pera M, Alcolea D, Sánches-Valle R et al (2013) Distinct patterns of APP processing in the CNS in autosomal-dominant and sporadic Alzheimer disease. Acta Neuropathol 125:201–213
5. Kumar VB, Franko M, Banks WA et al (2009) Increase in presenilin 1 (PS1) levels in senescence-accelerated mice (SAMP8) may indirectly impair memory by affecting amyloid precursor protein (APP) processing. J Exp Biol 212:494–498
6. Li T, Li YM, Ahn K et al (2011) Increased expression of PS1 is sufficient to elevate the level and activity of γ-secretase in vivo. PLoS One 6:e28179
7. Childlow G, Ebneter A, Wood JP et al (2011) The optic nerve head is the site of axonal transport disruption, axonal cytoskeleton damage and putative axonal regeneration failure in a rat model of glaucoma. Acta Neuropathol 121:737–751
8. Goldblum D, Kipfer-Kauer A, Sarra GM et al (2007) Distribution of amyloid precursor protein and amyloid- beta immunoreactivity in DBA/2J glaucomatous mouse retinas. Invest Ophthalmol Vis Sci 48:5085–5090
9. Guo L, Salt TE, Luong V et al (2007) Targeting amyloid-β in glaucoma treatment. Proc Natl Acad Sci U S A 104:13444–13449
10. Bach-Holm D, Kessing SV, Mogensen U et al (2012) Normal tension glaucoma and Alzheimer disease: comorbidity? Acta Ophthalmol 90:683–685
11. Ou Y, Grossman DS, Lee PP et al (2012) Glaucoma, Alzheimer disease and other dementia: a longitudinal analysis. Ophthalmic Epidemiol 19:285–292
12. Wostyn P, Audenaert K, De Deyn PP (2009) Alzheimer's disease and glaucoma: is there a causal relationship? Br J Ophthalmol 93:1557–1559
13. Liu YH, Tian T (2011) Hypothesis of optineurin as a new common risk factor in normal-tension glaucoma and Alzheimer's disease. Med Hypotheses 77:591–592
14. Inoue T, Kawaji T, Tanihara H (2013) Elevated levels of multiple biomarkers of Alzheimer's disease in the aqueous humor of eyes with open-angle glaucoma. Invest Ophthalmol Vis Sci 54:5353–5358
15. Paganelli R, Di Iorio A, Patricelli L et al (2002) Proinflammatory cytokines in sera of elderly patients with dementia: levels in vascular injury are higher than those of mild-moderate Alzheimer's disease patients. Exp Gerontol 37:257–263
16. Alvarez A, Cacabelos R, Sanpedro C et al (2007) Serum TNF-alpha levels are increased and correlate negatively with free IGF-I in Alzheimer disease. Neurobiol Aging 28:533–536
17. Swardfager W, Lanctôt K, Rothenburg L et al (2010) A meta-analysis of cytokines in Alzheimer's disease. Biol Psychiatry 68:930–941
18. Tweedie D, Ferguson RA, Fishman K et al (2012) Tumor necrosis factor-α synthesis inhibitor 3,6′-dithiothalidomide attenuates markers of inflammation, Alzheimer pathology and behavioral deficits in animal models of neuroinflammation and Alzheimer's disease. J Neuroinflammation 9:106
19. McAlpine FE, Lee JK, Harms AS et al (2009) Inhibition of soluble TNF signaling in a mouse model of Alzheimer's disease prevents pre-plaque amyloid-associated neuropathology. Neurobiol Dis 34:163–177
20. Tezel G (2008) TNF-α signaling in glaucomatous neurodegeneration. Prog Brain Res 173:409–421
21. Nakazawa T, Nakazawa C, Matsubara A et al (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J Neurosci 26:12633–12641
22. Munemasa Y, Kitaoka Y, Kuribayashi J et al (2010) Modulation of mitochondria in the axon and soma of retinal ganglion cells in a rat glaucoma model. J Neurochem 115:1508–1519
23. Sawada H, Fukuchi T, Tanaka T et al (2010) Tumor necrosis factor-α concentrations in the aqueous humor of patients with glaucoma. Invest Ophthalmol Vis Sci 51:903–906

24. Yang X, Luo C, Cai J et al (2011) Neurodegenerative and inflammatory pathway components linked to TNF-α/TNFR1 signaling in the glaucomatous human retina. Invest Ophthalmol Vis Sci 52:8422–8454
25. Xin X, Gao L, Wu T et al (2013) Roles of tumor necrosis factor alpha gene polymorphisms, tumor necrosis factor alpha level in aqueous humor, and the risks of open angle glaucoma: a meta-analysis. Mol Vis 19:526–535
26. Kojima K, Kitaoka Y, Munemasa Y et al (2012) Axonal protection via modulation of the amyloidogenic pathway in tumor necrosis factor-induced optic neuropathy. Invest Ophthalmol Vis Sci 53:7675–7683
27. Kitaoka Y, Kitaoka Y, Kwong JM et al (2006) TNF-alpha-induced optic nerve degeneration and nuclear factor-kappaB p65. Invest Ophthalmol Vis Sci 47:1448–1457
28. Mikulca JA, Nguyen V, Gajdosik DA et al (2014) Potential novel targets for Alzheimer pharmacotherapy: II. Update on secretase inhibitors and related approaches. J Clin Pharm Ther 39:25–37
29. Bateman RJ, Siemers ER, Mawuenyega KG et al (2009) A gamma-secretase inhibitor decreases amyloid-beta production in the central nervous system. Ann Neurol 66:48–54
30. Yan P, Bero AW, Cirrito JR et al (2009) Characterizing the appearance and growth of amyloid plaques in APP/PS1 mice. J Neurosci 29:10706–10714
31. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. Science 298:789–791
32. Lesné S, Koh MT, Kotilinek L et al (2006) A specific amyloid-beta protein assembly in the brain impairs memory. Nature 440:352–357
33. Busche MA, Chen X, Henning HA et al (2012) Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. Proc Natl Acad Sci USA 109:8740–8745
34. Liebscher S, Page RM, Käfer K et al (2013) Chronic γ-secretase inhibition reduces amyloid plaque-associated instability of pre- and postsynaptic structures. Mol Psychiatry 1–10 doi:10.1038/mp.2013.122
35. Singhal S, Bhatia B, Jayaram H et al (2012) Human Müller glia with stem cell characteristics differentiate into retinal ganglion cell (RGC) precursors in vitro and partially restore RGC function in vivo following transplantation. Stem Cells Transpl Med 1:188–199
36. Williams PA, Thirgood RA, Oliphant H et al (2013) Retinal ganglion cell dendritic degeneration in a mouse model of Alzheimer's disease. Neurobiol Aging 34:1799–1806
37. El Bejjani R, Hammarlund M (2012) Notch signaling inhibits axon regeneration. Neuron 73:268–278
38. Mizushima N, Levine B, Cuervo AM et al (2008) Autophagy fights disease through cellular self-digestion. Nature 451:1069–1075
39. Wang QJ, Ding Y, Kohtz DS et al (2006) Induction of autophagy in axonal dystrophy and degeneration. J Neurosci 26:8057–8068
40. Komatsu M, Wang QJ, Holstein GR et al (2007) Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc Natl Acad Sci U S A 104:14489–14494
41. Yang Y, Fukui K, Koike T et al (2007) Induction of autophagy in neurite degeneration of mouse superior cervical ganglion neurons. Eur J Neurosci 26:2979–2988
42. Katsumata K, Nishiyama J, Inoue T et al (2010) Dynein- and activity-dependent retrograde transport of autophagosomes in neuronal axons. Autophagy 6:378–385
43. Kim SH, Munemasa Y, Kwong JM et al (2008) Activation of autophagy in retinal ganglion cells. J Neurosci Res 86:2943–2951
44. Rodríguez-Muela N, Germain F, Mariño G et al (2012) Autophagy promotes survival of retinal ganglion cells after optic nerve axotomy in mice. Cell Death Differ 19:162–169
45. Park HYL, Kim JH, Park CK (2012) Activation of autophagy induces retinal ganglion cell death in a chronic hypertensive glaucoma model. Cell Death Dis 3:e290
46. Deng S, Wang M, Yan Z et al (2013) Autophagy in retinal ganglion cells in a rhesus monkey chronic hypertensive glaucoma model. PLoS One 8:e77100

47. Knöferle J, Koch JC, Ostendorf T et al (2010) Mechanisms of acute axonal degeneration in the optic nerve in vivo. Proc Natl Acad Sci U S A 107:6064–6069
48. Kitaoka Y, Munemasa Y, Kojima K et al (2013) Axonal protection by Nmnat3 overexpression with involvement of autophagy in optic nerve degeneration. Cell Death Dis 4:e860
49. Smith CM, Mayer JA, Duncan ID (2013) Autophagy promotes oligodendrocyte survival and function following dysmyelination in a long-lived myelin mutant. J Neurosci 33:8088–8100
50. Chen A, Xiong LJ, Tong Y et al (2013) Neuroprotective effect of brain-derived neurotrophic factor mediated by autophagy through the PI3K/Akt/mTOR pathway. Mol Med Rep 8:1011–1016
51. Fujino H, Kitaoka Y, Hayashi Y et al (2009) Axonal protection by brain-derived neurotrophic factor associated with CREB phosphorylation in tumor necrosis factor-alpha-induced optic nerve degeneration. Acta Neuropathol 117:75–84
52. Seibenhener ML, Babu JR, Geetha T et al (2004) Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. Mol Cell Biol 24:8055–8068
53. Ichimura Y, Komatsu M (2010) Selective degradation of p62 by autophagy. Semin Immunopathol 32:431–436
54. Tanabe F, Yone K, Kawabata N et al (2011) Accumulation of p62 in degenerated spinal cord under chronic mechanical compression: functional analysis of p62 and autophagy in hypoxic neuronal cells. Autophagy 7:1462–1471
55. Liang CC, Wang C, Peng X et al (2010) Neural-specific deletion of FIP200 leads to cerebellar degeneration caused by increased neuronal death and axon degeneration. J Biol Chem 285:3499–3509
56. Johansen T, Lamark T (2011) Selective autophagy mediated by autophagic adapter proteins. Autophagy 7:279–296
57. Mizushima N, Yoshimori T, Levine B (2010) Methods in mammalian autophagy research. Cell 140:313–326
58. Xu HD, Wu D, Gu JH et al (2013) The pro-survival role of autophagy depends on Bcl-2 under nutrition stress conditions. PLoS One 8:e63232

Chapter 10
Axonal Transport

Yuji Takihara and Masaru Inatani

Abstract Glaucomatous optic neuropathy is characterized by degeneration of retinal ganglion cell (RGC) axons and RGC death. Recent studies have suggested that glaucoma is a multifactorial disease that involves several molecular mechanisms. RGCs have long axons that provide spatial separation between the cell body and synapses. This anatomical structure has allowed us to use tracer injections to study axonal transport in RGCs. Increased intraocular pressure has been demonstrated to induce disturbances in axonal transport of tracers in RGCs. On the other hand, neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF), have been demonstrated to support the survival of damaged RGCs in vitro and in vivo. Therefore, disturbances in retrograde axonal transport of BDNF may be associated with RGC death in glaucomatous optic neuropathy. Here we summarize the current understanding of axonal transport and BDNF in glaucomatous optic neuropathy. In addition, we introduce our approach to glaucomatous optic neuropathy, live imaging of axonal transport in RGCs, which may lead to the prediction of RGC death in glaucoma patients.

Keywords Axonal transport • BDNF • Live imaging

Y. Takihara (✉) • M. Inatani
Department of Ophthalmology, Faculty of Medical Science, University of Fukui, 23-3 Shimoaizuki, Matsuoka, Eiheiji, Yoshida, Fukui 910-1193, Japan
e-mail: takihara@u-fukui.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_10,

10.1 Pathomechanisms of Glaucomatous Optic Neuropathy

Glaucomatous optic neuropathy is characterized by axonal degeneration in retinal ganglion cells (RGCs) and ultimately RGC death. In recent decades, research on glaucomatous optic neuropathy has made substantial progress and has indicated that glaucoma is a multifactorial disease.

In 1858, two hypotheses were proposed to explain pathomechanisms of glaucomatous optic neuropathy; these include the mechanical and vascular theories. The mechanical theory, which was proposed by Muller, hypothesized that increased intraocular pressure (IOP) compressed RGC axons and induced neuronal death. Approximately one million RGC axons project to the optic nerve head (ONH). Quigley et al. have used electron microscopy (EM) to evaluate human glaucomatous eyes and described several histological and structural findings [1]. First, they reported that an early increase in cup size preceded visual field losses and resulted from a loss of RGC axons. Second, they reported that as glaucomatous excavation increased in ONH, the backward and outward rotation of the laminar beams progressed. Finally, they reported that increased IOP led to a laminar distortion and subsequent compression of RGC axons.

On the other hand, the vascular theory, proposed by Von Jaeger, hypothesized that vascular dysfunction resulted in glaucoma. Interestingly, Kawasaki et al. have reported that retinal arteriolar narrowing is associated with a 10-year incidence of glaucoma [2].

In 1968, Lampert et al. proposed that interrupted axonal transport contributed to glaucomatous optic neuropathy [3]. Although this corroborates the mechanical theory, it does not exclude the vascular theory. Radius reported that in primates, occlusion of the short posterior ciliary circulation results in the interruption of axonal transport [4] and that the accumulation of a tracer of axonal transport was inversely proportional to the perfusion pressure [5].

10.2 Axonal Transport in Glaucomatous Optic Neuropathy

10.2.1 Basic Mechanisms of Axonal Transport

Neurons have complex structures that include axons. RGC axons are particularly long and must pass through the lamina cribrosa, which is vulnerable to IOP stress. Because neurons synthesize most proteins in the soma, they must send proteins to their synapses through axonal transport. The primary components of the neuron's cytoskeleton include microtubules, microfilaments, and neurofilaments. Especially, microtubules serve as the primary rail of axonal transport. Microtubules are made of α- and β-tubulin dimmers, which undergo polymerization and depolymerization. Microtubules have polarity that includes a fast-extending plus end and a slow-extending

minus end. Proximal dendrites include a mixed polarity of microtubules, whereas axons have a regular polarity with the plus end positioned toward the axon terminals. We have demonstrated that axonal transport of the brain-derived neurotrophic factor (BDNF) differs between axons and dendrites and these differences may result from the differences in microtubule polarity between dendrites and axons [6].

The quick-freeze, deep-etch EM method has helped to progress research on molecular motors. This method has revealed that molecular motors have a motor domain that connects with microtubules and a tail domain that connects with membranous vesicles. Kinesins and dyneins are responsible for microtubule-based axonal transport and use ATP for chemical energy. Most kinesins are responsible for anterograde axonal transport, which go toward the plus end of the microtubules. Most cytoplasmic dyneins are responsible for retrograde axonal transport, which go toward the minus end of the microtubules. Axonal transport is composed of fast transport and slow transport. The velocity of fast transport is 50–400 mm/day. By fast anterograde transport, synaptic vesicles, neurotransmitters, and mitochondria are mainly transported. By fast retrograde transport, endosomes and mitochondria are mainly transported. The velocity of slow transport is <8 mm/day. By slow transport, tubulins, actins, and neurofilaments are mainly transported. Slow transport is responsible for axonal maintenance and cytoskeleton repair. However, the molecular motors responsible for slow transport on microtubules have been not fully identified.

10.2.2 Studies of Axonal Transport in Glaucomatous Optic Neuropathy

Axonal transport in RGCs has been the focus of several glaucoma studies. RGCs are advantageous for studying axonal transport because they have long axons and their cell bodies and synapses are spatially separated. Anterograde axonal transport has been studied in RGCs using intravitreal tracer injections. Retrograde axonal transport of RGCs has been analyzed using tracers injected in central targets for RGC axons. The study by Anderson et al. indicated that axonal transport of tritiated leucine is blocked by an elevated IOP in the lamina cribrosa of primates [7]. Interestingly, Quigley et al. reported that by normalizing IOP after a 4 h period of increased IOP, leucine that had accumulated in the lamina cribrosa almost completely disappeared [8]. This reversal of axonal transport blockage suggests that axonal transport of leucine is disturbed in alive RGCs. In addition, this reversal suggests that RGCs with axonal transport disturbances may be a therapeutic target. Dandona et al. reported that in primates with chronic increased IOP, anterograde axonal transport from RGCs to the magnocellular layers in the dorsal lateral geniculate nucleus was less, compared with that to the parvocellular layers in the dorsal lateral geniculate nucleus [9]. Using immunohistochemistry, Pease et al. reported that BDNF and its tropomyosin-related kinase (TrkB) receptor had

accumulated in ONH of primates with chronic glaucoma [10]. Quigley et al. reported that acute increase in IOP in rats decreases retrograde transport of ^{125}I-BDNF from the superior colliculus [11]. Martin et al. reported that cytoplasmic dynein, which is responsible for fast retrograde axonal transport, accumulates in ONH with increased IOP in rats with experimental glaucoma [12]. This suggests that increased IOP could cease molecular motor movement. Buckingham et al. reported that retrograde axonal transport of tracers was impaired in RGCs long before axonal loss and neuronal death in DBA/2J mice [13]. Consistent with the findings reported by Buckingham et al. Salinas-Navarro et al. reported that the number of Brn-3-positive RGCs is greater, compared with the number of RGCs labeled by a retrograde axonal transport tracer 8 days after laser treatment [14]. This result suggests that in part of Brn-3-positive living RGCs, disturbances in retrograde axonal transport occur in mice with experimental glaucoma. Crish et al. reported that anterograde transport deficits of a tracer to the superior colliculus occurred and progressed in a distal-to-proximal manner in DBA/2J mice [15]. Moreover, they proposed that anterograde transport primarily decreases by aging, with increased IOP as an additional factor.

10.3 BDNF in Glaucomatous Optic Neuropathy

10.3.1 Basic Understanding of BDNF

However, how or whether axonal transport failure causes RGC death in adult mammals remains unknown. During retinal development, only RGCs that receive sufficient target-derived neurotrophic factors survive. This suggests that disrupted retrograde axonal transport of neurotrophic factors and neurotrophic factor deprivation induces RGC death in glaucomatous optic neuropathy. The neurotrophins are the most characterized family among neurotrophic factors. Neurotrophins, which are expressed in almost all neurons, play major roles in neurite growth, synaptic plasticity, and neuronal survival. In mammals, neurotrophins include nerve growth factor (NGF), BDNF, neurotrophin-3, and neurotrophin-4/5. In the early 1950s, Levi-Montalcini et al. [16] reported that the mouse sarcoma secretes a soluble factor that could stimulate growth of sensory and sympathetic neurons in chick embryos; this factor was later identified as NGF. In 1982, BDNF was purified from pig brain and was demonstrated to promote survival of chick sensory neurons [17]. BDNF binds to TrkB, a receptor in the tyrosine kinase family, and the p75 receptor. Because the first identified neurotrophin NGF is secreted from postsynaptic neurons and is retrogradely transported, BDNF was thought to be transported retrogradely alone. However, considerable evidence has indicated that BDNF is anterogradely transported. In 2001, Kohara et al. used BDNF tagged with green fluorescent protein (BDNF-GFP) to demonstrate that BDNF is anterogradely transported and transferred to postsynaptic neurons in an activity-dependent manner [18].

The BDNF gene includes five exons. After translation in ribosomes of the endoplasmic reticulum, the pre-pro-protein of BDNF is transported to the Golgi and the trans-Golgi network. BDNF is secreted by two pathways: the constitutive and regulated pathways. In the constitutive pathway, the prosequence of BDNF is cleaved by the trans-Golgi network-resident protein convertases. Vesicle release in the constitutive pathway does not rely on intracellular Ca^{2+}. In the regulated pathway, the prosequence of BDNF is cleaved after budding from the trans-Golgi network. Vesicle release in the regulated pathway is dependent on intracellular Ca^{2+}. We have demonstrated that BDNF is expressed in RGCs in a vesicular pattern in both axons and dendrites. BDNF vesicles produced in the neuron cell body are anterogradely transported to the synapse by the fast axonal transport. On the other hand, after BDNF that is released by postsynaptic neurons or via autocrine mechanisms binds to the TrkB or p75 receptor, the endocytotic vesicles are retrogradely transported.

10.3.2 BDNF in Glaucomatous Optic Neuropathy

Pearson reported that it takes several months for RGCs to be lost by selective degeneration of neurons in the lateral geniculate nucleus of adult cats using kainic acid [19]. This finding suggests that adult RGCs do not completely depend on neurotropic factors derived from their central targets. However, several studies have reported that BDNF promotes RGC survival. Johnson et al. reported that BDNF supports the survival of cultured RGCs in vitro [20]. Mansour–Robaey et al. reported that in rats with optic nerve transection, RGC survival (42 %) increases with intraocular injection of BDNF when compared with that (20 %) observed with an intraocular injection of vehicle 2 weeks after optic nerve transaction [21]. Interestingly, Cheng et al. [22] reported that in adult rats with optic nerve transection, the RGC survival rate (76 %) increased 2 weeks after optic nerve transection with TrkB gene transfer using adeno-associated virus combined with intravitreal injection of BDNF when compared with controls (10 %). Pease and Quigley proposed that retrograde axonal transport of BDNF is disturbed in glaucoma [10, 11]. Therefore, a disturbance of BDNF retrograde axonal transport in RGCs may be associated with RGC death in glaucomatous optic neuropathy.

10.4 Live Imaging of Axonal Transport in RGCs in Glaucomatous Optic Neuropathy

Recent studies on glaucoma suggest that glaucoma is a multifactorial disease that involves several molecular mechanisms and other types of retinal cells. Importantly, glaucoma is defined by degeneration of RGC axons and final RGC death. Here we introduce our approach for predicting RGC death in glaucoma [6]. We hypothesize that evaluating axonal transport in RGCs will allow us to detect

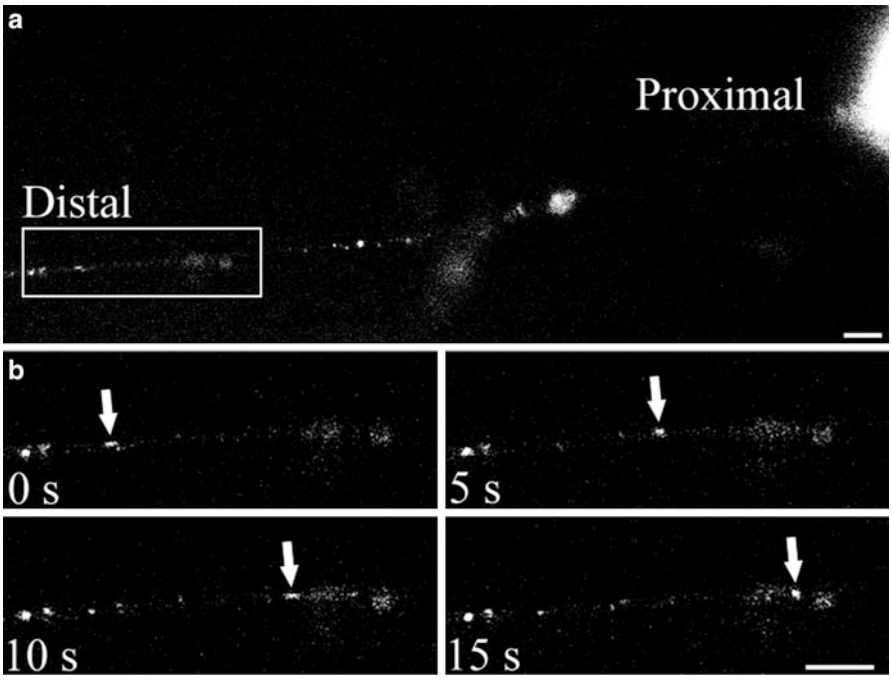


Fig. 10.1 Live imaging of retrograde axonal transport of BDNF-GFP in RGCs (**a**) RGC body is on the right side of Fig. 10.1a. (**b**) Magnification of Fig. 10.1a demonstrated a retrogradely transported BDNF-GFP vesicle. Scale bar, 5 μm. Data reproduced from Takihara et al. [6]

early axonal damage before RGC death in glaucoma. Interestingly, the data reported by Quigley et al. in a glaucoma model suggest that increased IOP disrupts axonal transport in alive RGCs and that by normalizing IOP after increased IOP, axonal transport blockage disappears before RGC death [8]. However, evaluation of axonal transport in RGCs has been limited to observations using fixed sections.

To visualize axonal transport of BDNF in living RGCs, we purified rat RGCs using a two-step immunopanning procedure and transfected purified RGCs with a plasmid encoding BDNF-GFP. To evaluate axonal transport of BDNF-GFP in living RGCs, time-lapse imaging was conducted in a chamber maintained at 37 °C and 5 % CO_2. Time-lapse imaging of the transfected RGCs revealed anterograde and retrograde axonal transport of BDNF-GFP (Figs. 10.1 and 10.2). To detect disturbances in axonal transport of BDNF-GFP vesicles resulting from axonal damage, we conducted time-lapse imaging of the transfected RGCs after treatment with colchicine, which disrupts the assembly of microtubules. The number of BDNF-GFP vesicles transported in RGC axons decreased 2 and 3 h after the colchicine treatment, as compared with controls (Fig. 10.3). BDNF-GFP vesicles which could not move smoothly were observed 3 h after the treatment (Fig. 10.3). Until 3 h after colchicine treatment, no RGCs were positive for

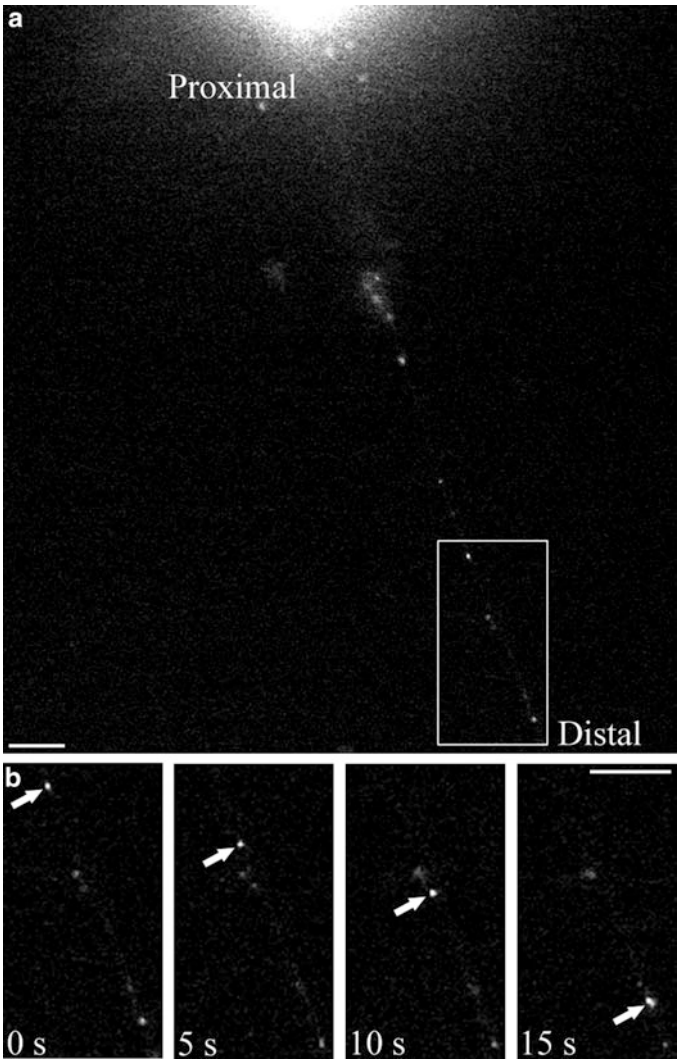


Fig. 10.2 Live imaging of anterograde axonal transport of BDNF-GFP in RGCs (**a**) RGC body is on the upper side of Fig. 10.2a. (**b**) Magnification of Fig. 10.2a demonstrated an anterogradely transported BDNF-GFP vesicle. Scale bar, 5 μm. Data reproduced from Takihara et al. [6]

ethidium homodimer-1. These results suggest that axonal transport of BDNF vesicles in damaged RGC axons is disturbed before RGC death.

Our approach may be useful in predicting RGC death in glaucoma patients. We believe that one problem in clinical practice with glaucoma patients is that IOP at which visual field loss does not progress differs among patients. In Japan, approximately 70 % patients with glaucoma have normal-tension glaucoma.

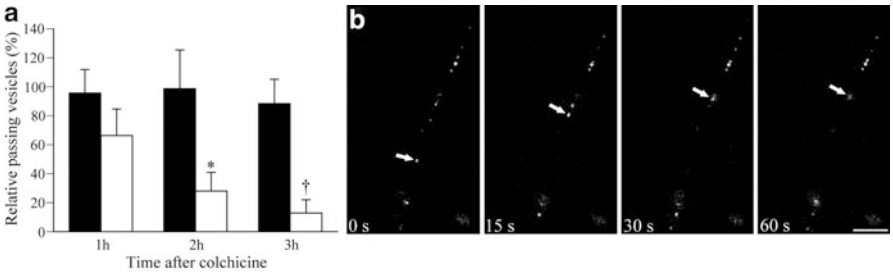


Fig. 10.3 Disturbance in axonal transport of BDNF-GFP after colchicine treatment (**a**) The number of BDNF-GFP vesicles significantly decreased 2 and 3 h after colchicine treatment (*$P = 0.003$, †$P = 0.0002$). (**b**) Representative time-lapse images of BDNF-GFP vesicles 3 h after colchicine treatment. The BDNF-GFP vesicle was not smoothly transported. Scale bar, 5 μm. Data reproduced from Takihara et al. [6]

We frequently experience patients whose visual field loss progresses even when IOP is less than 15 mmHg. The Collaborative Normal-Tension Glaucoma Study revealed that some patients experience a progression in visual field loss although their IOP was therapeutically decreased. In addition, these findings suggest that glaucoma is a group which has common glaucomatous optic neuropathy, but each of which have the heterogeneous main pathomechanisms. However, if we can quantify the stress level of RGCs with live imaging of axonal transport in RGCs, we may then be able to successfully predict RGC death in patients with glaucoma because the retina is the only part of the central nervous system that is directly visible without invasive techniques. Although there are many challenges in live imaging of axonal transport in RGCs of patients with glaucoma, the study of live imaging of axonal transport of BDNF in vitro suggests that live imaging of axonal transport in RGCs may be useful for predicting the future loss of RGCs in glaucoma and ultimately lead to the development of personalized medicine for patients with glaucoma.

References

1. Quigley HA, Addicks EM, Green WR, Maumenee AE (1981) Optic nerve damage in human glaucoma. II. The site of injury and susceptibility to damage. Arch Ophthalmol 99(4):635–649
2. Kawasaki R, Wang JJ, Rochtchina E, Lee AJ, Wong TY, Mitchell P (2013) Retinal vessel caliber is associated with the 10-year incidence of glaucoma: the Blue Mountains Eye Study. Ophthalmology 120(1):84–90. doi:10.1016/j.ophtha.2012.07.007
3. Lampert PW, Vogel MH, Zimmerman LE (1968) Pathology of the optic nerve in experimental acute glaucoma. Electron microscopic studies. Invest Ophthalmol 7(2):199–213
4. Radius RL (1980) Optic nerve fast axonal transport abnormalities in primates. Occurrence after short posterior ciliary artery occlusion. Arch Ophthalmol 98(11):2018–2022
5. Radius RL, Bade B (1981) Pressure-induced optic nerve axonal transport interruption in cat eyes. Arch Ophthalmol 99(12):2163–2165
6. Takihara Y, Inatani M, Hayashi H, Adachi N, Iwao K, Inoue T, Iwao M, Tanihara H (2011) Dynamic imaging of axonal transport in living retinal ganglion cells in vitro. Invest Ophthalmol Vis Sci 52(6):3039–3045. doi:10.1167/iovs.10-6435

7. Anderson DR, Hendrickson A (1974) Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve. Invest Ophthalmol 13(10):771–783
8. Quigley H, Anderson DR (1976) The dynamics and location of axonal transport blockade by acute intraocular pressure elevation in primate optic nerve. Invest Ophthalmol 15(8):606–616
9. Dandona L, Hendrickson A, Quigley HA (1991) Selective effects of experimental glaucoma on axonal transport by retinal ganglion cells to the dorsal lateral geniculate nucleus. Invest Ophthalmol Vis Sci 32(5):1593–1599
10. Pease ME, McKinnon SJ, Quigley HA, Kerrigan-Baumrind LA, Zack DJ (2000) Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. Invest Ophthalmol Vis Sci 41(3):764–774
11. Quigley HA, McKinnon SJ, Zack DJ, Pease ME, Kerrigan-Baumrind LA, Kerrigan DF, Mitchell RS (2000) Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest Ophthalmol Vis Sci 41(11):3460–3466
12. Martin KR, Quigley HA, Valenta D, Kielczewski J, Pease ME (2006) Optic nerve dynein motor protein distribution changes with intraocular pressure elevation in a rat model of glaucoma. Exp Eye Res 83(2):255–262. doi:10.1016/j.exer.2005.11.025
13. Buckingham BP, Inman DM, Lambert W, Oglesby E, Calkins DJ, Steele MR, Vetter ML, Marsh-Armstrong N, Horner PJ (2008) Progressive ganglion cell degeneration precedes neuronal loss in a mouse model of glaucoma. J Neurosci 28(11):2735–2744. doi:10.1523/jneurosci.4443-07.2008
14. Salinas-Navarro M, Alarcon-Martinez L, Valiente-Soriano FJ, Ortin-Martinez A, Jimenez-Lopez M, Aviles-Trigueros M, Villegas-Perez MP, de la Villa P, Vidal-Sanz M (2009) Functional and morphological effects of laser-induced ocular hypertension in retinas of adult albino Swiss mice. Mol Vis 15:2578–2598
15. Crish SD, Sappington RM, Inman DM, Horner PJ, Calkins DJ (2010) Distal axonopathy with structural persistence in glaucomatous neurodegeneration. Proc Natl Acad Sci U S A 107 (11):5196–5201. doi:10.1073/pnas.0913141107
16. Levi-Montalcini R, Hamburger V (1951) Selective growth stimulating effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J Exp Zool 116(2):321–361
17. Barde YA, Edgar D, Thoenen H (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J 1(5):549–553
18. Kohara K, Kitamura A, Morishima M, Tsumoto T (2001) Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. Science 291(5512):2419–2423. doi:10.1126/science.1057415, United States
19. Pearson HE, Stoffler DJ (1992) Retinal ganglion cell degeneration following loss of postsynaptic target neurons in the dorsal lateral geniculate nucleus of the adult cat. Exp Neurol 116(2):163–171
20. Johnson JE, Barde YA, Schwab M, Thoenen H (1986) Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. J Neurosci 6(10):3031–3038
21. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A 91(5):1632–1636
22. Cheng L, Sapieha P, Kittlerova P, Hauswirth WW, Di Polo A (2002) TrkB gene transfer protects retinal ganglion cells from axotomy-induced death in vivo. J Neurosci 22(10):3977–3986

Chapter 11
Interaction Between RGC Bodies and Glia

Kenji Kashiwagi

Abstract The interactions between glial cells and retinal ganglion cells (RGC) in glaucoma are complicated and are variable depending on the cell types and the status of cell activity. Structural stability is only one function of glial cells. Other important roles of glial cells include intertalking with neurons, other glial cells, and extracellular matrix through direct or indirect communication methods. The transition from quiescent status to reactive status drastically changes the activity and characteristics of glial cells in glaucoma. Glial cells exert both neuroprotective and neurotoxic effects on RGC and effect not only cell survival but also cell morphology, synapse connectivity, and cell functions. Investigations into glial cell modulation and their roles in glaucoma could provide new strategies for glaucoma therapy that could complement intraocular pressure reduction therapy. These new strategies could rescue RGC from death, promote optic nerve regeneration, preserve dendritic morphology and synaptic connectivity, and inhibit the pathological reformation of extracellular matrix in the optic nerve.

Keywords Astrocyte • Microglia • Oligodendrocyte • Reactive glia • Retinal ganglion cell

11.1 Introduction

Glaucoma is a disease of progressive retinal ganglion cell (RGC) death that is primarily caused by the pathological elevation of intraocular pressure (IOP). Many studies have revealed that glial cells surrounding the RGC had a deeply involved glaucomatous neuropathology. New roles of glial cells have been unveiled through

K. Kashiwagi (✉)
Department of Ophthalmology, University of Yamanashi, 1110 Shimokato Chuo, Yamanashi 409-3898, Japan
e-mail: kenjik@yamanashil.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_11, © Springer Japan 2014

extensive investigations [1]. The primary role of glial cells involves maintaining the activity of RGC and providing structural support. However, glial cells have a biphasic relationship with RGC, namely through their neuroprotective action and neurotoxic action. There are several types of glial cells in human ocular tissues. Interestingly, different subtypes of glial cells exert different effects on RGC. Glaucomatous optic neuropathy is considered to be accompanied by dramatic alterations in the functional properties and distribution of glial cells in both the retina and the optic nerve head (ONH). Unfortunately, the relationship between glial cells and RGC death remains unknown. In this chapter, the interactions between RGC and glial cells are discussed with a focus on the pathology and treatment of glaucoma.

11.2 Glial Cells in Ocular Tissue

The somata of RGC form a layer in the inner sensory retina, and a demyelinated retinal nerve fiber lays on the surface of the retina and travels to the optic disc. The demyelinated axon fibers are myelinated around the post-lamina cribrosa region in the human eye. RGC is associated with different types of glial cells depending on the location.

11.2.1 Optic Nerve Head (Figs. 11.1 and 11.2)

In the optic nerve, glial cells include astrocyte, oligodendrocyte, and microglia. There are two subpopulations of astrocytes in normal optic nerves. Astrocytes are the primary glial cell type in nonmyelinated ONH in most mammals and provide cellular support to the axons, forming an interface between connective tissue surfaces and the surrounding blood vessels [3]. Type 1A astrocyte expresses only glial fibrillary acidic protein (GFAP), and type 1B astrocytes express both GFAP and neural cell adhesion molecules (N-CAM). Type 1A astrocyte is found throughout the optic nerve, but type IB astrocyte is the predominantly type in the optic nerve head. Type lA astrocyte provides structural support for the axons, while type 1B cell provides a physiological interface between the vitreous connective and vascular tissues. In the lamina cribrosa, astrocytes form lamellae that are oriented perpendicular to the axons, while the astrocytes themselves surround the blood vessels in the nerve fiber layer of the retina. Astrocyte processes occupy the prelaminar region and the absence of glial columns. In the lamina cribrosa, the nerve bundle area is occupied by cell bodies of astrocytes. Within the lamina

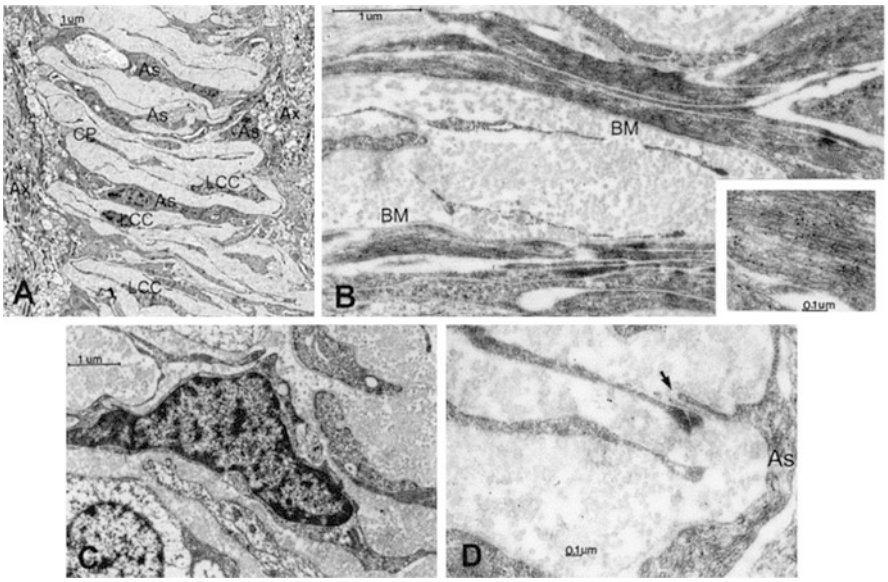


Fig. 11.1 Electron micrographs of glial cells in the lamina cribrosa of a normal adult monkey. (**a**). Low power view of the cribriform plates (CP) oriented perpendicular to the axons (Ax) of the retinal ganglion cells. Astrocytes (As) are elongated and extend thin processes into the ECM of the plates. Lamina cribrosa cells (LCC) are star-shaped cells inside the ECM of the plates. Bar 1 μm. (**b**). High magnification of the cribriform plates showing astrocytes forming lamellae. The basement membrane (BM) that surrounds the astrocyte cell body and processes. The *inset* shows the cytoplasm of an astrocyte stained with antihuman GFAP and immunogold labeling. (**c**). Lamina cribrosa cell in the core of the plates. Bar 1 μm. (**d**). Detail of the cell/cell contacts (*arrow*) between processes of the astrocytes in the core of the plates. (Adapted from Hernandez et al. [1])

cribrosa of monkeys and humans, where each capillary is embedded within a connective tissue sheath, the astrocyte envelops the axon beams.

Oligodendrocyte lies along the long axis of the nerve in a group of five or more cells. Solitary astrocyte occupies in a space between the groups of oligodendrocytes. Primary processes of oligodendrocyte separate axon bundles orthogonally and radially into fascicles. The major function of oligodendrocyte is myelinating multiple axons for axonal integrity and nodal function in addition to supporting for neurons as other glial cells [2].

Microglia, the resident immune cell, has important roles in innate immunity and inflammatory neuropathology. Microglia forming stellate shape with thin, ramified processes exists as a quiescent form in human normal optic nerve heads. These cells were found throughout the normal ONH in the walls of large blood vessels and surrounding capillaries in the glial columns and the cribriform plates [4].

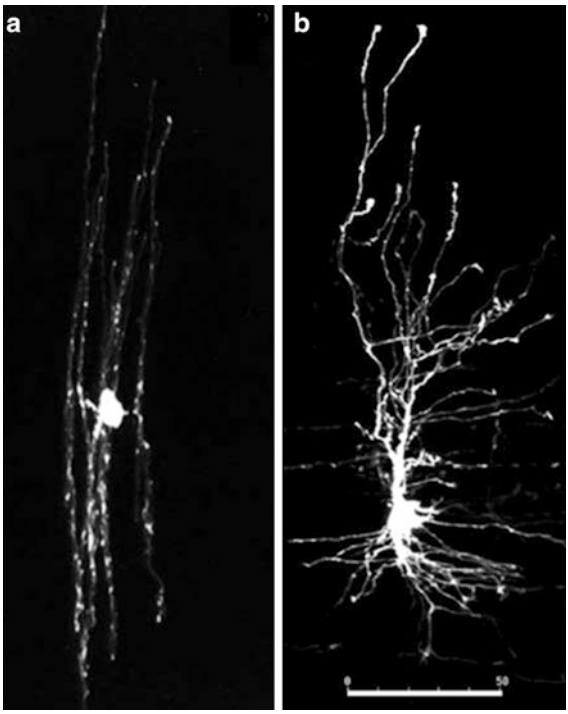


Fig. 11.2 Confocal images of rat optic nerve glia. (**a**) Oligodendrocytes have 5–50 parallel processes connecting to the internodal myelin sheath. (**b**) Astrocytes extend thick processes perpendicular and parallel to the axonal axis, which terminate in bulbous swellings or end feet at the pia and on blood vessels. Scale bar, 50 μm. (Adapted and modified from Butt et al. [2])

11.2.2 Retina (Fig. 11.3)

The retina contains two types of macroglial cells, namely Müller cell and astrocytes, and microglia. Müller cell is a type of astrocyte and is the main glial cell of the neuronal retina. The human retina contains 8–10 million Müller cells. Müller cell spans the entire thickness of the retina and makes contact with the retinal neurons. Although Müller cell was classically considered to primarily provide structural stability to the retina, it also plays an important role in supporting photoreceptor and ganglion cell survival. Astrocyte is important in the development, maintenance, and functioning of retinal neurons and blood vessels. Astrocyte possesses an elongated type and star-shaped type, with somata located in the ganglion cell layer and nerve fiber layer of the retina. Processes of astrocyte wrap around the blood vessels of the superficial plexus and the basal lamina of the vitreal surface in conjunction with Müller cell processes. Astrocyte

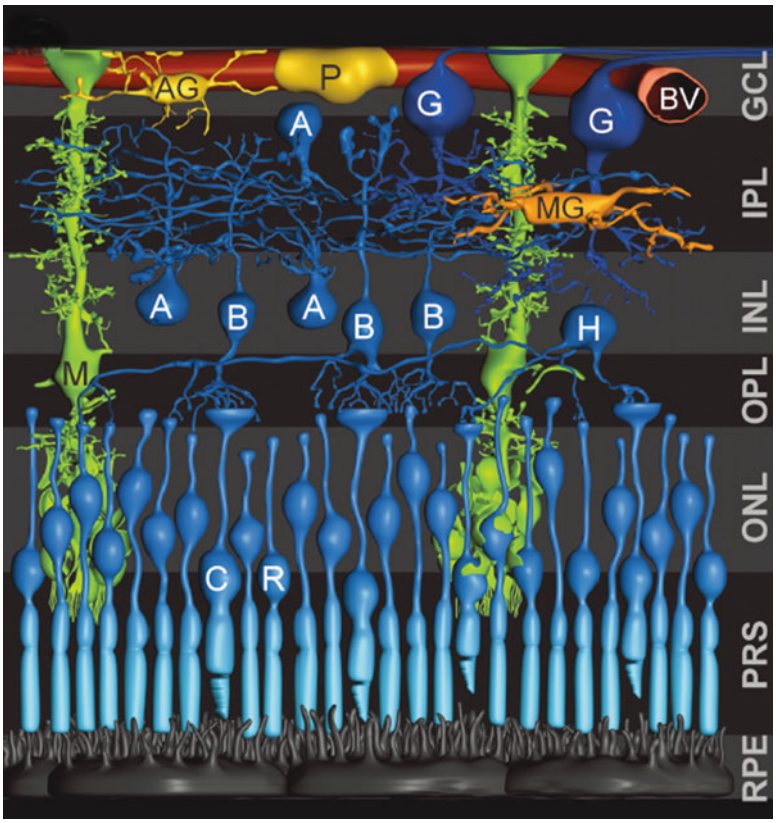


Fig. 11.3 Glial cells in the retina. Schematic drawing of the cellular constituents of a human retina. The perikarya of Müller cells are localized in the inner nuclear layer (INL). The funnel-shaped end feet of Müller cells form the inner surface of the retina. In the outer (OPL) and inner plexiform layers (IPL), side branches which form perisynaptic membrane sheaths originate at the stem processes. Both astrocytes (AG) and Müller cells contact the superficial blood vessels and the inner surface of the retina. In the outer nuclear layer (ONL), the stem process of Müller cells forms membrane sheaths which envelop the perikarya of rods (R) and cones (C). Microvilli of Müller cells extent into the subretinal space which surround the photoreceptor segments (PRS). Microglial (MG) cells are located in both plexiform layers and the ganglion cell layer (GCL). *A* amacrine cell, *B* bipolar cell, *G* ganglion cell, *H* horizontal cell, *P* pericyte, *RPE* retinal pigment epithelium. (Adapted from Reichenbach et al. [5])

is absent in an avascular retina and is only associated with blood vessels in vascularized retina. Astrocyte possesses a stellate morphology with somata located in the ganglion cell layer and nerve fiber layer of the retina. Microglia is specialized innate immune cells that reside in the retina and is sensitive to changes in their microenvironment.

11.3 Interaction Between Glial Cells and RGC Under Physiological Conditions

The major role of glial cells is involved in maintaining normal neuronal activity through bidirectional communication with neurons, which has a wide range of properties, such as production of neurotrophic factors, provision of basic metabolic support for neurons, regulation of the local concentration of some molecules including potentially neurotoxic, and modulation of the ionic balance of the extracellular space surrounding neurons.

11.3.1 Optic Nerve

In the optic nerve, glial cells insulate neurons, provide physical support, and supplement them with several metabolites and growth factors. These neurosupportive cells also play important roles in axon guidance and the control of synaptogenesis [6–8]. Astrocytes provide a variety of growth factors for neuron survival. In addition, astrocytes play many roles: supplying energy substrate to axons, maintaining extracellular pH and ion homeostasis, axon-glial signaling, and extracellular matrix (ECM) remodeling. Tenascin C is an astrocytic multifunctional ECM glycoprotein that potentially promotes or inhibits neurite outgrowth [9]. An excitatory neurotransmitter glutamate exerts neurotoxic action with high dose. Oligodendrocytes terminate the activity of glutamate by uptake into their cell body, which contributes alleviation of neuronal damage.

11.3.2 Retina

Müller cells perform a wide range of functions. They provide structural stability to the retina and are also considered to support photoreceptor and ganglion cell survival. Under normal conditions, Müller cell is believed to maintain the extracellular homeostasis of relevant ions through ion channels, glucose, and other metabolites; water primarily via aquaporins; and pH and neurotransmitters such as glutamate and gamma-aminobutyric acid (GABA) through transporters. Müller cells are involved in maintaining the inner blood-retinal barrier and in recycling neurotransmitters. Each Müller cell forms a functional unit with retinal neurons and interacts with the neurons of its column in a symbiotic relationship; Müller cells are also responsible for the functional and metabolic support of their associated neurons. These cells are also involved in recycling neurotransmitters, thus serving an important role in the regulation of neuronal activity. Müller cells generate neurotransmitter precursors such as glutamine, lactate, alanine, and α-ketoglutarate. They also release gliotransmitters with other neuroactive substances such as glutamate, D-serine, adenosine triphosphate (ATP), adenosine, and purinergic receptor agonists.

Astrocytes are important in the development, maintenance, and functioning of retinal neurons and blood vessels and act in roles similar to those of Müller cells.

Microglial cells are the resident immune cells responding sensitively to microenvironment changes arising from any neurodegeneration. Macroglia are electrically and chemically coupled to each other, thus integrating hemodynamics with neurodynamics.

Glial cells are involved in neurotransmission among glial cells. Ca^{2+} waves between neighboring astrocytes, between Müller cells, or between astrocytes and Müller cells are often coupled to neuronal activity and spread via the release of ATP or through internal messengers. Macroglia could be more directly involved in neurodynamics, as Müller cells serve as optical fibers and both classes of macroglia can release gliotransmitters. They also enhance synaptic transmission by wrapping around ganglion cell axons and modifying the expression of neurotransmitter transporters.

11.4 Interaction Between Glial Cells and RGC in Glaucoma

11.4.1 Optic Nerve

Glial cells transform from quiescent status to reactive status arising by glaucoma or experimental glaucoma models, and this transition drastically changes the activity and characteristics of glial cells.

Reactive astrocytes are considered to play a main role in glaucomatous optic neuropathy. Increased IOP, hypoperfusion, or other stimuli may trigger to transform astrocyte to reactive status. In the primate model of ocular hypertension, astrocytes round up and migrate from the core of the cribriform plates and express tenascin, an ECM molecule, as evidence of their activation [10, 11]. Astrocytes from genetically prepared mouse glaucoma model show thickening of processes, simplification of their processes, and localized hypertrophy of processes [12].

In human glaucoma, the organization of astrocytes in the anterior part of the optic nerve shows marked disruption. Astrocytes in the prelaminar ONH gather and migrate, abandoning their columnar organization in glaucoma or experimental glaucoma models [8] (Fig. 11.4).

The roles of quiescent and reactive glia in the process of ECM remodeling are dramatically different. Reactive glial cells accelerate the remodeling and disorganization of collagens I and III, basement membrane collagen IV, microfibrillar collagen VI, tenascin C, and elastic ECM [10–14]. To model glaucoma, ONH astrocytes have been exposed to hydrostatic pressure or mechanical stretching in vitro, resulting in the upregulation of many of these ECM components. Axonal debris is phagocytosed and the neural area contracts and is replaced by a GFAP-positive astrocytic scar [1, 15]. Activated glial cells in either the optic nerve or the

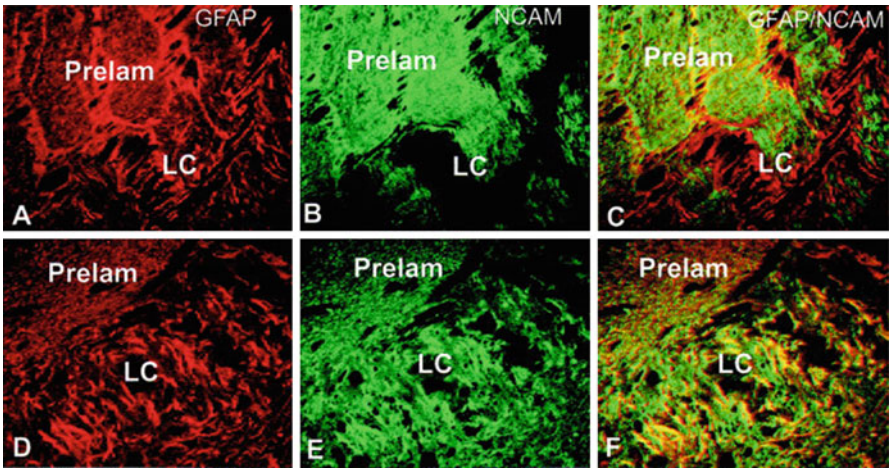


Fig. 11.4 Morphological changes in the optic nerve head of monkey experimental glaucoma model. (**a**–**c**) are sagittal sections from a normal eye. (**d**–**f**) are sagittal sections of the contralateral eye with chronic elevated IOP. (**a**) GFAP staining (*red*) is localized to astrocytes forming the glial columns in the prelaminar region (Prelam) and to astrocytes in the lamina cribrosa (LC) of the optic nerve head. (**b**) NCAM staining (*green*) is localized to astrocytes in the glial columns and the lamina cribrosa. NCAM also stains the axons of the nerve head. (**c**) Co-localization of both epitopes to astrocytes. (**d**, **e**) GFAP and NCAM staining in hypertrophied astrocytes in the lamina cribrosa. Note that astrocyte processes occupy the prelaminar region and the absence of glial columns. In the lamina cribrosa the nerve bundle area is occupied by cell bodies of astrocytes. Note that NCAM staining of the axons is noticeably decreased. (**f**) Co-localization of NCAM and GFAP to astrocytes. Magnification 750. (Adapted and modified from Hernandez et al. [8])

retina show different characteristics. The proliferation of astrocytes is only observed in astrocytes in the optic nerve. Glaucoma and experimental glaucoma models show that retinal and ONH astrocytes respond differently to damage.

In addition to modulating the ONH environment, astrocytes are also known to produce and/or respond to neurotoxic molecules including nitric oxide, tissue necrotic factor-α (TNF-α), interleukin 6 (IL-6)-type cytokines, and endothelins [16–20].

Hypertrophy, reorganization, or increases in the density of microglia have been observed in the optic nerve, which transforms microglial cell from quiescent to reactive [4]. Reactive microglia cells change the amount of secretion of a number of cytokines and trophic factors, which can be protective or destructive. Reactive microglia demonstrate increased immunolabeling for TNF-α, TNF receptor-1, TGFβ2, proliferating cell nuclear antigen, cyclooxygenase 2, and MMP and TIMP proteins [21]. They are also implicated as sources of reactive oxygen species, as well as initiators of both protective and destructive immune responses.

In the central nerve system, oligodendrocytes are exceptionally sensitive to any insults to the neurons, such as injury, ischemia, or inflammation, which result in the loss of oligodendrocytes and myelin, and eventually secondary axon degeneration. However, the role of oligodendrocytes in glaucoma is controversial. Nakazawa et al.

had reported that oligodendrocyte death is not only an early event in glaucoma but also a central mechanism through which the axons are damaged leading to the subsequent death of RGC [22]. Oligodendrocytes negatively regulate axonal promotion through semaphorin and Sema5A [23]. In contrast, Son et al. had found only a modest loss of oligodendrocytes that occurred after axons had already degenerated. They found a large increase in astrocyte reactivity occurred early in both animal models [24]. In human glaucoma, a marked proliferation of astrocytes, which is a sign of advanced gliosis, had been reported [25]. The characteristic response of astrocytes and oligodendrocyte progenitor cells is a reactive gliosis and the formation of a protective glial scar. However, the glial scar is also highly inhibitory, and oligodendrocyte progenitor cell is one of the most potent inhibitors of axon regeneration.

11.4.2 Retina

Practically, glaucomatous changes are limited to characteristic changes in the optic nerve head and loss of retinal nerve fiber and RGCs in the retina. Many studies revealed that glaucoma results in some morphological and functional changes in the retina and that retinal glia makes an important role of these. Reactive Müller cell could increase the susceptibility of RGCs to stress signals and contribute to disease progression.

11.5 Role of Glia in Glaucomatous RGC Death

Recent studies indicate that glial cells are actively involved in the initiation of RGC apoptosis. Increased retinal expression of GFAP appears to be an early event in the pathogenesis of glaucoma (Fig. 11.5). The exclusive co-localization of caspases with GFAP in the retina was observed in a rodent glaucoma model. Increased Müller cell reactivity for Fas-associated death domain, an important component for Fas-caspase-mediated apoptosis, indicates that glial cells contribute to RGC apoptosis from elevated IOP. Retinal glial cells are a major source of extracellular ATP. Müller cells release ATP in response to mechanical stimuli. In experimental glaucoma, extracellular ATP mediates the apoptotic death of retinal ganglion cells [27].

11.5.1 Axon Change and Synaptic Connectivity

There are clear evidences of RGC dendritic alterations in glaucoma. Early changes, including thinning and reduced arbor complexity, occurred in the RGC dendrites of

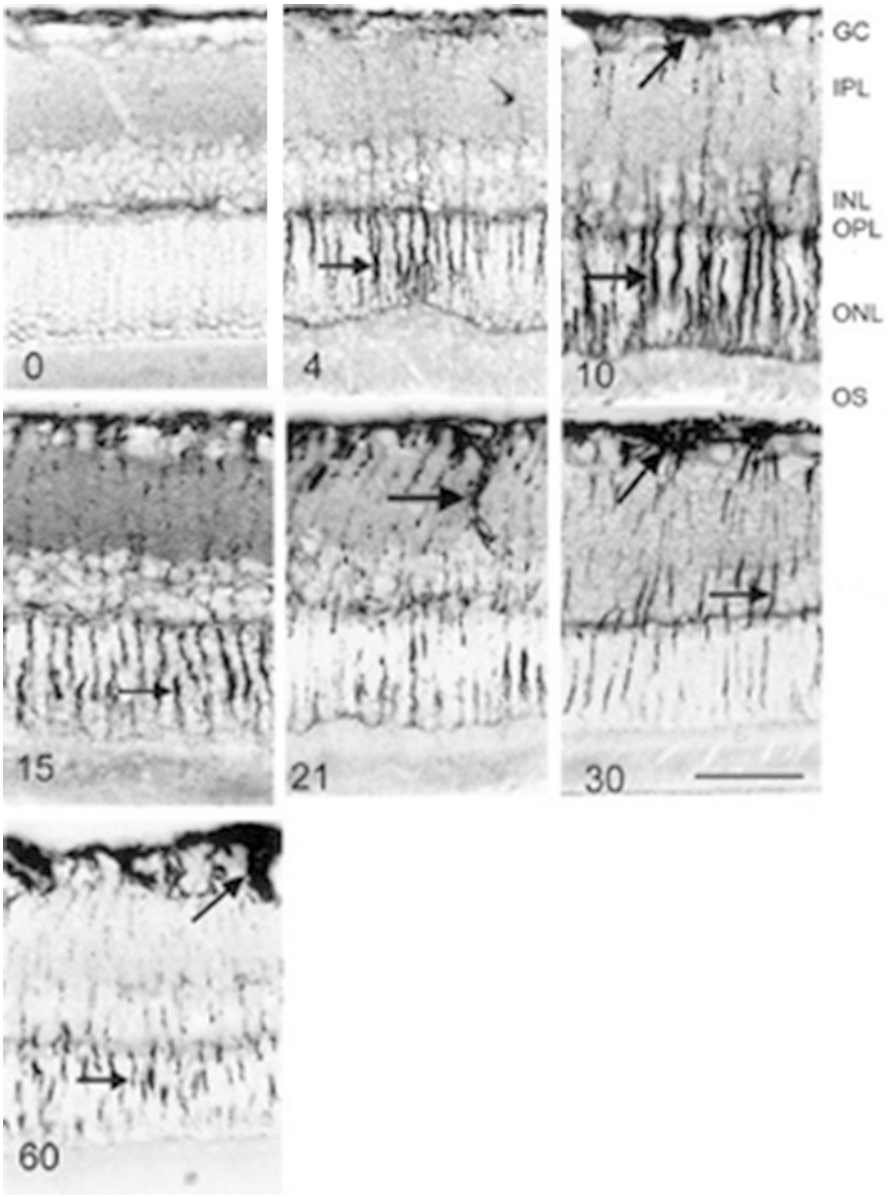


Fig. 11.5 Changes in expression of GFAP in retina after elevation of intraocular pressure (IOP). Expression of GFAP is enhanced by a time-related manner. Numbers indicate day(s) after IOP elevation. Note the immunostaining of the Müller cells after (*arrows*). Bar, 50 μm. (Adapted and modified from Woldemussue et al. [26])

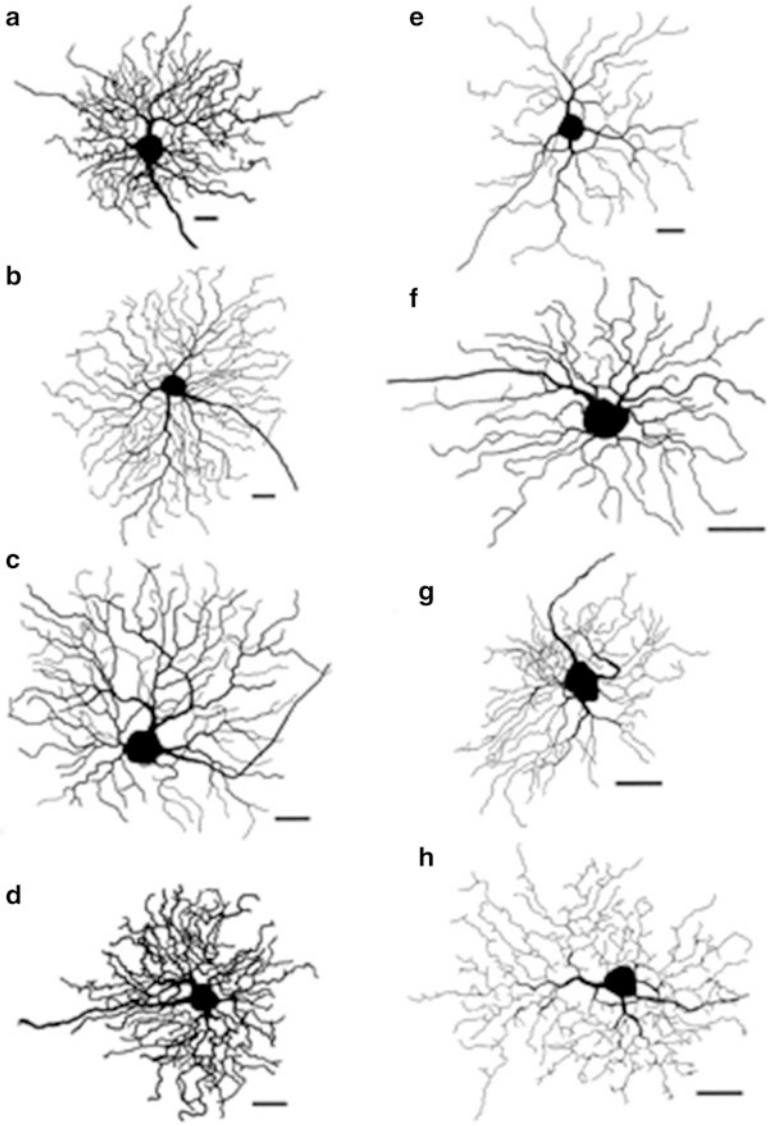


Fig. 11.6 Morphologic change in experimental glaucoma monkey eye. Compared to the with parasol cells from normal eye (**a**–**d**), neurons from glaucomatous eye contain fewer dendritic processes, a less complex dendritic tree, and greater variation of individual dendrites in thickness along their lengths (**e**–**h**). Scale bars, 25 μm (Adapted from Weber et al. [29])

a primate glaucoma model [28]. Parasol cells from glaucomatous eye contain fewer dendritic processes, a less complex dendritic tree, and greater variation of individual dendrites in thickness along their lengths [29] (Fig. 11.6). Dendritic arbor retraction and remodeling have also been described in other experimental glaucoma

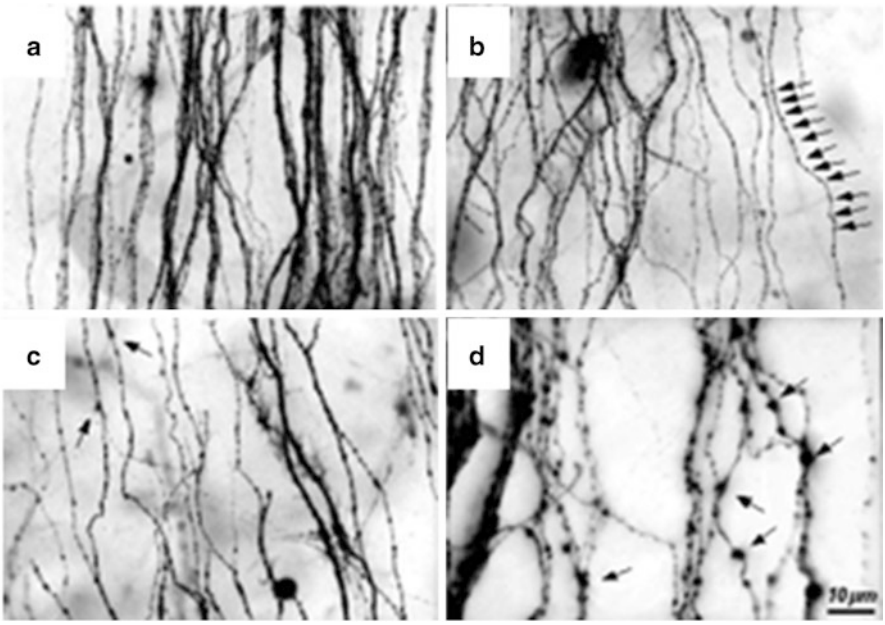


Fig. 11.7 Morphological changes in RGC axons of human glaucoma eye. Compared with the central axons (**a**) and peripheral axons (**b**) in a normal retina, glaucoma axons show much more enlarged beads along the axons in both the central (**c**) and peripheral (**d**). (Adapted and modified from Pavlidis et al. [25])

models [29, 30]. These morphological changes occurred prior to RGC shrinkage and axon atrophy, suggesting that dendritic defects could be an early sign of glaucomatous neurodegeneration. Synaptic connections may be also damaged at early stage of glaucoma. The levels of c-Fos, a marker of neuronal connectivity, declined early after laser-induced ocular hypertension in rats [31].

A substantial reduction in the bifurcation and the diameter of the dendritic trees in RGC was reported in a postmortem study of glaucomatous human retinas, though the number of remaining RGCs was extremely low due to the advanced stage of the disease [25] (Figs. 11.7 and 11.8). Although it is not fully understood, retinal glia is considered resulting in synapse elimination by stimulating a complement cascade observed in the central nerve system.

11.5.2 Role of the Glial Cell Modulation of Glutamate in Glaucoma

Alterations in the activity and levels of glutamine synthetase have been reported in models of ocular hypertension [32, 33]. Reactive Müller cells could impair RGC functioning in glaucoma by malfunction in the potassium buffering system, water

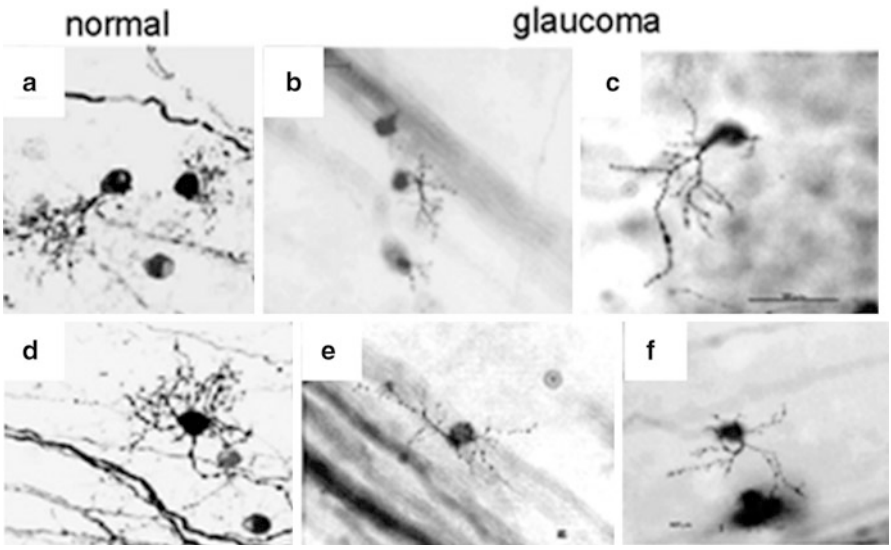


Fig. 11.8 Morphological changes in midget and parasol cells of the human glaucoma eye. Midget cells within glaucomatous retinas (**b**, **c**) show increase of eccentricity and reduction of the bifurcation frequency compared with control (**a**). Parasol cells within glaucomatous retinas (**e**, **f**) show marked decrease of the arborization and reduction of branching frequency compared with control (**d**) (Adapted from Pavlidis et al. [25])

clearance or production of antioxidant molecules. Reactive Müller cells also upregulate NF-κB activity and release neurotoxic factors, including TNF-α, NO, and alpha2-macroglobulin, that could directly contribute to RGC loss in glaucoma [16–20, 34–37]. The enhanced Müller cell-derived production of polyamines, a family of arginine-derived co-activators of NMDAR, exacerbated the excitotoxic death of RGCs [38]. The reduced expression of Müller cell proteins that typically play a supportive role for RGCs could trigger death signals in these neurons.

11.5.3 Reactive Gliosis of Glia

Gliotic Müller cells increase in stiffness due to the upregulation of intermediate filaments, in particular GFAP [39]. This inhibits neurite growth and could be one reason for aberrant retinal tissue repair after retinal injury. Additionally, gliotic Müller cells increase the expression of ECM and cell adhesion molecules; these molecules function as chemical inhibitors of axonal growth and neuronal regeneration. Retinal gliosis occurs early in the astrocytes and Müller cells of genetically prepared mouse glaucoma model. Gliosis of glaucoma is different from that of CNS

injury and disease in which the proliferation and hypertrophy of glial cells such as astrocytes and microglia occurs. Müller cells do not proliferate, but microglia proliferates during glaucoma development [40].

11.5.4 Role of Microglia in Glaucoma

As in the ONH, microglia overactivation often results in the excess production of pro-inflammatory and neurotoxic factors such as TNF-α, interleukin-6, NO, endothelin, complement proteins, and superoxide [41–43]. Reactive microglia have been reported in the retina and optic nerves in animal glaucoma models [11, 24, 40, 44–46] and in human glaucoma [4, 21]. The microglial response is considered as an early event in glaucomatous neuropathology [47, 48].

11.6 Strategy for Glial Cell-Induced RGC Rescue or Regeneration

There are sufficient evidences that ocular glial cells are deeply involved in RGC damage in glaucoma, which indicates that glial cells could be a good target to rescue RGC from glaucomatous insult. Strategies for glial cell-related RGC protection include rescue from RGC death, the promotion of optic nerve regeneration, the preservation of dendritic morphology and synaptic connectivity, and the inhibition of the pathological reformation of ECM in the optic nerve.

Reactive glial cells in the retina and/or optic nerve could protect RGCs from cell death via various mechanisms, including the secretion of neurotrophic and growth factors, particularly the basic fibroblast growth factor (bFGF) and the glial cell line-derived neurotrophic factor (GDNF). Retinal glial cells mediate RGC death by modulating extracellular ATP [27]. Retinal astrocytes transformed RGCs into an active regenerative status through the upregulation of CNTF [49]. For the preservation of dendritic morphology and synaptic connectivity, neurotrophic factors also serve an important role in optic nerve regeneration either directly or indirectly through retinal astrocyte/Müller cells. They induce axon growth through the regulated intramembranous proteolysis of p75 neurotrophic factor receptors. Glial-related neurite outgrowth appears to be mediated via apolipoprotein E (ApoE), which is secreted from activated retinal astrocytes and Müller cell [50].

The ECM in the ONH are modulated through the release of metalloproteinases (MMP) and plasminogen activators from RGC axons, as well as tissue inhibitors of metalloproteinases from the optic nerve glia. Therefore, the regulation of MMP and TIMP expression could be effective in preventing the progression of glaucomatous ONH cupping [51].

The role of microglia in the onset or progression of glaucoma has not been unequivocally established; however, strategies that prevent microglia activation or function have proven to be beneficial for injured RGCs [47, 52, 53]. In their activated state, microglia possess beneficial functions including the clearance of toxic debris and the release of neurotrophic and anti-inflammatory factors. The modulation of microglia activation via the deletion of the phagocytic microglial receptor CD11b may be neuroprotective in experimental glaucoma [22]. Microglia with the upregulation of GFAP expression promotes the neural process [54]. The inhibition of microglia activation was achieved using minocycline-delayed RGC death in models of optic nerve injury [55, 56].

11.7 Summary

Many previous studies revealed that glial cells are deeply involved in RGC survival and activity. The roles of glial cells in RGC survival are very complicated and variable depending on the degree of activation status. It is clear that glial cells are not simple passive structural elements but interact with neurons in a dynamic manner, which is important for coupling neuronal and glial functions.

In glaucoma, RGC showed morphological and functional changes prior to cell death, and glial cells are deeply involved in this. It is challenging to regenerate RGC so far, but it is possible to rescue RGC that show morphological and functional changes prior to their death by modulating glial cells; this may represent a new strategy for glaucoma treatment in addition to IOP reduction.

References

1. Hernandez MR (2000) The optic nerve head in glaucoma: role of astrocytes in tissue remodeling. Prog Retin Eye Res 19:297–321
2. Butt AM, Pugh M, Hubbard P, James G (2004) Functions of optic nerve glia: axoglial signalling in physiology and pathology. Eye (Lond) 18:1110–1121
3. Anderson DA (1969) Ultrastructure of human and monkey lamina cribrosa and optic nerve head. Arch Ophthalmol 82:800–814
4. Neufeld AH (1999) Microglia in the optic nerve head and the region of parapapillary chorioretinal atrophy in glaucoma. Arch Ophthalmol 117:1050–1056
5. Reichenbach A, Bringmann A (2013) New functions of Muller cells. Glia 61:651–678
6. Crawford Downs J, Roberts MD, Sigal IA (2011) Glaucomatous cupping of the lamina cribrosa: a review of the evidence for active progressive remodeling as a mechanism. Exp Eye Res 93:133–140
7. Burgoyne CF (2011) A biomechanical paradigm for axonal insult within the optic nerve head in aging and glaucoma. Exp Eye Res 93:120–132
8. Hernandez MR, Miao H, Lukas T (2008) Astrocytes in glaucomatous optic neuropathy. Prog Brain Res 173:353–373

9. Siddiqui S, Horvat-Brocker A, Faissner A (2008) The glia-derived extracellular matrix glycoprotein tenascin-C promotes embryonic and postnatal retina axon outgrowth via the alternatively spliced fibronectin type III domain TNfnD. Neuron Glia Biol 4:271–283
10. Varela HJ, Hernandez MR (1997) Astrocyte responses in human optic nerve head with primary open-angle glaucoma. J Glaucoma 6:303–313
11. Johnson EC, Jia L, Cepurna WO, Doser TA, Morrison JC (2007) Global changes in optic nerve head gene expression after exposure to elevated intraocular pressure in a rat glaucoma model. Invest Ophthalmol Vis Sci 48:3161–3177
12. Lye-Barthel M, Sun D, Jakobs TC (2013) Morphology of astrocytes in a glaucomatous optic nerve. Invest Ophthalmol Vis Sci 54:909–917
13. Fukuchi T, Sawaguchi S, Hara H, Shirakashi M, Iwata K (1992) Extracellular matrix changes of the optic nerve lamina cribrosa in monkey eyes with experimentally chronic glaucoma. Graefes Arch Clin Exp Ophthalmol 230:421–427
14. Fukuchi T, Sawaguchi S, Yue BY, Iwata K, Hara H, Kaiya T (1994) Sulfated proteoglycans in the lamina cribrosa of normal monkey eyes and monkey eyes with laser-induced glaucoma. Exp Eye Res 58:231–243
15. Almasieh M, Wilson AM, Morquette B, Cueva Vargas JL, Di Polo A (2012) The molecular basis of retinal ganglion cell death in glaucoma. Prog Retin Eye Res 31:152–181
16. Cho KJ, Kim JH, Park HY, Park CK (2011) Glial cell response and iNOS expression in the optic nerve head and retina of the rat following acute high IOP ischemia-reperfusion. Brain Res 1403:67–77
17. Yuan L, Neufeld AH (2001) Activated microglia in the human glaucomatous optic nerve head. J Neurosci Res 64:523–532
18. Chidlow G, Wood JP, Ebneter A, Casson RJ (2012) Interleukin-6 is an efficacious marker of axonal transport disruption during experimental glaucoma and stimulates neuritogenesis in cultured retinal ganglion cells. Neurobiol Dis 48:568–581
19. Johnson EC, Doser TA, Cepurna WO, Dyck JA, Jia L, Guo Y et al (2011) Cell proliferation and interleukin-6-type cytokine signaling are implicated by gene expression responses in early optic nerve head injury in rat glaucoma. Invest Ophthalmol Vis Sci 52:504–518
20. Prasanna G, Krishnamoorthy R, Yorio T (2011) Endothelin, astrocytes and glaucoma. Exp Eye Res 93:170–177
21. Yuan L, Neufeld AH (2000) Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. Glia 32:42–50
22. Nakazawa T, Nakazawa C, Matsubara A, Noda K, Hisatomi T, She H et al (2006) Tumor necrosis factor-alpha mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J Neurosci 26:12633–12641
23. Goldberg JL, Vargas ME, Wang JT, Mandemakers W, Oster SF, Sretavan DW et al (2004) An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. J Neurosci 24:4989–4999
24. Son JL, Soto I, Oglesby E, Lopez-Roca T, Pease ME, Quigley HA et al (2010) Glaucomatous optic nerve injury involves early astrocyte reactivity and late oligodendrocyte loss. Glia 58:780–789
25. Pavlidis M, Stupp T, Naskar R, Cengiz C, Thanos S (2003) Retinal ganglion cells resistant to advanced glaucoma: a postmortem study of human retinas with the carbocyanine dye DiI. Invest Ophthalmol Vis Sci 44:5196–5205
26. Woldemussie E, Wijono M, Ruiz G (2004) Muller cell response to laser-induced increase in intraocular pressure in rats. Glia 47:109–119
27. Resta V, Novelli E, Vozzi G, Scarpa C, Caleo M, Ahluwalia A et al (2007) Acute retinal ganglion cell injury caused by intraocular pressure spikes is mediated by endogenous extracellular ATP. Eur J Neurosci 25:2741–2754
28. Weber AJ, Kaufman PL, Hubbard WC (1998) Morphology of single ganglion cells in the glaucomatous primate retina. Invest Ophthalmol Vis Sci 39:2304–2320

29. Weber AJ, Harman CD (2005) Structure-function relations of parasol cells in the normal and glaucomatous primate retina. Invest Ophthalmol Vis Sci 46:3197–3207
30. Shou T, Liu J, Wang W, Zhou Y, Zhao K (2003) Differential dendritic shrinkage of alpha and beta retinal ganglion cells in cats with chronic glaucoma. Invest Ophthalmol Vis Sci 44:3005–3010
31. Fu QL, Li X, Shi J, Xu G, Wen W, Lee DH et al (2009) Synaptic degeneration of retinal ganglion cells in a rat ocular hypertension glaucoma model. Cell Mol Neurobiol 29:575–581
32. Chen CT, Alyahya K, Gionfriddo JR, Dubielzig RR, Madl JE (2008) Loss of glutamine synthetase immunoreactivity from the retina in canine primary glaucoma. Vet Ophthalmol 11:150–157
33. Moreno MC, Sande P, Marcos HA, de Zavalia N, Keller Sarmiento MI, Rosenstein RE (2005) Effect of glaucoma on the retinal glutamate/glutamine cycle activity. FASEB J 19:1161–1162
34. Lebrun-Julien F, Duplan L, Pernet V, Osswald I, Sapieha P, Bourgeois P et al (2009) Excitotoxic death of retinal neurons in vivo occurs via a non-cell-autonomous mechanism. J Neurosci 29:5536–5545
35. Shi Z, Rudzinski M, Meerovitch K, Lebrun-Julien F, Birman E, Di Polo A et al (2008) Alpha2-macroglobulin is a mediator of retinal ganglion cell death in glaucoma. J Biol Chem 283:29156–29165
36. Becquet F, Courtois Y, Goureau O (1997) Nitric oxide in the eye: multifaceted roles and diverse outcomes. Surv Ophthalmol 42:71–82
37. Tezel G, Wax MB (2000) Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. J Neurosci 20:8693–8700
38. Pernet V, Bourgeois P, Di Polo A (2007) A role for polyamines in retinal ganglion cell excitotoxic death. J Neurochem 103:1481–1490
39. Schuettauf F, Rejdak R, Walski M, Frontczak-Baniewicz M, Voelker M, Blatsios G et al (2004) Retinal neurodegeneration in the DBA/2J mouse-a model for ocular hypertension. Acta Neuropathol 107:352–358
40. Inman DM, Horner PJ (2007) Reactive nonproliferative gliosis predominates in a chronic mouse model of glaucoma. Glia 55:942–953
41. Howell GR, Macalinao DG, Sousa GL, Walden M, Soto I, Kneeland SC et al (2011) Molecular clustering identifies complement and endothelin induction as early events in a mouse model of glaucoma. J Clin Invest 121:1429–1444
42. Lam TT, Kwong JM, Tso MO (2003) Early glial responses after acute elevated intraocular pressure in rats. Invest Ophthalmol Vis Sci 44:638–645
43. Sappington RM, Calkins DJ (2006) Pressure-induced regulation of IL-6 in retinal glial cells: involvement of the ubiquitin/proteasome pathway and NFkappaB. Invest Ophthalmol Vis Sci 47:3860–3869
44. Ebneter A, Casson RJ, Wood JP, Chidlow G (2010) Microglial activation in the visual pathway in experimental glaucoma: spatiotemporal characterization and correlation with axonal injury. Invest Ophthalmol Vis Sci 51:6448–6460
45. Naskar R, Wissing M, Thanos S (2002) Detection of early neuron degeneration and accompanying microglial responses in the retina of a rat model of glaucoma. Invest Ophthalmol Vis Sci 43:2962–2968
46. Wang X, Sam-Wah Tay S, Ng YK (2000) Nitric oxide, microglial activities and neuronal cell death in the lateral geniculate nucleus of glaucomatous rats. Brain Res 878:136–147
47. Bosco A, Crish SD, Steele MR, Romero CO, Inman DM, Horner PJ et al (2012) Early reduction of microglia activation by irradiation in a model of chronic glaucoma. PLoS One 7:e43602
48. Bosco A, Steele MR, Vetter ML (2011) Early microglia activation in a mouse model of chronic glaucoma. J Comp Neurol 519:599–620
49. Leibinger M, Muller A, Andreadaki A, Hauk TG, Kirsch M, Fischer D (2009) Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal

ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. J Neurosci 29:14334–14341
50. Lorber B, Berry M, Douglas MR, Nakazawa T, Logan A (2009) Activated retinal glia promote neurite outgrowth of retinal ganglion cells via apolipoprotein E. J Neurosci Res 87:2645–2652
51. Surgucheva I, Chidambaram K, Willoughby DA, Surguchov A (2010) Matrix metalloproteinase 9 expression: new regulatory elements. J Ocul Biol Dis Infor 3:41–52
52. Chiu K, Yeung SC, So KF, Chang RC (2010) Modulation of morphological changes of microglia and neuroprotection by monocyte chemoattractant protein-1 in experimental glaucoma. Cell Mol Immunol 7:61–68
53. Zhang S, Wang H, Lu Q, Qing G, Wang N, Wang Y et al (2009) Detection of early neuron degeneration and accompanying glial responses in the visual pathway in a rat model of acute intraocular hypertension. Brain Res 1303:131–143
54. Toops KA, Hagemann TL, Messing A, Nickells RW (2012) The effect of glial fibrillary acidic protein expression on neurite outgrowth from retinal explants in a permissive environment. BMC Res Notes 5:693
55. Baptiste DC, Hartwick AT, Jollimore CA, Baldridge WH, Seigel GM, Kelly ME (2004) An investigation of the neuroprotective effects of tetracycline derivatives in experimental models of retinal cell death. Mol Pharmacol 66:1113–1122
56. Bosco A, Inman DM, Steele MR, Wu G, Soto I, Marsh-Armstrong N et al (2008) Reduced retina microglial activation and improved optic nerve integrity with minocycline treatment in the DBA/2J mouse model of glaucoma. Invest Ophthalmol Vis Sci 49:1437–1446

Chapter 12
Aquaporin in Optic Neuropathies

Akiyasu Kanamori

Abstract The aquaporins (AQPs) are plasma membrane water-transporting proteins. Many AQPs have been identified as playing essential roles in the nervous system and in ocular functions. The AQPs are potential drug targets for several neurological conditions. Additionally, recent evidence has demonstrated that some AQPs are involved in the pathophysiology of optic neuropathies. Notably, AQP4, a pure water channel expressed at astrocytes in the optic nerve, is the first target of neuromyelitis optica (NMO) in optic neuritis. AQP9 facilitates the transport of lactate for neural energy metabolism and may be considered an essential factor for retinal ganglion cell survival. This review summarizes the expression and known functions of the ocular AQPs related to optic neuropathies.

Keywords Aquaporin • Astrocytes • Müller cell • Retinal ganglion cell • Water channel

12.1 Introduction

Water is an important molecule involved in all biochemical processes in living cells and diffuses rapidly through water channels known as aquaporins (AQPs). In neural cells, water is an essential factor for life that plays important roles in development and the spreading of electrical activity [1]. The AQPs are a family of integral membrane transport proteins; the family functions as water channels with broad permeability that includes water, gases (CO_2, nitric oxide), ions (K^+ and Cl^-), and other small solutes (e.g., glycerol, urea) [2]. Fourteen AQPs have been identified in humans and rodents, and at least eight of these facilitate water movement

A. Kanamori (✉)
Division of Ophthalmology, Department of Surgery, Graduate School of Medicine, Kobe University, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
e-mail: kanaaki@med.kobe-u.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_12, © Springer Japan 2014

Table 12.1 Localization of aquaporins in the nervous system and the ocular tissues

Isoform	Group	Localization in the nervous system	Localization in the ocular tissues
AQP1	Pure water channel	Astrocytes, choroid plexus epithelial cells, Schwann cells, non-myelinated dorsal root ganglion neurons	Corneal stroma and endothelium, ciliary epithelium, trabecular meshwork, lens epithelium, RPE cells
AQP2	Pure water channel	Ependymal cells, Schwann cells	Inner limiting membrane, RPE cells
AQP3	Aquaglyceroporin	Astrocytes	RPE cells
AQP4	Pure water channel	Astrocytes, ependymal cells	Ciliary epithelium, trabecular meshwork, Müller cell, astrocytes
AQP5	Pure water channel	Astrocytes	RPE cells
AQP6	Pure water channel		Müller cells
AQP7	Aquaglyceroporin		Inner limiting membrane, Outer limiting membrane, RPE cells
AQP8	Pure water channel	Astrocytes, ependymal cells, oligodendrocytes	
AQP9	Aquaglyceroporin	Astrocytes, ependymal cells, tanycytes	Astrocytes, amacrine cells, RGCs, photoreceptors, RPE cells

(Table 12.1). The AQP family is subdivided into the following three groups according to sequence homology and permeability: (1) AQPs 0, 1, 2, 4, 5, 6, and 8 belong to the "pure water channel family" and are primarily permeable only to water; however, AQP6 is permeable to anions, and AQP8 is permeable to urea; (2) the "aquaglyceroporin" subgroup is composed of AQP3, 7, 9, and 10 and facilitates the diffusion of water and some monocarboxylates, such as lactate and beta-hydroxybutyrate (AQP9) [3]; and (3) the third "super-aquaporins" group is composed of AQP11 and 12, which are localized in the cytoplasm; however, their permeability has not been determined, and these aquaporins might be involved in intracellular water transport and the regulation of organelle volume [4].

Recent studies have demonstrated the involvement of AQPs in central nervous system (CNS) disorders. Notably, it has recently been established that neuromyelitis optica (NMO) is an AQP4-related CNS disease in which astrocytes are damaged by AQP4-specific antibodies. Additionally, AQPs are expressed in various cell types in ocular tissues. Accumulating evidence indicates the involvement of AQPs in optic neuropathies in which retinal ganglion cells (RGCs) are impaired. This chapter will focus on the recent discoveries on AQPs in optic neuropathies.

12.2 AQP Expression in the Nerve System and the Ocular Tissues

AQP expression has been studied for the past two decades. It has been revealed that various cell types in the ocular tissues express AQPs. The expression pattern and location of AQPs in the nervous system and the ocular tissues are summarized in Table 12.1.

AQP1 is expressed in the plasma membrane of choroid plexus epithelial cells and plays a role as a channel for cerebrospinal fluid [5]. Additionally, some evidence has demonstrated the upregulation of AQP1 under pathological conditions. AQP1 is found in reactive astrocytes accumulating in subarachnoid hemorrhage lesions [6], contusions [7], cerebral infarctions, multiple sclerosis [8], and Alzheimer's disease [8]. In the spinal cord, reactive astrocytes expressing AQP1 surround the lesion [9]. These observations have demonstrated a pathological role for AQP1 in the abnormal regulation of water transport. In the eye, the corneal stroma and corneal endothelium express AQP1 [10], suggesting a function in fluid transport in the cornea. AQP1 is present in the non-pigmented ciliary body epithelium [11, 12], the endothelium of the trabecular meshwork, and the endothelium of the canal of Schlemm [13]. AQP1 controls the production and outflow facility of aqueous humor [14, 15]. In the retina, amacrine cells, photoreceptor cells, and retinal pigment epithelium (RPE) cells express AQP1 [15–17]. AQP1 also facilitates efficient transepithelial water transport across the RPE.

AQP4 is the most abundant water channel in the CNS [18–20]. Although AQP4 is widely expressed in the astrocyte cell plasma membrane, it is primarily located at specific lesions, such as astrocyte foot processes [21, 22]. The subpial processes with AQP4 form the glial limiting membrane with the perivascular astrocyte end feet and the basolateral membrane of the ependymal cells and subependymal astrocytes in the brain. This expression of AQP4 at the borders between the brain and the water-containing components suggests that AQP4 plays a key role in the flow of water into and out of the brain. Furthermore, AQP4 is primarily localized in the perivascular astrocyte and foot processes and in the glial limiting membrane in the spinal cord. In the ocular tissues, many cells express AQP4, including the basolateral membranes of non-pigmented ciliary epithelium, the endothelium of the trabecular meshwork, Müller cells, and astrocytes in the retina and optic nerve [13, 20, 23, 24]. AQP4 expression in Müller cells shows a polarized distribution with predominant expression in the end feet membranes [23]. This expression pattern suggests that Müller cells have a role in water control in the retina and direct osmotically driven water flux to the vitreous body and vessels [23]. Additionally, AQP4 expression in Müller cells has a similar distribution pattern as the inwardly rectifying potassium channel Kir4.1 [23]. Astrocytic AQP4 has the potential to facilitate the clearance of K^+ following neural activity [25].

AQP6 is expressed in the membranes of Müller cells that surround the ribbon synapses and extend into the photoreceptor layer. AQP6 functions as an anion channel with a high permeability for nitrate [26, 27]. Thus, it is possible that AQP6

in Müller cells regulates the cell-mediated regulation of synaptic ion concentrations [27].

AQP9 is weakly expressed in the brain [28], but it has been found to be localized in a subset of GFAP-positive ependymal cells lacking cilia, called tanycytes [29]. However, there are conflicting reports about AQP9 expression in the subset of ciliated ependymal cells [30, 31]. AQP9 is also expressed in the glia limitans and white matter tracts in the brain and spinal cord [19, 30]. Apparent AQP9 expression is found in the retina; amacrine cells, RGCs, and astrocyte processes surrounding retinal capillaries; and the cytoplasm of the inner segments of the photoreceptors and RPE cells [26, 32–35]. The characteristic pattern of AQP9 expression may be observed in the optic nerve head. Retinal nerve fibers and optic nerve fibers arise from the same RGC axon, but they are fundamentally different; the RGC axons that course through the retina are not myelinated, whereas the optic nerve fibers beyond the retina become myelinated at the optic nerve head. AQP9 is expressed in astrocytes at both the optic nerve head and the retrobulbar regions in the human and monkey, especially in the laminar portion. In contrast, AQP4 expression was found only in the retrobulbar regions [36]. These findings were also observed in rodents. The rodent optic nerve head can be divided into three regions: the neck region, located at the level of the sclera; the transition region, an extension zone between the neck region and the unmyelinated retrobulbar optic nerve; and the retrobulbar region, characterized by myelinated axons. In the rat neck and transition regions, AQP9 expression, not AQP4 expression, was found to co-localize with GFAP (Fig. 12.1) [33]. This evidence suggests that AQP9 expressed in astrocytes in the optic nerve head with unmyelinated axons may have different roles than AQP4.

12.3 AQPs in Optic Neuropathies

12.3.1 Neuromyelitis Optica (NMO)

Neuromyelitis optica (NMO) is an autoimmune inflammatory disease of the CNS that is associated with demyelinating lesions mainly in the optic nerve and spinal cord and leads to blindness and paralysis [37, 38]. Although NMO had been classified as a subtype of multiple sclerosis (MS) for many years, it is now clear that it has distinct clinical and pathological features. Neuropathologic studies have demonstrated that NMO is characterized by a distinctive vasculocentric pathology with prominent perivascular immunoglobulin deposition and evidence of the activation of the complement lytic pathway invoking B cell participation in the disease process [39, 40]. In 2004, Lennon and colleagues described an IgG, the presence of which was 73 % sensitive and 91 % specific for clinically defined NMO [41]. The majority of NMO patients are seropositive for NMO-specific serum autoantibodies, collectively known as NMO-IgG [41, 42]. Recently, the target antigen of NMO-IgG has been identified as the mercurial-insensitive water channel protein AQP4, which

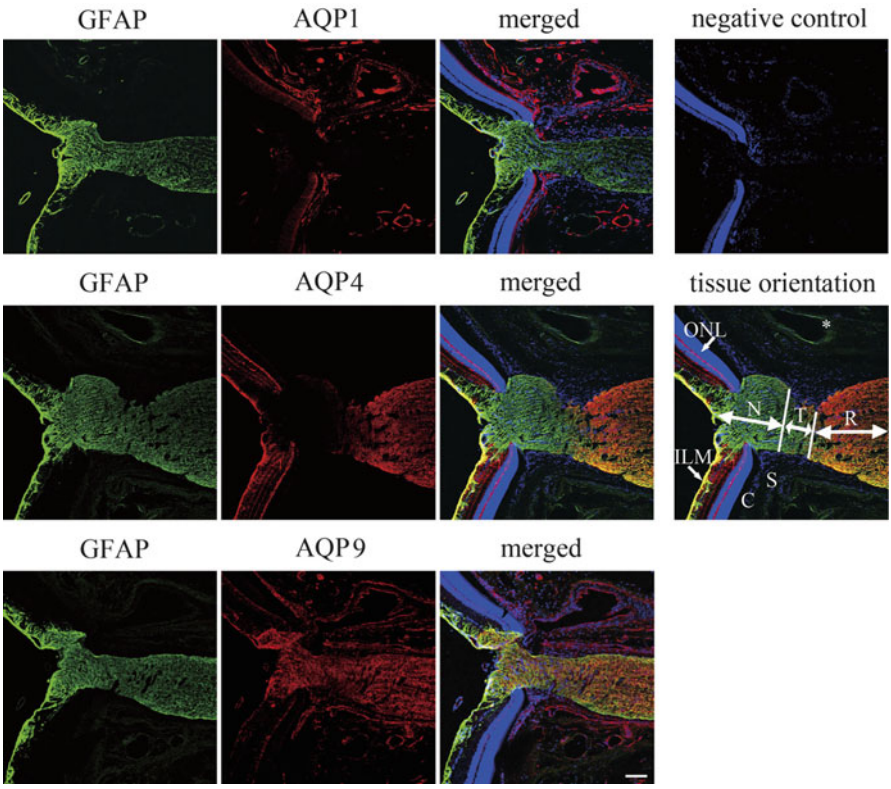


Fig. 12.1 Immunohistochemistry for AQP1, 4, and 9 (*red*) in the optic nerve head. GFAP (*green*) and nuclei (*blue*) were also stained. An intense honeycomb pattern of AQP9 immunoreactivity was detected throughout the optic nerve from the vitreo-optic nerve head interface to the retrobulbar region where a myelinated axon existed. In contrast, although the neck and the transition regions, each of which consisted of unmyelinated axons, lacked AQP4 immunoreactivity, the retrobulbar myelinated optic nerve exhibited intense AQP4 immunoreactivity. *ILM* inner limiting membrane, *S* sclera, *C* choroid, *N* neck region, *T* transition region, *R* retrobulbar region. *Asterisk*: extraocular vein

is the dominant water channel within the CNS [43]. Misu and colleagues reported an immunohistochemical analysis that revealed the loss of AQP4 in 90 % of acute and chronic NMO lesions, which were more pronounced in the active perivascular lesions where immunoglobulins and complement were deposited [42]. In addition, Lucchinetti and Roemer and their colleagues have independently performed a detailed comparative study of both NMO and MS lesions; in particular, in contrast to NMO, they revealed that the AQP4 immunoreactivity was variable in the MS lesions [39, 44]. Now, the loss of AQP4 in the absence of demyelination or necrosis suggests that the binding of an antibody to AQP4 may be the initial pathogenic event in the NMO lesions. In an experimental mouse model, Saadoun and colleagues demonstrated that the intracerebral administration of IgG from NMO

patients produced lesions with NMO-like characteristics, including inflammation, loss of AQP4 and GFAP immunoreactivity, and myelin loss [45]. Two ex vivo studies of NMO using the optic nerve have been performed. Zhang and colleagues have demonstrated that recombinant NMO-IgG with human complement reduced AQP4 immunoreactivity [46]. In another study, Marignier et al. showed that IgG from NMO patients without human complement damaged the oligodendrocytes in the optic nerve [47]. Matsumoto and colleagues recently developed rat optic neuritis model in NMO [48]. They founded that the serum corrected from AQP4-positive NMO patients directly damaged astrocytes in the optic nerve and finally reduced nerve fibers and RGCs. This recent evidence focusing on the mechanism of optic neuritis in NMO and other studies may help to develop new strategies for NMO treatment. Biological and small-molecule therapeutics have been developed that block AQP4-specific IgG [49, 50]. Additionally, IgG-selective enzymatic deglycosylation neutralizes AQP4-specific IgG pathogenicity and produces a therapeutic blocking antibody [51]. We believe that such translational studies will improve visual performance in optic neuritis in NMO patients.

The new findings in clinical research using imaging systems have been demonstrated in the NMO retina. Optical coherence tomography (OCT) can evaluate the thicknesses of the retinal layers. Recent developments in OCT technology have revealed an increased thickness of the inner nuclear layer (INL) in patients with NMO when compared with those in MS patients [52]. The cytoplasm of a Müller cell with abundant AQP4 expression is located at the INL. Additionally, microcystic macular edema in the INL could be observed in NMO patients [53, 54], and this occurs significantly more frequently in NMO patients than in MS patients [55]. AQP4 channels are the conduit for facilitating fluid homeostasis in the inner retina [25]. The deletion of AQP4 has also been implicated as a cause of swollen Müller cells in mice [56]. In one study, a hypo-osmolar solution induced rapid swelling and sodium influx into the Müller cells in AQP4 null mice but not in the wild-type mice, suggesting that water flux through AQP4 is involved in rapid Müller cell volume regulation in response to osmotic stress and that deletion of AQP4 results in swelling of the glial cells of the retinal tissue [56]. Further studies using imaging technology may help understand the structural dysfunction associated with AQP4 damage in the retina with NMO.

12.3.2 Glaucoma

Glaucoma is a progressive optic neuropathy that affects the optic nerve head and RGCs. It is thought that the first trigger of glaucoma is axonal damage at the optic nerve head. The most important risk factor for glaucoma is an elevated intraocular pressure (IOP). There are several lines of evidence demonstrating that an elevated IOP causes a downregulation of AQP9 expression. In the optic nerve, AQP9 is expressed on astrocytes in the optic nerve head, as mentioned above. Both a rodent

model and a monkey model of chronic high IOP have revealed reduced AQP9 expression at the optic nerve head (Fig. 12.1) [33, 36]. This was consistent with the findings in human donor eyes with glaucoma [36]. AQP4 is expressed in astrocytes at the retrobulbar optic nerve with myelinated axons. The change in AQP4 expression is controversial in different animal models of glaucoma. Two reports have demonstrated essentially unchanged AQP4 expression in the optic nerves of glaucomatous human and monkey eyes and in rats with chronic IOP elevation [33, 36]. However, AQP4 expression in the optic nerve was increased in a mouse ocular hypertension model [57].

In the retina with an elevated IOP, the reduced AQP9 expression in the RGCs was observed in the rat glaucoma model [33]. In the mouse retina with ocular hypertension, AQP4 expression was reduced [57]. Importantly, retinal function and cell survival were improved in the AQP4-deficient mice with an elevated IOP [58]. This evidence suggests that AQP4 and AQP9 are involved in the pathophysiology of glaucoma.

12.3.3 Optic Nerve Injury

The optic nerve injury model induced by crush or transection is considered an axonal injury model that occurs in traumatic optic neuropathy and has been used as an acute glaucoma model. In the rat model of optic nerve crush, reduced AQP4 and Kir4.1 expression was found in the retina [59]. This suggests the impairment of ion homeostasis and K^+ spatial buffering. Additionally, AQP9 expression in the RGCs was reduced in the rat retina with optic nerve transection [34]. Importantly, AQP9 in the RGCs was decreased before cell death in this model (Fig. 12.2) [34]. In addition, the downregulation of AQP9 by RNA interference significantly increased apoptosis, reactive oxygen species (ROS) accumulation, and the NAD/NADH ratio in the RGC-5 cells. These findings suggest that AQP9 loss adversely affects the survival of RGCs, at least partly because of decreased transport of lactate as a substrate for energy and/or a ROS scavenger [34]. This series of experiments suggested to us the importance of AQP9 in neuronal function. Glucose is converted into lactate in astrocytes, and the lactate is transferred to neurons through a monocarboxylate transporter (MCT) as a fuel for neurons [60]. In both astrocytes and neurons, AQP9 at the plasma membrane may facilitate the diffusion of lactate in conjunction with MCTs [28, 60]. In the retina, a part of the lactate produced in the astrocytes is taken into Müller cells and then transferred to neural cells (Fig. 12.3) [61]. The observed reduction in AQP9 levels before the RGC loss after optic nerve transection has implications in the potential role of AQP9 as a lactate transporter in the retina and for the significance of the astrocyte-to-neuron lactate shuttle in maintaining the physiological functions of RGCs and their axons. Lactate plays another critical role in enabling neural tissue to respond adequately to

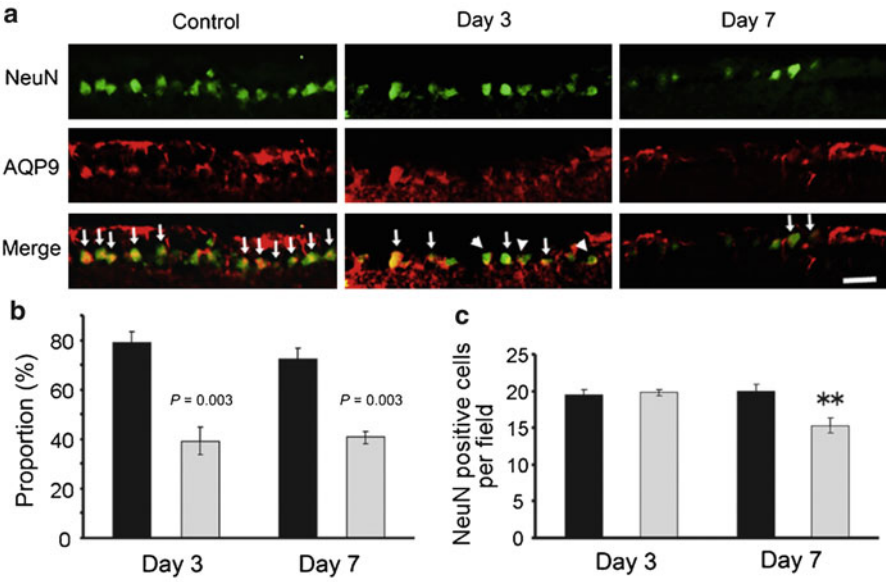


Fig. 12.2 Reduction of somatic AQP9 immunoreactivity in ganglion cell layer (GCL) after optic nerve transection. (**a**) Immunolabeling for NeuN (*green*) and AQP9 (*red*) in the GCL and the retinal nerve fiber layer of control eyes and of eyes at days 3 and 7 after the transection. The *upper side* of the retinal nerve fiber layer faces the vitreous cavity, and the *lower side* faces the inner plexiform layer. NeuN-immunopositive cells exhibit AQP9 expression in the centers of their somata (*arrows*) or only at the edges of their somata (*arrowheads*). (**b** and **c**) The proportion of cells immunoreactive for both AQP9 and NeuN at the center of the somata relative to the total of NeuN-immunoreactive cells in the ganglion cell layer (**b**) and the mean number of NeuN-immunoreactive cells in the same field (**c**) in the sham-operated controls (*black bars*) and eyes with optic nerve transection (*gray bars*). The reduction of AQP9 immunoreactivity in the somata of cells in the ganglion cell layer preceded the reduction in cell death

glutamate excitation and to recover after neuronal excitation [62]. The NADH generated during the lactate-to-pyruvate conversion in the mitochondria can be used as an endogenous scavenger of ROS to protect the neurons from ROS-induced damage [62, 63]. AQP9 is known to be located in the mitochondria, where it acts as a monocarboxylate channel and presumably works with the MCT to transport lactate [6, 28, 64, 65]. The downregulation of AQP9 expression therefore likely impairs the transfer of the lactate generated in the cytoplasm into the mitochondrial membrane. The impaired lactate transfer between these organelles may not only reduce energy production but may also reduce the cellular content of the ROS scavenger NADH, resulting in the increased death of neural cells. These lines of evidence might enable us to develop new therapeutic strategies involving AQPs for neuroprotection in the RGCs.

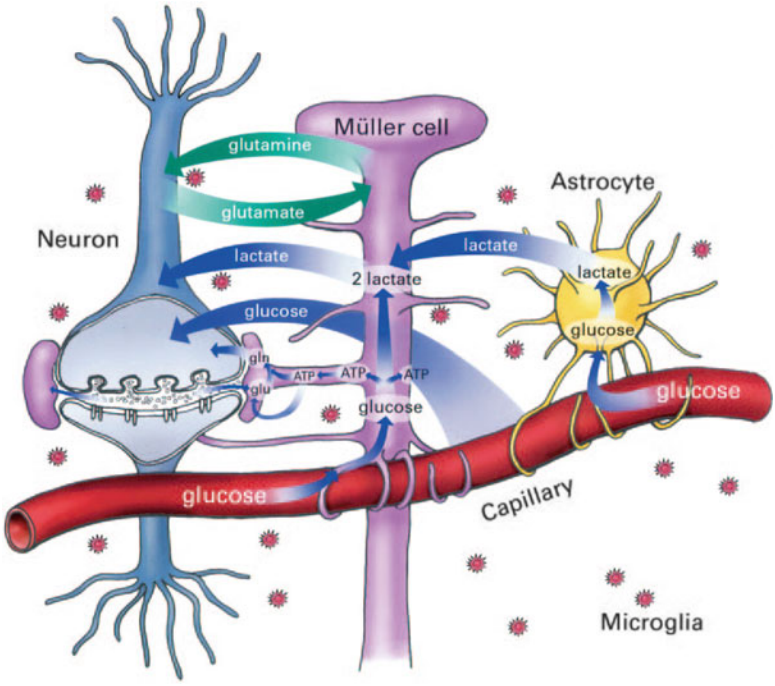


Fig. 12.3 A schema for the astrocyte-to-neuron lactate shuttle hypothesis in the retina. Glucose is directly transported from the capillary to neurons. Glucose is not oxidized in astrocytes and Müller cells; instead, it is converted to lactate. This lactate is also transported from these glial cells to neurons. Glutamate and glutamine are interconverted between the glia and neurons. This cycle requires ATP, which is produced during the conversion of glucose to lactate

12.4 Conclusions

Research in the field of AQPs has advanced remarkably in the past decade. However, there remain many questions about the exact functions and regulation of the AQP families in the nervous system. Regrettably, AQP-selective inhibitors are not available at the present time. This makes the expansion of AQP research difficult. However, it is now clear that the AQP families are expressed in many ocular tissues and play important roles in neural and glial activity. The most striking finding in AQP research has been that the AQP4 protein in the astrocytes of the optic nerve is the first target of optic neuritis in NMO. These scientific advances have furthered our understanding of NMO and are providing new treatments for this disease. Additionally, AQP9 protein is involved in RGC survival in glaucoma and axonal injury. We hope that AQP9-targeted therapy could be a new neuroprotective treatment in the future.

References

1. Tait MJ, Saadoun S, Bell BA, Papadopoulos MC (2008) Water movements in the brain: role of aquaporins. Trends Neurosci 31(1):37–43. doi:10.1016/j.tins.2007.11.003
2. Gonen T, Walz T (2006) The structure of aquaporins. Q Rev Biophys 39(4):361–396. doi:10.1017/S0033583506004458
3. Badaut J, Brunet JF, Regli L (2007) Aquaporins in the brain: from aqueduct to "multi-duct". Metab Brain Dis 22(3–4):251–263. doi:10.1007/s11011-007-9057-2
4. Nozaki K, Ishii D, Ishibashi K (2008) Intracellular aquaporins: clues for intracellular water transport? Pflugers Arch 456(4):701–707. doi:10.1007/s00424-007-0373-5
5. Oshio K, Watanabe H, Song Y, Verkman AS, Manley GT (2005) Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1. FASEB J 19(1):76–78. doi:10.1096/fj.04-1711fje
6. Amiry-Moghaddam M, Ottersen OP (2003) The molecular basis of water transport in the brain. Nat Rev Neurosci 4(12):991–1001. doi:10.1038/nrn1252
7. Suzuki R, Okuda M, Asai J, Nagashima G, Itokawa H, Matsunaga A, Fujimoto T, Suzuki T (2006) Astrocytes co-express aquaporin-1, -4, and vascular endothelial growth factor in brain edema tissue associated with brain contusion. Acta Neurochir Suppl 96:398–401
8. Satoh J, Tabunoki H, Yamamura T, Arima K, Konno H (2007) Human astrocytes express aquaporin-1 and aquaporin-4 in vitro and in vivo. Neuropathology 27(3):245–256
9. Nesic O, Lee J, Unabia GC, Johnson K, Ye Z, Vergara L, Hulsebosch CE, Perez-Polo JR (2008) Aquaporin 1 - a novel player in spinal cord injury. J Neurochem 105(3):628–640. doi:10.1111/j.1471-4159.2007.05177.x
10. Fischbarg J (2003) On the mechanism of fluid transport across corneal endothelium and epithelia in general. J Exp Zool A Comp Exp Biol 300(1):30–40. doi:10.1002/jez.a.10306
11. Hasegawa H, Lian SC, Finkbeiner WE, Verkman AS (1994) Extrarenal tissue distribution of CHIP28 water channels by in situ hybridization and antibody staining. Am J Physiol 266(4 Pt 1):C893–C903
12. Yamaguchi Y, Watanabe T, Hirakata A, Hida T (2006) Localization and ontogeny of aquaporin-1 and -4 expression in iris and ciliary epithelial cells in rats. Cell Tissue Res 325 (1):101–109. doi:10.1007/s00441-005-0122-z
13. Hamann S, Zeuthen T, La Cour M, Nagelhus EA, Ottersen OP, Agre P, Nielsen S (1998) Aquaporins in complex tissues: distribution of aquaporins 1-5 in human and rat eye. Am J Physiol 274(5 Pt 1):C1332–C1345
14. Goel M, Picciani RG, Lee RK, Bhattacharya SK (2010) Aqueous humor dynamics: a review. Open Ophthalmol J 4:52–59. doi:10.2174/1874364101004010052
15. Stamer WD, Peppel K, O'Donnell ME, Roberts BC, Wu F, Epstein DL (2001) Expression of aquaporin-1 in human trabecular meshwork cells: role in resting cell volume. Invest Ophthalmol Vis Sci 42(8):1803–1811
16. Kang TH, Choi YK, Kim IB, Oh SJ, Chun MH (2005) Identification and characterization of an aquaporin 1 immunoreactive amacrine-type cell of the mouse retina. J Comp Neurol 488 (3):352–367. doi:10.1002/cne.20589
17. Iandiev I, Pannicke T, Reichel MB, Wiedemann P, Reichenbach A, Bringmann A (2005) Expression of aquaporin-1 immunoreactivity by photoreceptor cells in the mouse retina. Neurosci Lett 388(2):96–99. doi:10.1016/j.neulet.2005.06.046
18. Papadopoulos MC, Manley GT, Krishna S, Verkman AS (2004) Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J 18(11):1291–1293. doi:10.1096/fj.04-1723fje
19. Oshio K, Binder DK, Yang B, Schecter S, Verkman AS, Manley GT (2004) Expression of aquaporin water channels in mouse spinal cord. Neuroscience 127(3):685–693. doi:10.1016/j.neuroscience.2004.03.016

20. Nagelhus EA, Veruki ML, Torp R, Haug FM, Laake JH, Nielsen S, Agre P, Ottersen OP (1998) Aquaporin-4 water channel protein in the rat retina and optic nerve: polarized expression in Muller cells and fibrous astrocytes. J Neurosci 18(7):2506–2519
21. Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S (1998) Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. Proc Natl Acad Sci U S A 95(20):11981–11986
22. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 17(1):171–180
23. Nagelhus EA, Horio Y, Inanobe A, Fujita A, Haug FM, Nielsen S, Kurachi Y, Ottersen OP (1999) Immunogold evidence suggests that coupling of K^+ siphoning and water transport in rat retinal Muller cells is mediated by a coenrichment of Kir4.1 and AQP4 in specific membrane domains. Glia 26(1):47–54
24. Tenckhoff S, Hollborn M, Kohen L, Wolf S, Wiedemann P, Bringmann A (2005) Diversity of aquaporin mRNA expressed by rat and human retinas. Neuroreport 16(1):53–56
25. Goodyear MJ, Crewther SG, Junghans BM (2009) A role for aquaporin-4 in fluid regulation in the inner retina. Vis Neurosci 26(2):159–165. doi:10.1017/S0952523809090038
26. Hollborn M, Dukic-Stefanovic S, Pannicke T, Ulbricht E, Reichenbach A, Wiedemann P, Bringmann A, Kohen L (2011) Expression of aquaporins in the retina of diabetic rats. Curr Eye Res 36(9):850–856. doi:10.3109/02713683.2011.593108
27. Iandiev I, Dukic-Stefanovic S, Hollborn M, Pannicke T, Hartig W, Wiedemann P, Reichenbach A, Bringmann A, Kohen L (2011) Immunolocalization of aquaporin-6 in the rat retina. Neurosci Lett 490(2):130–134. doi:10.1016/j.neulet.2010.12.042
28. Badaut J, Petit JM, Brunet JF, Magistretti PJ, Charriaut-Marlangue C, Regli L (2004) Distribution of Aquaporin 9 in the adult rat brain: preferential expression in catecholaminergic neurons and in glial cells. Neuroscience 128(1):27–38. doi:10.1016/j.neuroscience.2004.05.042
29. Tsukaguchi H, Shayakul C, Berger UV, Mackenzie B, Devidas S, Guggino WB, van Hoek AN, Hediger MA (1998) Molecular characterization of a broad selectivity neutral solute channel. J Biol Chem 273(38):24737–24743
30. Badaut J, Hirt L, Granziera C, Bogousslavsky J, Magistretti PJ, Regli L (2001) Astrocyte-specific expression of aquaporin-9 in mouse brain is increased after transient focal cerebral ischemia. J Cereb Blood Flow Metab 21(5):477–482. doi:10.1097/00004647-200105000-00001
31. Elkjaer M, Vajda Z, Nejsum LN, Kwon T, Jensen UB, Amiry-Moghaddam M, Frokiaer J, Nielsen S (2000) Immunolocalization of AQP9 in liver, epididymis, testis, spleen, and brain. Biochem Biophys Res Commun 276(3):1118–1128. doi:10.1006/bbrc.2000.3505
32. Dibas A, Yang MH, Bobich J, Yorio T (2007) Stress-induced changes in neuronal Aquaporin-9 (AQP9) in a retinal ganglion cell-line. Pharmacol Res 55(5):378–384. doi:10.1016/j.phrs.2007.01.021
33. Naka M, Kanamori A, Negi A, Nakamura M (2010) Reduced expression of aquaporin-9 in rat optic nerve head and retina following elevated intraocular pressure. Invest Ophthalmol Vis Sci 51(9):4618–4626. doi:10.1167/iovs.09-4712
34. Miki A, Kanamori A, Negi A, Naka M, Nakamura M (2013) Loss of aquaporin 9 expression adversely affects the survival of retinal ganglion cells. Am J Pathol 182(5):1727–1739. doi:10.1016/j.ajpath.2013.01.027
35. Tran TL, Bek T, Holm L, la Cour M, Nielsen S, Prause JU, Rojek A, Hamann S, Heegaard S (2013) Aquaporins 6–12 in the human eye. Acta Ophthalmol 91(6):557–563. doi:10.1111/j.1755-3768.2012.02547.x
36. Mizokami J, Kanamori A, Negi A, Nakamura M (2011) A preliminary study of reduced expression of aquaporin-9 in the optic nerve of primate and human eyes with glaucoma. Curr Eye Res 36(11):1064–1067. doi:10.3109/02713683.2011.611610

37. Jarius S, Wildemann B (2010) AQP4 antibodies in neuromyelitis optica: diagnostic and pathogenetic relevance. Nat Rev Neurol 6(7):383–392. doi:10.1038/nrneurol.2010.72
38. Kitley J, Leite MI, Nakashima I, Waters P, McNeillis B, Brown R, Takai Y, Takahashi T, Misu T, Elsone L, Woodhall M, George J, Boggild M, Vincent A, Jacob A, Fujihara K, Palace J (2012) Prognostic factors and disease course in aquaporin-4 antibody-positive patients with neuromyelitis optica spectrum disorder from the United Kingdom and Japan. Brain 135 (Pt 6):1834–1849. doi:10.1093/brain/aws109
39. Lucchinetti CF, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM, Trebst C, Weinshenker B, Wingerchuk D, Parisi JE, Lassmann H (2002) A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 125(Pt 7):1450–1461
40. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG (2006) Revised diagnostic criteria for neuromyelitis optica. Neurology 66(10):1485–1489. doi:10.1212/01.wnl.0000216139.44259.74
41. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, Nakashima I, Weinshenker BG (2004) A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 364(9451):2106–2112. doi:10.1016/S0140-6736(04)17551-X
42. Misu T, Fujihara K, Nakamura M, Murakami K, Endo M, Konno H, Itoyama Y (2006) Loss of aquaporin-4 in active perivascular lesions in neuromyelitis optica: a case report. Tohoku J Exp Med 209(3):269–275
43. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 202(4):473–477. doi:10.1084/jem.20050304
44. Roemer SF, Parisi JE, Lennon VA, Benarroch EE, Lassmann H, Bruck W, Mandler RN, Weinshenker BG, Pittock SJ, Wingerchuk DM, Lucchinetti CF (2007) Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. Brain 130(Pt 5):1194–1205. doi:10.1093/brain/awl371
45. Saadoun S, Waters P, Bell BA, Vincent A, Verkman AS, Papadopoulos MC (2010) Intracerebral injection of neuromyelitis optica immunoglobulin G and human complement produces neuromyelitis optica lesions in mice. Brain 133(Pt 2):349–361. doi:10.1093/brain/awp309
46. Zhang H, Bennett JL, Verkman AS (2011) Ex vivo spinal cord slice model of neuromyelitis optica reveals novel immunopathogenic mechanisms. Ann Neurol 70(6):943–954. doi:10.1002/ana.22551
47. Marignier R, Nicolle A, Watrin C, Touret M, Cavagna S, Varrin-Doyer M, Cavillon G, Rogemond V, Confavreux C, Honnorat J, Giraudon P (2010) Oligodendrocytes are damaged by neuromyelitis optica immunoglobulin G via astrocyte injury. Brain 133(9):2578–2591. doi:10.1093/brain/awq177
48. Matsumoto Y, Kanamori A, Nakamura M, Takahashi T, Nakashima I, Negi A (2014) Sera from patients with seropositive neuromyelitis optica spectral disorders caused the degeneration of rodent optic nerve. Exp Eye Res 119:61–69. doi:10.1016/j.exer.2013.12.010
49. Tradtrantip L, Zhang H, Anderson MO, Saadoun S, Phuan PW, Papadopoulos MC, Bennett JL, Verkman AS (2012) Small-molecule inhibitors of NMO-IgG binding to aquaporin-4 reduce astrocyte cytotoxicity in neuromyelitis optica. FASEB J 26(5):2197–2208. doi:10.1096/fj.11-201608
50. Tradtrantip L, Zhang H, Saadoun S, Phuan PW, Lam C, Papadopoulos MC, Bennett JL, Verkman AS (2012) Anti-aquaporin-4 monoclonal antibody blocker therapy for neuromyelitis optica. Ann Neurol 71(3):314–322. doi:10.1002/ana.22657
51. Tradtrantip L, Ratelade J, Zhang H, Verkman AS (2013) Enzymatic deglycosylation converts pathogenic neuromyelitis optica anti-aquaporin-4 immunoglobulin G into therapeutic antibody. Ann Neurol 73(1):77–85. doi:10.1002/ana.23741
52. Fernandes DB, Raza AS, Nogueira RG, Wang D, Callegaro D, Hood DC, Monteiro ML (2013) Evaluation of inner retinal layers in patients with multiple sclerosis or neuromyelitis optica

using optical coherence tomography. Ophthalmology 120(2):387–394. doi:10.1016/j.ophtha.2012.07.066
53. Sotirchos ES, Saidha S, Byraiah G, Mealy MA, Ibrahim MA, Sepah YJ, Newsome SD, Ratchford JN, Frohman EM, Balcer LJ, Crainiceanu CM, Nguyen QD, Levy M, Calabresi PA (2013) In vivo identification of morphologic retinal abnormalities in neuromyelitis optica. Neurology 80(15):1406–1414. doi:10.1212/WNL.0b013e31828c2f7a
54. Gelfand JM, Cree BA, Nolan R, Arnow S, Green AJ (2013) Microcystic inner nuclear layer abnormalities and neuromyelitis optica. JAMA Neurol 70(5):629–633. doi:10.1001/jamaneurol.2013.1832
55. Kaufhold F, Zimmermann H, Schneider E, Ruprecht K, Paul F, Oberwahrenbrock T, Brandt AU (2013) Optic neuritis is associated with inner nuclear layer thickening and microcystic macular edema independently of multiple sclerosis. PLoS One 8(8):e71145. doi:10.1371/journal.pone.0071145
56. Pannicke T, Wurm A, Iandiev I, Hollborn M, Linnertz R, Binder DK, Kohen L, Wiedemann P, Steinhauser C, Reichenbach A, Bringmann A (2010) Deletion of aquaporin-4 renders retinal glial cells more susceptible to osmotic stress. J Neurosci Res 88(13):2877–2888. doi:10.1002/jnr.22437
57. Dibas A, Yang MH, He S, Bobich J, Yorio T (2008) Changes in ocular aquaporin-4 (AQP4) expression following retinal injury. Mol Vis 14:1770–1783
58. Da T, Verkman AS (2004) Aquaporin-4 gene disruption in mice protects against impaired retinal function and cell death after ischemia. Invest Ophthalmol Vis Sci 45(12):4477–4483. doi:10.1167/iovs.04-0940
59. Dibas A, Oku H, Fukuhara M, Kurimoto T, Ikeda T, Patil RV, Sharif NA, Yorio T (2010) Changes in ocular aquaporin expression following optic nerve crush. Mol Vis 16:330–340
60. van Hall G, Stromstad M, Rasmussen P, Jans O, Zaar M, Gam C, Quistorff B, Secher NH, Nielsen HB (2009) Blood lactate is an important energy source for the human brain. J Cereb Blood Flow Metab 29(6):1121–1129. doi:10.1038/jcbfm.2009.35
61. Antonetti DA, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, Kester M, Kimball SR, Krady JK, LaNoue KF, Norbury CC, Quinn PG, Sandirasegarane L, Simpson IA, Group JDRC (2006) Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. Diabetes 55(9):2401–2411. doi:10.2337/db05-1635
62. Schurr A, Gozal E (2011) Aerobic production and utilization of lactate satisfy increased energy demands upon neuronal activation in hippocampal slices and provide neuroprotection against oxidative stress. Front Pharmacol 2:96. doi:10.3389/fphar.2011.00096
63. Schurr A, Payne RS (2007) Lactate, not pyruvate, is neuronal aerobic glycolysis end product: an in vitro electrophysiological study. Neuroscience 147(3):613–619. doi:10.1016/j.neuroscience.2007.05.002
64. Mylonakou MN, Petersen PH, Rinvik E, Rojek A, Valdimarsdottir E, Zelenin S, Zeuthen T, Nielsen S, Ottersen OP, Amiry-Moghaddam M (2009) Analysis of mice with targeted deletion of AQP9 gene provides conclusive evidence for expression of AQP9 in neurons. J Neurosci Res 87(6):1310–1322. doi:10.1002/jnr.21952
65. Amiry-Moghaddam M, Lindland H, Zelenin S, Roberg BA, Gundersen BB, Petersen P, Rinvik E, Torgner IA, Ottersen OP (2005) Brain mitochondria contain aquaporin water channels: evidence for the expression of a short AQP9 isoform in the inner mitochondrial membrane. FASEB J 19(11):1459–1467. doi:10.1096/fj.04-3515com

Chapter 13
Microglia

Yuki Fujita and Toshihide Yamashita

Abstract Microglia are known as the resident immune cells in the central nervous system (CNS), including the retina. Under normal physiological conditions, they are involved in the surveillance of the CNS and are required for the construction of neural circuitry during development. Accumulating evidences have shown that microglia change to a reactive phenotype under pathological conditions. Microglial activation is associated with various CNS diseases such as glaucoma. When microglia are activated, they form an amoeboid shape and release inflammatory cytokines. Excessive microglial activation causes retinal tissue damage and contributes to apoptosis. In contrast, microglia act as phagocytes that remove apoptotic neurons, pathogens, and cellular debris. This may proceed to protect retinal neurons. Thus, microglia show dual functions in neurodegeneration. Although it is under debate whether microglia are good or bad for CNS diseases, both effects appear to occur in glaucoma. This chapter reviews the roles of microglia in the retina, with a particular focus on their protective and destructive roles in glaucoma.

Keywords Amoeboid • Glaucoma • Microglia • Ramified • Retina

13.1 Introduction

Glaucoma is a neurodegenerative disease affecting the entire visual pathway and is characterized by progressive, irreversible loss of retinal ganglion cells (RGC) and optic nerve axons. These neurodegenerative processes sometimes cause the loss of visual field sensitivity. It is well established that retinal glial cells react to neuronal injury in glaucoma, suggesting the involvement of glial cells in cellular fate

Y. Fujita • T. Yamashita (✉)
Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
e-mail: yamashita@molneu.med.osaka-u.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_13,

decisions in RGC. Among the three glial cell types localized in the retina, including microglia, astrocytes, and Müller cells, microglia are involved in immune defense in the central nervous system (CNS). This chapter focuses on the role of microglia in the retinal degeneration process. Microglia show the morphological and functional changes at the injury site. These microglial "reactive" states promote glaucomatous optic nerve degeneration, whereas they also contribute to protect neurons from cell death after injury. Thus, microglia seem to have bidirectional effects on glaucoma. Recent studies have provided the underlying mechanisms of these diverse roles of microglia. Here, we discuss the current knowledge concerning the neuroprotective or neurodestructive roles of microglia in glaucoma.

13.2 Origin and Distribution of Microglia in the Retina

Based on Cajal's silver carbonate staining, del Rio-Hortega first demonstrated the concept of a mesodermal origin of microglia [1, 2]. Since then, there has been much debate regarding the origin of microglia [3, 4]. There have been three main theories as to this: (1) mesodermal/mesenchymal tissues [5, 6], (2) neuroectoderm (similar to neurons, astrocytes, and oligodendrocytes) [7, 8], and (3) circulating blood monocytes [9]. Recent studies show that microglia are derived from myeloid progenitors in the mouse embryonic yolk sac at embryonic day (E) 7.5 [10–13]. Then, the cells enter the brain at E9.5 [4, 10–15].

It has been shown that microglia are already present in E11.5 mouse retinas, primarily in their central site [16]. The amount of microglia increases at E12.5 and continues to increase until the end of embryonic development. At E12.5, microglia show a macrophage-like rounded shape and they are also observed in the peripheral retina. These amoeboid-shaped microglia frequently contain cell debris, thereby suggesting a phagocytic function. A previous study using an antibody against the macrophage-specific surface glycoprotein F4/80 showed that amoeboid-shaped microglia are colocalized with pyknotic nuclei of dying cells [17]. It has been established that the overproduction of neurons and selective programmed cell death occurs during retinal development [18, 19]. These observations indicate that amoeboid-shaped microglia remove cellular debris from apoptotic neurons in the developing retina. At the end of embryonic development, microglia localize in the nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), and neuroblastic layer (NbL). The density of microglia in the retina is reduced at birth, whereas it increases again during the first postnatal week. The distribution of microglia in the mouse retina suggests that microglia infiltrate the retina through the ciliary margin and vitreous body [16]. Microglial infiltration across the ciliary margin has also been observed in the retinas of other species such as human and avian [20–22].

It is under debate whether resident microglia in the CNS including the retina originate from in situ proliferation and/or the recruitment of circulating blood monocytes [4, 23–26]. Enhanced green fluorescent protein (EGFP)-transgenic

mice and microglia-specific GFP-expressing mice ($Cx3cr1^{+/GFP}$ mice) were used to address this controversy in the retina [27–29]. EGFP-expressing bone marrow cells were transplanted into lethally irradiated mice and the fate of the donor-derived cells in the retinal flat mounts was evaluated at various time points. EGFP-expressing bone marrow cells were transplanted into lethally irradiated mice and evaluated concerning the fate of the donor-derived cells in the retinal flat mounts at various time points. After 8 weeks, the EGFP-expressing bone marrow-derived cells infiltrated the retina, and after 6 months they had replaced the microglia in the retina [27]. In this study, the authors mentioned that the irradiation did not cause retinal damage that might affect cell recruitment. Further studies to confirm the data on microglial precursor recruitment have also been reported [28, 29]. These studies using $Cx3cr1^{+/GFP}$ mice as bone marrow donors enabled the clear visualization of newly recruited monocyte-derived cells. The earliest recruitment of GFP-expressing donor cells into the host retina occurred at 4 weeks [28].

In contrast, other researchers suggest that only a small amount of bone marrow-derived cells exist in the uninjured normal retina [30]. In addition, parabiosis experiments demonstrated that the CNS microglia are not replenished by bone marrow-derived progenitors. Since parabiosis studies do not require irradiation or transplantation, experimental confounds such as perturbation of the blood-retinal barrier can be excluded [31, 32]. The replacement of microglia by circulating microglial precursors did not occur under physiological conditions, suggesting that there is a specific origin of microglia.

13.3 The Function of Microglia in the Healthy Retina

At least two different subtypes of microglia exist in the adult CNS including the ramified "resting" microglia and the amoeboid-shaped microglia. In the adult retina, the dominant subtype is the ramified microglia, which are found in the inner and outer plexiform layers. The ramified microglia serve several functions in immune surveillance [33, 34]. Although ramified microglia are called "resting" microglia, recent studies using transgenic mice demonstrate they are not dormant [35, 36]. Studies based on two-photon microscopy in $Cx3cr1^{+/GFP}$ mice revealed that ramified microglia have highly mobile processes and arborizations. These motile processes connect microglia to the retinal environment. In addition, microglia can monitor the retinal microenvironment via their surface proteins including receptors for cytokines, chemokines, antibodies, complement, and adhesion molecules [37]. They play roles in immune recognition and immune defense as the only cell type of immune cells in the CNS. Furthermore, ramified microglia not only exhibit immune surveillance functions, but also seem to be involved in the control of neuronal activity and the maintenance of neuronal homeostasis [35].

It has been shown that fractalkine (CX3CL1)–CX3CR1 signaling and the P2Y12 purinergic receptor are important for microglial functions in the healthy CNS [38]. CX3CR1 is expressed in monocytes, dendritic cells, and subsets of T cells

and natural killer cells in the circulation and by microglia in the CNS. Fractalkine is synthesized as a transmembrane glycoprotein and can be proteolytically released from healthy neurons [39, 40]. Binding of fractalkine to CX3CR1 on microglia seems to regulate microglial homeostasis. *Cx3cr1*$^{-/-}$ mice show increased microglial activation and enhanced neuronal damage [41]. In addition, fractalkine (CX3CL1)–CX3CR1 signaling seems to be required for synaptic remodeling. Synaptic remodeling is important for refining the neuronal circuit. In a common process of the neural development throughout vertebrate CNS, axons initially form exuberant synaptic connections, and then they are pruned [42]. In vivo imaging by two-photon microscopy revealed that microglial processes make contacts with synapses in a neuronal activity-dependent manner [43]. Further, microglial processes localize around dendritic spines in the juvenile visual cortex, and the spines were typically disappeared over 2 days. The deprivation of visual experience induces microglia to become less motile and localize near dendritic spines [44]. These observations suggest that microglia regulate neural circuit formation by eliminating excess synapses.

Adenosine triphosphate (ATP)–P2X4/P2Y12 signaling is also important for microglial functions. The P2Y12 purinergic receptors transduce their signals by coupling to Gi-adenylyl cyclase pathways [45, 46] and are highly expressed on resting microglia in the brain, but not on other tissue macrophages [47]. Microglia derived from *P2Y12*–/– mice demonstrated normal morphology and distribution, whereas they could not polarize, migrate, or extend their processes upon stimulation with adenosine diphosphate (ADP) or ATP. Further, the P2Y12 receptor is robustly expressed in "resting" microglia, but this expression is reduced after microglial activation. Although most of these functions were observed in the brain, similar mechanisms seem to occur in retinal microglia, since P2Y receptor subtypes have been detected in the retina [48, 49].

13.4 Activation of Microglia in Retinal Degeneration

Early microglial activation in the retina is a common response to progressive neurodegeneration [50]. When microglia react to injury, their processes immediately become shorter and they change morphologically from a ramified to an amoeboid-shaped macrophage-like phenotype. Several surface markers such as F4/80, complement receptor 3 (CD11b/CD18, OX42), MHC-II (OX6), CD68, and *Griffonia simplicifolia* isolectin B4 have been used to detect the activation of microglia by immunofluorescence staining [51, 52]. After reactivation, microglia exhibit strongly enhanced proliferation, motility, phagocytosis, and production of a variety of bioactive molecules. Reactive microglia migrate to the injury site where they interact with damaged cells [53]. Then, amoeboid phagocytic microglia clean the cellular debris [54, 55]. Thus, the reactive microglia play important roles in maintaining a stable environment in the CNS [56]. However, they may also

exacerbate neuronal diseases, such as age-related macular degeneration or glaucoma [57–60].

It has been shown that many molecules can trigger the conversion of microglia from the resting to reactivated phenotype [34]. In vitro culture models with primary cells and immortalized cell lines such as BV-2, HMO6, and CHME were used to identify the early signals in microglial activation [61]. It is well established that LPS induce the activation of microglia. Further, many molecules have been shown to stimulate or enhance microglial activation [62].

13.5 The Role of Microglia in Glaucoma

Since microglia clear cellular debris from the CNS, this minimizes neuronal exposure to pathogens and the proinflammatory response. In glaucoma, microglia also remove debris from degenerating RGC, thereby protecting retinal cells. However, microglia can produce neurotoxic molecules including proinflammatory cytokines, which may mediate neuronal damage in glaucoma [63–65]. Further, microglia are also involved in initiating immune processes through antigen presentation and mediate neurodegeneration in the CNS [66–68]. The following sections summarize both the neuroprotective and neurodestructive functions of microglia in glaucomatous optic nerve degeneration (Fig. 13.1).

13.5.1 Neurodestructive Role of Microglia in Glaucoma

In the normal physiological state, microglia recognize and defend against infections and facilitate regenerative processes. In contrast, microglia are abnormally activated in neurodegeneration, and evidence supporting the neurotoxicity of reactive microglia is well established [69, 70]. In their reactive state, microglia express and release various inflammatory proteins such as tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS), or interleukin-1 (IL-1), which lead to the production of nitric oxide and reactive oxygen species [33, 71]. Excessive amounts of these compounds can damage the surrounding CNS parenchyma. Microarray analysis revealed the retinal gene expression profile in a model of glaucoma [72, 73]. It has been reported that similar microglia-derived inflammatory proteins are increased in glaucoma. Several studies support the hypothesis that inhibition of reactive microglia provides a neuroprotective effect against glaucoma. Early reduction of microglial activation by irradiation was associated with reduced neurodegeneration in the DBA/2J mouse model of glaucoma [74]. Other studies demonstrated that inhibition of reactive microglia by anti-inflammatory drugs such as minocycline also promote the survival of RGC in the DBA/2J model or N-methyl-D-aspartate (NMDA)-induced excitotoxic damage [75, 76].

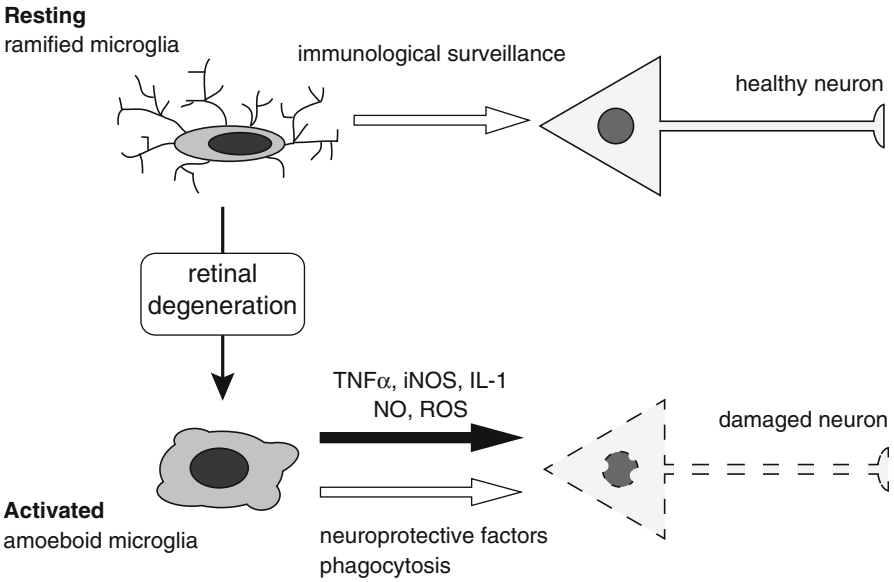


Fig. 13.1 The association between the activity state of microglia and neuronal damage. Ramified "resting" microglia are involved in immunological surveillance of the CNS and maintenance of CNS homeostasis. Microglia transform their shape to amoeboid type during CNS injury. These reactive microglia have both neuroprotective and destructive roles in damaged neurons

Inhibition of the signaling initiated by microglial activation may also lead to neuroprotection. Among the various microglia-derived inflammatory proteins, TNF-α is specially focused in glaucoma. TNF-α is a proinflammatory cytokine and is released by microglia in response to infection or injury. In addition, it is engaged in various neurodegenerative diseases, including glaucoma [77, 78]. TNF-α has been shown to be involved in RGC death during the pathogenic process of glaucoma [79, 80]. Although TNF-α stimulated several survival signals, it could also mediate apoptotic signaling in RGC [81]. In the CNS, TNF-α is predominantly expressed by reactive microglia and astrocytes. TNF-α and its receptors TNFR1a and TNFR1b are increased in the retina of glaucoma patients [79, 82, 83]. In these patients, increased expression of TNF receptors was predominantly observed in the optic nerve heads. In the damaged optic nerve heads, RGC axons express TNFR1 [83]. In the rat glaucoma animal model of chronic ocular hypertension, the number of reactive microglia was increased, accompanied by elevated TNF-α [84]. This study also implies that microglia are the major source of TNF-α expression after ocular hypertension [84].

A truncated tropomyosin receptor kinase (Trk) neurotrophin receptor isoform, TrkC.T1, lacking the kinase domain, is involved in glaucomatous RGC death through TNF-α production [85]. TrkC.T1 expression was upregulated in the glaucomatous retina. Deletion of TrkC.T1 reduced RGC death and the production

of retinal TNF-α in the experimental glaucoma model. These results suggest that glaucoma-induced expression of TrkC.T1 and the production of TNF-α result in RGC death.

Several polymorphisms in the promoter region of TNF-α have been identified [86]. It has been reported that the occurrence of single-nucleotide polymorphisms of the *TNF-α* gene seems significantly higher in high-tension glaucoma patients [87]. Taken together, these observations indicate that TNF-α expression has a role in the degenerative process of glaucomatous optic nerve degeneration.

13.5.2 Neuroprotective Role of Microglia in Glaucoma

As mentioned above, the suppression of reactive microglia seems to protect RGC from glaucomatous damage. However, reactive microglia are required for cleaning cellular and axonal debris, which inhibits regenerative processes [56]. This suggests that microglia mediate not only degenerative processes but also protective processes.

Microglial phagocytosis is regulated by signals received from their environment. It is reported that the complement components C1q and C3 are involved in the elimination of inappropriate synapses [88]. Knockout of C1q or the downstream complement C3 showed defects in synapse elimination, and lateral geniculate nucleus (LGN) neurons remained multiply innervated. Microglia express the complement C3 receptor (Mac-1) and initiate a phagocytic response when the complement binds to this receptor. Thus, microglia eliminate excess synapses tagged by the complement. A similar mechanism is observed in the glaucoma model [88]. It has been demonstrated that the expression of C1q is increased in the retina at early and moderate glaucoma in this animal model. C1q was expressed at disease onset and marked the synapses, axons, and cell bodies before significant synapse loss and RGC death occurred. These findings suggest that neurons and synapses, which are supposed to be removed by microglial phagocytosis, are initially marked by complement in glaucoma. Thus, clearance mechanisms via classical complement signaling, which are involved in removing CNS synapses during development, became reactivated in the adult retina during the neurodegenerative phase of glaucoma.

Furthermore, various neuroprotective molecules generated by reactive microglia contribute to the beneficial effect. It has been shown that microglia express insulin-like growth factor 1 (IGF-1) and ciliary neurotrophic factor (CNTF) [89–91]. Moreover, microglia were required for the neuroprotective effect of IGF-1 in the dystrophic retina of the *rd10* mouse model. The depletion of microglia with clodronate liposomes reduced the neuroprotective effect of IGF-1 in photoreceptor cells [89]. Intravitreal injection of CNTF recruited macrophages into the eye, and these cells contributed to CNTF-induced RGC survival and axonal regeneration after optic nerve injury [90]. These results suggest that the growth factors derived from microglia protect RGC from neurodegenerative damage. Reactive microglia

are also required to promote the Müller cell-derived production of growth factors such as glial cell-line-derived neurotrophic factor (GDNF) and leukemia inhibitory factor (LIF) [92]. Thus, reactive microglia can also attenuate glaucomatous damages through phagocytosis and the production of neuroprotective factors.

13.6 Conclusions

Microglia have dual functions in neurodegeneration such as glaucoma. The balance between beneficial and deleterious roles of microglia may be critical for the treatment of neurodegenerative diseases. The development of drugs that can shift the immune response towards neuroprotection would be an effective therapeutic approach for glaucoma.

Acknowledgments This work was supported by a Grants-in-Aid for Scientific Research (S) from the Japan Society for the Promotion of Science (JSPS) to TY.
Conflict of Interest Statement The authors declare that they have no conflict of interest.

References

1. del Rio-Hortega P (1919) El “tercer elemento” de los centros nerviosus. Bol Soc Esp Biol 9:69–120
2. del Rio-Hortega P (1932) Microglia, vol 2. Cytology and cellular pathology of the nervous system. Hoeber, New York
3. Rezaie P, Male D (2002) Mesoglia & microglia–a historical review of the concept of mononuclear phagocytes within the central nervous system. J Hist Neurosci 11(4):325–374. doi:10.1076/jhin.11.4.325.8531
4. Chan WY, Kohsaka S, Rezaie P (2007) The origin and cell lineage of microglia: new concepts. Brain Res Rev 53(2):344–354. doi:10.1016/j.brainresrev.2006.11.002
5. Ashwell K (1991) The distribution of microglia and cell death in the fetal rat forebrain. Brain Res Dev Brain Res 58(1):1–12
6. Boya J, Calvo J, Prado A (1979) The origin of microglial cells. J Anat 129(Pt 1):177–186
7. Kitamura T, Miyake T, Fujita S (1984) Genesis of resting microglia in the gray matter of mouse hippocampus. J Comp Neurol 226(3):421–433. doi:10.1002/cne.902260310
8. Paterson JA, Privat A, Ling EA, Leblond CP (1973) Investigation of glial cells in semithin sections. 3. Transformation of subependymal cells into glial cells, as shown by radioautography after 3H-thymidine injection into the lateral ventricle of the brain of young rats. J Comp Neurol 149(1):83–102. doi:10.1002/cne.901490106
9. Ling EA (1982) Influence of cortisone on amoeboid microglia and microglial cells in the corpus callosum in postnatal rats. J Anat 134(Pt 4):705–717
10. Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res Dev Brain Res 117(2):145–152
11. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult

microglia derive from primitive macrophages. Science 330(6005):841–845. doi:10.1126/science.1194637
12. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW, Frampton J, Liu KJ, Geissmann F (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336(6077):86–90. doi:10.1126/science.1219179
13. Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, Wieghofer P, Heinrich A, Riemke P, Holscher C, Muller DN, Luckow B, Brocker T, Debowski K, Fritz G, Opdenakker G, Diefenbach A, Biber K, Heikenwalder M, Geissmann F, Rosenbauer F, Prinz M (2013) Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 16(3):273–280. doi:10.1038/nn.3318
14. Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, Capecchi MR (2010) Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. Cell 141(5):775–785. doi:10.1016/j.cell.2010.03.055
15. Herbomel P, Thisse B, Thisse C (2001) Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Dev Biol 238(2):274–288. doi:10.1006/dbio.2001.0393
16. Santos AM, Calvente R, Tassi M, Carrasco MC, Martin-Oliva D, Marin-Teva JL, Navascues J, Cuadros MA (2008) Embryonic and postnatal development of microglial cells in the mouse retina. J Comp Neurol 506(2):224–239. doi:10.1002/cne.21538
17. Hume DA, Perry VH, Gordon S (1983) Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. J Cell Biol 97(1):253–257
18. Oppenheim RW (1991) Cell death during development of the nervous system. Annu Rev Neurosci 14:453–501. doi:10.1146/annurev.ne.14.030191.002321
19. Buss RR, Sun W, Oppenheim RW (2006) Adaptive roles of programmed cell death during nervous system development. Annu Rev Neurosci 29:1–35. doi:10.1146/annurev.neuro.29.051605.112800
20. Diaz-Araya CM, Provis JM, Penfold PL, Billson FA (1995) Development of microglial topography in human retina. J Comp Neurol 363(1):53–68. doi:10.1002/cne.903630106
21. Provis JM, Diaz CM, Penfold PL (1996) Microglia in human retina: a heterogeneous population with distinct ontogenies. Perspect Dev Neurobiol 3(3):213–222
22. Marin-Teva JL, Calvente R, Cuadros MA, Almendros A, Navascues J (1999) Circumferential migration of ameboid microglia in the margin of the developing quail retina. Glia 27(3):226–238
23. Kaur C, Hao AJ, Wu CH, Ling EA (2001) Origin of microglia. Microsc Res Tech 54(1):2–9. doi:10.1002/jemt.1114
24. Soulet D, Rivest S (2008) Bone-marrow-derived microglia: myth or reality? Curr Opin Pharmacol 8(4):508–518. doi:10.1016/j.coph.2008.04.002
25. Ransohoff RM, Perry VH (2009) Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol 27:119–145. doi:10.1146/annurev.immunol.021908.132528
26. Graeber MB, Streit WJ (2010) Microglia: biology and pathology. Acta Neuropathol 119(1):89–105. doi:10.1007/s00401-009-0622-0
27. Xu H, Chen M, Mayer EJ, Forrester JV, Dick AD (2007) Turnover of resident retinal microglia in the normal adult mouse. Glia 55(11):1189–1198. doi:10.1002/glia.20535
28. Kezic J, McMenamin PG (2008) Differential turnover rates of monocyte-derived cells in varied ocular tissue microenvironments. J Leukoc Biol 84(3):721–729. doi:10.1189/jlb.0308166
29. Kezic J, Xu H, Chinnery HR, Murphy CC, McMenamin PG (2008) Retinal microglia and uveal tract dendritic cells and macrophages are not CX3CR1 dependent in their recruitment and distribution in the young mouse eye. Invest Ophthalmol Vis Sci 49(4):1599–1608. doi:10.1167/iovs.07-0953

30. Kaneko H, Nishiguchi KM, Nakamura M, Kachi S, Terasaki H (2008) Characteristics of bone marrow-derived microglia in the normal and injured retina. Invest Ophthalmol Vis Sci 49 (9):4162–4168. doi:10.1167/iovs.08-1738
31. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci 10(12):1538–1543. doi:10.1038/nn2014
32. Ransohoff RM (2007) Microgliosis: the questions shape the answers. Nat Neurosci 10 (12):1507–1509. doi:10.1038/nn1207-1507
33. Hanisch UK (2002) Microglia as a source and target of cytokines. Glia 40(2):140–155. doi:10.1002/glia.10161
34. Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394. doi:10.1038/nn1997
35. Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308(5726):1314–1318. doi:10.1126/science.1110647
36. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8 (6):752–758. doi:10.1038/nn1472
37. Streit WJ (2002) Microglia as neuroprotective, immunocompetent cells of the CNS. Glia 40 (2):133–139. doi:10.1002/glia.10154
38. van Rossum D, Hanisch UK (2004) Microglia. Metab Brain Dis 19(3–4):393–411
39. Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN, Adhikari S, Thompson DA, Botti P, Bacon KB, Feng L (1998) Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 95(18):10896–10901
40. Cook DN, Chen SC, Sullivan LM, Manfra DJ, Wiekowski MT, Prosser DM, Vassileva G, Lira SA (2001) Generation and analysis of mice lacking the chemokine fractalkine. Mol Cell Biol 21(9):3159–3165. doi:10.1128/MCB.21.9.3159-3165.2001
41. Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9(7):917–924. doi:10.1038/nn1715
42. Luo L, O'Leary DD (2005) Axon retraction and degeneration in development and disease. Annu Rev Neurosci 28:127–156. doi:10.1146/annurev.neuro.28.061604.135632
43. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29(13):3974–3980. doi:10.1523/JNEUROSCI.4363-08.2009
44. Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8(11):e1000527. doi:10.1371/journal.pbio.1000527
45. Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V, Yang RB, Nurden P, Nurden A, Julius D, Conley PB (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature 409(6817):202–207. doi:10.1038/35051599
46. Zhang FL, Luo L, Gustafson E, Lachowicz J, Smith M, Qiao X, Liu YH, Chen G, Pramanik B, Laz TM, Palmer K, Bayne M, Monsma FJ Jr (2001) ADP is the cognate ligand for the orphan G protein-coupled receptor SP1999. J Biol Chem 276(11):8608–8615. doi:10.1074/jbc.M009718200
47. Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D (2006) The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci 9 (12):1512–1519. doi:10.1038/nn1805
48. Chen M, Forrester JV, Xu H (2011) Dysregulation in retinal para-inflammation and age-related retinal degeneration in CCL2 or CCR2 deficient mice. PLoS One 6(8):e22818. doi:10.1371/journal.pone.0022818

49. Fries JE, Wheeler-Schilling TH, Guenther E, Kohler K (2004) Expression of P2Y1, P2Y2, P2Y4, and P2Y6 receptor subtypes in the rat retina. Invest Ophthalmol Vis Sci 45(10):3410–3417. doi:10.1167/iovs.04-0141
50. Schuetz E, Thanos S (2004) Microglia-targeted pharmacotherapy in retinal neurodegenerative diseases. Curr Drug Targets 5(7):619–627
51. Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19(8):312–318
52. Streit WJ, Walter SA, Pennell NA (1999) Reactive microgliosis. Prog Neurobiol 57 (6):563–581
53. Barron KD (1995) The microglial cell. A historical review. J Neurol Sci 134(Suppl):57–68
54. Joly S, Francke M, Ulbricht E, Beck S, Seeliger M, Hirrlinger P, Hirrlinger J, Lang KS, Zinkernagel M, Odermatt B, Samardzija M, Reichenbach A, Grimm C, Reme CE (2009) Cooperative phagocytes: resident microglia and bone marrow immigrants remove dead photoreceptors in retinal lesions. Am J Pathol 174(6):2310–2323. doi:10.2353/ajpath.2009.090023
55. Wohl SG, Schmeer CW, Witte OW, Isenmann S (2010) Proliferative response of microglia and macrophages in the adult mouse eye after optic nerve lesion. Invest Ophthalmol Vis Sci 51 (5):2686–2696. doi:10.1167/iovs.09-4537
56. Neumann H, Kotter MR, Franklin RJ (2009) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain 132(Pt 2):288–295. doi:10.1093/brain/awn109
57. Chao CC, Hu S, Molitor TW, Shaskan EG, Peterson PK (1992) Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. J Immunol 149(8):2736–2741
58. Gupta N, Brown KE, Milam AH (2003) Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. Exp Eye Res 76 (4):463–471
59. Neufeld AH (1999) Microglia in the optic nerve head and the region of parapapillary chorioretinal atrophy in glaucoma. Arch Ophthalmol 117(8):1050–1056
60. Santos AM, Martin-Oliva D, Ferrer-Martin RM, Tassi M, Calvente R, Sierra A, Carrasco MC, Marin-Teva JL, Navascues J, Cuadros MA (2010) Microglial response to light-induced photoreceptor degeneration in the mouse retina. J Comp Neurol 518(4):477–492. doi:10.1002/cne.22227
61. Nagai A, Mishima S, Ishida Y, Ishikura H, Harada T, Kobayashi S, Kim SU (2005) Immortalized human microglial cell line: phenotypic expression. J Neurosci Res 81(3):342–348. doi:10.1002/jnr.20478
62. Nakamura Y (2002) Regulating factors for microglial activation. Biol Pharm Bull 25 (8):945–953
63. Levin LA (1999) Direct and indirect approaches to neuroprotective therapy of glaucomatous optic neuropathy. Surv Ophthalmol 43(Suppl 1):S98–S101
64. Neufeld AH, Sawada A, Becker B (1999) Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. Proc Natl Acad Sci U S A 96(17):9944–9948
65. Tezel G, Li LY, Patil RV, Wax MB (2001) TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. Invest Ophthalmol Vis Sci 42(8):1787–1794
66. Mitrovic B, Martin FC, Charles AC, Ignarro LJ, Anton PA, Shanahan F, Merrill JE (1994) Neurotransmitters and cytokines in CNS pathology. Prog Brain Res 103:319–330
67. Streit WJ (1996) The role of microglia in brain injury. Neurotoxicology 17(3–4):671–678
68. Stoll G, Jander S (1999) The role of microglia and macrophages in the pathophysiology of the CNS. Prog Neurobiol 58(3):233–247
69. Kim SU, de Vellis J (2005) Microglia in health and disease. J Neurosci Res 81(3):302–313. doi:10.1002/jnr.20562
70. Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8(1):57–69. doi:10.1038/nrn2038

71. Taylor DL, Jones F, Kubota ES, Pocock JM (2005) Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced neurotoxicity in concert with microglial-derived Fas ligand. J Neurosci 25(11):2952–2964. doi:10.1523/JNEUROSCI.4456-04.2005
72. Ahmed F, Brown KM, Stephan DA, Morrison JC, Johnson EC, Tomarev SI (2004) Microarray analysis of changes in mRNA levels in the rat retina after experimental elevation of intraocular pressure. Invest Ophthalmol Vis Sci 45(4):1247–1258
73. Steele MR, Inman DM, Calkins DJ, Horner PJ, Vetter ML (2006) Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma. Invest Ophthalmol Vis Sci 47 (3):977–985. doi:10.1167/iovs.05-0865
74. Bosco A, Crish SD, Steele MR, Romero CO, Inman DM, Horner PJ, Calkins DJ, Vetter ML (2012) Early reduction of microglia activation by irradiation in a model of chronic glaucoma. PLoS One 7(8):e43602. doi:10.1371/journal.pone.0043602
75. Bosco A, Inman DM, Steele MR, Wu G, Soto I, Marsh-Armstrong N, Hubbard WC, Calkins DJ, Horner PJ, Vetter ML (2008) Reduced retina microglial activation and improved optic nerve integrity with minocycline treatment in the DBA/2J mouse model of glaucoma. Invest Ophthalmol Vis Sci 49(4):1437–1446. doi:10.1167/iovs.07-1337
76. Singhal S, Lawrence JM, Salt TE, Khaw PT, Limb GA (2010) Triamcinolone attenuates macrophage/microglia accumulation associated with NMDA-induced RGC death and facilitates survival of Muller stem cell grafts. Exp Eye Res 90(2):308–315. doi:10.1016/j.exer.2009.11.008
77. de Kozak Y, Cotinet A, Goureau O, Hicks D, Thillaye-Goldenberg B (1997) Tumor necrosis factor and nitric oxide production by resident retinal glial cells from rats presenting hereditary retinal degeneration. Ocul Immunol Inflamm 5(2):85–94
78. Shohami E, Ginis I, Hallenbeck JM (1999) Dual role of tumor necrosis factor alpha in brain injury. Cytokine Growth Factor Rev 10(2):119–130
79. Tezel G (2008) TNF-alpha signaling in glaucomatous neurodegeneration. Prog Brain Res 173:409–421. doi:10.1016/S0079-6123(08)01128-X
80. Yang X, Luo C, Cai J, Powell DW, Yu D, Kuehn MH, Tezel G (2011) Neurodegenerative and inflammatory pathway components linked to TNF-alpha/TNFR1 signaling in the glaucomatous human retina. Invest Ophthalmol Vis Sci 52(11):8442–8454. doi:10.1167/iovs.11-8152
81. Tezel G, Yang X, Yang J, Wax MB (2004) Role of tumor necrosis factor receptor-1 in the death of retinal ganglion cells following optic nerve crush injury in mice. Brain Res 996 (2):202–212
82. Yan X, Tezel G, Wax MB, Edward DP (2000) Matrix metalloproteinases and tumor necrosis factor alpha in glaucomatous optic nerve head. Arch Ophthalmol 118(5):666–673
83. Yuan L, Neufeld AH (2000) Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. Glia 32(1):42–50
84. Roh M, Zhang Y, Murakami Y, Thanos A, Lee SC, Vavvas DG, Benowitz LI, Miller JW (2012) Etanercept, a widely used inhibitor of tumor necrosis factor-alpha (TNF-alpha), prevents retinal ganglion cell loss in a rat model of glaucoma. PLoS One 7(7):e40065. doi:10.1371/journal.pone.0040065
85. Bai Y, Shi Z, Zhuo Y, Liu J, Malakhov A, Ko E, Burgess K, Schaefer H, Esteban PF, Tessarollo L, Saragovi HU (2010) In glaucoma the upregulated truncated TrkC.T1 receptor isoform in glia causes increased TNF-alpha production, leading to retinal ganglion cell death. Invest Ophthalmol Vis Sci 51(12):6639–6651. doi:10.1167/iovs.10-5431
86. Allen RD (1999) Polymorphism of the human TNF-alpha promoter–random variation or functional diversity? Mol Immunol 36(15–16):1017–1027
87. Fan BJ, Liu K, Wang DY, Tham CC, Tam PO, Lam DS, Pang CP (2010) Association of polymorphisms of tumor necrosis factor and tumor protein p53 with primary open-angle glaucoma. Invest Ophthalmol Vis Sci 51(8):4110–4116. doi:10.1167/iovs.09-4974

88. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SW, Barres BA (2007) The classical complement cascade mediates CNS synapse elimination. Cell 131(6):1164–1178. doi:10.1016/j.cell.2007.10.036
89. Arroba AI, Alvarez-Lindo N, van Rooijen N, de la Rosa EJ (2011) Microglia-mediated IGF-I neuroprotection in the rd10 mouse model of retinitis pigmentosa. Invest Ophthalmol Vis Sci 52 (12):9124–9130. doi:10.1167/iovs.11-7736
90. Cen LP, Luo JM, Zhang CW, Fan YM, Song Y, So KF, van Rooijen N, Pang CP, Lam DS, Cui Q (2007) Chemotactic effect of ciliary neurotrophic factor on macrophages in retinal ganglion cell survival and axonal regeneration. Invest Ophthalmol Vis Sci 48(9):4257–4266. doi:10.1167/iovs.06-0791
91. Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T (2013) Layer V cortical neurons require microglial support for survival during postnatal development. Nat Neurosci 16(5):543–551. doi:10.1038/nn.3358
92. Wang M, Ma W, Zhao L, Fariss RN, Wong WT (2011) Adaptive Muller cell responses to microglial activation mediate neuroprotection and coordinate inflammation in the retina. J Neuroinflammation 8:173. doi:10.1186/1742-2094-8-173

Part II
Neuroprotection for Age-Related Macular Degeneration (AMD), Retinal Pigmentary Degeneration

Chapter 14
Neuroprotection for Photoreceptors

Toshiaki Abe and Nobuhiro Nagai

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

T. Abe (✉) • N. Nagai
Division of Clinical Cell Therapy, United Centers for Advanced Research and Translational Medicine (ART), School of Medicine, Tohoku University Graduate, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
e-mail: toshi@oph.med.tohoku.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_14, © Springer Japan 2014

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.

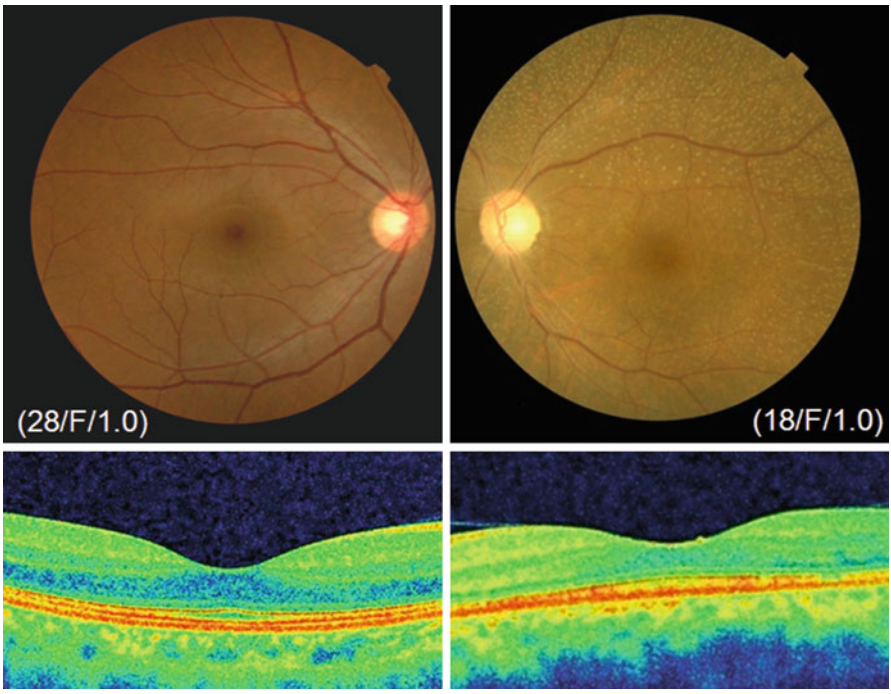


Fig. 14.1 Optical coherence tomography (*OCT*) has become the gold standard for assessing anatomical abnormalities in retinal diseases, especially for the macular area. RP patients with good visual acuity (18-year-old woman in the right column) show a relatively reserved macular structure, although the 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-α 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].

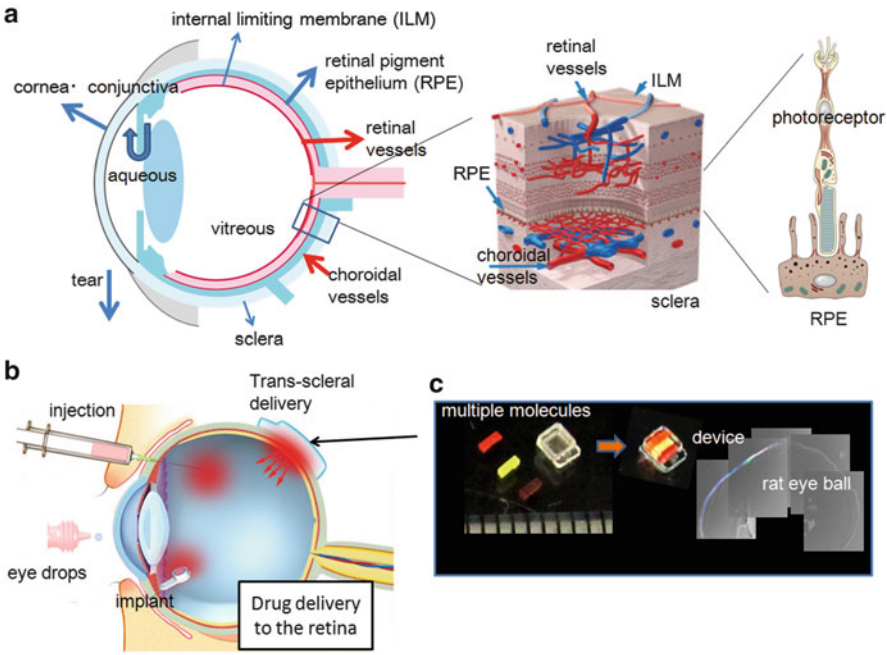


Fig. 14.2 Retina is protected by many tissue barriers (**a**), which is why development of retinal therapy has been difficult. Recently, we reported a novel trans-scleral drug delivery device placed on the sclera that consisted of a drug-releasing semipermeable membrane and impermeable membranes acting 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 trophic 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 was 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 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 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 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 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 K^+ channels in nerve cells effectively regulates cell membrane potentials. Large-conductance voltage- and Ca^{++}-dependent K^+ (max-K or BK) channels react to increased intracellular Ca^{++} and membrane depolarization and show rapid hyperpolarization of the membrane and reduce voltage-dependent 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 system**s** (not only drug**s**, but also gene**s**) may allow new insights into strategies for photoreceptor protection.

References

1. Murakami Y, Notomi S, Hisatomi T, Nakazawa T, Ishibashi T, Miller JW, Vavvas DG (2013) Photoreceptor cell death and rescue in retinal detachment and degenerations. Prog Retin Eye Res 37:114–140. doi:10.1016/j.preteyeres.2013.08.001
2. Komeima K, Rogers BS, Lu L, Campochiaro PA (2006) Antioxidants reduce cone cell death in a model of retinitis pigmentosa. Proc Natl Acad Sci USA 103(30):11300–11305. doi:10.1073/pnas.0604056103
3. Ohnaka M, Miki K, Gong YY, Stevens R, Iwase T, Hackett SF, Campochiaro PA (2012) Long-term expression of glial cell line-derived neurotrophic factor slows, but does not stop retinal degeneration in a model of retinitis pigmentosa. J Neurochem 122(5):1047–1053. doi:10.1111/j.1471-4159.2012.07842.x
4. Hisatomi T, Sakamoto T, Murata T, Yamanaka I, Oshima Y, Hata Y, Ishibashi T, Inomata H, Susin SA, Kroemer G (2001) Relocalization of apoptosis-inducing factor in photoreceptor apoptosis induced by retinal detachment in vivo. Am J Pathol 158(4):1271–1278. doi:10.1016/S0002-9440(10)64078-3
5. Piano I, Novelli E, Gasco P, Ghidoni R, Strettoi E, Gargini C (2013) Cone survival and preservation of visual acuity in an animal model of retinal degeneration. Eur J Neurosci 37(11):1853–1862. doi:10.1111/ejn.12196
6. van Soest S, Westerveld A, de Jong PT, Bleeker-Wagemakers EM, Bergen AA (1999) Retinitis pigmentosa: defined from a molecular point of view. Surv Ophthalmol 43(4):321–334

7. Makiyama Y, Ooto S, Hangai M, Takayama K, Uji A, Oishi A, Ogino K, Nakagawa S, Yoshimura N (2013) Macular cone abnormalities in retinitis pigmentosa with preserved central vision using adaptive optics scanning laser ophthalmoscopy. PLoS One 8(11): e79447. doi:10.1371/journal.pone.0079447
8. Lockshin RA, Williams CM (1965) Programmed cell death–I. Cytology of degeneration in the intersegmental muscles of the pernyi silkmoth. J Insect Physiol 11:123–133
9. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26(4):239–257
10. Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J (1993) Induction of apoptosis in fibroblasts by IL-1 beta-converting enzyme, a mammalian homolog of the C. elegans cell death gene ced-3. Cell 75(4):653–660
11. Cory S, Adams JM (2002) The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2(9):647–656. doi:10.1038/nrc883
12. Punzo C, Kornacker K, Cepko CL (2009) Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. Nat Neurosci 12(1):44–52. doi:10.1038/nn.2234
13. Kunchithapautham K, Rohrer B (2007) Apoptosis and autophagy in photoreceptors exposed to oxidative stress. Autophagy 3(5):433–441
14. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G (2007) Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol 8(9):741–752. doi:10.1038/nrm2239
15. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J (2000) Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 1(6):489–495. doi:10.1038/82732
16. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, Chan FK (2009) Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 137(6):1112–1123. doi:10.1016/j.cell.2009.05.037
17. Sato K, Li S, Gordon WC, He J, Liou GI, Hill JM, Travis GH, Bazan NG, Jin M (2013) Receptor interacting protein kinase-mediated necrosis contributes to cone and rod photoreceptor degeneration in the retina lacking interphotoreceptor retinoid-binding protein. J Neurosci Official J Soc Neurosci 33(44):17458–17468. doi:10.1523/JNEUROSCI.1380-13.2013
18. Nakazawa T, Kayama M, Ryu M, Kunikata H, Watanabe R, Yasuda M, Kinugawa J, Vavvas D, Miller JW (2011) Tumor necrosis factor-alpha mediates photoreceptor death in a rodent model of retinal detachment. Invest Ophthalmol Vis Sci 52(3):1384–1391. doi:10.1167/iovs.10-6509
19. Eskelinen EL (2008) To be or not to be? Examples of incorrect identification of autophagic compartments in conventional transmission electron microscopy of mammalian cells. Autophagy 4(2):257–260
20. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119(3):493–501
21. Chang B, Hawes NL, Hurd RE, Davisson MT, Nusinowitz S, Heckenlively JR (2002) Retinal degeneration mutants in the mouse. Vision Res 42(4):517–525
22. Hartong DT, Berson EL, Dryja TP (2006) Retinitis pigmentosa. Lancet 368(9549):1795–1809. doi:10.1016/S0140-6736(06)69740-7
23. Testa F, Maguire AM, Rossi S, Pierce EA, Melillo P, Marshall K, Banfi S, Surace EM, Sun J, Acerra C, Wright JF, Wellman J, High KA, Auricchio A, Bennett J, Simonelli F (2013) Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2. Ophthalmology 120(6):1283–1291. doi:10.1016/j.ophtha.2012.11.048
24. Cideciyan AV, Jacobson SG, Beltran WA, Hauswirth WW, Aguirre GD (2013) Reply to Townes-Anderson: RPE65 gene therapy does not alter the natural history of retinal degeneration. Proc Natl Acad Sci USA 110(19):E1706

25. Montana CL, Kolesnikov AV, Shen SQ, Myers CA, Kefalov VJ, Corbo JC (2013) Reprogramming of adult rod photoreceptors prevents retinal degeneration. Proc Natl Acad Sci USA 110(5):1732–1737. doi:10.1073/pnas.1214387110
26. Aloe L (2004) Rita Levi-Montalcini: the discovery of nerve growth factor and modern neurobiology. Trends Cell Biol 14(7):395–399. doi:10.1016/j.tcb.2004.05.011
27. Tao W, Wen R, Goddard MB, Sherman SD, O'Rourke PJ, Stabila PF, Bell WJ, Dean BJ, Kauper KA, Budz VA, Tsiaras WG, Acland GM, Pearce-Kelling S, Laties AM, Aguirre GD (2002) Encapsulated cell-based delivery of CNTF reduces photoreceptor degeneration in animal models of retinitis pigmentosa. Invest Ophthalmol Vis Sci 43(10):3292–3298
28. Okoye G, Zimmer J, Sung J, Gehlbach P, Deering T, Nambu H, Hackett S, Melia M, Esumi N, Zack DJ, Campochiaro PA (2003) Increased expression of brain-derived neurotrophic factor preserves retinal function and slows cell death from rhodopsin mutation or oxidative damage. J Neurosci Official J Soc Neurosci 23(10):4164–4172
29. Lavail MM (2005) Survival factors for treatment of retinal degenerative disorders: preclinical gains and issues for translation into clinical studies. Retina 25(8 Suppl):S25–S26
30. MacDonald IM, Sauve Y, Sieving PA (2007) Preventing blindness in retinal disease: ciliary neurotrophic factor intraocular implants. Can J Ophthalmol 42(3):399–402. doi:10.3129/can j ophthalmol.i07-039
31. Birch DG, Weleber RG, Duncan JL, Jaffe GJ, Tao W (2013) Randomized trial of ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for retinitis pigmentosa. Am J Ophthalmol 156(2):283–292. doi:10.1016/j.ajo.2013.03.021, e281
32. Rhee KD, Nusinowitz S, Chao K, Yu F, Bok D, Yang XJ (2013) CNTF-mediated protection of photoreceptors requires initial activation of the cytokine receptor gp130 in Muller glial cells. Proc Natl Acad Sci USA 110(47):E4520–E4529. doi:10.1073/pnas.1303604110
33. Yu DY, Cringle SJ (2005) Retinal degeneration and local oxygen metabolism. Exp Eye Res 80 (6):745–751. doi:10.1016/j.exer.2005.01.018
34. Ahuja-Jensen P, Johnsen-Soriano S, Ahuja S, Bosch-Morell F, Sancho-Tello M, Romero FJ, Abrahamson M, van Veen T (2007) Low glutathione peroxidase in rd1 mouse retina increases oxidative stress and proteases. Neuroreport 18(8):797–801. doi:10.1097/WNR. 0b013e3280c1e344
35. Bresnick GH (1970) Oxygen-induced visual cell degeneration in the rabbit. Invest Ophthalmol 9(5):372–387
36. Tezel G (2006) Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. Prog Retin Eye Res 25(5):490–513. doi:10.1016/j.preteyeres.2006.07.003
37. Berson EL (1993) Retinitis pigmentosa. The Friedenwald lecture. Invest Ophthalmol Vis Sci 34(5):1659–1676
38. Berson EL, Rosner B, Sandberg MA, Weigel-DiFranco C, Willett WC (2012) Omega-3 intake and visual acuity in patients with retinitis pigmentosa receiving vitamin A. Arch Ophthalmol 130(6):707–711. doi:10.1001/archophthalmol.2011.2580
39. Trifunovic D, Sahaboglu A, Kaur J, Mencl S, Zrenner E, Ueffing M, Arango-Gonzalez B, Paquet-Durand F (2012) Neuroprotective strategies for the treatment of inherited photoreceptor degeneration. Curr Mol Med 12(5):598–612
40. Jin ZB, Okamoto S, Osakada F, Homma K, Assawachananont J, Hirami Y, Iwata T, Takahashi M (2011) Modeling retinal degeneration using patient-specific induced pluripotent stem cells. PLoS One 6(2):e17084. doi:10.1371/journal.pone.0017084
41. Rhee KD, Ruiz A, Duncan JL, Hauswirth WW, Lavail MM, Bok D, Yang XJ (2007) Molecular and cellular alterations induced by sustained expression of ciliary neurotrophic factor in a mouse model of retinitis pigmentosa. Invest Ophthalmol Vis Sci 48(3):1389–1400. doi:10. 1167/iovs.06-0677
42. Arshavsky VY, Wensel TG (2013) Timing is everything: GTPase regulation in phototransduction. Invest Ophthalmol Vis Sci 54(12):7725–7733. doi:10.1167/iovs.13-13281

43. Mendes HF, van der Spuy J, Chapple JP, Cheetham ME (2005) Mechanisms of cell death in rhodopsin retinitis pigmentosa: implications for therapy. Trends Mol Med 11(4):177–185. doi:10.1016/j.molmed.2005.02.007
44. Semple-Rowland SL, Cheng KM (1999) rd and rc chickens carry the same GC1 null allele (GUCY1*). Exp Eye Res 69(5):579–581. doi:10.1006/exer.1999.0743
45. Fabian E, Reglodi D, Mester L, Szabo A, Szabadfi K, Tamas A, Toth G, Kovacs K (2012) Effects of PACAP on intracellular signaling pathways in human retinal pigment epithelial cells exposed to oxidative stress. J Mol Neurosci 48(3):493–500. doi:10.1007/s12031-012-9812-7
46. Delcuve GP, Rastegar M, Davie JR (2009) Epigenetic control. J Cell Physiol 219(2):243–250. doi:10.1002/jcp.21678
47. Sancho-Pelluz J, Alavi MV, Sahaboglu A, Kustermann S, Farinelli P, Azadi S, van Veen T, Romero FJ, Paquet-Durand F, Ekstrom P (2010) Excessive HDAC activation is critical for neurodegeneration in the rd1 mouse. Cell Death Disease 1:e24. doi:10.1038/cddis.2010.4
48. Uehara N, Miki K, Tsukamoto R, Matsuoka Y, Tsubura A (2006) Nicotinamide blocks N-methyl-N-nitrosourea-induced photoreceptor cell apoptosis in rats through poly (ADP-ribose) polymerase activity and Jun N-terminal kinase/activator protein-1 pathway inhibition. Exp Eye Res 82(3):488–495. doi:10.1016/j.exer.2005.08.006
49. Herrup K, Yang Y (2007) Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? Nat Rev Neurosci 8(5):368–378. doi:10.1038/nrn2124
50. Paquet-Durand F, Johnson L, Ekstrom P (2007) Calpain activity in retinal degeneration. J Neurosci Res 85(4):693–702. doi:10.1002/jnr.21151
51. Oka T, Walkup RD, Tamada Y, Nakajima E, Tochigi A, Shearer TR, Azuma M (2006) Amelioration of retinal degeneration and proteolysis in acute ocular hypertensive rats by calpain inhibitor ((1S)-1-((((1S)-1-benzyl-3-cyclopropylamino-2,3-di-oxopropyl)amino)carbonyl)-3-me thylbutyl)carbamic acid 5-methoxy-3-oxapentyl ester. Neuroscience 141 (4):2139–2145. doi:10.1016/j.neuroscience.2006.05.060
52. Wingrave JM, Sribnick EA, Wilford GG, Matzelle DD, Mou JA, Ray SK, Hogan EL, Banik NL (2004) Higher calpastatin levels correlate with resistance to calpain-mediated proteolysis and neuronal apoptosis in juvenile rats after spinal cord injury. J Neurotrauma 21(9):1240–1254. doi:10.1089/neu.2004.21.1240
53. Paquet-Durand F, Sanges D, McCall J, Silva J, van Veen T, Marigo V, Ekstrom P (2010) Photoreceptor rescue and toxicity induced by different calpain inhibitors. J Neurochem 115 (4):930–940. doi:10.1111/j.1471-4159.2010.06983.x
54. Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1 (8):623–634
55. Gribkoff VK, Starrett JE Jr, Dworetzky SI (1997) The pharmacology and molecular biology of large-conductance calcium-activated (BK) potassium channels. Adv Pharmacol 37:319–348
56. Gribkoff VK, Starrett JE Jr, Dworetzky SI, Hewawasam P, Boissard CG, Cook DA, Frantz SW, Heman K, Hibbard JR, Huston K, Johnson G, Krishnan BS, Kinney GG, Lombardo LA, Meanwell NA, Molinoff PB, Myers RA, Moon SL, Ortiz A, Pajor L, Pieschl RL, Post-Munson DJ, Signor LJ, Srinivas N, Taber MT, Thalody G, Trojnacki JT, Wiener H, Yeleswaram K, Yeola SW (2001) Targeting acute ischemic stroke with a calcium-sensitive opener of maxi-K potassium channels. Nat Med 7(4):471–477. doi:10.1038/86546
57. Dal-Cim T, Martins WC, Santos AR, Tasca CI (2011) Guanosine is neuroprotective against oxygen/glucose deprivation in hippocampal slices via large conductance Ca(2)+-activated K^+ channels, phosphatidilinositol-3 kinase/protein kinase B pathway activation and glutamate uptake. Neuroscience 183:212–220. doi:10.1016/j.neuroscience.2011.03.022
58. Tsuruma K, Tanaka Y, Shimazawa M, Mashima Y, Hara H (2011) Unoprostone reduces oxidative stress- and light-induced retinal cell death, and phagocytotic dysfunction, by activating BK channels. Mol Vis 17:3556–3565
59. Cuppoletti J, Malinowska DH, Tewari KP, Chakrabarti J, Ueno R (2007) Cellular and molecular effects of unoprostone as a BK channel activator. Biochim Biophys Acta 1768 (5):1083–1092. doi:10.1016/j.bbamem.2006.12.015

60. Tawada A, Sugawara T, Ogata K, Hagiwara A, Yamamoto S (2013) Improvement of central retinal sensitivity 6 months after topical isopropyl unoprostone in patients with retinitis pigmentosa. Indian J Ophthalmol 61(3):95–99. doi:10.4103/0301-4738.109377
61. Tokuda T, Asano R, Sugitani S, Terasawa Y, Nunoshita M, Nakauchi K, Fujikado T, Tano Y, Ohta J (2007) In vivo stimulation on rabbit retina using CMOS LSI-based multi-chip flexible stimulator for retinal prosthesis. Conf Proc IEEE Eng Med Biol 2007:5791–5794. doi:10.1109/IEMBS.2007.4353663
62. Feucht M, Laube T, Bornfeld N, Walter P, Velikay-Parel M, Hornig R, Richard G (2005) Development of an epiretinal prosthesis for stimulation of the human retina. Der Ophthalmologe (Zeitschrift der Deutschen Ophthalmol Gesellschaft) 102(7):688–691. doi:10.1007/s00347-005-1186-6
63. Sahni JN, Angi M, Irigoyen C, Semeraro F, Romano MR, Parmeggiani F (2011) Therapeutic challenges to retinitis pigmentosa: from neuroprotection to gene therapy. Curr Genom 12 (4):276–284. doi:10.2174/138920211795860062
64. Abe T, Yoshida M, Yoshioka Y, Wakusawa R, Tokita-Ishikawa Y, Seto H, Tamai M, Nishida K (2007) Iris pigment epithelial cell transplantation for degenerative retinal diseases. Prog Retin Eye Res 26(3):302–321. doi:10.1016/j.preteyeres.2007.01.003
65. Jin ZB, Takahashi M (2012) Generation of retinal cells from pluripotent stem cells. Prog Brain Res 201:171–181. doi:10.1016/B978-0-444-59544-7.00008-1
66. West EL, Gonzalez-Cordero A, Hippert C, Osakada F, Martinez-Barbera JP, Pearson RA, Sowden JC, Takahashi M, Ali RR (2012) Defining the integration capacity of embryonic stem cell-derived photoreceptor precursors. Stem Cells 30(7):1424–1435. doi:10.1002/stem.1123
67. Grisanti S, Tatar O (2008) The role of vascular endothelial growth factor and other endogenous interplayers in age-related macular degeneration. Prog Retin Eye Res 27(4):372–390. doi:10.1016/j.preteyeres.2008.05.002
68. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM (1990) Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. Nature 347(6288):83–86. doi:10.1038/347083a0
69. Pilli S, Kotsolis A, Spaide RF, Slakter J, Freund KB, Sorenson J, Klancnik J, Cooney M (2008) Endophthalmitis associated with intravitreal anti-vascular endothelial growth factor therapy injections in an office setting. Am J Ophthalmol 145(5):879–882. doi:10.1016/j.ajo.2007.12.036
70. Nagai N, Kaji H, Onami H, Ishikawa Y, Nishizawa M, Osumi N, Nakazawa T, Abe T (2013) A polymeric device for controlled trans-scleral multi-drug delivery to the posterior segment of the eye. Acta Biomater 10:680–687. doi:10.1016/j.actbio.2013.11.004

Chapter 15
Retinal Photooxidative Stress and Its Modifiers

Masaki Tanito

Abstract Retinal damage is classified into three types based on the mechanisms of the damage, i.e., photothermal, photomechanical, and photochemical, with the last likely the most common form of light-induced damage, since the natural range of light energy sufficient causative. Photochemical damage is caused by reactive oxygen species and free radicals generated by light exposure, also called photooxidative stress. Although different types of photooxidative stress have been proposed, they sometimes cannot be differentiated clearly in various experimental settings. Epidemiologic studies have suggested a correlation between environmental light exposure and development/progression of human retinal degeneration such as age-related macular degeneration and retinitis pigmentosa. The double bonds in long-chain polyunsaturated fatty acids (PUFAs), which are highly enriched in the retinal membranes, could be target substrates to propagate photooxidative stress in the photoreceptors. Posttranslational modification of retinal proteins by PUFA-degraded molecules, such as 4-hydroxynonenal and 4-hydroxyhexenal, causes retinal degeneration and neuroprotection depending on the timing and level of formation.

Keywords 4-Hydroxynonenal (4-HNE) • Age-related macular degeneration (AMD) • Docosahexaenoic acid (DHA22:6*n*-3) • Nuclear-factor-E2-related factor 2 (Nrf2) • Polyunsaturated fatty acids (PUFAs) • Retinal photooxidative stress

M. Tanito (✉)
Division of Ophthalmology, Matsue Red Cross Hospital, 200 Horomachi, Matsue, Shimane 690-8506, Japan
e-mail: tanito-oph@umin.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_15, © Springer Japan 2014

15.1 Types of Light-Induced Retinal Damage

Retinal damage is classified into three types based on the mechanisms [1]. Photothermal damage is induced by at least a 10 °C increase in ambient temperature in the retinal tissue originating from transfer of radiant energy from photons to the tissue during an exposure duration between 0.1 and 1.0 s [2–4]. Thermal damage results from a tissue temperature of 55–58 °C that causes cellular apoptosis, temperature of 60–68 °C that causes apoptosis and necrosis, and temperatures of 72 °C or higher that cause immediate cell death [1]. Laser photocoagulation, transpupillary thermotherapy, and micropulse diode laser are clinical applications that cause thermal damage. Photomechanical damage, as that caused by Nd:YAG laser, is induced by rapid introduction of compressive or tensile forces on retinal tissues by exposure to light with high irradiances (typically mega- or terawatts/cm^2) during a very short time (typically between nano- and picoseconds) [5, 6]. Photochemical damage is independent of either photothermal or photomechanical damage and is thought to be the most common mechanism of light-induced retinal damage, since the natural range of light causes this type of damage [7]. Theoretically, photochemical damage, as that caused by photodynamic therapy, is caused by reactive oxygen species and free radicals generated by light exposure; thus, this is also referred to as photooxidative stress.

15.2 Action Spectrum of Retinal Damage Induced by Photooxidative Stress

15.2.1 Light Properties

Among the electromagnetic waves, the wavelengths between 400 (380 nm) and 800 nm (760 nm) are called visible light because they reach the retina and are the source of visual information. Ultraviolet (UV) light is that with wavelengths shorter than visible light. Among the UV wavelengths, UVB (wavelengths between 290 and 320 nm) and UVA (wavelengths between 320 and 400 nm) are absorbed by the cornea and crystalline lens, respectively, do not reach the retina, and are not used for visual sensing in humans. Infrared light with a wavelength longer than visible light also is absorbed by the crystalline lens. In phakic human eyes, photooxidative stress is thought to result mainly from visible light wavelengths (Fig 15.1).

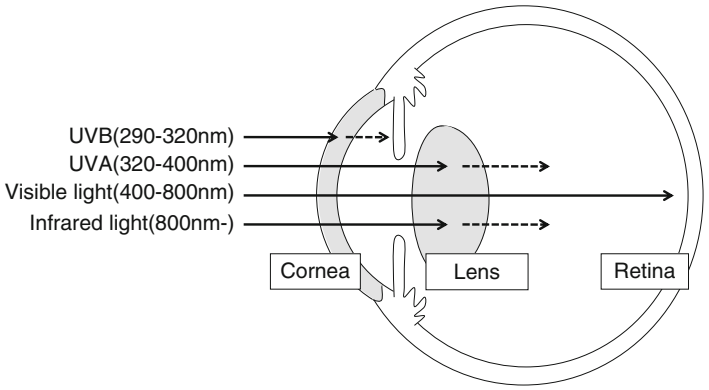


Fig. 15.1 Transmission of light through the cornea and crystalline lens. Light wavelengths that are shorter (ultraviolet) or longer (infrared) than visible light are absorbed by the cornea or lens and do not reach the retina

15.2.2 Types of Photooxidative Stress

Previous studies have suggested two possible mechanisms of retinal photooxidative stress [8, 9]. Noell et al. first reported retinal damage induced by constant green light (wavelengths, 490–580 nm) at relatively low retinal irradiance (<1 mW/cm^2) during a long exposure time (at least 1.5 h, typically >8 h) in rats [7]. The action spectrum of this damage is similar to the absorption spectrum of rhodopsin. This type of photooxidative stress is referred to as Noell's damage or class I damage. Noell's spectrum of retinal damage has been reported subsequently in mice [10], macaque monkeys [11], fish [12], and chickens [13]. Ham et al. first reported retinal damage induced by visible light [14] and later by UV radiation [14] at higher retinal irradiance and shorter (<5 h) exposure times than Noell's damage in monkeys. In Ham's experiments, the susceptibility of retinal damage decreased monotonically from short to long wavelengths (Fig 15.2). This type of photooxidative stress is referred to as Ham's damage, class II damage, or blue-light hazard. Ham et al. later reported the spectrum of retinal damage in rabbits [15], rats [16, 17], and squirrels [18]. Currently, Ham's action spectrum serves as the basis for international standards or guidelines for protection against retinal damage by lasers and other light sources [19, 20] (Fig 15.3); the spectrum of Noell et al. is not included because it is considered to be exclusive to rodents [8]. These guidelines are used mainly to protect eyes from acute light damage such as that induced by sunlight [21] or to determine the upper limits of light exposure in a work environment. Thus, no reliable standards yet have been established to protect eyes from chronic light exposure.

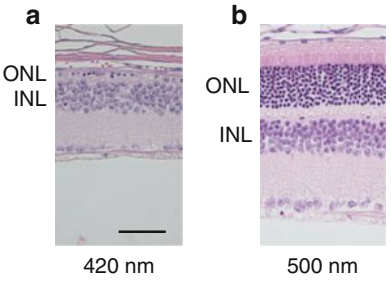


Fig. 15.2 Wavelength dependence of retinal light damage. Albino mice are exposed to light with a narrow bandwidth. The eyes are nucleated 1 week after light exposure. (**a**) A retinal section from a mouse exposed to 420-nm wavelength light at 500 J/cm^2. Most of the ONL has disappeared, suggesting severe retinal damage resulting from light exposure. (**b**) A retinal section from a mouse exposed to 500-nm wavelength light at 500 J/cm^2. Most of the ONL is preserved, suggesting mild retinal damage from light exposure. *INL* inner nuclear layer. Scale bar = 50 μm

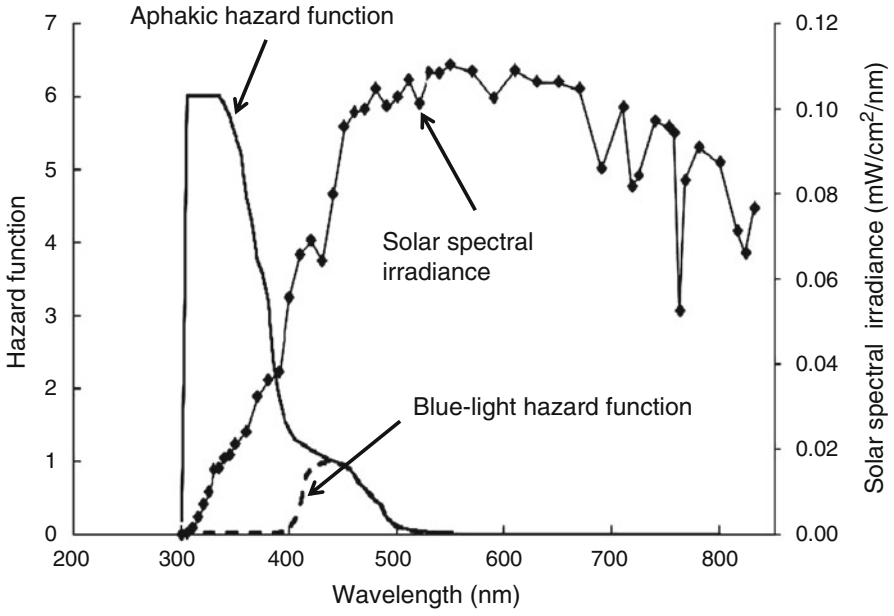


Fig. 15.3 Aphakic hazard function, blue-light hazard function, and solar spectral irradiance. The aphakic hazard function (*solid line*) and the blue-light function (*dashed line*) show the effectiveness of optical radiation in producing photochemical retinal damage as a function of wavelength (*left axis*). The solar spectral irradiance shows the distribution of the radiant power of sunlight over a 37° tilted hemispheric surface with an air mass coefficient of 1.5 (*right axis*). This figure was generated from the data reported in References 19, 20, and 21

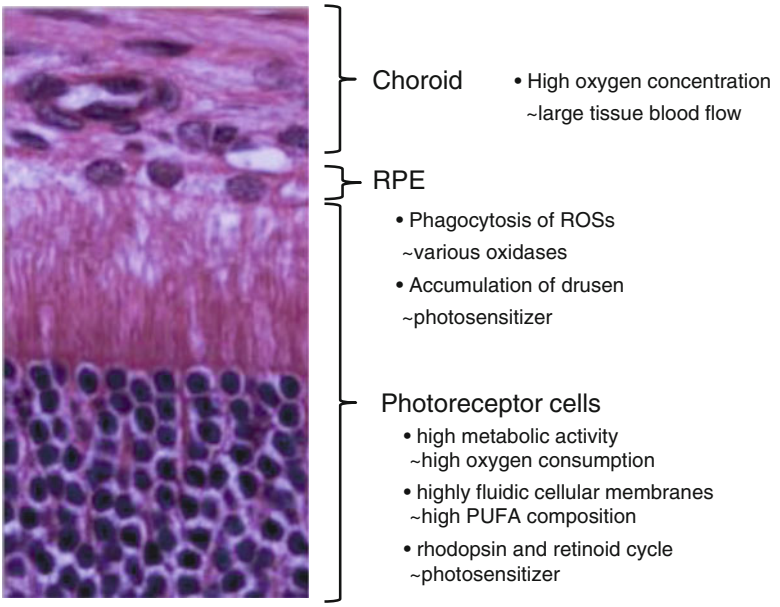


Fig. 15.4 Conditions around the outer retina. Various conditions around the outer retina are suitable for this location to be susceptible to photooxidation damage. *PUFA* polyunsaturated fatty acids

15.2.3 Target of Photooxidative Stress

Across species, photoreceptors are the primary target of retinal damage caused by UV light and the shortest visible violet light (Fig 15.4). With Noell's spectrum of damage, damage is observed in the photoreceptor cells with exposure to light for 1.5–48 h and in both photoreceptors and retinal pigment epithelium (RPE) cells with longer (8–50 days) durations [22], indicating that sustained visual cycle turnover and accumulation of rhodopsin-related molecules including retinoids and intermediate products of the visual cycle are the primary suspects in the initiation of damage [23]. With Ham's spectrum of damage by visible light, the rod and cone photoreceptors are not expected to be primary targets of damage since rhodopsin and cone opsin are breached during a relatively short exposure time. The damage occurs predominantly in the RPE [24], indicating that changes in molecules in the RPE, including lipofuscin, melanin, and intermediate products of the visual cycle, are primary suspects for damage initiation [9]. Photooxidation of visual cycle-related molecules (Fig 15.5), such as all-*trans*-retinal [25], all-*trans*-retinol [26], and all-*trans*-retinyl ester [27], are thought to be sensitizers of retinal damage. Accumulation of all-*trans*-retinal leads to light-induced retinal damage [28], which can be decreased by various types of antioxidants such as ascorbate [29],

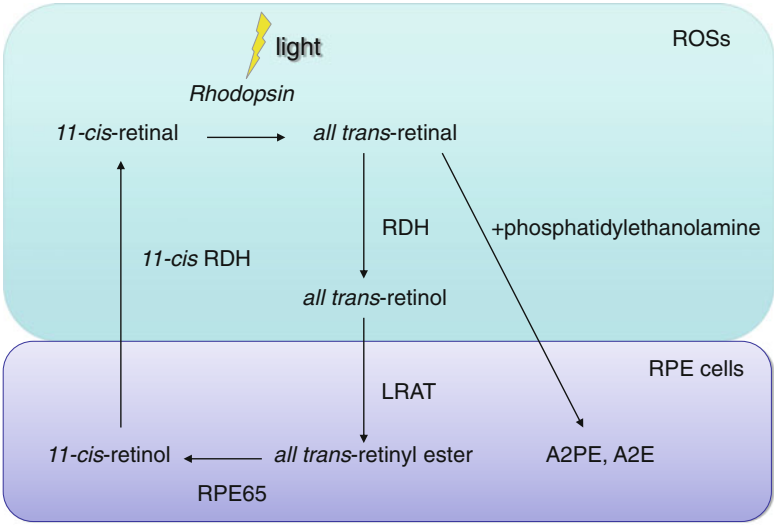


Fig. 15.5 Visual cycle. *RDH* all-*trans*-retinol dehydrogenase, *LRAT* lecithin retinol acyltransferase, *RPE65* retinal pigment epithelium-specific 65 kDa protein, *11-cis RDH* 11-*cis* retinol dehydrogenase, *A2PE* phosphatidyl-pyridinium bisretinoid, *A2E* pyridinium bisretinoid

dimethylthiourea [30], thioredoxin [31, 32], 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPOL) derivatives [33, 34], and phenyl-*N*-*tert*-butylnitrone (PBN) [35, 36] (Fig 15.6). Inhibitors or modulators of the visual cycle prevent retinal light damage [37] and may be therapies for dry age-related macular degeneration (AMD) in humans [38].

15.2.4 Fate of Photoreceptor/RPE Cells in Photooxidative Stress

Although two different types of photooxidative mechanisms have been proposed, dataset analyses in previous studies have not clearly distinguished both types of mechanisms in various experimental settings [8]. In either pathway, the apoptosis is the main pathway of light-induced cell death [39] (Fig 15.7). Apoptosis of the photoreceptors also is thought to occur in retinitis pigmentosa (RP) and AMD. The susceptibility to retinal damage differs greatly among animal species. Generally, albino animals sustain light damage more easily than pigmented animals, and albino rats are more susceptible to retinal light damage than albino mice. Exposure to white fluorescent light at 2,700 lx for 6 h causes devastating apoptotic

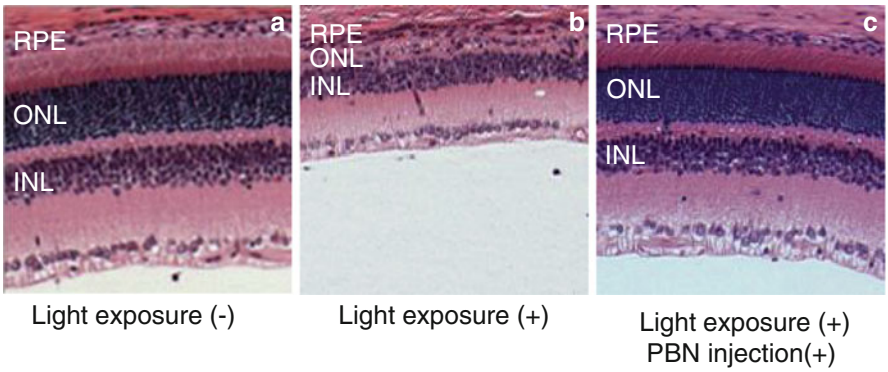


Fig. 15.6 Inhibition of retinal light damage by a free radical scavenger. (**a**) A retinal section from an albino rat not exposed to light. (**b**) A retinal section from a rat exposed to 5 k lx white fluorescent light for 6 h. The eye was enucleated 7 days after light exposure. (**c**) A retinal section from rat preinjected with PBN intraperitoneally 30 min before and then exposed to 5 k lx white fluorescent light for 6 h. The eye was enucleated 7 days after light exposure. *INL* inner nuclear layer

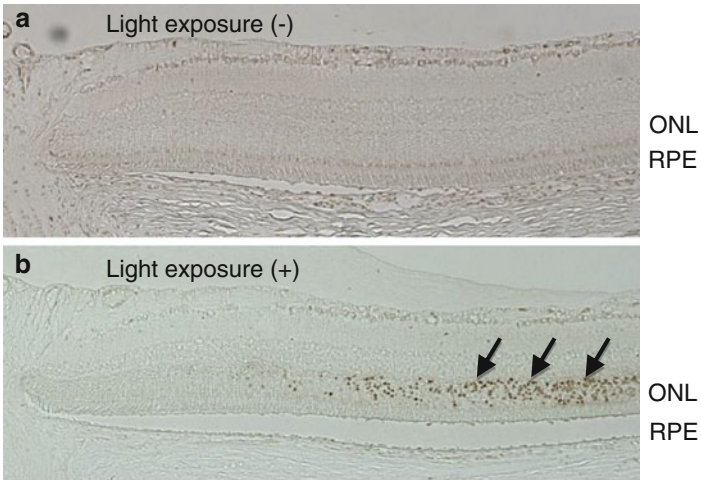


Fig. 15.7 Detection of apoptotic photoreceptor cells after light exposure by terminal deoxynucleotidyl transferase dUTP nick-end labeling (*TUNEL*) staining. (**a**) A retinal section from an albino rat not exposed to light. (**b**) A retinal section from a rat exposed to 5 k lx white fluorescent light for 3 h. The eye was enucleated 24 h after light exposure. TUNEL-positive photoreceptor cells are observed in the ONL (*arrows*)

photoreceptor cell loss in albino rats raised in a dim cyclic light environment [34], whereas exposure to white fluorescent light at 8,000 lx for 2 h only promotes oxidative stress in pigmented mice [40].

15.3 Role of Membrane Fatty Acids and Photooxidative Stress

15.3.1 Fatty Acids in the Retina

N-3 and *n*-6 polyunsaturated fatty acids (PUFAs), which contain two or more methylene interrupted *cis* double bonds, are major families of fatty acids in mammalian cells. Through a series of oxidation (desaturation) and chain elongation reactions, *n*-3 and *n*-6 fatty acids are synthesized from essential fatty acids 18:3*n*-3 and 18:2, respectively. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol comprise, respectively, 40–50 %, 30–35 %, 5–15 %, and 3–6 % of the PUFAs in the retinal photoreceptor phospholipids [41]. Thus, the highest levels of PUFAs in any tissues are in the phospholipids in the retinal membranes. The most abundant retinal fatty acid is docosahexaenoic acid (DHA, 22:6*n*-3); its levels in the rod outer segment (ROS) membrane phospholipids are 40–50 % of the total fatty acids [41]. *n*-3 PUFA deprivation resulting from *n*-3-deficient diets resulted in only modest changes in retinal DHA levels; thus, the retina retains DHA and other *n*-3 PUFAs [42]. More substantial changes were seen during the last trimester of pregnancy and throughout the nursing period when an *n*-3-deficient diet was fed to pregnant rats and when the same diet was given to weaning rats for 10–12 weeks [43]. Under these conditions, the DHA levels in the ROS decreased by 50 % and were replaced by nearly equal amounts of 22:5*n*-6. The electroretinography responses decreased in animals that were DHA deficient, especially the *a*-wave amplitudes in rats [44], guinea pigs [45], and monkeys [46]. *n*-3 deficiency reduces the activation of rhodopsin, reduces and delays rhodopsin-transducin coupling, and decreases cGMP-phosphodiesterase activity in biochemical assays [47].

15.3.2 Fatty Acids: A Molecular Target of Photooxidative Stress

With exposure to damaging light, mice with lower *n*6/*n*3 ratios and higher DHA levels in ROS had greater retinal damage [48], which suggested a positive correlation between the DHA level and retinal vulnerability to photooxidative stress (Fig 15.8). As already mentioned, both types of photooxidative stress are initiated by light absorption by the target molecules. Such chromophores, called

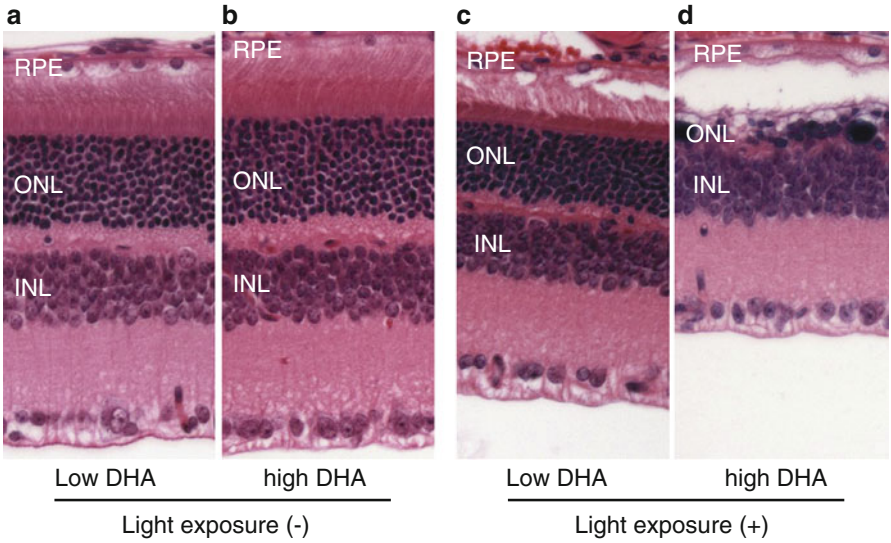


Fig. 15.8 Effect of retinal fatty acid composition on susceptibility to retinal light damage. Albino mice with low (**a**, **c**; *n*-6/*n*-3 PUFA ratio in ROSs, 1.6) or normal DHA (**b**, **d**; *n*-6/*n*-3 PUFA ratio in ROSs, 0.2) levels in the retina are exposed to white fluorescent light for 24 h. The eyes were enucleated 7 days after light exposure. (**a**, **b**) With no light exposure, the difference in the retinal DHA level does not result in marked morphologic changes. A retinal section from an albino rat not exposed to light. (**c**, **d**) After light exposure, more severe loss of the ONL is seen in retinas from mice with high DHA level than those with low DHA levels, suggesting that retinal DHA can be a molecular target of photooxidation. *INL* inner nuclear layer

photosensitizers, are photoexcited by light, undergo intersystem crossing, and produce free radicals. The free radicals then can directly attack the PUFAs and initiate free radical chain reactions that cause lipid peroxidation in cellular membranes and generation of lipid radical species that cause PUFA degradation into oxidized products, including aldehydes. Thus, PUFAs in ROS are target substrates to propagate oxidative stress. Some extremely reactive aldehydes potentially can damage intracellular and extracellular molecules that are a distance from the initial site of free radical attack; this occurs because aldehydes are relatively longer lived than free radicals [49, 50]. Damaging aldehydes include 4-hydroxyalkenals, i.e., 4-hydroxynonenal (4-HNE) and 4-hydroxyhexenal (4-HHE), α, β-unsaturated aldehydes that are end products of lipid peroxidation of PUFAs. 4-HNE forms from *n*-6 PUFAs, such as linoleic acid and AA [51], and 4-HHE forms from *n*-3 PUFAs such as DHA, eicosapentaenoic acid, and linolenic acid [52] via several nonenzymatic steps. These highly reactive aldehydes can react readily with histidine, cysteine, or lysine residues of proteins to form stable Michael adducts with a hemiacetal structure [53]. They exhibit cytopathologic effects, e.g., inhibition of enzyme activity and protein, RNA, and DNA synthesis; cell-cycle arrest; and apoptosis [49]. The aldehyde-modified proteins accumulate in the photoreceptor cells by 3 h after light exposure (Fig. 15.9). The retinal location of these proteins corresponds

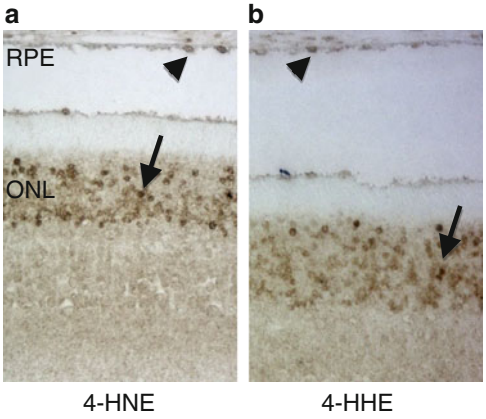


Fig. 15.9 Initiation of reactive aldehyde-modified proteins by damaging light exposure in the retina. The eyes are enucleated 3 h after the 5 k lx white fluorescent light exposure for 3 h. Positive immunoreactivity against 4-HNE- and 4-HHE-modifided proteins is seen in the ONL (*arrows*) and RPE (*arrowheads*) layers. 4-HNE, 4-hydroxynonenal; 4-HHE, 4-hydroxyhexenal

well with the locations with increased terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining at 24 h and severely decreases the outer nuclear layer (ONL) thickness 7 days after exposure. The posttranslational protein modifications caused by these aldehydes occur early before apoptosis and subsequent photoreceptor cellular loss [54].

15.4 Role of Environmental Light and Photooxidative Stress

15.4.1 Light-Adaptation Neuroprotective Phenomenon

Environmental light may regulate the cellular or tissue tolerance to photoreceptor cell damage induced by more intense light exposure. Albino mice and rats raised in bright (300–800 lx) cyclic light were resistant to light (1,700–3,000 lx for 24–72 h)-induced photoreceptor cell apoptosis compared with animals raised in dim (5 lx) cyclic light [55, 56] (Fig. 15.10). There is 6.5 times more DHA compared with palmitic acid in the ROS of rats raised in dim (<10 lx) light and only 0.6 in bright (400 lx) light. This is accompanied by shorter ROS, lower concentrations of rhodopsin, and altered rates of rhodopsin regeneration [56]. Albino rats exhibit a "photostasis phenomenon," by which they adapt to environmental light to capture a constant number of photons daily [57]. Therefore, retinal survival mechanisms against harmful bright light may include control of photon capture and the efficacy of visual transduction through control of the ROS DHA level [58].

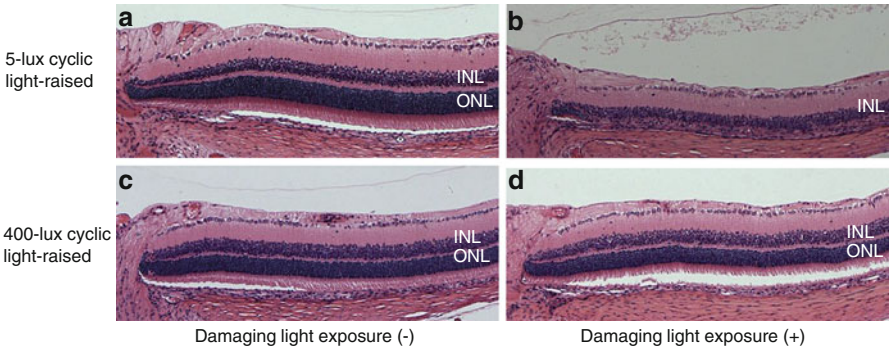


Fig. 15.10 Effect of environmental light on susceptibility to retinal light damage. Albino rats born and raised for 4 weeks in dim (5 lx; **a**, **b**) or bright (400 lx; **c**, **d**) cyclic light are exposed to damaging light (3,000 lx, 6 h; **b**, **d**). The eyes were enucleated 7 days after light exposure (**b**, **d**). (**a**, **c**) Without damaging light exposure, mild loss of the ONL is observed in rats raised in *bright light* (**c**) compared to rats raised in *dim light* (**a**). (**b**, **d**) After damaging light exposure, the ONL thickness is markedly reduced in rats raised in *dim light* (**b**) compared to those raised in *bright light* (**d**), indicating the presence of a light adaptation neuroprotective phenomenon. *INL* inner nuclear layer

15.4.2 Endogenous Defense Mechanisms Against Photooxidative Stress

Rats raised in bright cyclic light had higher retinal levels of endogenous antioxidant enzymes glutathione (GSH) peroxidase [59], GSH reductase [59], GSH S-transferase [59], thioredoxin (Trx) [60], Trx reductase [60], and proteins modified by 4-HNE compared with those raised in dim cyclic light [60]. Rats raised in bright cyclic light also had more DNA binding of transcription factor nuclear-factor-E2-related factor 2 (Nrf2) to the antioxidant-responsive element (ARE) [60]. A sublethal dose of 4-HNE in vitro upregulated the Trx system via the Nrf2-ARE pathway and protected cells from H_2O_2-induced damage. Thus, in the early stages of cellular stress, generation of 4-HNE at low concentrations plays an important role in cell signal transduction and gene expression, and Nrf2-ARE-driven gene regulation is involved in the molecular mechanism of the retinal neuroprotection phenomenon [60]. Trx inducers, e.g., geranylgeranylacetone, an antigastric ulcer drug, or sulforaphane, a component of broccoli sprout, effectively upregulate endogenous Trx in retinal tissues [61, 62]. These compounds mimic an adaptive response mediated by bright cyclic light. Accordingly, modulating the Trx system via activation of the Nrf2/ARE pathway may be a molecular target to prevent photooxidative stress-related retinal diseases such as AMD, RP, and photic maculopathy.

15.5 Light Stress and Retinal Degeneration in Humans

Epidemiologic studies have suggested that excessive light enhances the progression and severity of AMD and some forms of RP [63, 64]. Twelve of 14 publications in a recent meta-analysis reported that light exposure, including longer/intense outdoor activity and sunbathing, can be a risk factor for AMD; six of those articles reported a significant association [65]. Hirakawa et al. measured the extent facial wrinkles, which is associated with a history of sunlight exposure, in patients with age-related maculopathy (ARM) [66] and found that the wrinkles were more severe in patients with late ARM than in those with early ARM. This suggested that lifetime sunlight exposure is an important factor in the progression of late ARM. Subretinal accumulation of drusen is a major risk factor for development of AMD (Fig. 15.11). Drusen, which contains esterified cholesterol-rich, apolipoprotein B-containing lipoprotein particles, are constitutively produced by the RPE [67] and modified bisretinoid (A2E), which forms because of a reaction between all-*trans*-retinal and phosphatidylethanolamine [68]. Thus, drusen may be a waste product of RPE

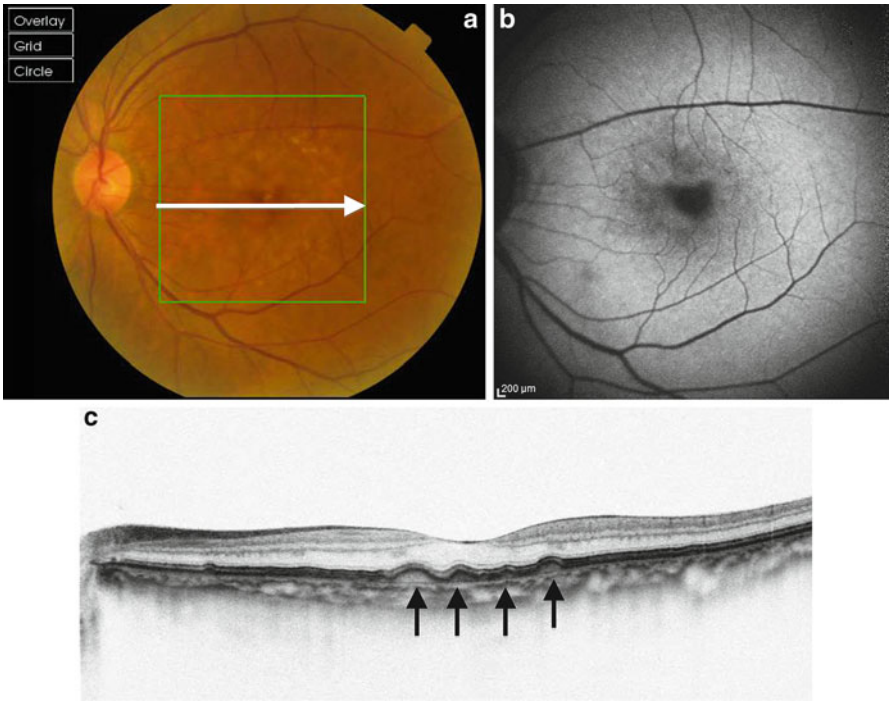


Fig. 15.11 Accumulation of drusen in AMD. Fundus photograph (**a**), fundus autofluorescence image (**b**), and optical coherence tomography (**c**) image of human macular degeneration show intense subretinal accumulations of yellowish-white autofluorescent-positive drusen under the RPE layer (**c**, *arrows*). The *arrow* in (**a**) indicates the scanning direction in (**c**)

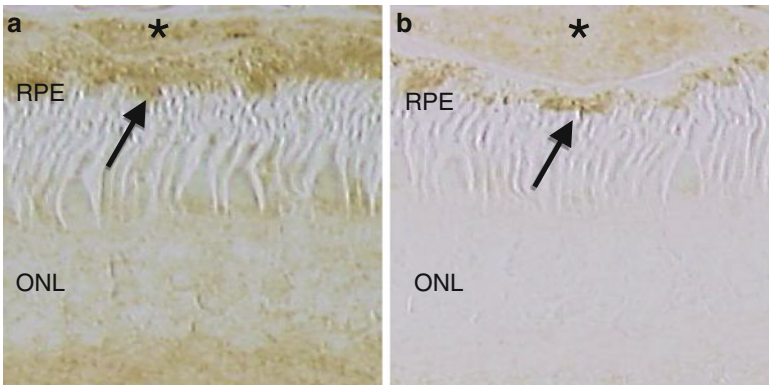


Fig. 15.12 Detection of reactive aldehyde-modified proteins in monkey drusen. Positive immunoreactivity against 4-HNE-modified (**a**) and 4-HHE-modified (**b**) proteins is seen in the RPE (*arrows*) and drusen (*asterisks*)

phagocytosis and retinoid cycle (Fig. 15.5). A proteomic approach to the study of damaging light-exposed rat retinal specimens found that intense light exposure increased 4-HNE-protein modifications in specific retinal proteins from several functional categories including energy metabolism, glycolysis, chaperone, phototransduction, and RNA processing [69]. Many of these proteins are common components among the accumulated proteins in drusen from monkey [70] (Fig. 15.12) and human [71] eyes. Accordingly, drusen form as a result of subretinal/RPE accumulation of degenerated macromolecules such as proteins and lipids, and photooxidation of fatty acids in photoreceptor cells triggers these pathological steps (Fig. 15.13).

15.6 Filtering of Short-Wavelength Visible Light

15.6.1 Presence of Macular Pigment and Supplemental Lutein/Zeaxanthin

The retinal defense mechanism against photooxidative stress includes superoxide dismutase, catalase, glutathione peroxidase, and vitamins E and C. The pigment concentrated in the macula lutea also is part of this defense system (Fig. 15.14). In the 1980s, the carotenoids lutein ((3R3′R,6′R)-lutein), zeaxanthin ((3R,3′R)-zeaxanthin), and meso-zeaxanthin ((3R,3′S;meso)-zeaxanthin) were identified as the primary components in macular pigment [72, 73]. Meso-zeaxanthin, a stereoisomer of zeaxanthin not in the natural diet, is synthesized enzymatically in the retina [74].

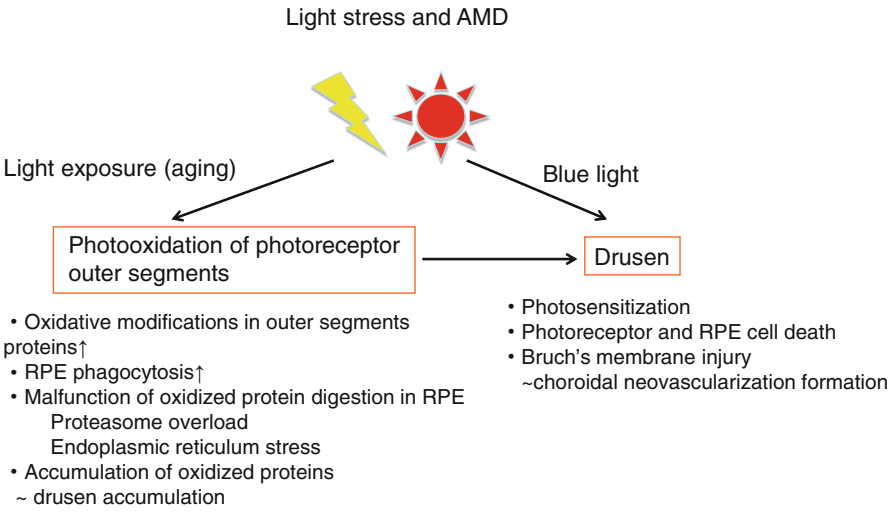


Fig. 15.13 Possible mechanisms between chronic light exposure and AMD. Lifelong exposure to environmental light causes malfunction of the RPE cell machinery of the photoreceptor outer segment waste, resulting in drusen accumulation. Accumulated drusen can act as a photosensitizer of *blue light*, which enhances photoreceptor and RPE cell damage via photooxidative stress

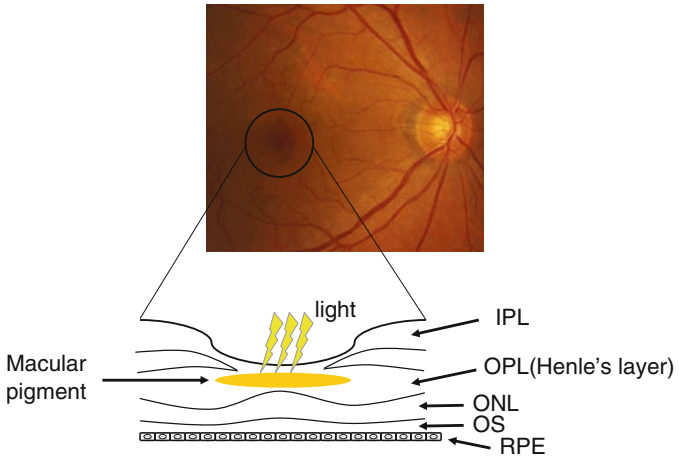


Fig. 15.14 Schematic distribution of macular pigments in a human macula. The highest concentration of macular pigments (*yellow oval*) accumulates in the light path to the foveal photoreceptor cells. *IPL* inner plexiform layer, *OPL* outer plexiform layer, *OS* outer segments

These three carotenoids absorb blue light and act as a filter that may attenuate photochemical damage from short-wavelength visible light, since yellow filters blue from white light (Fig. 15.15). These carotenoids also are antioxidants that may protect against light-induced oxidative retinal damage by quenching oxygen

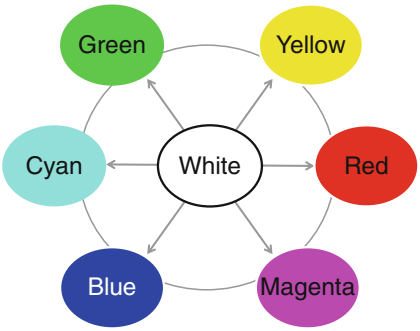


Fig. 15.15 Color circle. A pair of opposite ends of each diameters of the circle are complimentary colors. *Yellow* specifically filters *blue* from *white* light

radicals [75, 76]. A pathological study of autopsy eyes [77], measurements of macular pigment optical density (MPOD) by resonance Raman spectroscopy [78, 79], and other methods [80] have shown that the concentration of macular pigment in patients with AMD is significantly lower compared with normal, healthy eyes. The MPOD levels decrease in light irises and in association with tobacco smoking, low uptake of lutein and zeaxanthin, excessive light exposure [81–83], and aging [78, 79, 84]. Growing evidence has suggested that intake of these carotenoids protects against visual loss from AMD [85–88], although a large-scale randomized control study failed to find a positive effect of adding lutein/zeaxanthin to a nutrient formulation that included vitamins C and E, beta carotene, and zinc in reducing the risk of progression to advanced AMD [89].

15.6.2 Yellowing of Crystalline Lens with Aging and Implantation of Yellow-Tinted Intraocular Lens During Cataract Surgery

The absorption properties of the cornea and crystalline lens help protect the retina from light damage. Yellowing of the crystalline lens with aging causes a progressive increase in absorbance of visible light in the blue range (Fig. 15.16); thus, the aging human crystalline lens also blocks phototoxic blue light [90–92]. Removing the crystalline lens during cataract surgery increases the amount of optical radiation that reaches the retina, and implanting an intraocular lens (IOL) lowers the ocular defenses against photic retinopathy [93]. IOLs with UVR-blocking dye bonded to optic polymers were introduced in the early 1980s [93] and are commonly implanted during cataract surgery. The transmission properties of the colorless UVR-blocking IOLs may not be comparable to those of the aging crystalline lens in absorbance of blue light, which causes Ham's-type retinal phototoxicity [14]. Epidemiologic studies have suggested that the odds ratios of AMD prevalence are 1.7–3.8 in eyes after cataract surgery [94–96] (Fig. 15.17). In prospective studies that spanned 5–10 years, the Beaver Dam Eye Study and Blue Mountains

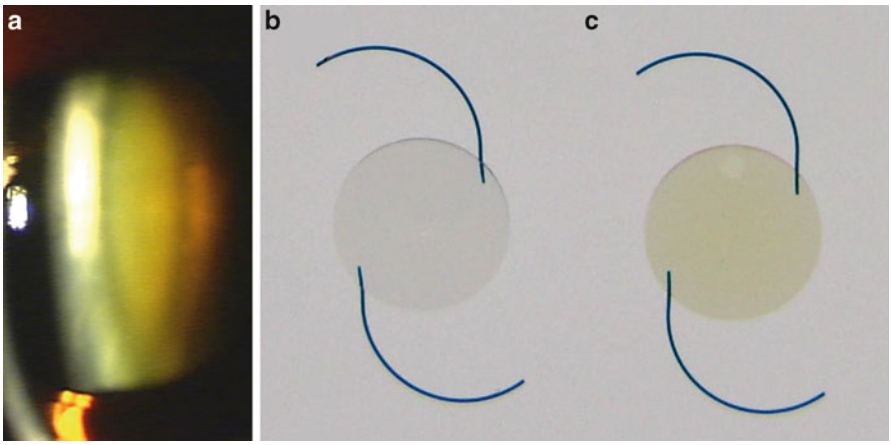


Fig. 15.16 Cataract and IOLs. (**a**) Yellowing of the crystalline lens with aging. (**b**) UVR-blocking clear IOL. (**c**) UVR- and blue-light-blocking yellow-tinted IOL

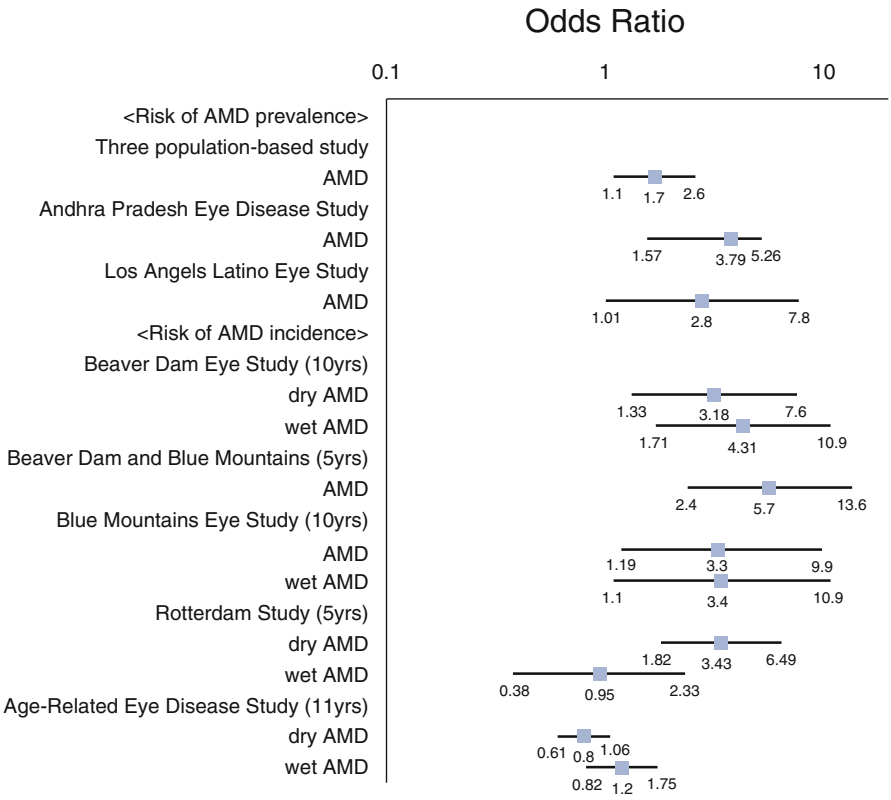


Fig. 15.17 Risk of cataract surgery in AMD prevalence and incidence. Each *bar* indicates the 95 % confidence interval of the odds ratio

Eye Study reported a significant relation between cataract surgery and increased risk of AMD [97–100]. However, the Age-Related Eye Disease Study did not find a significant association between cataract surgery and development of the dry- or wet-type of AMD [101] (Fig. 15.17). To compensate for reduced filtering of blue light by the colorless UVR-blocking IOLs, blue-light and UVR-absorbing yellow-tinted IOLs made of rigid polymethylmethacrylate were introduced in the 1990s [102]; later these were made of foldable silicone or soft acrylic material (Fig. 15.16). Yellow-tinted IOLs that were evaluated in experimental studies confer a protective effect against retinal photooxidative stress in photosensitizing A2E-laden RPE cells exposed to blue light [103] and artificial sunlight [104] in vitro and in rats [105], mice [106], and rabbits [107] exposed to blue or white light in vivo. Obana et al. reported higher levels of macular pigment in eyes implanted with yellow-tinted IOL than in eyes implanted with clear IOLs 1 year and longer after cataract surgery [108]. A large clinical trial should evaluate the effects of blue-blocking filters on AMD development and progression and identify the best balance of filtering spectra for visual function.

Disclosure Chapters 3, 4, and 5 include text extracts from Reference [109] that were used with permission of Future Medicine Ltd.

References

1. Youssef PN, Sheibani N, Albert DM (2011) Retinal light toxicity. Eye (Lond) 25:1–14
2. Crochet JJ, Gnyawali SC, Chen Y, Lemley EC, Wang LV, Chen WR (2006) Temperature distribution in selective laser-tissue interaction. J Biomed Optics 11:34031
3. Birngruber R, Gabel VP, Hillenkamp F (1983) Experimental studies of laser thermal retinal injury. Health Phys 44:519–531
4. Birngruber R, Gabel VP (1983) Thermal versus photochemical damage in the retina–thermal calculations for exposure limits. Trans Ophthalmol Soc UK 103(Pt 4):422–427
5. Vogel A, Capon MR, Asiyo-Vogel MN, Birngruber R (1994) Intraocular photodisruption with picosecond and nanosecond laser pulses: tissue effects in cornea, lens, and retina. Invest Ophthalmol Vis Sci 35:3032–3044
6. Vogel A, Busch S, Jungnickel K, Birngruber R (1994) Mechanisms of intraocular photodisruption with picosecond and nanosecond laser pulses. Laser Surg Med 15:32–43
7. Noell WK, Walker VS, Kang BS, Berman S (1966) Retinal damage by light in rats. Invest Ophthalmol 5:450–473
8. van Norren D, Gorgels TG (2011) The action spectrum of photochemical damage to the retina: a review of monochromatic threshold data. Photochem Photobiol 87:747–753
9. Hunter JJ, Morgan JI, Merigan WH, Sliney DH, Sparrow JR, Williams DR (2012) The susceptibility of the retina to photochemical damage from visible light. Prog Retin Eye Res 31:28–42
10. Reme CE (2005) The dark side of light: rhodopsin and the silent death of vision the proctor lecture. Invest Ophthalmol Vis Sci 46:2671–2682
11. Sperling HG, Harwerth RS (1971) Red-green cone interactions in the increment-threshold spectral sensitivity of primates. Science 172:180–184
12. Penn JS (1985) Effects of continuous light on the retina of a fish, *Notemigonus crysoleucas*. J Comp Neurol 238:121–127

13. Machida M, Dubousset J, Imamura Y et al (1994) Pathogenesis of idiopathic scoliosis: SEPs in chicken with experimentally induced scoliosis and in patients with idiopathic scoliosis. J Pediatr Orthop B 14:329–335
14. Ham WT Jr, Mueller HA, Sliney DH (1976) Retinal sensitivity to damage from short wavelength light. Nature 260:153–155
15. Hoppeler T, Hendrickson P, Dietrich C, Reme C (1988) Morphology and time-course of defined photochemical lesions in the rabbit retina. Curr Eye Res 7:849–860
16. van Norren D, Schellekens P (1990) Blue light hazard in rat. Vision Res 30:1517–1520
17. Gorgels TG, van Norren D (1995) Ultraviolet and green light cause different types of damage in rat retina. Invest Ophthalmol Vis Sci 36:851–863
18. Collier RJ, Waldron WR, Zigman S (1989) Temporal sequence of changes to the gray squirrel retina after near-UV exposure. Invest Ophthalmol Vis Sci 30:631–637
19. American_Conference_of_Governmental_Industrial_Hygienists (2008) Light and near-infrared radiation. TLVs and BEIs Cincinnati, USA, pp 1–7
20. ICNIRP_Guidelines (International Commission on Non-Ionizing Radiation Protection) (1997) Guidelines on limits of exposure to broad-band incoherent optical radiation (0.38 to 3 microM). Health Phys 73:539–554
21. American_Society_for_Testing_and_Materials (1998) ASTM G159-98 standard tables for references solar spectral irradiance at air mass 1.5: direct normal and hemispherical for a 37° tilted surface. http://www.astm.org/
22. Noell WK (1980) Possible mechanisms of photoreceptor damage by light in mammalian eyes. Vision Res 20:1163–1171
23. Maeda A, Maeda T, Imanishi Y et al (2006) Retinol dehydrogenase (RDH12) protects photoreceptors from light-induced degeneration in mice. J Biol Chem 281:37697–37704
24. Ham WT Jr, Ruffolo JJ Jr, Mueller HA, Clarke AM, Moon ME (1978) Histologic analysis of photochemical lesions produced in rhesus retina by short-wave-length light. Invest Ophthalmol Vis Sci 17:1029–1035
25. Delmelle M (1977) Retinal damage by light: possible implication of singlet oxygen. Biophys Struct Mech 3:195–198
26. Noell WK, Albrecht R (1971) Irreversible effects on visible light on the retina: role of vitamin A. Science 172:76–79
27. Lamb LE, Zareba M, Plakoudas SN, Sarna T, Simon JD (2001) Retinyl palmitate and the blue-light-induced phototoxicity of human ocular lipofuscin. Arch Biochem Biophys 393:316–320
28. Maeda A, Maeda T, Golczak M et al (2009) Involvement of all-*trans*-retinal in acute light-induced retinopathy of mice. J Biol Chem 284:15173–15183
29. Organisciak DT, Wang HM, Li ZY, Tso MO (1985) The protective effect of ascorbate in retinal light damage of rats. Invest Ophthalmol Vis Sci 26:1580–1588
30. Organisciak DT, Darrow RM, Jiang YI, Marak GE, Blanks JC (1992) Protection by dimethylthiourea against retinal light damage in rats. Invest Ophthalmol Vis Sci 33:1599–1609
31. Tanito M, Masutani H, Nakamura H, Ohira A, Yodoi J (2002) Cytoprotective effect of thioredoxin against retinal photic injury in mice. Invest Ophthalmol Vis Sci 43:1162–1167
32. Tanito M, Masutani H, Nakamura H, Oka S, Ohira A, Yodoi J (2002) Attenuation of retinal photooxidative damage in thioredoxin transgenic mice. Neurosci Lett 326:142–146
33. Tanito M, Li F, Anderson RE (2010) Protection of retinal pigment epithelium by OT-551 and its metabolite TEMPOL-H against light-induced damage in rats. Exp Eye Res 91:111–114
34. Tanito M, Li F, Elliott MH, Dittmar M, Anderson RE (2007) Protective effect of TEMPOL derivatives against light-induced retinal damage in rats. Invest Ophthalmol Vis Sci 48:1900–1905
35. Ranchon I, LaVail MM, Kotake Y, Anderson RE (2003) Free radical trap phenyl-*N*-tert-butylnitrone protects against light damage but does not rescue P23H and S334ter rhodopsin transgenic rats from inherited retinal degeneration. J Neurosci 23:6050–6057

36. Tomita H, Kotake Y, Anderson RE (2005) Mechanism of protection from light-induced retinal degeneration by the synthetic antioxidant phenyl-*N*-tert-butylnitrone. Invest Ophthalmol Vis Sci 46:427–434
37. Maeda A, Maeda T, Golczak M et al (2006) Effects of potent inhibitors of the retinoid cycle on visual function and photoreceptor protection from light damage in mice. Mol Pharmacol 70:1220–1229
38. Kubota R, Boman NL, David R, Mallikaarjun S, Patil S, Birch D (2012) Safety and effect on rod function of ACU-4429, a novel small-molecule visual cycle modulator. Retina 32:183–188
39. Hafezi F, Steinbach JP, Marti A et al (1997) The absence of c-fos prevents light-induced apoptotic cell death of photoreceptors in retinal degeneration in vivo. Nat Med 3:346–349
40. Tanito M, Nishiyama A, Tanaka T et al (2002) Change of redox status and modulation by thiol replenishment in retinal photooxidative damage. Invest Ophthalmol Vis Sci 43:2392–2400
41. Fliesler SJ, Anderson RE (1983) Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res 22:79–131
42. Anderson RE, Maude MB (1972) Lipids of ocular tissues. 8. The effects of essential fatty acid deficiency on the phospholipids of the photoreceptor membranes of rat retina. Arch Biochem Biophys 151:270–276
43. Wiegand RD, Koutz CA, Chen H, Anderson RE (1995) Effect of dietary fat and environmental lighting on the phospholipid molecular species of rat photoreceptor membranes. Exp Eye Res 60:291–306
44. Wheeler TG, Benolken RM, Anderson RE (1975) Visual membranes: specificity of fatty acid precursors for the electrical response to illumination. Science 188:1312–1314
45. Weisinger HS, Vingrys AJ, Sinclair AJ (1996) Effect of dietary *n*-3 deficiency on the electroretinogram in the guinea pig. Ann Nutr Metab 40:91–98
46. Neuringer M, Connor WE, Lin DS, Anderson GJ, Barstad L (1991) Dietary omega-3 fatty acids: effects on retinal lipid composition and function in primates. In: Anderson RE, Hollyfield JG, LaVail MM (eds) Retinal degenerations. CRC, New York, pp 1–13
47. Niu SL, Mitchell DC, Lim SY et al (2004) Reduced G protein-coupled signaling efficiency in retinal rod outer segments in response to *n*-3 fatty acid deficiency. J Biol Chem 279:31098–31104
48. Tanito M, Brush RS, Elliott MH, Wicker LD, Henry KR, Anderson RE (2009) High levels of retinal membrane docosahexaenoic acid increase susceptibility to stress-induced degeneration. J Lipid Res 50:807–819
49. Awasthi YC, Yang Y, Tiwari NK et al (2004) Regulation of 4-hydroxynonenal-mediated signaling by glutathione S-transferases. Free Radic Biol Med 37:607–619
50. Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 11:81–128
51. Esterbauer H (1993) Cytotoxicity and genotoxicity of lipid-oxidation products. Am J Clin Nutr 57:779S–785S, Discussion 785S–786S
52. Van Kuijk FJ, Holte LL, Dratz EA (1990) 4-Hydroxyhexenal: a lipid peroxidation product derived from oxidized docosahexaenoic acid. Biochim Biophys Acta 1043:116–118
53. Uchida K, Stadtman ER (1992) Modification of histidine residues in proteins by reaction with 4-hydroxynonenal. Proc Natl Acad Sci USA 89:4544–4548
54. Tanito M, Elliott MH, Kotake Y, Anderson RE (2005) Protein modifications by 4-hydroxynonenal and 4-hydroxyhexenal in light-exposed rat retina. Invest Ophthalmol Vis Sci 46:3859–3868
55. Kaldi I, Martin RE, Huang H, Brush RS, Morrison KA, Anderson RE (2003) Bright cyclic rearing protects albino mouse retina against acute light-induced apoptosis. Mol Vis 9:337–344
56. Penn JS, Anderson RE (1987) Effect of light history on rod outer-segment membrane composition in the rat. Exp Eye Res 44:767–778

57. Penn JS, Williams TP (1986) Photostasis: regulation of daily photon-catch by rat retinas in response to various cyclic illuminances. Exp Eye Res 43:915–928
58. Anderson RE, Penn JS (2004) Environmental light and heredity are associated with adaptive changes in retinal DHA levels that affect retinal function. Lipids 39:1121–1124
59. Penn JS, Naash MI, Anderson RE (1987) Effect of light history on retinal antioxidants and light damage susceptibility in the rat. Exp Eye Res 44:779–788
60. Tanito M, Agbaga MP, Anderson RE (2007) Upregulation of thioredoxin system via Nrf2-antioxidant responsive element pathway in adaptive-retinal neuroprotection in vivo and in vitro. Free Radic Biol Med 42:1838–1850
61. Tanito M, Kwon YW, Kondo N et al (2005) Cytoprotective effects of geranylgeranylacetone against retinal photooxidative damage. J Neurosci 25:2396–2404
62. Tanito M, Masutani H, Kim YC, Nishikawa M, Ohira A, Yodoi J (2005) Sulforaphane induces thioredoxin through the antioxidant-responsive element and attenuates retinal light damage in mice. Invest Ophthalmol Vis Sci 46:979–987
63. Cideciyan AV, Hood DC, Huang Y et al (1998) Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. Proc Natl Acad Sci USA 95:7103–7108
64. Cruickshanks KJ, Klein R, Klein BE (1993) Sunlight and age-related macular degeneration. Beaver Dam Eye Study Arch Ophthalmol 111:514–518
65. Sui GY, Liu GC, Liu GY et al (2013) Is sunlight exposure a risk factor for age-related macular degeneration? A systematic review and meta-analysis. Br J Ophthalmol 97:389–394
66. Hirakawa M, Tanaka M, Tanaka Y et al (2008) Age-related maculopathy and sunlight exposure evaluated by objective measurement. Br J Ophthalmol 92:630–634
67. Curcio CA, Johnson M, Huang JD, Rudolf M (2009) Aging, age-related macular degeneration, and the response-to-retention of apolipoprotein B-containing lipoproteins. Prog Retin Eye Res 28:393–422
68. Sparrow JR, Wu Y, Kim CY, Zhou J (2010) Phospholipid meets all-*trans*-retinal: the making of RPE bisretinoids. J Lipid Res 51:247–261
69. Tanito M, Haniu H, Elliott MH, Singh AK, Matsumoto H, Anderson RE (2006) Identification of 4-hydroxynonenal-modified retinal proteins induced by photooxidative stress prior to retinal degeneration. Free Radic Biol Med 41:1847–1859
70. Umeda S, Suzuki MT, Okamoto H et al (2005) Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of macular degeneration in cynomolgus monkey (Macaca fascicularis). FASEB J 19:1683–1685
71. Crabb JW, Miyagi M, Gu X et al (2002) Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci USA 99:14682–14687
72. Bone RA, Landrum JT, Tarsis SL (1985) Preliminary identification of the human macular pigment. Vision Res 25:1531–1535
73. Snodderly DM, Brown PK, Delori FC, Auran JD (1984) The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. Invest Ophthalmol Vis Sci 25:660–673
74. Johnson EJ, Neuringer M, Russell RM, Schalch W, Snodderly DM (2005) Nutritional manipulation of primate retinas, III: Effects of lutein or zeaxanthin supplementation on adipose tissue and retina of xanthophyll-free monkeys. Invest Ophthalmol Vis Sci 46:692–702
75. Rapp LM, Maple SS, Choi JH (2000) Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. Invest Ophthalmol Vis Sci 41:1200–1209
76. Sommerburg OG, Siems WG, Hurst JS, Lewis JW, Kliger DS, van Kuijk FJ (1999) Lutein and zeaxanthin are associated with photoreceptors in the human retina. Curr Eye Res 19:491–495

77. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE (2001) Macular pigment in donor eyes with and without AMD: a case-control study. Invest Ophthalmol Vis Sci 42:235–240
78. Bernstein PS, Gellermann W (2002) Measurement of carotenoids in the living primate eye using resonance Raman spectroscopy. Methods Mol Biol 196:321–329
79. Obana A, Hiramitsu T, Gohto Y et al (2008) Macular carotenoid levels of normal subjects and age-related maculopathy patients in a Japanese population. Ophthalmology 115:147–157
80. Berendschot TT, Willemse-Assink JJ, Bastiaanse M, de Jong PT, van Norren D (2002) Macular pigment and melanin in age-related maculopathy in a general population. Invest Ophthalmol Vis Sci 43:1928–1932
81. Beatty S, Boulton M, Henson D, Koh HH, Murray IJ (1999) Macular pigment and age related macular degeneration. Br J Ophthalmol 83:867–877
82. Hammond BR Jr, Caruso-Avery M (2000) Macular pigment optical density in a Southwestern sample. Invest Ophthalmol Vis Sci 41:1492–1497
83. Mellerio J, Ahmadi-Lari S, van Kuijk F, Pauleikhoff D, Bird A, Marshall J (2002) A portable instrument for measuring macular pigment with central fixation. Curr Eye Res 25:37–47
84. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME (2001) Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. Invest Ophthalmol Vis Sci 42:439–446
85. Krinsky NI, Johnson EJ (2005) Carotenoid actions and their relation to health and disease. Mol Aspects Med 26:459–516
86. Krinsky NI, Landrum JT, Bone RA (2003) Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr 23:171–201
87. Landrum JT, Bone RA (2001) Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 385:28–40
88. SanGiovanni JP, Chew EY, Clemons TE et al (2007) The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. Arch Ophthalmol 125:1225–1232
89. Age-Related Eye Disease Study 2 Research Group (2013) Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the age-related eye disease study 2 (AREDS2) randomized clinical trial. JAMA 309:2005–2015
90. Bron AJ, Vrensen GF, Koretz J, Maraini G, Harding JJ (2000) The ageing lens. Ophthalmologica 214:86–104
91. Mellerio J (1987) Yellowing of the human lens: nuclear and cortical contributions. Vision Res 27:1581–1587
92. Weale RA (1988) Age and the transmittance of the human crystalline lens. J Physiol 395:577–587
93. Mainster MA, Sparrow JR (2003) How much blue light should an IOL transmit? Br J Ophthalmol 87:1523–1529
94. Fraser-Bell S, Choudhury F, Klein R, Azen S, Varma R (2010) Ocular risk factors for age-related macular degeneration: the Los Angeles latino eye study. Am J Ophthalmol 149:735–740
95. Freeman EE, Munoz B, West SK, Tielsch JM, Schein OD (2003) Is there an association between cataract surgery and age-related macular degeneration? Data from three population-based studies. Am J Ophthalmol 135:849–856
96. Krishnaiah S, Das T, Nirmalan PK et al (2005) Risk factors for age-related macular degeneration: findings from the Andhra Pradesh eye disease study in South India. Invest Ophthalmol Vis Sci 46:4442–4449
97. Cugati S, Mitchell P, Rochtchina E, Tan AG, Smith W, Wang JJ (2006) Cataract surgery and the 10-year incidence of age-related maculopathy: the Blue Mountains Eye Study. Ophthalmology 113:2020–2025
98. Ho L, Boekhoorn SS, Liana et al (2008) Cataract surgery and the risk of aging macula disorder: the rotterdam study. Invest Ophthalmol Vis Sci 49:4795–4800

99. Klein R, Klein BE, Wong TY, Tomany SC, Cruickshanks KJ (2002) The association of cataract and cataract surgery with the long-term incidence of age-related maculopathy: the Beaver Dam eye study. Arch Ophthalmol 120:1551–1558
100. Wang JJ, Klein R, Smith W, Klein BE, Tomany S, Mitchell P (2003) Cataract surgery and the 5-year incidence of late-stage age-related maculopathy: pooled findings from the Beaver Dam and Blue Mountains eye studies. Ophthalmology 110:1960–1967
101. Chew EY, Sperduto RD, Milton RC et al (2009) Risk of advanced age-related macular degeneration after cataract surgery in the Age-Related Eye Disease Study: AREDS report 25. Ophthalmology 116:297–303
102. Niwa K, Yoshino Y, Okuyama F, Tokoro T (1996) Effects of tinted intraocular lens on contrast sensitivity. Ophthalmic Physiol Opt 16:297–302
103. Sparrow JR, Miller AS, Zhou J (2004) Blue light-absorbing intraocular lens and retinal pigment epithelium protection in vitro. J Cataract Refract Surg 30:873–878
104. Mukai K, Matsushima H, Sawano M, Nobori H, Obara Y (2009) Photoprotective effect of yellow-tinted intraocular lenses. Jpn J Ophthalmol 53:47–51
105. Tanito M, Kaidzu S, Anderson RE (2006) Protective effects of soft acrylic yellow filter against blue light-induced retinal damage in rats. Exp Eye Res 83:1493–1504
106. Kurihara T, Omoto M, Noda K et al (2009) Retinal phototoxicity in a novel murine model of intraocular lens implantation. Mol Vis 15:2751–2761
107. Nilsson SE, Textorius O, Andersson BE, Swenson B (1989) Clear PMMA versus yellow intraocular lens material. An electrophysiologic study on pigmented rabbits regarding "the blue light hazard.". Prog Clin Biol Res 314:539–553
108. Obana A, Tanito M, Gohto Y, Gellermann W, Okazaki S, Ohira A (2011) Macular pigment changes in pseudophakic eyes quantified with resonance raman spectroscopy. Ophthalmology 118:1852–1858
109. Tanito M, Anderson RE (2009) Dual roles of polyunsaturated fatty acids in retinal physiology and pathophysiology associated with retinal degeneration. Clin Lipidol 4:1838–1850

Chapter 16
Roles of the Retinal Pigment Epithelium in Neuroprotection

Atsuhiro Kanda and Susumu Ishida

Abstract The retinal pigment epithelium (RPE), located between the photoreceptors and Bruch's membrane, is a vital tissue for the maintenance of photoreceptor functions. Numerous proteins expressed in the RPE regulate the transport of nutrients and waste products to and from the photoreceptors, which contributes to phagocytosis of shed photoreceptor outer segments and protects from the excessive light and oxygen reactive species to maintain retinal homeostasis. During aging, the RPE is observed to undergo characteristic changes including cell loss, loss of intact melanin granules, metabolic changes, and the intracellular accumulation of lipofuscin. Those changes adversely affect the RPE functions and associate with various diseases of the neural retina and the choroid. Given a number of studies reporting the RPE functions which serve for the retina and the choroid, it is essential to understand the physiological functions of the RPE in order to decipher disease- and age-related changes in vision.

Keywords Neuroprotection • Retina • Retinal pigment epithelium

16.1 Introduction

The retinal pigment epithelium (RPE) consists of highly polarized and specialized single-layered epithelial cells, sandwiched between the neural retina and the choroid, and plays a key role in the maintenance and survival of photoreceptors. It is a low cuboidal epithelium containing long thin and sheetlike microvilli on its apical surface that faces the tips of the photoreceptor outer segments [1, 2]. The RPE

A. Kanda • S. Ishida (✉)
Laboratory of Ocular Cell Biology and Visual Science, Department of Ophthalmology, Graduate School of Medicine, Hokkaido University, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan
e-mail: ishidasu@med.hokudai.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_16,

supports the long-term preservation of retinal integrity and visual functions including absorption of stray light, supply of nutrients, secretion of growth factors, regeneration of visual pigment, and phagocytosis of the photoreceptor-shed outer segments. In the human eye, there are approximately five million RPE cells, and the cell density increases steadily in the macular area during development. One RPE cell maintenances about 45 photoreceptors, which shed daily ~5 % of their outer segment mass [1]. Several factors (e.g., aging, inflammation, oxidative stress, lipid metabolism, and genetic susceptibility variants) affect fundamental functions of the RPE and lead to the impairment of retinal cells. Those processes contribute to the pathogenesis of retinal diseases such as retinitis pigmentosa, proliferative vitreoretinopathy, Stargardt's disease, Leber's congenital amaurosis, and age-related macular degeneration (AMD). In the following sections these functions will be described in more detail.

16.2 Cellular Organization of the RPE

The RPE positions a functionally important site in the eye, located between the light-sensitive outer segments of the photoreceptors and blood supply of the choroid (Fig. 16.1a, b). It is a hexagonally packed monolayer sheet of cells containing pigment granules. The interlocking hexagonal cells are bound together with tight junctions, which block the free passage of water and ions [2]. This junction barrier is the comparable of the blood-retinal barrier formed by the capillary endothelium of the intrinsic retinal vasculature. The number of photoreceptors that overlie RPE cells is about 30–50 photoreceptors per RPE cell, and each RPE cell is metabolically responsible for providing support and maintenance functions to the overlying photoreceptors [1].

RPE cells have a characteristic organelle distribution and cellular appearance (Fig. 16.1c, d). The RPE cell cytoplasm contains largely smooth and relatively little rough endoplasmic reticulum, a characteristic of cells actively involved in lipid metabolism. In RPE cells, the Golgi complex, with a function critical for the maintenance of cell polarity, is small and difficult to distinguish from the other tubules and vesicles of the endoplasmic reticulum. The majority of mitochondria are located basally near the nucleus and close to the basal infoldings of the plasma membrane (Fig. 16.1d). Catalase-containing microperoxisomes that function in the conversion of hydrogen peroxide to water are abundant in RPE cells [3].

The RPE, which derives from the same neural anlage as the neural retina, is developmentally the first pigmented tissue in the body, and melanogenesis continues throughout life [4]. However, many melanin granules during aging frequently fuse with lysosomes and break down. As a result, the elderly fundus typically appears less pigmented [5]. The pigment absorbs stray light and minimizes scatter within the eye and protects the retinas from oxidative stress and detoxification of peroxides. The RPE possesses two types of pigment, melanin and lipofuscin. Melanin, an insoluble high-molecular-weight polymer derived from

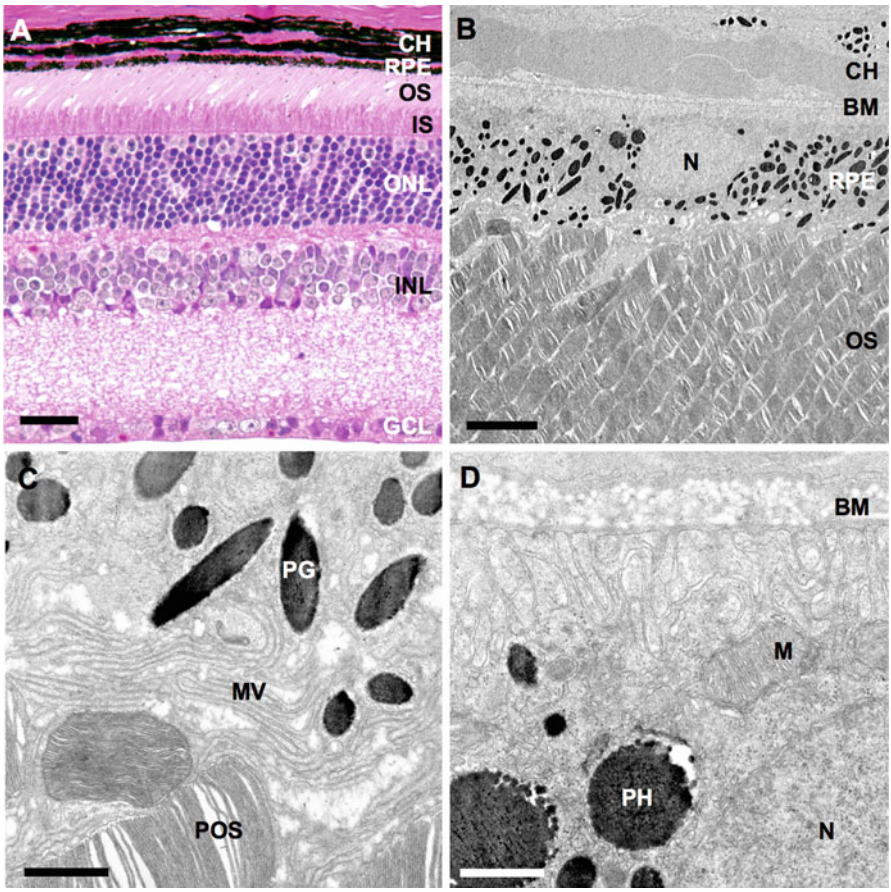


Fig. 16.1 Photomicrograph and electron micrograph of mouse retina. (**a**) Hematoxylin and eosin staining of C57BL/6J mouse retina at 2 months. *CH* choroid, *RPE* retinal pigment epithelium, *OS* outer segments, *IS* inner segments, *ONL* outer nuclear layer, *INL* inner nuclear layer, *GCL* ganglion cell layer. Scale bar = 50 μm. (**b**) Transmission electron micrograph of RPE and photoreceptor cells in the mouse eye at 2 months. *BM* Bruch's membrane, *N* nucleus. Scale bar = 5 μm. (**c**) Observation at higher magnification showed bounder of photoreceptor outer segment and RPE (RPE apical surface). *MV* microvilli, *PG* pigment granules, *POS* photoreceptor outer segments. Scale bar = 1 μm. (**d**) Observation at higher magnification showed bounder of Bruch's membrane and RPE (RPE basal surface). *M* mitochondria, *N* nucleus, *PH* phagosomes. Scale bar = 1 μm

the enzymatic oxidation of tyrosine and dihydroxyphenylalanine, is linked to proteins and contained in membrane-limited granules in the RPE melanosomes and serves as a free radical stabilizer [6, 7]. It can also bind toxins and retinotoxic drugs such as chloroquine and thioridazine, although it is unclear whether this effect is beneficial or harmful. Another important function of melanin is the binding of zinc, since melanin is the main source of zinc in the eye, and zinc plays an essential role

in the metabolism of the retina [5, 8]. Lipofuscin, an undegradable fluorescent material, is a heterogeneous material composed of a mixture of lipids and different fluorescent compounds, the main fluorophore of which has been identified as the pyridinium bis-retinoid, *N*-retinylidene-*N*-retinylethanolamine (A2E), a derivative of vitamin A [6, 9, 10]. Lipofuscin pigment is intracellular yellow-brown autofluorescent granules exhibiting sudanophilic, osmiophilic, argyrophilic, and periodic acid-Schiff-positive and acid-fast staining characteristics [11]. RPE lipofuscin is produced as a by-product mainly from the phagocytosed photoreceptor outer segments.

RPE apical microvilli play important roles in the maintenance of photoreceptor and retinal attachment [2, 12]. To maintain attachment, RPE cells develop elongated apical microvilli of 5–7 μm in length, forming sheaths that involve in the phagocytosis of photoreceptor outer segments (Fig. 16.1c). Any disruption of the relationship between photoreceptors and the RPE will lead to pathological consequences such as a retinal detachment. On the basolateral side, RPE cells display highly convoluted infoldings that attach to Bruch's basement membrane, an acellular layer separating the RPE from the choriocapillaris. The RPE's basal surface participates in extensive metabolic exchanges of nutrients and signaling molecules with blood vessels in the underlying choriocapillaris [13].

16.3 Biological Functions of the RPE

RPE cells play important roles in the process of highly specialized metabolic and transport functions essential for homeostasis of the neural retina [2]. These include phagocytosis of photoreceptor-shed outer segments, transport of nutrients into and removal of waste products from photoreceptor cells, and retinoid transport and regeneration. The phagocytic process occurs through engulfment by the apical membrane of the RPE. Although the RPE is capable of slow, nonspecific phagocytosis of a diversity of large and small particles, the daily, specific phagocytosis of photoreceptor outer segment disks is one of the important properties of RPE cells for the maintenance of visual function [14]. The phagocytosed outer segments undergo enzymatic digestion within the RPE, and after a phagosome has formed following internalization, fusion occurs with lysosomes. Inhibition of lysosomal proteases causes engorgement with undegraded phagosomes, rapidly [15]. Two thousand photoreceptor disks in the parafovea, 3500 in the perifovea, and about 4,000 in the periphery of the monkey eye are shed by RPE phagocytic load per day. Phagocytosis by the RPE results in the complete turnover of the photoreceptor outer segments once every 8–13 days [16].

RPE cells produce and secrete various growth factors essential for the maintenance of the structural integrity of the retina and choriocapillaris. A number of reports have been conducted to show that the RPE is part of a complex system of cellular cross talk that controls vascular supply, permeability, growth, repair, and other processes vital to retinal functions. The RPE is able to secrete ATP; ciliary

neurotrophic factor (CNTF) [17], which influences cell survival, proliferation, and differentiation; fibroblast growth factors (FGF-1, FGF-2, and FGF-5), which can be neurotropic [18]; insulin-like growth factor 1 (IGF-1), which involves in transformation events [19]; lens epithelium-derived growth factor (LEDGF), which is a growth and survival factor [20]; pigment epithelium-derived factor (PEDF), which acts as a neuroprotectant and vascular inhibitor [21]; platelet-derived growth factor (PDGF), which modulates cell growth and healing [22]; transforming growth factor-β (TGF-β), which moderates inflammation [23]; tissue inhibitors of matrix metalloprotease (TIMPs), which remodel the extracellular matrix [24]; vascular endothelial growth factor (VEGF), which can stimulate vascular permeability and/or growth [25, 26]; and members of the interleukin family, which regulate immune response [27, 28]. These factors are activated when the retina or the RPE is exposed to pathological environments including hypoxia, oxidative stress, and metabolic stress. For example, VEGF and TIMPs are secreted from the basolateral side of the RPE to the choroid, which are required for stabilizing the fenestrated structure of the choroidal endothelium [25]. On the apical/retinal side of the RPE, PEDF is secreted to stabilize the neuronal retina in preventing apoptosis [29–31].

The retina is the tissue with highest cell density in the body. The photoreceptors, neurons, and Müller glial cells produce a large amount of metabolic water that accumulates in the retina because the retina has a high metabolic turnover rate. The RPE transports ions and water from the subretinal space/apical side to the blood/basolateral side, and tight junctions of RPE cells establish the basis for the blood-retinal barrier between the retina and choroidal circulation [13]. The RPE has a characteristic distribution of the membrane proteins containing a number of ion channels, as well as active or facilitative transport systems for ions and for metabolites such as glucose and amino acids. Different channels and transporters are present on the apical and basal membranes (e.g., an electrogenic sodium-potassium pump only on the apical side, a chloride-bicarbonate exchange transporter on the basal side). Water transport is driven by a transepithelial transport of chloride from subretinal space to the blood side through the RPE. The chloride transport is an active transport driven by the activity of the apically localized sodium-potassium ATPase [32]. Thus, tight junctions of RPE cells are required to maintain the neural environment of the retina, forming a nearly impermeable seal between RPE cells to regulate intercellular diffusion via RPE cells.

16.4 Aging of the RPE

Aging is a universal process that is associated with the decline of cellular functions and susceptibility to degenerative diseases. During aging, the number of neurons from all kinds of the neuronal cells decreases, and photoreceptor cells become shortened, resulting in overall thinning of the retina. The density of RPE cells has been reported to decrease by about 0.3 % per year with increasing age, which was greater in the macula compared with the periphery due to higher metabolic activity

in the central retina [33]. Both of funduscopic and morphologic examinations exhibit significant pigmentary changes in the RPE with increasing age. The accumulation of lipofuscin in RPE cells may be due in large part to incomplete digestion of shed photoreceptor disks and affect the antioxidant properties of melanin [34, 35]. Photobleaching with aging damages melanosomes, which also diminishes the antioxidant efficiency of melanin [36]. The aged lipid A2E (present in lipofuscin) and mitochondrial dysfunction synergistically lead to disorder of RPE phagocytosis and stimulate the complement system [37, 38]. The advanced glycation end products (AGEs) were reported to accumulate within Bruch's membrane and the RPE and affect lysosomal enzyme functions in RPE cells associated with lipofuscin [39, 40]. Increase of those products in the RPE is associated with AMD, Leber's amaurosis, Best's disease, and Stargardt's disease. The RPE during aging displays numerous other functional and structural changes such as formation of drusen and disorganization of RPE microvilli and basal infoldings [41–44]. Drusen are debris-like insoluble lipid-rich deposits that accumulate between the basal lamina of the RPE and the inner collagenous layer of Bruch's membrane. Biochemical compositional analysis of drusen has identified glyco-conjugates, vitronectin, apolipoproteins B and E, alpha-crystallin, complement proteins, and lipids [45–47]. Small drusen are commonly observed in older individuals and usually do not interfere with vision. However, the presence of large and many drusen increases the risk of diseases such as AMD. Several reports have suggested that local inflammation plays a critical role in drusen formation [48–50]. The process of drusen deposition is a similar to other age-related diseases such as Alzheimer's disease and atherosclerosis, both of which have accumulation of extracellular plaques and a local chronic inflammatory event due to deposits. The RPE microvilli involve several antioxidant enzymes (e.g., lactate dehydrogenase, glutathione S-transferase, peroxiredoxin, ceruloplasmin, and superoxide dismutase) [51], suggesting that impairment of RPE microvilli also affects several of the key functions carried out by the apical surface.

16.5 Disease of the RPE

AMD is the most common complex disorder in the elderly population in industrialized countries that primarily affects the central region of the retina (macula) and is the leading cause of legal blindness. AMD affects over 1.7 million people in the United States alone, and 30 % of individuals >75 years of age show some signs of the disease. This number is expected to reach three million by the year 2020 [52]. With increased life expectancy, this devastating disease will continue to have a significant public health impact on the quality of life worldwide [53, 54]. Early signs of the disease are characterized by the presence of ophthalmoscopically visible soft drusen, areas of hyperpigmentation, and depigmented areas (Fig. 16.2a, b), whereas later stages manifest either choroidal neovascularization or atrophy of photoreceptors and the RPE [52, 55, 56]. Neovascular/exudative/wet

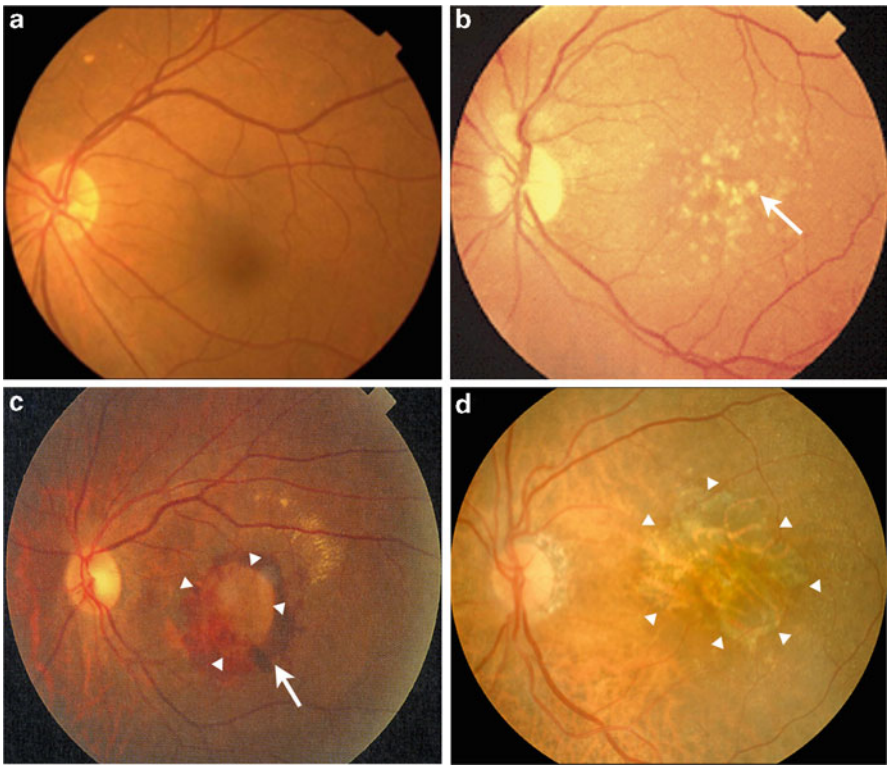


Fig. 16.2 Fundus photograph of human eyes. (**a**) Fundus photograph of a healthy control subject. (**b**) Fundus photograph of a patient with soft drusen (*arrow*). (**c**) Fundus photograph of a patient with neovascular/exudative/wet AMD, the neovascular form with retinal hard exudates (*yellow waxy lesions*, *arrowheads*), and subretinal hemorrhage (*arrow*). (**d**) Fundus photograph of a patient with atrophic/dry form of AMD, an area of atrophy with the loss of photoreceptor, RPE, and the choriocapillaris (*arrowheads*)

AMD is characterized by abnormal capillary growth from the choroid beyond Bruch's membrane and often the RPE and by subsequent exudation of fluid, lipid, and blood, resulting eventually in a disciform scar in the macula and is responsible for severe visual loss (Fig. 16.2c). Atrophic/dry AMD, known as geographic atrophy, is characterized by progressive degeneration of the outer neurosensory retina, the RPE, and the choriocapillaris (Fig. 16.2d). The dysfunction and cell death of the RPE is considered to happen primarily, followed by secondary loss of neighboring photoreceptors and choriocapillaris. AMD harbors a multifactorial trait involving both genetic [57, 58] and environmental factors [59], although the precise etiology of AMD remains elusive. Inflammation, oxidative damage, cholesterol metabolism, and/or impaired function of RPE also have been implicated in AMD development, although aging is a key event that contributes to the pathogenesis of

AMD [60, 61]. Moreover, dysfunction of the RPE is involved in the pathogenesis of several other retinal diseases such as retinitis pigmentosa, proliferative vitreoretinopathy, Stargardt's disease, and Leber's congenital amaurosis. Gene mutations also are associated with retinal diseases, for example, Stargardt's disease caused by mutations in the *ABCA4* (ATP-binding cassette, subfamily A, member 4) gene that encodes an ATP-binding cassette transporter, which lead to an increase of A2E within the RPE [38, 62].

16.6 Conclusions

Located between light-sensitive outer segments of photoreceptors and blood vessels of the choriocapillaris, the RPE plays critical roles in the development and maintenance of retinal functions that closely interact with photoreceptors and the choroid. Several pathological microenvironmental changes including aging, oxidative stress, and inflammatory responses impair physiological functions of the RPE. Functional impairment of the RPE has clearly been identified as the primary cause of certain retinal disorders and secondarily plays further pathological roles in many more. Clarification of the connections among those multiple events in the RPE will provide an approach for the elucidation of the pathogenesis of many degenerative retinal diseases leading to blindness such as AMD. The accumulation of novel knowledge will become translated into therapeutic tools/drugs for the prevention and treatment of vision loss and thus will bring new hope to those whose sight is compromised.

Acknowledgments Our research is supported by the Takeda Science Foundation, Mishima Saiichi Memorial Ophthalmic Research Japan Foundation, Charitable Trust Fund for Ophthalmic Research in Commemoration of Santen Pharmaceutical's Founder, Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program, and a grant-in-aid from the Ministry of Education, Science, and Culture of Japan (A.K. #24791823, S.I. #24390392).

References

1. Zinn K, Benjamin-Henkind J (1979) Anatomy of the human retinal pigment epithelium. In: The retinal pigment epithelium. Harvard University Press, Cambridge, pp 3–31
2. Bok D (1993) The retinal pigment epithelium: a versatile partner in vision. J Cell Sci Suppl 17:189–195
3. Marmorstein AD (2001) The polarity of the retinal pigment epithelium. Traffic 2(12):867–872
4. Marmorstein AD, Finnemann SC, Bonilha VL, Rodriguez-Boulan E (1998) Morphogenesis of the retinal pigment epithelium: toward understanding retinal degenerative diseases. Ann N Y Acad Sci 857:1–12
5. Schraermeyer U, Heimann K (1999) Current understanding on the role of retinal pigment epithelium and its pigmentation. Pigment Cell Res 12(4):219–236

6. Feeney L (1978) Lipofuscin and melanin of human retinal pigment epithelium. Fluorescence, enzyme cytochemical, and ultrastructural studies. Invest Ophthalmol Vis Sci 17(7):583–600
7. Rozanowska M, Sarna T, Land EJ, Truscott TG (1999) Free radical scavenging properties of melanin interaction of eu- and pheo-melanin models with reducing and oxidising radicals. Free Radic Biol Med 26(5–6):518–525
8. Samuelson DA, Smith P, Ulshafer RJ, Hendricks DG, Whitley RD, Hendricks H, Leone NC (1993) X-ray microanalysis of ocular melanin in pigs maintained on normal and low zinc diets. Exp Eye Res 56(1):63–70. doi:10.1006/exer.1993.1009
9. Kennedy CJ, Rakoczy PE, Constable IJ (1995) Lipofuscin of the retinal pigment epithelium: a review. Eye 9(Pt 6):763–771. doi:10.1038/eye.1995.192
10. Sparrow JR, Boulton M (2005) RPE lipofuscin and its role in retinal pathobiology. Exp Eye Res 80(5):595–606. doi:10.1016/j.exer.2005.01.007
11. Strehler BL (1964) On the histochemistry and ultrastructure of age pigment. Adv Gerontol Res 21:343–384
12. Marmor MF (1990) Control of subretinal fluid: experimental and clinical studies. Eye 4 (Pt 2):340–344. doi:10.1038/eye.1990.46
13. Hughes BA, Gallemore RP, Miller SS (1998) Transport mechanisms in the retinal pigment epithelium. In: Marmor MF, Wolfensberger TJ (eds) The retinal pigment epithelium: function and disease. Oxford University Press, New York, pp 103–134
14. Besharse JC, Defoe DM (1998) Role of the retinal pigment epithelium in photoreceptor membrane turnover. In: Marmor MF, Wolfensberger TJ (eds) The retinal pigment epithelium: function and disease. Oxford University Press, New York, pp 152–172
15. Katz ML (1989) Incomplete proteolysis may contribute to lipofuscin accumulation in the retinal pigment epithelium. Adv Exp Med Biol 266:109–116, Discussion 116–108
16. Young RW (1971) The renewal of rod and cone outer segments in the rhesus monkey. J Cell Biol 49(2):303–318
17. Cao W, Wen R, Li F, Lavail MM, Steinberg RH (1997) Mechanical injury increases bFGF and CNTF mRNA expression in the mouse retina. Exp Eye Res 65(2):241–248. doi:10.1006/exer.1997.0328
18. Bost LM, Aotaki-Keen AE, Hjelmeland LM (1994) Cellular adhesion regulates bFGF gene expression in human retinal pigment epithelial cells. Exp Eye Res 58(5):545–552
19. Takagi H, Yoshimura N, Tanihara H, Honda Y (1994) Insulin-like growth factor-related genes, receptors, and binding proteins in cultured human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 35(3):916–923
20. Ahuja P, Caffe AR, Holmqvist I, Soderpalm AK, Singh DP, Shinohara T, van Veen T (2001) Lens epithelium-derived growth factor (LEDGF) delays photoreceptor degeneration in explants of rd/rd mouse retina. Neuroreport 12(13):2951–2955
21. Ogata N, Tombran-Tink J, Nishikawa M, Nishimura T, Mitsuma Y, Sakamoto T, Matsumura M (2001) Pigment epithelium-derived factor in the vitreous is low in diabetic retinopathy and high in rhegmatogenous retinal detachment. Am J Ophthalmol 132(3):378–382
22. Campochiaro PA, Hackett SF, Vinores SA, Freund J, Csaky C, LaRochelle W, Henderer J, Johnson M, Rodriguez IR, Friedman Z et al (1994) Platelet-derived growth factor is an autocrine growth stimulator in retinal pigmented epithelial cells. J Cell Sci 107 (Pt 9):2459–2469
23. Khaliq A, Patel B, Jarvis-Evans J, Moriarty P, McLeod D, Boulton M (1995) Oxygen modulates production of bFGF and TGF-beta by retinal cells in vitro. Exp Eye Res 60 (4):415–423
24. Alexander JP, Bradley JM, Gabourel JD, Acott TS (1990) Expression of matrix metalloproteinases and inhibitor by human retinal pigment epithelium. Invest Ophthalmol Vis Sci 31 (12):2520–2528
25. Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO (2003) Vascular endothelial growth factors and angiogenesis in eye disease. Prog Retin Eye Res 22(1):1–29

26. Adamis AP, Shima DT, Yeo KT, Yeo TK, Brown LF, Berse B, D'Amore PA, Folkman J (1993) Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. Biochem Biophys Res Commun 193 (2):631–638
27. Planck SR, Dang TT, Graves D, Tara D, Ansel JC, Rosenbaum JT (1992) Retinal pigment epithelial cells secrete interleukin-6 in response to interleukin-1. Invest Ophthalmol Vis Sci 33 (1):78–82
28. Elner VM, Burnstine MA, Strieter RM, Kunkel SL, Elner SG (1997) Cell-associated human retinal pigment epithelium interleukin-8 and monocyte chemotactic protein-1: immunochemical and in-situ hybridization analyses. Exp Eye Res 65(6):781–789. doi:10.1006/exer.1997.0380
29. Becerra SP, Fariss RN, Wu YQ, Montuenga LM, Wong P, Pfeffer BA (2004) Pigment epithelium-derived factor in the monkey retinal pigment epithelium and interphotoreceptor matrix: apical secretion and distribution. Exp Eye Res 78(2):223–234
30. Ogata N, Wang L, Jo N, Tombran-Tink J, Takahashi K, Mrazek D, Matsumura M (2001) Pigment epithelium derived factor as a neuroprotective agent against ischemic retinal injury. Curr Eye Res 22(4):245–252
31. Cayouette M, Smith SB, Becerra SP, Gravel C (1999) Pigment epithelium-derived factor delays the death of photoreceptors in mouse models of inherited retinal degenerations. Neurobiol Dis 6(6):523–532. doi:10.1006/nbdi.1999.0263
32. Strauss O (2005) The retinal pigment epithelium in visual function. Physiol Rev 85(3):845–881. doi:10.1152/physrev.00021.2004
33. Panda-Jonas S, Jonas JB, Jakobczyk-Zmija M (1996) Retinal pigment epithelial cell count, distribution, and correlations in normal human eyes. Am J Ophthalmol 121(2):181–189
34. Steinbrook R (2006) The price of sight–ranibizumab, bevacizumab, and the treatment of macular degeneration. N Engl J Med 355(14):1409–1412. doi:10.1056/NEJMp068185
35. Boulton M, Dontsov A, Jarvis-Evans J, Ostrovsky M, Svistunenko D (1993) Lipofuscin is a photoinducible free radical generator. J Photochem Photobiol B Biol 19(3):201–204
36. Sarna T, Burke JM, Korytowski W, Rozanowska M, Skumatz CM, Zareba A, Zareba M (2003) Loss of melanin from human RPE with aging: possible role of melanin photooxidation. Exp Eye Res 76(1):89–98
37. Vives-Bauza C, Anand M, Shirazi AK, Magrane J, Gao J, Vollmer-Snarr HR, Manfredi G, Finnemann SC (2008) The age lipid A2E and mitochondrial dysfunction synergistically impair phagocytosis by retinal pigment epithelial cells. J Biol Chem 283(36):24770–24780. doi:10.1074/jbc.M800706200
38. Zhou J, Jang YP, Kim SR, Sparrow JR (2006) Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. Proc Natl Acad Sci USA 103(44):16182–16187. doi:10.1073/pnas.0604255103
39. Glenn JV, Mahaffy H, Wu K, Smith G, Nagai R, Simpson DA, Boulton ME, Stitt AW (2009) Advanced glycation end product (AGE) accumulation on Bruch's membrane: links to age-related RPE dysfunction. Invest Ophthalmol Vis Sci 50(1):441–451. doi:10.1167/iovs.08-1724
40. Schutt F, Bergmann M, Holz FG, Kopitz J (2003) Proteins modified by malondialdehyde, 4-hydroxynonenal, or advanced glycation end products in lipofuscin of human retinal pigment epithelium. Invest Ophthalmol Vis Sci 44(8):3663–3668
41. Boulton M, Dayhaw-Barker P (2001) The role of the retinal pigment epithelium: topographical variation and ageing changes. Eye 15(Pt 3):384–389. doi:10.1038/eye.2001.141
42. Katz ML, Robison WG Jr (1984) Age-related changes in the retinal pigment epithelium of pigmented rats. Exp Eye Res 38(2):137–151
43. Lai YL, Rana MW (1986) A study of photoreceptor-retinal pigment epithelium complex: age-related changes in monkeys. Proc Soc Exp Biol Med 181(3):371–381
44. Weisse I (1995) Changes in the aging rat retina. Ophthalmic Res 27(Suppl 1):154–163

45. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn ME, Salomon RG, Hollyfield JG (2002) Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci USA 99(23):14682–14687. doi:10.1073/pnas.222551899
46. Malek G, Li CM, Guidry C, Medeiros NE, Curcio CA (2003) Apolipoprotein B in cholesterol-containing drusen and basal deposits of human eyes with age-related maculopathy. Am J Pathol 162(2):413–425. doi:10.1016/S0002-9440(10)63836-9
47. Mullins RF, Russell SR, Anderson DH, Hageman GS (2000) Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 14 (7):835–846
48. Anderson DH, Mullins RF, Hageman GS, Johnson LV (2002) A role for local inflammation in the formation of drusen in the aging eye. Am J Ophthalmol 134(3):411–431
49. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF (2001) An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res 20(6):705–732
50. Johnson LV, Leitner WP, Staples MK, Anderson DH (2001) Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. Exp Eye Res 73(6):887–896. doi:10.1006/exer.2001.1094
51. Bonilha VL, Bhattacharya SK, West KA, Sun J, Crabb JW, Rayborn ME, Hollyfield JG (2004) Proteomic characterization of isolated retinal pigment epithelium microvilli. Mol Cell Proteomics 3(11):1119–1127. doi:10.1074/mcp.M400106-MCP200
52. Friedman DS, O'Colmain BJ, Munoz B, Tomany SC, McCarty C, de Jong PT, Nemesure B, Mitchell P, Kempen J, Eye Diseases Prevalence Research G (2004) Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol 122(4):564–572. doi:10.1001/archopht.122.4.564
53. Abecasis GR, Yashar BM, Zhao Y, Ghiasvand NM, Zareparsi S, Branham KE, Reddick AC, Trager EH, Yoshida S, Bahling J, Filippova E, Elner S, Johnson MW, Vine AK, Sieving PA, Jacobson SG, Richards JE, Swaroop A (2004) Age-related macular degeneration: a high-resolution genome scan for susceptibility loci in a population enriched for late-stage disease. Am J Hum Genet 74(3):482–494. doi:10.1086/382786
54. Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuchi A, Hong S, Pravda EA, Majchrzak S, Carper D, Hellstrom A, Kang JX, Chew EY, Salem N Jr, Serhan CN, Smith LE (2007) Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med 13(7):868–873. doi:10.1038/nm1591
55. Sarks SH (1976) Ageing and degeneration in the macular region: a clinico-pathological study. Br J Ophthalmol 60(5):324–341
56. Fine SL, Berger JW, Maguire MG, Ho AC (2000) Age-related macular degeneration. N Engl J Med 342(7):483–492. doi:10.1056/NEJM200002173420707
57. Chen W, Stambolian D, Edwards AO, Branham KE, Othman M, Jakobsdottir J, Tosakulwong N, Pericak-Vance MA, Campochiaro PA, Klein ML, Tan PL, Conley YP, Kanda A, Kopplin L, Li Y, Augustaitis KJ, Karoukis AJ, Scott WK, Agarwal A, Kovach JL, Schwartz SG, Postel EA, Brooks M, Baratz KH, Brown WL, Complications of Age-Related Macular Degeneration Prevention Trial Research G, Brucker AJ, Orlin A, Brown G, Ho A, Regillo C, Donoso L, Tian L, Kaderli B, Hadley D, Hagstrom SA, Peachey NS, Klein R, Klein BE, Gotoh N, Yamashiro K, Ferris Iii F, Fagerness JA, Reynolds R, Farrer LA, Kim IK, Miller JW, Corton M, Carracedo A, Sanchez-Salorio M, Pugh EW, Doheny KF, Brion M, Deangelis MM, Weeks DE, Zack DJ, Chew EY, Heckenlively JR, Yoshimura N, Iyengar SK, Francis PJ, Katsanis N, Seddon JM, Haines JL, Gorin MB, Abecasis GR, Swaroop A (2010) Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci USA 107(16):7401–7406. doi:10.1073/pnas.0912702107

58. Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, Thorleifsson G, Zack DJ, Arakawa S, Cipriani V, Ripke S, Igo RP Jr, Buitendijk GH, Sim X, Weeks DE, Guymer RH, Merriam JE, Francis PJ, Hannum G, Agarwal A, Armbrecht AM, Audo I, Aung T, Barile GR, Benchaboune M, Bird AC, Bishop PN, Branham KE, Brooks M, Brucker AJ, Cade WH, Cain MS, Campochiaro PA, Chan CC, Cheng CY, Chew EY, Chin KA, Chowers I, Clayton DG, Cojocaru R, Conley YP, Cornes BK, Daly MJ, Dhillon B, Edwards AO, Evangelou E, Fagerness J, Ferreyra HA, Friedman JS, Geirsdottir A, George RJ, Gieger C, Gupta N, Hagstrom SA, Harding SP, Haritoglou C, Heckenlively JR, Holz FG, Hughes G, Ioannidis JP, Ishibashi T, Joseph P, Jun G, Kamatani Y, Katsanis N, NK C, Khan JC, Kim IK, Kiyohara Y, Klein BE, Klein R, Kovach JL, Kozak I, Lee CJ, Lee KE, Lichtner P, Lotery AJ, Meitinger T, Mitchell P, Mohand-Said S, Moore AT, Morgan DJ, Morrison MA, Myers CE, Naj AC, Nakamura Y, Okada Y, Orlin A, Ortube MC, Othman MI, Pappas C, Park KH, Pauer GJ, Peachey NS, Poch O, Priya RR, Reynolds R, Richardson AJ, Ripp R, Rudolph G, Ryu E, Sahel JA, Schaumberg DA, Scholl HP, Schwartz SG, Scott WK, Shahid H, Sigurdsson H, Silvestri G, Sivakumaran TA, Smith RT, Sobrin L, Souied EH, Stambolian DE, Stefansson H, Sturgill-Short GM, Takahashi A, Tosakulwong N, Truitt BJ, Tsironi EE, Uitterlinden AG, van Duijn CM, Vijaya L, Vingerling JR, Vithana EN, Webster AR, Wichmann HE, Winkler TW, Wong TY, Wright AF, Zelenika D, Zhang M, Zhao L, Zhang K, Klein ML, Hageman GS, Lathrop GM, Stefansson K, Allikmets R, Baird PN, Gorin MB, Wang JJ, Klaver CC, Seddon JM, Pericak-Vance MA, Iyengar SK, Yates JR, Swaroop A, Weber BH, Kubo M, Deangelis MM, Leveillard T, Thorsteinsdottir U, Haines JL, Farrer LA, Heid IM, Abecasis GR, Consortium AMDG (2013) Seven new loci associated with age-related macular degeneration. Nat Genet 45(4):433–439. doi:10.1038/ng.2578, 439e431–432
59. Evans JR (2001) Risk factors for age-related macular degeneration. Prog Retin Eye Res 20 (2):227–253
60. Winkler BS, Boulton ME, Gottsch JD, Sternberg P (1999) Oxidative damage and age-related macular degeneration. Mol Vis 5:32
61. Zarbin MA (2004) Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol 122(4):598–614. doi:10.1001/archopht.122.4.598
62. Mata NL, Weng J, Travis GH (2000) Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. Proc Natl Acad Sci USA 97(13):7154–7159. doi:10.1073/pnas.130110497

Chapter 17
Oxidative Stress in the RPE and Its Contribution to AMD Pathogenesis: Implication of Light Exposure

Yoko Ozawa

Abstract The retinal pigment epithelium (RPE) is a single layer of cuboidal cells that develops from the neural tube as well as neural retina does, and is therefore considered part of the central nervous system. While the neural retina receives light and converts it to electrical signals to process information and transfer it to the brain, the RPE acts as an essential coordinator of the retinal microenvironment for appropriate retinal neural function. Reactive oxygen species (ROS) are generated during basal cellular metabolic processes. Moreover, daily exposure to light stimuli enhances the production of ROS in the RPE and the neural retina, the excessive accumulation of which contributes to retinal pathogenesis. In this section, oxidative stress in the RPE and its proposed contribution to age-related macular degeneration (AMD) are discussed, reviewing recent in vivo and in vitro studies that support the role of oxidative stress in the development and progression of AMD.

Keywords Inflammation • Light exposure • Reactive oxygen species (ROS) • Retinal pigment epithelium (RPE) • Tight junction

17.1 Roles of the RPE and Oxidative Stress

17.1.1 Physiological Roles of the RPE

The retinal pigment epithelium (RPE) is a multifunctional regulator of the retinal microenvironment. Its physiological and cellular functions include the recycling of retinal pigment during the visual cycle, the directional transport of oxygen and

Y. Ozawa (✉)
Laboratory of Retinal Cell Biology, Department of Ophthalmology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
e-mail: ozawa@a5.keio.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_17,

nutrients, the formation of the blood–retinal barrier (BRB) which regulates the infiltration of molecules and cells, and the polarized secretion of cytokines and chemokines [1, 2].

The RPE plays a central role in regulating the visual cycle of rhodopsin. During phototransduction, the rhodopsin chromophore 11-*cis* retinal is converted into all-*trans* retinol, which accumulates in the outer segments of photoreceptor cells. The RPE cells phagocytose the outer segments containing all-*trans* retinol, which is then sequentially metabolized to regenerate 11-*cis* retinal and the rhodopsin pigment. During this process, *N*-retinylidene-*N*-retinylethanolamine (A2E) is generated, which can accumulate and lead to lipofuscin formation in the aging RPE. A2E also induces production of reactive oxygen species (ROS) in response to blue light [3], which can result in RPE cell death [4]. An abnormality in the gene encoding a transmembrane transporter, ATP-binding cassette, subfamily A (ABC1), member 4 (ABCA4), causes A2E and lipofuscin accumulation, leading to the retinal degenerative condition known as Stargardt disease [5]. Light-induced photoreceptor cell death is suppressed in the absence of rhodopsin [6] or in the presence of a mutation in an enzyme of the visual cycle, RPE65 [7]. These findings suggest that cellular metabolism during the visual cycle in the RPE contributes to increases in ROS and the development of tissue damage in the retina.

The RPE controls the flow of fluid which contains oxygen and nutrients from the highly vascularized choroid into the outer retina. Together the vascular and epithelial components of the BRB maintain the specialized environment of the neural retina [8]. The tight junctions and adherens junctions (Fig. 17.1) form connections between cells and regulate the localization of membrane proteins and cell polarity, and in the retinal environment, these structures are important for establishing the BRB. Thus, breakdown of the BRB can result in abnormal infiltrations of molecules and/or cells, including vascular endothelial cells and inflammatory cells, which may contribute to the development and/or progression of AMD lesions in the subretinal space.

The RPE secretes vascular endothelial growth factor (VEGF), which is indispensable for maintaining the choroidal vessels that provide oxygen and nutrients to the outer neural retina, mainly the photoreceptor cells [9, 10]. In mice that have been engineered to express only the membrane-bound, non-soluble isoform of VEGF (VEGF188), vessels in the choroid undergo degeneration, followed by photoreceptor cell apoptosis [9]. Targeted depletion of VEGF in the adult RPE also causes the similar changes [10]. The RPE also secretes a neuroprotective and vascular suppressive factor, pigment epithelium-derived factor (PEDF), which inhibits excessive VEGF signaling; insufficient PEDF may also be associated with AMD pathogenesis [11, 12]. In addition to these physiological cytokines, the RPE also secretes monocyte chemotactic factor-1 (MCP-1), which can recruit inflammatory cells and promote AMD lesions [13, 14]. Various studies have shown that ROS cross talk with inflammatory reactions can lead to a vicious cycle [15–18] that contributes to the development of AMD [19, 20].

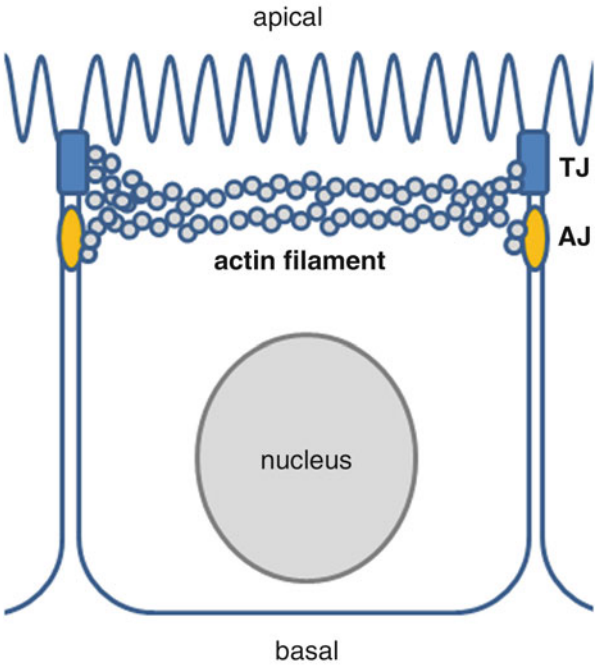


Fig. 17.1 Tight and adherens junctions in the RPE. Schematic illustration of the tight junction (TJ) and adherens junction (AJ) and their association with actin filaments

- O_2 (energy transfer by a photosensitizer) → singlet oxygen ($^1O_2$)
- $O_2 + e^- \rightarrow$ superoxide anion radical ($O^{\cdot -}$)
- $O^{\cdot -} + e^- + H^+ \rightarrow$ hydroxyl radical ($HO^{\cdot}$)
- $HO^{\cdot -} + e^- + H^+ \rightarrow$ hydrogen peroxide (H_2O_2)

Fig. 17.2 Generation of ROS. Reactions leading to ROS generation. The singlet oxygen ($1O_2$), superoxide anion radical (O_2^-), hydroxyl radical ($HO^{\cdot}$), and hydrogen peroxide (H_2O_2) are shown

17.1.2 ROS and Oxidative Stress

Oxidative stress occurs when excessive ROS accumulate and are not eliminated by the normal biological self-defense systems (see below), resulting in tissue/organ damage and various pathological conditions. ROS are highly reactive chemicals derived from oxygen following exposure to high-energy or electron-transferring chemical reactions [21, 22]. ROS include singlet oxygen ($^1O_2$), superoxide anion radical ($O^{\cdot -}$), hydroxyl radical ($HO^{\cdot}$), and hydrogen peroxide (H_2O_2) (Fig. 17.2). ROS are toxic to biological organisms due to their oxidation of lipids, proteins, DNA, and carbohydrates, which results in the loss of normal membrane, metabolic, and reproductive functions.

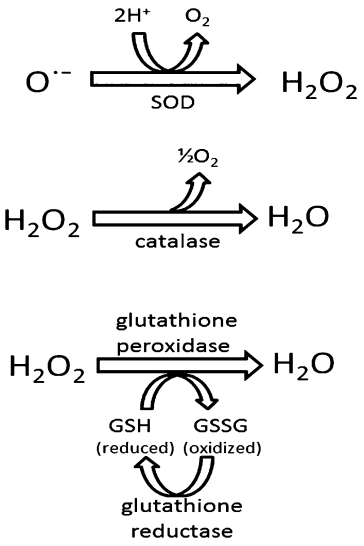


Fig. 17.3 Antioxidative reactions. SOD converts $O^{\cdot -}$ to H_2O_2, which is further converted to H_2O by catalase. H_2O_2 is also converted to H_2O by glutathione peroxidase. In this system, GSH acts as an antioxidant reducing H_2O_2 and is oxidized to become GSSG. GSSG is then reduced by glutathione reductase to replenish GSH. *SOD* superoxide dismutase, *GSH* reduced glutathione, *GSSG* oxidized glutathione representing glutathione-S-S-glutathione

17.1.3 Generation, Elimination, and Accumulation of ROS

ROS are unavoidable by-products of the biochemical pathways involved in energy production and storage, such as glycolysis, the electron transport chain, and photosynthesis [22]. Mitochondrial oxidants are formed predominantly at complexes I and III of the cytochrome chain when electrons initially derived from NADH or FADH2 react with oxygen to produce superoxide anions [23]. Nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases also generate ROS. The NADPH-dependent oxidases comprise a seven-member family of membrane-bound enzymes (Nox1–5 and Duox1–2) that are widely expressed and evolutionarily conserved [23]. NADPH-dependent oxidase-produced ROS directly eliminate invading pathogens and play a central role in the pathobiology of sepsis by regulating the bactericidal activity of phagocytes [24]. Additional cellular sources of ROS production include a number of intracellular enzymes, such as xanthine oxidase, cyclooxygenases, cytochrome p450 enzymes, and lipoxygenases, all of which produce oxidants as part of their normal enzymatic function [23].

ROS levels are regulated by several enzymes that eliminate ROS in the cytosol or mitochondria (Fig. 17.3). These enzymes include the superoxide dismutase (SOD) family. SOD1 (Cu–Zn SOD) is cytosolic, while SOD2 (Mn SOD) localizes

to the mitochondria, and SOD3 (a Cu–Zn SOD encoded by another gene and with a different protein structure compared to SOD1) localizes to the extracellular space. In the peroxisome, catalases are expressed that eliminate the H_2O_2 produced during the β-oxidation-mediated breakdown of long-chain fatty acids which are subsequently shuttled to mitochondria to generate ATP. One of the most important cellular antioxidants is glutathione, a cysteine-containing peptide found in most forms of aerobic life. Glutathione's antioxidant properties are due to a thiol group in its cysteine moiety that functions as a reducing agent and can be reversibly oxidized and reduced.

An additional means of regulating oxidation is by the uptake of exogenous antioxidative molecules. Examples of exogenous antioxidants include the carotenoid, lutein [17, 25–27], and vitamins. Lutein is not synthesized in humans and is considered to be a micronutrient found in certain foods, such as spinach and kale. Lutein is delivered to the retina and concentrated in the macula, the center of the retina; it also accumulates in the RPE [17, 28]. Two large clinical studies, the Age-Related Eye Disease Study (AREDS) [29] and AREDS 2 [30], demonstrated that lutein intake protects against the progression of AMD.

The cellular accumulation of ROS is determined by the net balance of ROS-generating and ROS-eliminating reactions. When ROS generation is excessive or when elimination fails, high levels of ROS will promote tissue/organ damage. Excessive ROS production can result from a metabolic imbalance induced by diabetes or in response to external environmental insults such as exposure to light or smoke, while the reduced elimination of ROS occurs when ROS-eliminating enzymes are disabled due to genetic abnormalities [31–33] or aging-related reductions in enzymatic activity [34]. For example, the SOD activity in mesenteric lymphatic vessels was shown to be reduced in 24- versus 9-month-old rats [34]. Interestingly, excessive ROS accumulation may lead to the reduced expression of antioxidative enzymes; Yuki et al. showed that oxidative stress caused by *N*-methyl-D-aspartate (NMDA) results in reduced SOD1 mRNA and protein levels in the retina [33]. Thus, ROS accumulation is mediated by multiple mechanisms.

17.2 Association of Oxidative Stress in the RPE with Aging and AMD

Although the ROS generated during normal metabolic processes are eliminated through the pathways described above, oxidative stress occurs constantly, and the effects of residual ROS that are not completely removed may contribute to the aging process [32, 35, 36]. The age-related increase in oxidative stress leads to an increased accumulation of autoxidative lipofuscin in the lysosomes of RPE cells, as well as drusen formation in the extracellular space between the RPE and Bruch's membrane [2, 37]. The accumulation of oxidized low-density lipoproteins and lipid

peroxidation end products reduces the degradation of phagocytosed photoreceptor outer segments and increases cellular stress in the RPE cells [38]. Excess ROS can lead to protein damage and unfolding, especially in age-related conditions [39]. Oxidative stress can also lead to mitochondrial DNA damage, which further increases ROS generation and reduces the metabolic capacity, thereby enhancing age-related degenerative tissue changes [2]. Moreover, these ROS-induced cellular changes may lead to the further production of ROS that are not properly eliminated, thus accelerating the ROS-induced pathogenic changes [32, 33].

These aging-related events are believed to lead to subsequent immunological responses, including the production of several types of inflammation-related molecules, the recruitment of leukocytes such as macrophages and dendritic cells, and the activation of complement pathways, and all of these processes are related to the pathogenesis of AMD [19]. AMD risk factors include smoking, metabolic syndromes (including hypertension and arteriosclerosis), single nucleotide polymorphisms (SNPs) [20, 40–42], and light exposure [43], all of which are proposed sources of ROS accumulation and can lead to consecutive and chronic inflammation. Recent studies indicate that the RPE is a primary target of oxidative stress and that changes in the RPE represent early changes in the pathogenesis of AMD. In the following sections, specific experimental studies are introduced.

17.3 In Vitro Analysis of Oxidative Stress Using the ARPE-19 Human RPE Cell Line

ARPE-19 is a spontaneously arising RPE cell line derived from the normal eyes of a 19-year-old male. To explore the underlying mechanisms of AMD, oxidative stress in the RPE is often studied using this cell line. ROS are induced by treating the cells with H_2O_2 or paraquat or by exposing them to ultraviolet or visible light [44, 45]. ARPE-19 cells exhibit barrier functions mediated by tight and adherens junctions and secrete cytokines, consistent with the RPE's functions in vivo. However, the ARPE-19 cells exhibit reduced transepithelial resistance (TER), reduced levels of secreted cytokines, and limited formation of the hexagonal shape, a distinctive characteristic of the RPE, compared with the primary RPE culture known as fetal human RPE (fhRPE) [11, 35].

Despite these limitations, ARPE-19 cells are still commonly used for studying the influence of oxidative stress on the RPE. When ARPE-19 cells are treated intermittently and repeatedly with tert-butylhydroperoxide (tert-BHP), an organic peroxide, they exhibit four well-known senescence biomarkers: hypertrophy, senescence-associated β-galactosidase activity, growth arrest, and cell-cycle arrest in G1. This chronic oxidative stress leads to modifications of the transcriptome and cellular functions, which are involved in the cellular aging phenotype and pathophysiology of AMD [46]. The cells under this treatment also show increased amyloidogenesis, which can contribute to drusen formation, and an angiogenic

molecular expression profile associated with AMD pathogenesis [47]. Alternatively, another group showed that treating ARPE-19 cells with A2E combined with blue light irradiation induces MCP-1, interleukin-8 (IL-8), and complement factor H (CFH), which are also proposed to be involved in AMD pathogenesis [48].

ARPE-19 cells have also been used to investigate the effects of oxidative stress on cell–cell junctions and their association with AMD pathogenesis [49, 50]. The cadherin proteins are essential components of the adherens junctions and have important roles in cell adhesion. Studies using ARPE-19 cells showed that ROS-mediated Src kinase activation increases the tyrosine phosphorylation of p120 catenin, a cellular protein that associates with and regulates cadherin turnover at the cell surface, thereby controlling the level of cadherin available for cell–cell adhesion. The induction of ROS rapidly triggers the translocation of p120 catenin and the internalization of *N*-cadherin from the cell–cell adhesion sites to an early endosomal compartment. The endosomal accumulation of p120 catenin results in stress fiber formation and cell–cell dissociation through activation of the Rho/Rho-associated protein kinase (ROCK) signaling pathway. Another group reported that white light exposure disrupts the expression of zona occludens (ZO-1), a component of tight junctions, in the ARPE19 cells and simultaneously activates the Wnt/β-catenin pathway [45]. These results are consistent with other studies showing that p120 and β-catenin bind to the intracellular domain of cadherin and are released when Wnt ligand binds to its receptor, Dvl, a human homolog of the Drosophila dishevelled. In fact, the oxidative stress-induced cytoskeletal remodeling and cell–cell dissociation were transient in their study, due to the induction of SOD and activation of a nuclear factor-κB (NF-κB)-induced negative feedback loop [49]; the ROS generation–elimination systems can be balanced under their study condition; therefore, the junction may be repaired.

Much of the research investigating the effects of oxidative stress on the RPE has been performed using ARPE-19 cells, in part because of the challenge in isolating sufficient primary RPE for mechanistic studies. However, since AMD pathogenesis involves whole ocular and systemic interactions, in vivo studies are indispensable for understanding this disease.

17.4 Proposed Mechanisms Linking Oxidative Stress in the RPE to the Pathogenesis of AMD

17.4.1 Analysis of AMD Pathogenesis in Animal Models

In vivo AMD pathogenesis studies are often performed with laser-induced choroidal neovascularization (CNV) models using wild-type or genetically modified mice [51–60]. These models are well accepted and have shed light on the inflammatory cytokines and signaling molecules contributing to neovascularization, including IL-6 [52], IL-17 [51], CCR3 [57], the renin–angiotensin system (including angiotensin II type 1 receptor [55], bradykinin [55], and the prorenin receptor [58]),

JNK [59], and hyaluronan-CD44 [54]. In contrast, IL-18 has been shown to be a protective factor that inhibits CNV development [61].

The impact of MCP-1 in generating CNV has been reported in a model of CNV generated by injecting oxidized phospholipids into the subretinal space [14]. Notably, the CNV induction in this model is not observed in MCP-1 knockout mice. The study also showed that mild, chronic light exposure for 6 months induces the accumulation of oxidized phospholipids in the retina (including the RPE), which induces CNV in 50 % of the time (they found CNVs in four eyes in the eight analyzed eyes), while MCP-1 knockout mice never show CNV generation under the same condition. Since light exposure is associated with AMD [43], this model may be more physiologically relevant than the artificially laser-induced CNV model.

There are certain gene-targeted mice which develop AMD-related pathogenesis in the absence of CNV development. Neprilysin-deficient mice, in which amyloid β accumulates, exhibit RPE vacuolization and VEGF–PEDF imbalance [47], and Dicer1-deficient mice accumulate Alu RNA and show subsequent RPE degeneration and geographic atrophy, which is characteristic of an AMD subtype [62].

Inflammation and accumulated materials are highly intertwined with oxidative stress that could be involved in the AMD pathogenesis [35].

17.4.2 Influence of Light-Induced ROS in the RPE

In addition to the abovementioned studies, the significance of ROS and the underlying molecular mechanism in the development of AMD-related pathological changes in the RPE was investigated by Narimatsu et al., using another model in which wild-type Balb/c mice are acutely exposed to moderate light [63]; Balb/c mice have a polymorphism in the RPE65 gene, which is required for the visual cycle, and are thus more susceptible to light-induced retinal degeneration than C57B/6J mice [7]. In this study, the examination of flat-mounted RPE samples revealed that light exposure leads to the disruption of cell–cell junctions and the actin cytoskeleton. The analysis of specific membrane-bound molecules, including ZO-1 (a tight junction marker), *N*-cadherin and *β*-catenin (both adherens junction markers), and *F*-actin (an actin cytoskeleton marker detected by phalloidin), revealed the disruption of cell junctions (Fig. 17.4). These observations suggested that light exposure leads to breakdown of the BRB, which may promote CNV invasion and related exudative changes, as well as an infiltration of inflammatory cells, from the choroid into the subretinal space.

In this model, analysis using DCF-DH, a fluorescent probe that detects hydroxyl and peroxyl radicals, and other ROS, after deacetylation by endogenous esterases, revealed that the ROS levels in the RPE–choroid are elevated immediately after light exposure. (Separation of the RPE and choroid was not technically feasible; thus the two tissues were analyzed as a complex.) To determine if the altered cell junctions and cytoskeletal disruptions are mediated by the excessive ROS accumulation, light-exposed mice were treated with the antioxidant

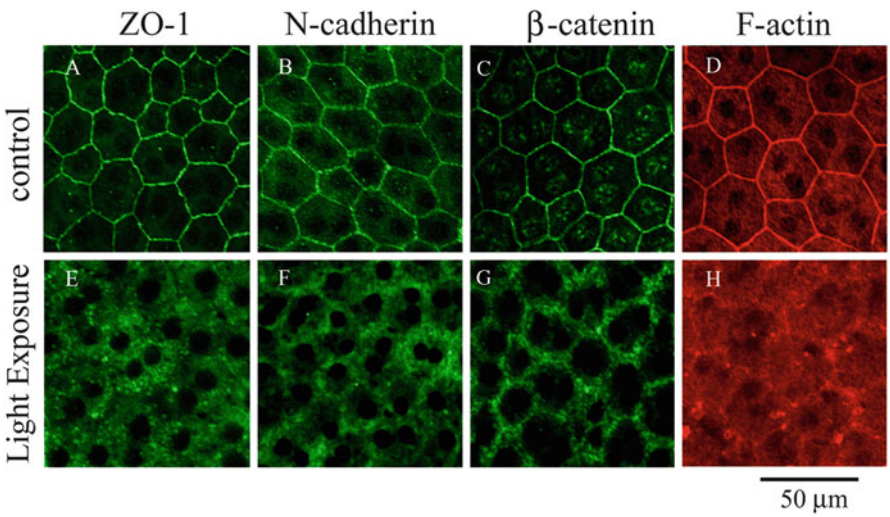


Fig. 17.4 Photodamage to the tight junction, adherens junction, and actin cytoskeleton. Light exposure disrupts the sub-membrane distribution of markers of the tight junction (ZO-1), adherens junction (*N*-cadherin and *β*-catenin), and actin cytoskeleton (*F*-actin) in the RPE, in vivo

N-acetylcysteine (NAC). NAC is a by-product of glutathione and plays a role in glutathione maintenance and metabolism [64]; it is also an approved pharmaceutical drug and nutritional supplement used primarily as a mucolytic agent and in the detoxification of paracetamol (acetaminophen) overdose. NAC treatment was shown to prevent the light-induced cell–cell junction and actin cytoskeletal disruption, as indicated by the attenuated loss of membrane-bound ZO-1, *N*-cadherin, *β*-catenin, and *F*-actin. These findings indicated that excessive ROS levels are responsible for these changes and implicate oxidative stress-induced breakdown of the BRB in the pathogenesis of AMD.

In general, cell–cell junctions are regulated by Rho/ROCK signaling [65]. ROCK, activated by GTP-bound RhoA, phosphorylates LIM kinase, myosin light chain (MLC), and MLC phosphatase, all of which are key regulators of actin organization. Notably, ROCK activity increased after light exposure, and NAC treatment reduced the ROCK activation, in the above-mentioned study, indicating that ROCK is activated by excessive ROS accumulation [63]. A ROCK inhibitor, Y27632, suppressed the light-induced cell junction and actin cytoskeleton disruption in the RPE. Taken together, these findings suggest that light-induced ROS accumulation leads to ROCK activation, which disrupts cell–cell junctions and the actin cytoskeleton, resulting in breakdown of the BRB, in vivo. This mechanism may be involved in AMD progression.

Inflammatory cytokines and macrophages have also been implicated in AMD pathogenesis [13, 14, 57]. To investigate the involvement of light-induced ROS in promoting cytokine expression in the RPE–choroid, Narimatsu et al. also analyzed cytokine mRNA and protein levels after light exposure [63]; MCP-1 is strongly

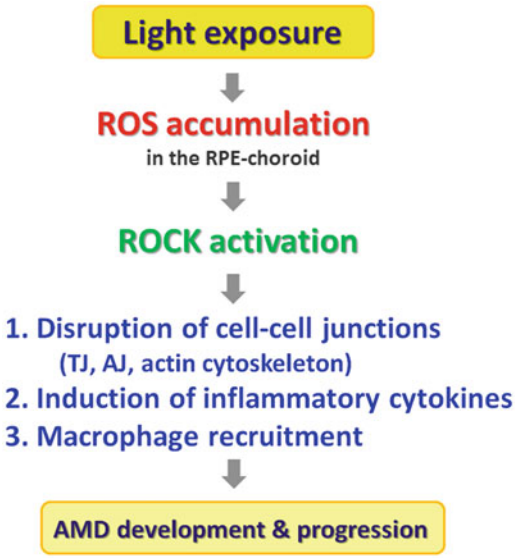


Fig. 17.5 Proposed model of the mechanism linking light exposure in the RPE–choroid to the pathogenesis of AMD. Intense light produces excessive ROS, thereby promoting ROCK activation, which leads to the disruption of cell–cell junctions and breakdown of the BRB, pathological cytokine production, and macrophage recruitment to the RPE and/or choroid. These changes may promote AMD development and progression

induced by light exposure at both the mRNA and protein levels, and macrophage infiltration is increased in the RPE–choroid, as shown by both F4/80 mRNA induction and the recruitment of F4/80-immunopositive cells to the choroid and subretinal space. The macrophage infiltration may result from the disrupted RPE and may contribute to CNV invasion into the subretinal space, since VEGF is secreted by macrophages. In addition, all of the inflammatory changes were found to be inhibited by NAC-mediated ROS suppression or Y27632-mediated ROCK inhibition. Another inflammatory cytokine, IL-6, which is also implicated in AMD pathogenesis [52], increases at the mRNA and protein levels in the RPE–choroid after light exposure, and these increases are suppressed by NAC or Y27632 treatment. Ccl-11, another AMD-related cytokine, was also found to increase after light exposure and to decrease by NAC administration, but was not regulated by ROCK activity. Taken together, these findings demonstrate that the light-induced inflammatory changes associated with AMD pathogenesis are mediated by ROS accumulation and in many cases ROCK activation.

Although light exposure is a widely accepted risk factor for AMD development, the underlying mechanisms contributing to AMD's pathogenesis have not been fully defined. However, the study by Narimatsu et al. suggests that light exposure acts on the RPE–choroid and increases the risk of AMD through ROS accumulation and ROCK activation (Fig. 17.5). To develop treatments for age-related retinal pathologies, it is important to better understand the biological effects of light exposure on the RPE, which plays a number of important roles in supporting retinal function but may cause AMD when oxidative stress accumulates. Therapeutic approaches designed to block the molecular mechanisms involved in light-induced retinal changes would be of great value. The study discussed here raises the

possibility that pharmaceuticals targeting ROS and ROCK signaling pathways may provide additional benefit when used in combination with light-blocking strategies [66, 67]. Further studies investigating the underlying molecular mechanisms and exploring potential therapeutic approaches are required.

17.5 Summary

It is well accepted that ROS accumulation in the RPE contributes to AMD pathogenesis. The studies reviewed here suggest that light stimuli may be one of the important factors that cause ROS accumulation. Light-induced ROS accelerate the pathological changes in the RPE, at least in part, by altering the BRB structure, the molecular expression of inflammatory molecules, and the subsequent recruitment of inflammatory cells. These in vivo cellular changes observed in mice and the underlying molecular mechanisms contribute to our understanding of the pathogenesis of AMD and to the investigation of new treatment targets. Further biological studies are warranted.

Acknowledgment I wish to thank all the members of the Laboratory of Retinal Cell Biology (RCB lab) and Professor Kazuo Tsubota for supporting the retinal research program.

References

1. Athanasiou D, Aguila M, Bevilacqua D, Novoselov SS, Parfitt DA, Cheetham ME (2013) The cell stress machinery and retinal degeneration. FEBS Lett 587(13):2008–2017. doi:10.1016/j.febslet.2013.05.020
2. Kaarniranta K, Sinha D, Blasiak J, Kauppinen A, Vereb Z, Salminen A, Boulton ME, Petrovski G (2013) Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration. Autophagy 9(7):973–984. doi:10.4161/auto.24546
3. Sparrow JR, Zhou J, Ben-Shabat S, Vollmer H, Itagaki Y, Nakanishi K (2002) Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE. Invest Ophthalmol Vis Sci 43(4):1222–1227
4. Maeda A, Maeda T, Golczak M, Palczewski K (2008) Retinopathy in mice induced by disrupted all-trans-retinal clearance. J Biol Chem 283(39):26684–26693. doi:10.1074/jbc.M804505200
5. Fujinami K, Zernant J, Chana RK, Wright GA, Tsunoda K, Ozawa Y, Tsubota K, Webster AR, Moore AT, Allikmets R, Michaelides M (2013) ABCA4 gene screening by next-generation sequencing in a British cohort. Invest Ophthalmol Vis Sci 54(10):6662–6674. doi:10.1167/iovs.13-12570
6. Grimm C, Wenzel A, Hafezi F, Yu S, Redmond TM, Reme CE (2000) Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. Nat Genet 25(1):63–66. doi:10.1038/75614
7. Wenzel A, Grimm C, Samardzija M, Reme CE (2003) The genetic modifier Rpe65Leu(450): effect on light damage susceptibility in c-Fos-deficient mice. Invest Ophthalmol Vis Sci 44(6):2798–2802

8. Runkle EA, Antonetti DA (2011) The blood-retinal barrier: structure and functional significance. Methods Mol Biol 686:133–148. doi:10.1007/978-1-60761-938-3_5
9. Saint-Geniez M, Kurihara T, Sekiyama E, Maldonado AE, D'Amore PA (2009) An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. Proc Natl Acad Sci USA 106(44):18751–18756. doi:10.1073/pnas.0905010106
10. Kurihara T, Westenskow PD, Bravo S, Aguilar E, Friedlander M (2012) Targeted deletion of Vegfa in adult mice induces vision loss. J Clin Invest 122(11):4213–4217. doi:10.1172/JCI65157
11. Ablonczy Z, Prakasam A, Fant J, Fauq A, Crosson C, Sambamurti K (2009) Pigment epithelium-derived factor maintains retinal pigment epithelium function by inhibiting vascular endothelial growth factor-R2 signaling through gamma-secretase. J Biol Chem 284 (44):30177–30186. doi:10.1074/jbc.M109.032391
12. Sonoda S, Sreekumar PG, Kase S, Spee C, Ryan SJ, Kannan R, Hinton DR (2010) Attainment of polarity promotes growth factor secretion by retinal pigment epithelial cells: relevance to age-related macular degeneration. Aging 2(1):28–42
13. Sakurai E, Anand A, Ambati BK, van Rooijen N, Ambati J (2003) Macrophage depletion inhibits experimental choroidal neovascularization. Invest Ophthalmol Vis Sci 44 (8):3578–3585
14. Suzuki M, Tsujikawa M, Itabe H, Du ZJ, Xie P, Matsumura N, Fu X, Zhang R, Sonoda KH, Egashira K, Hazen SL, Kamei M (2012) Chronic photo-oxidative stress and subsequent MCP-1 activation as causative factors for age-related macular degeneration. J Cell Sci 125 (Pt 10):2407–2415. doi:10.1242/jcs.097683
15. Miyake S, Takahashi N, Sasaki M, Kobayashi S, Tsubota K, Ozawa Y (2012) Vision preservation during retinal inflammation by anthocyanin-rich bilberry extract: cellular and molecular mechanism. Lab Invest J Tech Meth Pathol 92(1):102–109. doi:10.1038/labinvest.2011.132
16. Ozawa Y, Kurihara T, Sasaki M, Ban N, Yuki K, Kubota S, Tsubota K (2011) Neural degeneration in the retina of the streptozotocin-induced type 1 diabetes model. Exp Diabetes Res 2011:108328. doi:10.1155/2011/108328
17. Ozawa Y, Sasaki M, Takahashi N, Kamoshita M, Miyake S, Tsubota K (2012) Neuroprotective effects of lutein in the retina. Curr Pharm Des 18(1):51–56
18. Ozawa Y, Yuki K, Yamagishi R, Tsubota K, Aihara M (2013) Renin-angiotensin system involvement in the oxidative stress-induced neurodegeneration of cultured retinal ganglion cells. Jpn J Ophthalmol 57(1):126–132. doi:10.1007/s10384-012-0204-x
19. Grisanti S, Tatar O (2008) The role of vascular endothelial growth factor and other endogenous interplayers in age-related macular degeneration. Prog Retin Eye Res 27(4):372–390. doi:10.1016/j.preteyeres.2008.05.002
20. Ozawa Y, Ishida S, Tsubota K (2008) Age-related macular degeneration (AMD); from pathogenesis and approved therapies to proposed treatments for prevention. Anti-Aging Med 5:87–92, http://www.anti-aging.gr.jp/webjournal/thesis/vol0509/PDF/5-9r_2008.pdf
21. Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399. doi:10.1146/annurev.arplant.55.031903.141701
22. Rodriguez R, Redman R (2005) Balancing the generation and elimination of reactive oxygen species. Proc Natl Acad Sci USA 102(9):3175–3176. doi:10.1073/pnas.0500367102
23. Finkel T (2011) Signal transduction by reactive oxygen species. J Cell Biol 194(1):7–15. doi:10.1083/jcb.201102095
24. Kong X, Thimmulappa R, Kombairaju P, Biswal S (2010) NADPH oxidase-dependent reactive oxygen species mediate amplified TLR4 signaling and sepsis-induced mortality in Nrf2-deficient mice. J Immunol 185(1):569–577. doi:10.4049/jimmunol.0902315
25. Sasaki M, Ozawa Y, Kurihara T, Kubota S, Yuki K, Noda K, Kobayashi S, Ishida S, Tsubota K (2010) Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. Diabetologia 53(5):971–979. doi:10.1007/s00125-009-1655-6

26. Sasaki M, Ozawa Y, Kurihara T, Noda K, Imamura Y, Kobayashi S, Ishida S, Tsubota K (2009) Neuroprotective effect of an antioxidant, lutein, during retinal inflammation. Invest Ophthalmol Vis Sci 50(3):1433–1439. doi:10.1167/iovs.08-2493
27. Sasaki M, Yuki K, Kurihara T, Miyake S, Noda K, Kobayashi S, Ishida S, Tsubota K, Ozawa Y (2012) Biological role of lutein in the light-induced retinal degeneration. J Nutr Biochem 23 (5):423–429. doi:10.1016/j.jnutbio.2011.01.006
28. Ozawa Y, Sasaki M (2013) Lutein and oxidative stress-mediated retinal neurodegeneration in diabetes. In: Preedy V (ed) Diabetes: oxidative stress and dietary antioxidants. Academic, New York, pp 223–229
29. Age-Related Eye Disease Study Research G, SanGiovanni JP, Chew EY, Clemons TE, Ferris FL 3rd, Gensler G, Lindblad AS, Milton RC, Seddon JM, Sperduto RD (2007) The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS report No. 22. Arch Ophthalmol 125(9):1225–1232. doi:10.1001/archopht.125.9.1225
30. Age-Related Eye Disease Study 2 Research G (2013) Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. JAMA 309(19):2005–2015. doi:10.1001/jama.2013.4997
31. Hashizume K, Hirasawa M, Imamura Y, Noda S, Shimizu T, Shinoda K, Kurihara T, Noda K, Ozawa Y, Ishida S, Miyake Y, Shirasawa T, Tsubota K (2008) Retinal dysfunction and progressive retinal cell death in SOD1-deficient mice. Am J Pathol 172(5):1325–1331. doi:10.2353/ajpath.2008.070730
32. Yuki K, Ozawa Y, Yoshida T, Kurihara T, Hirasawa M, Ozeki N, Shiba D, Noda K, Ishida S, Tsubota K (2011) Retinal ganglion cell loss in superoxide dismutase 1 deficiency. Invest Ophthalmol Vis Sci 52(7):4143–4150. doi:10.1167/iovs.10-6294
33. Yuki K, Yoshida T, Miyake S, Tsubota K, Ozawa Y (2013) Neuroprotective role of superoxide dismutase 1 in retinal ganglion cells and inner nuclear layer cells against *N*-methyl-D-aspartate-induced cytotoxicity. Exp Eye Res 115:230–238. doi:10.1016/j.exer.2013.07.002
34. Thangaswamy S, Bridenbaugh EA, Gashev AA (2012) Evidence of increased oxidative stress in aged mesenteric lymphatic vessels. Lymphatic Res Biol 10(2):53–62. doi:10.1089/lrb.2011.0022
35. Ardeljan D, Chan CC (2013) Aging is not a disease: Distinguishing age-related macular degeneration from aging. Prog Retin Eye Res 37:68–89. doi:10.1016/j.preteyeres.2013.07.003
36. Gu X, Neric NJ, Crabb JS, Crabb JW, Bhattacharya SK, Rayborn ME, Hollyfield JG, Bonilha VL (2012) Age-related changes in the retinal pigment epithelium (RPE). PLoS One 7(6): e38673. doi:10.1371/journal.pone.0038673
37. Plafker SM, O'Mealey GB, Szweda LI (2012) Mechanisms for countering oxidative stress and damage in retinal pigment epithelium. Int Rev Cell Mol Biol 298:135–177. doi:10.1016/B978-0-12-394309-5.00004-3
38. Finnemann SC, Leung LW, Rodriguez-Boulan E (2002) The lipofuscin component A2E selectively inhibits phagolysosomal degradation of photoreceptor phospholipid by the retinal pigment epithelium. Proc Natl Acad Sci USA 99(6):3842–3847. doi:10.1073/pnas.052025899
39. Alexeyev MF (2009) Is there more to aging than mitochondrial DNA and reactive oxygen species? FEBS J 276(20):5768–5787. doi:10.1111/j.1742-4658.2009.07269.x
40. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA (2005) Complement factor H polymorphism and age-related macular degeneration. Science 308(5720):421–424. doi:10.1126/science.1110189
41. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA (2005) Complement factor H variant increases the risk of age-related macular degeneration. Science 308(5720):419–421. doi:10.1126/science.1110359
42. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J (2005) Complement

factor H polymorphism in age-related macular degeneration. Science 308(5720):385–389. doi:10.1126/science.1109557
43. Clemons TE, Milton RC, Klein R, Seddon JM, Ferris FL 3rd, Age-Related Eye Disease Study Research G (2005) Risk factors for the incidence of advanced age-related macular degeneration in the age-related eye disease study (AREDS) AREDS report no. 19. Ophthalmology 112 (4):533–539. doi:10.1016/j.ophtha.2004.10.047
44. Relvas LJ, Bouffioux C, Marcet B, Communi D, Makhoul M, Horckmans M, Blero D, Bruyns C, Caspers L, Boeynaems JM, Willermain F (2009) Extracellular nucleotides and interleukin-8 production by ARPE cells: potential role of danger signals in blood-retinal barrier activation. Invest Ophthalmol Vis Sci 50(3):1241–1246. doi:10.1167/iovs.08-1902
45. Iriyama A, Iriyama T, Tamaki Y, Yanagi Y (2008) Effects of white light on beta-catenin signaling pathway in retinal pigment epithelium. Biochem Biophys Res Comm 375(1):173–177. doi:10.1016/j.bbrc.2008.07.158
46. Glotin AL, Debacq-Chainiaux F, Brossas JY, Faussat AM, Treton J, Zubielewicz A, Toussaint O, Mascarelli F (2008) Prematurely senescent ARPE-19 cells display features of age-related macular degeneration. Free Radic Biol Med 44(7):1348–1361. doi:10.1016/j.freeradbiomed.2007.12.023
47. Yoshida T, Ohno-Matsui K, Ichinose S, Sato T, Iwata N, Saido TC, Hisatomi T, Mochizuki M, Morita I (2005) The potential role of amyloid beta in the pathogenesis of age-related macular degeneration. J Clin Invest 115(10):2793–2800. doi:10.1172/JCI24635
48. Bian Q, Gao S, Zhou J, Qin J, Taylor A, Johnson EJ, Tang G, Sparrow JR, Gierhart D, Shang F (2012) Lutein and zeaxanthin supplementation reduces photooxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells. Free Radic Biol Med 53(6):1298–1307. doi:10.1016/j.freeradbiomed.2012.06.024
49. Inumaru J, Nagano O, Takahashi E, Ishimoto T, Nakamura S, Suzuki Y, Niwa S, Umezawa K, Tanihara H, Saya H (2009) Molecular mechanisms regulating dissociation of cell–cell junction of epithelial cells by oxidative stress. Genes Cells Devoted Mol Cell Mech 14(6):703–716. doi:10.1111/j.1365-2443.2009.01303.x
50. Bailey TA (2004) Oxidative stress affects the junctional integrity of retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 45(2):675–684. doi:10.1167/iovs.03-0351
51. Hasegawa E, Sonoda KH, Shichita T, Morita R, Sekiya T, Kimura A, Oshima Y, Takeda A, Yoshimura T, Yoshida S, Ishibashi T, Yoshimura A (2013) IL-23-independent induction of IL-17 from gammadeltaT cells and innate lymphoid cells promotes experimental intraocular neovascularization. J Immunol 190(4):1778–1787. doi:10.4049/jimmunol.1202495
52. Izumi-Nagai K, Nagai N, Ohgami K, Satofuka S, Ozawa Y, Tsubota K, Umezawa K, Ohno S, Oike Y, Ishida S (2007) Macular pigment lutein is antiinflammatory in preventing choroidal neovascularization. Arterioscler Thromb Vasc Biol 27(12):2555–2562. doi:10.1161/ATVBAHA.107.151431
53. Lambert V, Lecomte J, Hansen S, Blacher S, Gonzalez ML, Struman I, Sounni NE, Rozet E, de Tullio P, Foidart JM, Rakic JM, Noel A (2013) Laser-induced choroidal neovascularization model to study age-related macular degeneration in mice. Nat Protoc 8(11):2197–2211. doi:10.1038/nprot.2013.135
54. Mochimaru H, Takahashi E, Tsukamoto N, Miyazaki J, Yaguchi T, Koto T, Kurihara T, Noda K, Ozawa Y, Ishimoto T, Kawakami Y, Tanihara H, Saya H, Ishida S, Tsubota K (2009) Involvement of hyaluronan and its receptor CD44 with choroidal neovascularization. Invest Ophthalmol Vis Sci 50(9):4410–4415. doi:10.1167/iovs.08-3044
55. Nagai N, Oike Y, Izumi-Nagai K, Koto T, Satofuka S, Shinoda H, Noda K, Ozawa Y, Inoue M, Tsubota K, Ishida S (2007) Suppression of choroidal neovascularization by inhibiting angiotensin-converting enzyme: minimal role of bradykinin. Invest Ophthalmol Vis Sci 48 (5):2321–2326. doi:10.1167/iovs.06-1296
56. Nagai N, Oike Y, Izumi-Nagai K, Urano T, Kubota Y, Noda K, Ozawa Y, Inoue M, Tsubota K, Suda T, Ishida S (2006) Angiotensin II type 1 receptor-mediated inflammation is required for

choroidal neovascularization. Arterioscler Thromb Vasc Biol 26(10):2252–2259. doi:10.1161/01.ATV.0000240050.15321.fe
57. Takeda A, Baffi JZ, Kleinman ME, Cho WG, Nozaki M, Yamada K, Kaneko H, Albuquerque RJ, Dridi S, Saito K, Raisler BJ, Budd SJ, Geisen P, Munitz A, Ambati BK, Green MG, Ishibashi T, Wright JD, Humbles AA, Gerard CJ, Ogura Y, Pan Y, Smith JR, Grisanti S, Hartnett ME, Rothenberg ME, Ambati J (2009) CCR3 is a target for age-related macular degeneration diagnosis and therapy. Nature 460(7252):225–230. doi:10.1038/nature08151
58. Satofuka S, Ichihara A, Nagai N, Noda K, Ozawa Y, Fukamizu A, Tsubota K, Itoh H, Oike Y, Ishida S (2008) (Pro)renin receptor promotes choroidal neovascularization by activating its signal transduction and tissue renin-angiotensin system. Am J Pathol 173(6):1911–1918. doi:10.2353/ajpath.2008.080457
59. Du H, Sun X, Guma M, Luo J, Ouyang H, Zhang X, Zeng J, Quach J, Nguyen DH, Shaw PX, Karin M, Zhang K (2013) JNK inhibition reduces apoptosis and neovascularization in a murine model of age-related macular degeneration. Proc Natl Acad Sci USA 110(6):2377–2382. doi:10.1073/pnas.1221729110
60. Sheu SJ, Liu NC, Ou CC, Bee YS, Chen SC, Lin HC, Chan JY (2013) Resveratrol stimulates mitochondrial bioenergetics to protect retinal pigment epithelial cells from oxidative damage. Invest Ophthalmol Vis Sci 54(9):6426–6438. doi:10.1167/iovs.13-12024
61. Doyle SL, Campbell M, Ozaki E, Salomon RG, Mori A, Kenna PF, Farrar GJ, Kiang AS, Humphries MM, Lavelle EC, O'Neill LA, Hollyfield JG, Humphries P (2012) NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. Nat Med 18(5):791–798. doi:10.1038/nm.2717
62. Kaneko H, Dridi S, Tarallo V, Gelfand BD, Fowler BJ, Cho WG, Kleinman ME, Ponicsan SL, Hauswirth WW, Chiodo VA, Kariko K, Yoo JW, Lee DK, Hadziahmetovic M, Song Y, Misra S, Chaudhuri G, Buaas FW, Braun RE, Hinton DR, Zhang Q, Grossniklaus HE, Provis JM, Madigan MC, Milam AH, Justice NL, Albuquerque RJ, Blandford AD, Bogdanovich S, Hirano Y, Witta J, Fuchs E, Littman DR, Ambati BK, Rudin CM, Chong MM, Provost P, Kugel JF, Goodrich JA, Dunaief JL, Baffi JZ, Ambati J (2011) DICER1 deficit induces Alu RNA toxicity in age-related macular degeneration. Nature 471(7338):325–330. doi:10.1038/nature09830
63. Narimatsu T, Ozawa Y, Miyake S, Kubota S, Hirasawa M, Nagai N, Shimmura S, Tsubota K (2013) Disruption of cell–cell junctions and induction of pathological cytokines in the retinal pigment epithelium of light-exposed mice. Invest Ophthalmol Vis Sci 54(7):4555–4562. doi:10.1167/iovs.12-11572
64. Kerksick C, Willoughby D (2005) The antioxidant role of glutathione and *N*-acetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr 2:38–44. doi:10.1186/1550-2783-2-2-38
65. Riento K, Ridley AJ (2003) Rocks: multifunctional kinases in cell behaviour. Nat Rev Mol Cell Biol 4(6):446–456. doi:10.1038/nrm1128
66. Narimatsu T, Ozawa Y, Miyake S, Kubota S, Yuki K, Nagai N, Tsubota K (2013) Biological effects of blocking blue and other visible light on the mouse retina. Clin Exp Ophthalmol. doi:10.1111/ceo.12253
67. Tanito M, Kaidzu S, Anderson RE (2006) Protective effects of soft acrylic yellow filter against blue light-induced retinal damage in rats. Exp Eye Res 83(6):1493–1504. doi:10.1016/j.exer.2006.08.006

Chapter 18
Interaction Between Photoreceptors and RPEs

Takeshi Yoshida and Kyoko Ohno-Matsui

Abstract The relationship between the retinal pigment epithelial cells (RPE) and photoreceptor cells is crucial to sight; this is evident from basic and clinical studies demonstrating that primary dysfunction of the RPE can result in photoreceptor cell death and blindness. RPE cells have many functions including the conversion and storage of retinoid, the phagocytosis of shed photoreceptor outer segment membrane, and the transport of ion and fluid between RPE and photoreceptors to keep homeostasis of the eye. The major cause of adult blindness in industrialized countries is the progressive dysfunction and death of the retinal photoreceptors including age-related macular degeneration (AMD) and retinitis pigmentosa (RP). Molecular interaction between the RPE and photoreceptors in the pathogenesis of the diseases is complicated. The pathogenesis of AMD and RP is strongly associated with metabolism, aging, and genetic disorder. In addition to the molecular interaction between the RPE and photoreceptors, we address the correlation between gene expression and pathogenesis of AMD and RP in this review.

Keywords Age-related macular degeneration • Retinal pigment epithelial cell • Photoreceptors • Retinitis pigmentosa

18.1 Molecular Basis of Retinal Pigment Epithelial Cells and Photoreceptors

The retinal pigment epithelium (RPE) forms a monolayer of highly specialized neuroectodermally derived pigmented cells located between the neurosensory retina and the vascular choroid. Most of the RPE cells have developed polarized

T. Yoshida (✉) • K. Ohno-Matsui
Department of Ophthalmology and Visual Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
e-mail: takeoph@tmd.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_18,

characteristics with cobblestone-like shape and apical microvilli that extend into the subretinal space. The polarized organization of the RPE is essential for the proper functioning of the neural retina. The apical microvilli of the RPE face and interdigitate with the outer segments (OS) of the photoreceptor cells, while its basal surface is attached to the underlying basement membrane (Bruch's membrane). The RPE mediates functions essential for normal outer retinal physiology, including participation in the visual cycle, phagocytosis of shed photoreceptor OS, maintenance of the blood-retinal barrier (BRB), secretion of neurotrophic, inflammatory, and vasculotrophic growth factors, water transport out of subretinal space, and regulation of bidirectional ion and metabolic transport between the retina and choroid [1]. The RPE is a primary site of pathology in major causes of blindness diseases, including age-related macular degeneration (AMD) and pigment epithelium degeneration.

Our visual experience is initiated by the rod and cone photoreceptors in the retina. The photoreceptors are a specialized type of neuron that is capable of phototransduction. The photoreceptors convert light into signals that can stimulate neuronal impulse transmission by triggering a change in the cell membrane potential after absorbing a photon. The rod and cone photoreceptors are located in the outer nuclear layer of the retina. The rod and cone photoreceptors are polarized sensory neurons that contain the protein machinery necessary to convert incident light into a signal that can be interpreted by the nervous system of brain. The rod functions primarily in dim light and provides black and white vision, while the cone supports daytime vision and is responsible for color perception. Both the rod and cone photoreceptors are highly polarized elongated cells that can be described as having four subcellular compartments: the outer segment (OS), the inner segment (IS), the nucleus, and the synaptic terminal. The OS is where photons are captured, and activation of the phototransduction cascade begins. The OS compartment contains all components necessary for phototransduction, which is a set of biochemical reaction that converts photon capture to a change in a cationic current at the plasma membrane. Unlike the upper layers of the retina, the photoreceptor layer does not contain blood vessels: oxygen and nutrients are instead provided by the choriocapillaris through the RPE. The photoreceptor IS is rich in mitochondria, which are needed to provide energy for these highly metabolically active cells. The photoreceptors consume more oxygen per gram of tissue weight than any cell in the body and have a tissue oxygen level close to zero in the dark [2]. Loss of visual cells by apoptosis is a key feature of retinitis pigmentosa (RP) and of AMD [3–5]. Thus, understanding the process of photoreceptor apoptosis might provide clues on how to interfere with photoreceptor loss and therefore loss of vision in these diseases.

18.1.1 Phagocytosis

The RPE cells play an important role in the integrity of the visual axis through their phagocytic function. The RPE apically extends microvilli that interdigitate with the

photoreceptor OS. This arrangement is critical to keeping vision since renewal of the phototransduction and maintenance of a constant OS length require the shedding of apical stacks of discs. The RPE cells phagocytose photoreceptor OS that are shed daily during renewal of the photoreceptors. Indeed, defects in this process in several models have demonstrated its importance for the function and longevity of the photoreceptors [6–8]. Degradation of the OS following phagocytosis by RPE takes place following the delivery of lysosomal enzymes to the phagosome and the activation of proteolytic enzymes during acidification of this compartment. Degradation products are then removed from the cell by transport to the blood. Some phagocytosed disc materials are recycled to the photoreceptors to replenish necessary components [1]. Considering their role in daily phagocytosis of shed discs from the photoreceptor cells, postmitotic RPE cells can be considered as some of the most active phagocytic cells in the body. Photoreceptor disc shedding and RPE-mediated phagocytosis are mediated by a complex sequence of steps by both the photoreceptor and RPE layers. It begins with shedding of the photoreceptor OS discs, which expose phosphatidylserine at the tips of the photoreceptors to facilitate binding to $\alpha_v\beta_5$ [9] and CD36 [10] receptors on the apical surface of RPE cells. Disruption of RPE phagocytosis has been linked to disease phenotypes such as retinitis pigmentosa and rod/cone dystrophies [11]; however, the precise mechanisms are still unclear.

18.1.2 Visual Cycle

Light perception in the retina is initiated by the reaction of photons with light-sensitive pigments that are part of the membranes of the photoreceptor OS. Cooperation between the RPE cells and the photoreceptor allows these pigments to be recycled through a complex series of oxidation-reduction reactions and transport mechanisms that are referred as the visual cycle. The RPE contains the visual cycle required for the enzymatic isomerization of vitamin A [all-*trans*-retinol] into the light-sensitive chromophore [11-*cis*-retinal] to regenerate photobleached pigments for optimal vision [12]. All-*trans*-retinol is transported through the interface of the photoreceptor OS and microvilli of RPE via interphotoreceptor matrix (IPM) and interphotoreceptor retinoid-binding protein (IRBP). IRBP is the important soluble protein that exists in the IPM and functions as the two-way carrier of retinoids, both from the RPE to the photoreceptors and from the photoreceptors back to the RPE [13, 14] (Fig. 18.1). Photoreceptors are dependent upon the RPE cells for survival, so after RPE cells drop out, overlying photoreceptors die. Defects in either process can have serious consequences for vision.

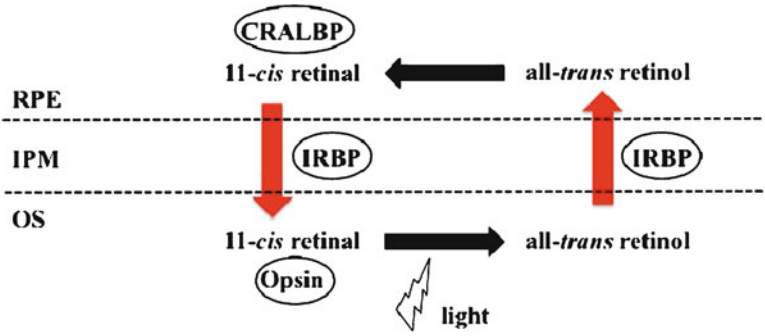


Fig. 18.1 Visual cycle between RPE and OS of photoreceptor. *RPE* retinal pigment epithelial. *IPM* interphotoreceptor matrix. *OS* outer segment. *IRBP* interphotoreceptor retinoid-binding protein. *CRALBP* cellular retinaldehyde-binding protein

18.1.3 Transport of Nutrients, Ions, and Water

The RPE constitutes the outer BRB. Tight junctions between the RPE cells and the endothelial cells of choroid are essential in the strict control of fluids and solutes that cross the BRB as well as in preventing the entrance of toxic molecules and plasma components into the retina. Therefore, this function is essential for the integrity of the retina [15]. In one direction, the RPE transports electrolytes and water from the subretinal space to the choroid, and in the other direction, the RPE transports glucose and other nutrients from the blood to the photoreceptors.

18.1.4 Transport from Choroid to Photoreceptors

The RPE takes up nutrients such as glucose, retinol, and fatty acids from the blood and delivers these nutrients to the photoreceptors. To transport glucose, the RPE contains high amounts of glucose transporters such as GLUT1 and GLUT3 in both the apical and the basolateral membranes [16]. Another important function of the RPE is the transport of retinol to the photoreceptors as mentioned in above. Delivery of fatty acids such as docosahexaenoic acid (DHA) to the photoreceptors is a third kind of transport of importance for visual function [17]. DHA is an essential omega-3 fatty acid that is required as structural element by the membranes of the photoreceptors. DHA is taken up in a concentration-dependent manner.

18.1.5 Transport from Subretinal Space to Blood

The RPE is responsible for transportation of ions and water from the apical side to the basolateral side of the RPE. The Na^+-K^+-ATPase, which is located in the apical membrane of the RPE, provides the energy for transepithelial transport [18]. A consequence of the large metabolic turnover in neurons and photoreceptors produce a large amount of water. This makes the need for the constant removal of water from the inner retina to the choriocapillaris. Water in the subretinal space is eliminated by the RPE. The transport of water is mainly driven by a transport of Cl^- and K^+ [19]. Tight junctions make a barrier between the subretinal space and the choriocapillaris. For this reason, water cannot pass through the paracellular transport route, and water transport occurs mainly by transcellular pathways facilitated by aquaporin-1 [20].

18.1.6 Secretion of Cytokines and Growth Factors

The RPE is known to produce and to secrete a variety of cytokines and growth factors [21] as well as factors that are essential for the maintenance of the structural integrity of the retina. The RPE produces molecules that support the survival of the photoreceptors and protect a structural basis for the optimal circulation and supply of nutrients. The RPE is able to secrete pigment epithelium-derived factor (PEDF) [22], vascular endothelial growth factor (VEGF) [23], fibroblast growth factors (FGF-1, FGF-2, and FGF-5) [24], transforming growth factor-β (TGF-β) [25], insulin-like growth factor-I (IGF-I) [26], nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), ciliary neurotrophic factor (CNTF) [27], platelet-derived growth factor (PDGF) [28], tumor necrosis factor-α (TNF-α), colony-stimulating factors (CSF), and different types of tissue inhibitor of matrix metalloproteinase (TIMP). Among these factors, the best known factors are VEGF and PEDF.

18.1.7 PEDF and VEGF

The RPE secretes PEDF [22], which helps to maintain the retinal as well as the choriocapillaris structure in two ways. PEDF was described as a neuroprotective factor because it was shown to protect neurons against glutamate-induced or hypoxia-induced apoptosis. In addition, PEDF was shown to function as an antiangiogenic factor that inhibited endothelial cell proliferation and stabilized the endothelium of the choriocapillaris. These effects on vascularization also play an important role in the embryonic development of the eye. Using PEDF-deficient (PEDF-/-) mice, it has been confirmed that PEDF is an important modulator of early

postnatal retinal vascularization and that in its absence retinal vascularization proceeds at a faster rate and is more susceptible to hyperoxia-mediated vessel obliteration. VEGF is secreted in low concentrations by the RPE where it prevents endothelial cell apoptosis and is essential for an intact endothelium of the choriocapillaris. VEGF also acts as a permeability factor stabilizing the fenestrations of the endothelium [29]. In a healthy eye, PEDF and VEGF are secreted at opposite sides of the RPE. PEDF is predominantly secreted from the apical surface where it promotes an antiangiogenic and neuroprotective environment for the photoreceptors, whereas most of VEGF is secreted to the basal side where it acts on the choroidal endothelium. These factors show dysregulated expression in many retinal diseases such as AMD and pigment epithelium degeneration including RP.

18.2 Correlation Between RPE and Photoreceptor in Age-Related Macular Degeneration and Retinitis Pigmentosa

Among the elderly, blindness is feared more than any illness. The major cause of adult blindness in industrialized countries is the progressive dysfunction and death of the retinal photoreceptors. Diseases caused by RPE malfunction, including AMD and some forms of RP, lead to photoreceptor degeneration. As the average lifespan increases, disease prevalence is expected to rise. Treatments such as ranibizumab show the capacity to slow the rate of vision loss, but have no more than a 10 % rate of effectiveness in all AMD cases [30]. On the other hand, no effective treatments are currently available to restore the vision of patients who suffer from RP.

18.2.1 Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a heterogeneous set of inherited retinopathies with many disease-causing genes, many known mutations, and highly varied clinical consequences. RP is also one of the main causes of acquired blindness in developed countries. RP is a slowly progressive inherited retinal disease, and patients with RP have reduced visual function mainly because of degeneration of the rod and cone photoreceptors and the RPE [31–33]. A clinical diagnosis of RP is made based on the family history, presence of nyctalopia, visual field constriction, characteristic pigmentary retinal changes, bone spicule pigmentation in the periphery, and reduction of the standard full-field electroretinograms (ERGs) consistent with rod-cone dystrophy [34]. The ERG changes are presumably due to a degeneration of the photoreceptors. Currently, there is no treatment that can lead to a recovery of the decreased vision or halt the progression of the disease process. Several genetic mutations have been found to cause RP, e.g., X-linked, autosomal dominant, and

autosomal recessive conditions. However, RP without a known family history can also occur [33]. In spite of genetic diversity, many RP patients have similar symptoms, signs, and retinal histopathology [33, 35].

In RP patient, the retinal degeneration usually starts with degeneration of the rod photoreceptors which leads to nyctalopia. With advancement, the retinal degeneration gives rise to a characteristic ring-shaped scotoma in the midperiphery. The scotoma can then expand to the periphery and macula. The earliest histopathologic change in all forms of RP is a shortening of the rod outer segments [31, 32]. As the disease progresses, the cone photoreceptors also degenerate which then reduces central vision [35].

18.2.1.1 Mutations Affecting the OS Phagocytosis in RPE

The photoreceptor outer segment is a crucial compound in the processes mediating vision. The daily renewal and shedding of outer segment disks is a delicate process that forms a metabolic burden to the photoreceptor and RPE cells. Disruption of the continuous disk renewal or overactive degradation may lead to shortened and malfunctioning outer segments. Deficient degradation of the shedded disk material will result in the formation of retinal deposits, obstructing the flow of metabolites that need to pass through the RPE to the photoreceptor. Obviously, defects in this balance of disk renewal and disk shedding may result in retinal degeneration. It has been described some evidences that a subset of genes mutated in RP affects this mechanism, which leads to phenotypes with similar features. These genes encode rhodopsin, peripherin/RDS, and ROM1. Among them, the peripherin/RDS mutation seems to strongly correlate with OS phagocytosis. Furthermore, the mutations in retinol (vitamin A), cellular retinaldehyde-binding protein (CRALBP), and retinal pigment epithelium protein 65 (RPE65) gene are described to be associated with OS phagocytosis and RPE.

18.2.1.2 Peripherin/Retinal Degeneration Slow (Peripherin/RDS)

The importance of peripherin/RDS to the development and structural maintenance of photoreceptor OS was demonstrated in the RDS mouse [36], a naturally occurring retinal degeneration caused by a mutation in the gene encoding peripherin/RDS [37]. In this mouse, a large insertion in the peripherin/RDS gene interferes with the transcription and translational processes so that no protein will be synthesized [38]. Mice homozygous for the mutation completely lack peripherin/RDS and develop no outer segments [39]. In heterozygous mice, with a 50 % reduction in peripherin/RDS, outer segments were present in normal numbers but were shortened and displayed structural disorganization. A slow progression of retinal degeneration has been observed in these mice. In humans, mutations in the peripherin/RDS gene are involved in degenerative disease of the retina as well. A subset of the observed mutations results in a premature truncation of the protein, which will often

result in complete absence of functional peripherin/RDS. Individuals carrying such mutations have some distinctive features in common. In early stages no photoreceptor abnormalities are observed. This is followed by functional loss of both rods and cones and the appearance of yellow deposits in the RPE. When the disease progresses, degeneration of the RPE and photoreceptors is observed [40]. It has been proposed that the 50 % reduction in the amount of functional peripherin/RDS causes the observed phenotype, similar to the observations in the RDS mouse [41]. Insufficient peripherin/RDS leads to instability of the ROS and affects the continuous internalization and degradation of outer segment fragments by the RPE, which poses a substantial burden on the RPE cell metabolism. Initially this may be visualized by the appearance of lipofuscin containing yellowish dots, whereas later stages present with RPE atrophy accompanied by the degeneration of overlying photoreceptor cells. Mutations that result in the synthesis of an aberrant protein have been observed in the peripherin/RDS gene as well. Extensive phenotypic variation is observed in individuals heterozygous for these mutations, which demonstrate dominant segregation within families. Affected individuals display either RP, pattern dystrophy of the RPE, or cone and cone-rod dystrophies. These phenotypes all result from the degeneration of photoreceptors, but differ in the extent of rod and/or cone involvement.

18.2.1.3 Mutations Affecting the Retinol (Vitamin A) Metabolism

The RPE plays an essential role in the shedding and degradation of ROS disks. The second major function of the RPE cells lies in the retinol metabolism. Retinol deficiency resulting from malnutrition or metabolic disorders results in a phenotype similar to RP. Characteristic symptoms include night blindness and abnormalities in the ERG and the visual field. In contrast to the pigment deposits characteristic of RP, small white deposits at the level of the RPE characterize retinol deficiency. Animal models have shown that lasting retinol (vitamin A) deficiency ultimately leads to photoreceptor degeneration [42, 43]. Recently mutations in genes involved in the regeneration of the light-sensitive retinol derivative 11-*cis*-retinal have been found in RP and allied disorders.

18.2.1.4 Cellular Retinaldehyde-Binding Protein

The CRALBP is expressed in the RPE cells and is one of the retinoid-binding proteins that play a role in the regeneration of 11-*cis*-retinal after bleaching of the photoreceptor cells. It acts as a substrate-carrier protein for 11-*cis*-retinol and 11-*cis*-retinal, thereby modulating the interaction between these proteins and visual cycle enzymes [44]. Mutations in this gene have been identified in an Indian pedigree from a consanguineous background. Patients experienced atypical RP with early onset of night blindness, macular degeneration, and visual loss, progressing toward legal blindness in the third decade. Numerous white dots were

scattered over the entire fundus, similar to the phenotype resulting from retinol deficiency. All affected individuals are homozygous for a mutation in a domain that is highly conserved among related retinoid-binding proteins. In human, this mutation probably results in a complete loss of function that severely disturbs or even completely blocks the regeneration of 11-*cis*-retinal and rhodopsin [45] (Fig. 18.1).

18.2.1.5 Retinal Pigment Epithelium Protein 65

RPE65 is a 61-kD protein specifically expressed in the RPE. It accounts for 10 % of the total amount of protein expressed in this tissue. Its exact function is not known, although it is supposed to be part of the retinoid metabolism, because it is associated with the plasma retinol-binding protein, 11-*cis*-retinol dehydrogenase, and a third unknown protein [46]. A number of pedigrees segregating autosomal recessive retinal degeneration resulting from mutations in the RPE65 gene have been described. The phenotypes varied from severe, early-onset RP to Leber's congenital amaurosis [47]. All mutations described are likely to result in a partial or complete loss of function of the RPE65 protein, which explains the early or even congenital onset of the disease.

The year 2011 brought a new advance in the treatment of macular degenerations, with the US Food and Drug Administration approving clinical trials using ES cell-derived RPE transplants [48]. The eye is an ideal testing ground for stem cell therapies; since it exhibits relative immune privilege, it is readily accessible for monitoring and imaging purposes; and in the event of serious complications, its removal is not a life-threatening event. Li et al. [49] tested both the safety of differentiating human iPS cells into RPE for implant and the efficacy of iPS transplantation in improving retinal function in the Rpe65rd12/Rpe65rd12 mice. In addition, to determine whether any rescue effects were due to surgical injury or feeder cells, control groups of Rpe65rd12/Rpe65rd12 mice received grafts of mitomycin-C-treated undifferentiated mouse ES cells. Encouragingly, the iPS cell-derived RPE cells expressed RPE markers, and the mice transplanted with these cells showed enhanced ERG responses compared with control groups.

18.2.2 Age-Related Macular Degeneration

AMD is the leading cause of blindness in the elderly [50]. There are two phenotypes of the disease: the first is "dry," which accounts for about 90 % of the cases. Dry AMD is characterized by extensive loss of RPE cells and the photoreceptors and choriocapillaris [51]. The second phenotype is "wet." This form of AMD is characterized by the presence of choroidal neovascularization (CNV). CNV breaks through Bruch's membrane to the neuroretina from choroid, causing fluid leakage, lipid accumulation, and ultimately resulting in fibrovascular scarring and loss of photoreceptors. Considering the significant medical, personal, social, and economic

costs of AMD, the novel therapeutic and preventative strategies for AMD are needed. Innovation in AMD pharmacotherapy, in turn, depends largely upon a thorough understanding of the molecular mechanisms underlying AMD pathogenesis.

18.2.2.1 Changes of the Photoreceptors and Association with RPE in AMD

Curcio et al. observed more rod loss than cone loss in dry AMD as well as in wet form [51]. They suggested that photoreceptor degeneration and loss occurs before disease in the RPE/Bruch's membrane complex. This is consistent with reports of impaired visual function in patients with dry AMD. Dunaief et al. observed the apoptotic photoreceptors, the RPE, and the inner nuclear layer cells in human dry and wet AMD, and they also observed TUNEL-positive RPE and photoreceptor cells that were present at the edges of geographic atrophy [52], in which RPE cells die first, followed by photoreceptor death due to loss of the RPE support functions [53]. Furthermore, increased Fas labeling in AMD photoreceptors indicates that the Fas/Fas ligand system may be involved in photoreceptor apoptosis [52]. Maeda et al. showed that the loss of photoreceptors was directly related to loss of the RPE after intravitreal ornithine-induced degeneration, suggesting the importance of the RPE in the photoreceptor viability [54]. In geographic atrophy, the photoreceptors loss was associated with RPE loss [55]. Degeneration of the RPE cells severely impairs the visual function of the photoreceptors.

18.2.2.2 Drusen

Early stages of the disease feature the deposition of extracellular deposit, known as drusen, from the basal side of the RPE into Bruch's membrane. Drusen are the clinical hallmark of AMD, which consist of pathological, round-shaped, and yellowish extracellular deposits. Under the ophthalmoscope, they can be divided into hard drusen and soft ones. The hard drusen are considered to be a consequence of normal aging and appear to be yellow dots and have sharp borders. Soft drusen, which are not considered a part of normal aging, are larger than hard drusen, with fuzzy borders [56]. The latter are high-risk factors for the development of AMD complications [57].

Drusen include various elements. Advanced glycation end products (AGEs), ubiquitin, integrins, lipoproteins, tissue inhibitor of metalloproteinase 3, beta-amyloid, fibronectin, vitronectin, apolipoprotein E (ApoE), factor X, immunoglobulin lambda chains, complement components like C1q, late-stage activated complement components like the C5b-9 complex, major histocompatibility complex (MHC) class II antigens, lipofuscin, and melanin have been identified in drusen [58–61]. Drusen represent not only waste but also the by-product of local active processes involving inflammation, the complement system, and the

immune-related mechanisms [60]. Among them, one of key components of drusen is beta-amyloid, which accumulates in the central nervous system with aging and in Alzheimer's disease. Increased accumulation of beta-amyloid with aging was found in drusen [62]. We showed that neprilysin gene-disrupted mice, which leads to an increased deposition of beta-amyloid in drusen, have a marked increase in VEGF as well as a marked decrease in PEDF in the RPE [63]. Since PEDF has a neuroprotective role, a decrease of PEDF secretion from the RPE may promote a degeneration of the photoreceptors. Other interesting molecules in drusen are lipofuscin and AGEs. Lipofuscin is a by-product of photoreceptor OS turnover and is seen within both healthy and pathological RPE [64]. Lipofuscin pigments are generated from random nonenzymatic reactions of retinaldehyde in photoreceptors throughout life and enter RPE via phagocytosis. One of the principal lipofuscin fluorophores, A2E, has been experimentally shown to mediate apoptosis in blue-light-exposed RPE cell culture [65] as well as induce activation of the complement system in vitro [66], providing a link to AMD pathogenesis. AGEs are oxidative protein modifications resulting from the Maillard nonenzymatic glycation reaction that have been associated with aging and age-related diseases [67]. AGEs communicate to cells via the receptors, receptor for advanced glycation end products (RAGE) on RPE surface. RAGE activation by AGEs has been shown to increase a secretion of VEGF through an activation of nuclear factor-kappaB (NF-kB) in the RPE [68]. They contribute to the pathogenesis of AMD. Dysfunction of the RPE in AMD may lead to photoreceptor loss and blindness.

18.2.2.3 Activation of the Complement Cascade

Recent studies have showed significant interest in the inflammatory event of AMD pathogenesis [69]. These studies have been buttressed by genetic studies identifying inflammation-associated single nucleotide polymorphisms (SNPs) that modulate AMD risk. These SNPs lie in genes encoding complement factors. A number of studies have described the presence of complement components and complement regulatory proteins in drusen and nearby RPE of AMD patients, suggesting a role for complement in AMD pathogenesis [70]. A major AMD-associated SNP is the Y402H (tyrosine to histidine substitution at amino acid 402: Tyr402His) variant of complement factor H (CFH) [71–74]. CFH is now widely accepted as an important AMD susceptibility gene. CFH is identified in normal RPE and choroid [74]. CFH mainly functions to control the alternative complement activation in sites of tissue inflammation. CFH is involved in inhibiting the inflammatory response mediated via C3b by acting as a cofactor for cleavage of C3b to its inactive form, iC3b, and by weakening the active complex that forms between C3b and factor B (Fig. 18.2). C-reactive protein (CRP) and glycosaminoglycans normally improve the ability of CFH to inhibit complement. The mutation in CFH (Tyr402His) reduces the affinity of CFH for CRP and seems to alter the ability of factor H to recognize specific glycosaminoglycans. This change could result in reduced ability of CFH to regulate the alternative pathway, which could be permissive for chronic inflammation [75].

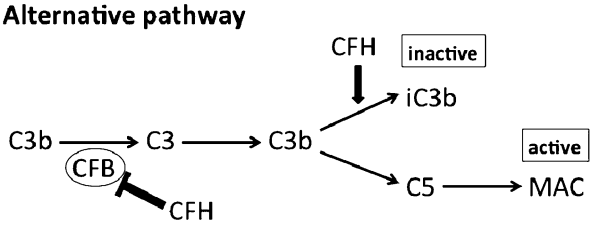


Fig. 18.2 Activation and inactivation of alternative complement pathway. *CFH* complement factor H. *CFB* complement factor B. *MAC* membrane attack complex

In addition to CFH, polymorphisms in complement factor B (CFB), complement factor I (CFI), factor 2 (C2), and factor 3 (C3) genes have been reported as novel risk factors for AMD [76–78]. An abundance of chronic inflammatory in RPE leads to dysfunction and cell death of the RPE cell, which finally induces loss of the photoreceptor.

18.2.2.4 Apolipoprotein E

ApoE is a structural component of plasma chylomicrons, very-low-density lipoproteins (VLDL), and a subclass of high-density lipoproteins (HDL) and is synthesized in many tissues. The primary physiological role of ApoE is to facilitate the binding of LDL to LDL receptors on cellular membranes, thereby regulating the uptake of cholesterol required by cells. Klaver et al. have speculated that an active biosynthesis of ApoE is required to support the high rate of photoreceptor renewal in the macular region [79]. The gene for ApoE is polymorphic, with three major allelic variants, ε2, ε3, and ε4 [80, 81]. Many previous studies suggest that the ApoE ε4 allele is associated with a lower risk of late AMD [82], whereas less consistently the ε2 allele seems to confer an increased risk [79].

18.2.2.5 Vascular Endothelial Growth Factor

VEGF is a key molecule in promoting angiogenesis in wet AMD [83]. VEGF also can potentially induce vascular leakage and inflammation by triggering the increased production and permeability of capillary endothelial cells. The VEGF family currently includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and placenta growth factor (PIGF) that bind in a distinct pattern to three structurally related receptor tyrosine kinases, denoted VEGF receptor-1, VEGF receptor-2, and VEGF receptor-3. Increased expression of VEGF-A in the RPE and in the outer nuclear layer is reported in postmortem maculae obtained from individuals with AMD [84]. VEGF plays a critical role in the pathogenesis of neovascular AMD, and recently, therapies targeting VEGF have shown promising

results of significant improvement of central vision and reduction of CNV [85]. Kurihara et al. determined that mice developed rapid changes in the subretinal vasculature following RPE-specific Cre-mediated conditionally knocking out VEGF-A, characterized by complete loss of the choriocapillaris and marked attenuation of choroid vessels. In addition, damage to the cone photoreceptors occurred as early as 3 days following Cre induction [86]. This result suggests VEGF-A may alternatively have direct neurotrophic effects on the cone photoreceptors. Indeed, photoreceptors have been shown to express VEGF receptors [87, 88]. Administration of anti-VEGF drugs is currently used for the treatment of patients with wet AMD, which aims to regress the CNV and retain visual acuity. These evidences indicate that anti-VEGF therapies should be administered with caution.

18.3 Conclusion

In summary the correlation within the photoreceptor and RPE is lost in RP and AMD. Loss of this functionally integrated relationship results in death and dysfunction of both of photoreceptors and RPE, which leads to visual loss. Restoration of the relationship can be accomplished therapeutically by targeting the initial insult. Anti-VEGF therapies are already having a profound effect on progression of wet AMD, but it is difficult to recover visual acuity in some cases. In RP, there is no effective treatment. The pathogenesis of RP and AMD is also associated with not only aging but also SNPs and gene mutation. For this reason, any new therapy such as gene therapy has emerged. Finally, controlling a certain genetic expression in RP and AMD could reduce the risk for AMD and RP and their progression.

References

1. Strauss O (2005) The retinal pigment epithelium in visual function. Physiol Rev 85 (3):845–881
2. Wangsa-Wirawan ND, Linsenmeier RA (2003) Retinal oxygen: fundamental and clinical aspects. Arch Ophthalmol 121(4):547–557
3. Portera-Cailliau C, Sung CH, Nathans J, Adler R (1994) Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa. Proc Natl Acad Sci USA 91(3):974–978
4. Reme CE, Grimm C, Hafezi F, Iseli HP, Wenzel A (2003) Why study rod cell death in retinal degenerations and how? Doc Ophthalmol (Adv Ophthalmol) 106(1):25–29
5. Suter M, Reme C, Grimm C, Wenzel A, Jaattela M, Esser P, Kociok N, Leist M, Richter C (2000) Age-related macular degeneration. The lipofusion component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. J Biol Chem 275(50):39625–39630
6. Duncan JL, LaVail MM, Yasumura D, Matthes MT, Yang H, Trautmann N, Chappelow AV, Feng W, Earp HS, Matsushima GK et al (2003) An RCS-like retinal dystrophy phenotype in mer knockout mice. Invest Ophthalmol Vis Sci 44(2):826–838

7. Edwards RB, Szamier RB (1977) Defective phagocytosis of isolated rod outer segments by RCS rat retinal pigment epithelium in culture. Science 197(4307):1001–1003
8. Nandrot EF, Finnemann SC (2006) Altered rhythm of photoreceptor outer segment phagocytosis in beta5 integrin knockout mice. Adv Exp Med Biol 572:119–123
9. Finnemann SC, Bonilha VL, Marmorstein AD, Rodriguez-Boulan E (1997) Phagocytosis of rod outer segments by retinal pigment epithelial cells requires alpha(v)beta5 integrin for binding but not for internalization. Proc Natl Acad Sci USA 94(24):12932–12937
10. Ryeom SW, Sparrow JR, Silverstein RL (1996) CD36 participates in the phagocytosis of rod outer segments by retinal pigment epithelium. J Cell Sci 109(Pt 2):387–395
11. Gal A, Li Y, Thompson DA, Weir J, Orth U, Jacobson SG, Apfelstedt-Sylla E, Vollrath D (2000) Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. Nat Genet 26(3):270–271
12. Travis GH, Golczak M, Moise AR, Palczewski K (2007) Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. Ann Rev Pharmacol Toxicol 47:469–512
13. Liou GI, Bridges CD, Fong SL, Alvarez RA, Gonzalez-Fernandez F (1982) Vitamin A transport between retina and pigment epithelium–an interstitial protein carrying endogenous retinol (interstitial retinol-binding protein). Vis Res 22(12):1457–1467
14. Wiggert B, Kutty G, Long KO, Inouye L, Gery I, Chader GJ, Aguirre GD (1991) Interphotoreceptor retinoid-binding protein (IRBP) in progressive rod-cone degeneration (prcd)–biochemical, immunocytochemical and immunologic studies. Exp Eye Res 53(3):389–398
15. Cunha-Vaz JG (1976) The blood-retinal barriers. Doc Ophthalmol (Adv Ophthalmol) 41 (2):287–327
16. Ban Y, Rizzolo LJ (2000) Regulation of glucose transporters during development of the retinal pigment epithelium. Brain Res Dev Brain Res 121(1):89–95
17. Bazan NG (1989) The metabolism of omega-3 polyunsaturated fatty acids in the eye: the possible role of docosahexaenoic acid and docosanoids in retinal physiology and ocular pathology. Prog Clin Biol Res 312:95–112
18. Caldwell RB, McLaughlin BJ (1984) Redistribution of Na-K-ATPase in the dystrophic rat retinal pigment epithelium. J Neurocytol 13(6):895–910
19. Mikami Y, Hara M, Yasukura T, Uyama M, Minato A, Inagaki C (1995) Atrial natriuretic peptide stimulates Cl-transport in retinal pigment epithelial cells. Curr Eye Res 14(5):391–397
20. Stamer WD, Bok D, Hu J, Jaffe GJ, McKay BS (2003) Aquaporin-1 channels in human retinal pigment epithelium: role in transepithelial water movement. Invest Ophthalmol Vis Sci 44 (6):2803–2808
21. Simo R, Carrasco E, Garcia-Ramirez M, Hernandez C (2006) Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. Curr Diabetes Rev 2(1):71–98
22. Tombran-Tink J, Shivaram SM, Chader GJ, Johnson LV, Bok D (1995) Expression, secretion, and age-related downregulation of pigment epithelium-derived factor, a serpin with neurotrophic activity. J Neurosci Official J Soc Neurosci 15(7 Pt 1):4992–5003
23. Lopez PF, Sippy BD, Lambert HM, Thach AB, Hinton DR (1996) Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. Invest Ophthalmol Vis Sci 37(5):855–868
24. Bost LM, Aotaki-Keen AE, Hjelmeland LM (1992) Coexpression of FGF-5 and bFGF by the retinal pigment epithelium in vitro. Exp Eye Res 55(5):727–734
25. Opas M, Dziak E (1989) Effects of TGF-beta on retinal pigmented epithelium in vitro. Exp Cell Biol 57(4):206–212
26. Rosenthal R, Wohlleben H, Malek G, Schlichting L, Thieme H, Bowes Rickman C, Strauss O (2004) Insulin-like growth factor-1 contributes to neovascularization in age-related macular degeneration. Biochem Biophys Res Comm 323(4):1203–1208
27. Ishida K, Yoshimura N, Yoshida M, Honda Y, Murase K, Hayashi K (1997) Expression of neurotrophic factors in cultured human retinal pigment epithelial cells. Curr Eye Res 16 (2):96–101

28. He S, Kumar SR, Zhou P, Krasnoperov V, Ryan SJ, Gill PS, Hinton DR (2010) Soluble EphB4 inhibition of PDGF-induced RPE migration in vitro. Invest Ophthalmol Vis Sci 51(1):543–552
29. Huang Q, Wang S, Sorenson CM, Sheibani N (2008) PEDF-deficient mice exhibit an enhanced rate of retinal vascular expansion and are more sensitive to hyperoxia-mediated vessel obliteration. Exp Eye Res 87(3):226–241
30. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY, Group MS (2006) Ranibizumab for neovascular age-related macular degeneration. New Engl J Med 355 (14):1419–1431
31. Heckenlively JR, Yoser SL, Friedman LH, Oversier JJ (1988) Clinical findings and common symptoms in retinitis pigmentosa. Am J Ophthalmol 105(5):504–511
32. Haim M (2002) Epidemiology of retinitis pigmentosa in Denmark. Acta Ophthalmol Scand Suppl 233:1–34
33. Berson EL (1993) Retinitis pigmentosa. The Friedenwald lecture. Invest Ophthalmol Vis Sci 34(5):1659–1676
34. Marmor MF, Holder GE, Seeliger MW, Yamamoto S, International Society for Clinical Electrophysiology of V (2004) Standard for clinical electroretinography (2004 update). Doc Ophthalmol (Adv Ophthalmol) 108(2):107–114
35. van Soest S, Westerveld A, de Jong PT, Bleeker-Wagemakers EM, Bergen AA (1999) Retinitis pigmentosa: defined from a molecular point of view. Surv Ophthalmol 43(4):321–334
36. Sanyal S, De Ruiter A, Hawkins RK (1980) Development and degeneration of retina in rds mutant mice: light microscopy. J Comp Neurol 194(1):193–207
37. Travis GH, Brennan MB, Danielson PE, Kozak CA, Sutcliffe JG (1989) Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (rds). Nature 338(6210):70–73
38. Martinez-Mir A, Bayes M, Vilageliu L, Grinberg D, Ayuso C, del Rio T, Garcia-Sandoval B, Bussaglia E, Baiget M, Gonzalez-Duarte R et al (1997) A new locus for autosomal recessive retinitis pigmentosa (RP19) maps to 1p13-1p21. Genomics 40(1):142–146
39. Cohen AI (1983) Some cytological and initial biochemical observations on photoreceptors in retinas of rds mice. Invest Ophthalmol Vis Sci 24(7):832–843
40. Jacobson SG, Cideciyan AV, Kemp CM, Sheffield VC, Stone EM (1996) Photoreceptor function in heterozygotes with insertion or deletion mutations in the RDS gene. Invest Ophthalmol Vis Sci 37(8):1662–1674
41. Bird AC (1995) Retinal photoreceptor dystrophies LI. Edward Jackson memorial lecture. Am J Ophthalmol 119(5):543–562
42. Hayes KC (1974) Retinal degeneration in monkeys induced by deficiencies of vitamin E or A. Invest Ophthalmol 13(7):499–510
43. Levy NS, Toskes PP (1974) Fundus albipunctatus and vitamin A deficiency. Am J Ophthalmol 78(6):926–929
44. Crabb JW, Gaur VP, Garwin GG, Marx SV, Chapline C, Johnson CM, Saari JC (1991) Topological and epitope mapping of the cellular retinaldehyde-binding protein from retina. J Biol Chem 266(25):16674–16683
45. Maw MA, Kennedy B, Knight A, Bridges R, Roth KE, Mani EJ, Mukkadan JK, Nancarrow D, Crabb JW, Denton MJ (1997) Mutation of the gene encoding cellular retinaldehyde-binding protein in autosomal recessive retinitis pigmentosa. Nat Genet 17(2):198–200
46. Bavik CO, Busch C, Eriksson U (1992) Characterization of a plasma retinol-binding protein membrane receptor expressed in the retinal pigment epithelium. J Biol Chem 267 (32):23035–23042
47. Gu SM, Thompson DA, Srikumari CR, Lorenz B, Finckh U, Nicoletti A, Murthy KR, Rathmann M, Kumaramanickavel G, Denton MJ et al (1997) Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. Nat Genet 17(2):194–197
48. Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, Mickunas E, Gay R, Klimanskaya I, Lanza R (2012) Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 379(9817):713–720

49. Li Y, Tsai YT, Hsu CW, Erol D, Yang J, Wu WH, Davis RJ, Egli D, Tsang SH (2012) Long-term safety and efficacy of human-induced pluripotent stem cell (iPS) grafts in a preclinical model of retinitis pigmentosa. Mol Med 18:1312–1319
50. Gehrs KM, Anderson DH, Johnson LV, Hageman GS (2006) Age-related macular degeneration–emerging pathogenetic and therapeutic concepts. Ann Med 38(7):450–471
51. Curcio CA, Medeiros NE, Millican CL (1996) Photoreceptor loss in age-related macular degeneration. Invest Ophthalmol Vis Sci 37(7):1236–1249
52. Dunaief JL, Dentchev T, Ying GS, Milam AH (2002) The role of apoptosis in age-related macular degeneration. Arch Ophthalmol 120(11):1435–1442
53. Sarks JP, Sarks SH, Killingsworth MC (1988) Evolution of geographic atrophy of the retinal pigment epithelium. Eye 2(Pt 5):552–577
54. Maeda H, Ogata N, Yi X, Takeuchi M, Ohkuma H, Uyama M (1998) Apoptosis of photoreceptor cells in ornithine-induced retinopathy. Graefe's Arch Clin Exp Ophthalmol (= Albrecht von Graefes Archiv fur klinische und Experimentelle Ophthalmologie) 236(3):207–212
55. Kim SY, Sadda S, Humayun MS, De Juan E Jr, Melia BM, Green WR (2002) Morphometric analysis of the macula in eyes with geographic atrophy due to age-related macular degeneration. Retina 22(4):464–470
56. Sarks SH (1980) Council lecture. Drusen and their relationship to senile macular degeneration. Aust J Ophthalmol 8(2):117–130
57. Macular Photocoagulation Study Group (1993) Five-year follow-up of fellow eyes of patients with age-related macular degeneration and unilateral extrafoveal choroidal neovascularization. Arch Ophthalmol 111(9):1189–1199
58. Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH (2000) A potential role for immune complex pathogenesis in Drusen formation. Exp Eye Res 70(4):441–449
59. Ishibashi T, Patterson R, Ohnishi Y, Inomata H, Ryan SJ (1986) Formation of drusen in the human eye. Am J Ophthalmol 101(3):342–353
60. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF (2001) An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. Prog Retin Eye Res 20(6):705–732
61. Anderson DH, Mullins RF, Hageman GS, Johnson LV (2002) A role for local inflammation in the formation of drusen in the aging eye. Am J Ophthalmol 134(3):411–431
62. Johnson LV, Leitner WP, Rivest AJ, Staples MK, Radeke MJ, Anderson DH (2002) The Alzheimer's A beta -peptide is deposited at sites of complement activation in pathologic deposits associated with aging and age-related macular degeneration. Proc Natl Acad Sci USA 99(18):11830–11835
63. Yoshida T, Ohno-Matsui K, Ichinose S, Sato T, Iwata N, Saido TC, Hisatomi T, Mochizuki M, Morita I (2005) The potential role of amyloid beta in the pathogenesis of age-related macular degeneration. J Clin Invest 115(10):2793–2800
64. Sparrow JR, Gregory-Roberts E, Yamamoto K, Blonska A, Ghosh SK, Ueda K, Zhou J (2012) The bisretinoids of retinal pigment epithelium. Prog Retin Eye Res 31(2):121–135
65. Sparrow JR, Nakanishi K, Parish CA (2000) The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. Invest Ophthalmol Vis Sci 41 (7):1981–1989
66. Zhou J, Jang YP, Kim SR, Sparrow JR (2006) Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. Proc Natl Acad Sci USA 103(44):16182–16187
67. Baynes JW (2001) The role of AGEs in aging: causation or correlation. Exp Gerontol 36 (9):1527–1537
68. Ma W, Lee SE, Guo J, Qu W, Hudson BI, Schmidt AM, Barile GR (2007) RAGE ligand upregulation of VEGF secretion in ARPE-19 cells. Invest Ophthalmol Vis Sci 48 (3):1355–1361
69. Klein R, Knudtson MD, Klein BE, Wong TY, Cotch MF, Liu K, Cheng CY, Burke GL, Saad MF, Jacobs DR Jr et al (2008) Inflammation, complement factor h, and age-related macular degeneration: the multi-ethnic study of atherosclerosis. Ophthalmology 115(10):1742–1749

70. Bora NS, Jha P, Bora PS (2008) The role of complement in ocular pathology. Semin Immunopathol 30(2):85–95
71. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA (2005) Complement factor H polymorphism and age-related macular degeneration. Science 308(5720):421–424
72. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM et al (2005) A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 102(20):7227–7232
73. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR et al (2005) Complement factor H variant increases the risk of age-related macular degeneration. Science 308(5720):419–421
74. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST et al (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308(5720):385–389
75. Johnson PT, Betts KE, Radeke MJ, Hageman GS, Anderson DH, Johnson LV (2006) Individuals homozygous for the age-related macular degeneration risk-conferring variant of complement factor H have elevated levels of CRP in the choroid. Proc Natl Acad Sci USA 103 (46):17456–17461
76. Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, Cramer K, Neel J, Bergeron J, Barile GR et al (2006) Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat Genet 38(4):458–462
77. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM (2007) Variation in complement factor 3 is associated with risk of age-related macular degeneration. Nat Genet 39 (10):1200–1201
78. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM (2009) Variation near complement factor I is associated with risk of advanced AMD. Eur J Hum Genet 17 (1):100–104
79. Klaver CC, Kliffen M, van Duijn CM, Hofman A, Cruts M, Grobbee DE, van Broeckhoven C, de Jong PT (1998) Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet 63(1):200–206
80. Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240(4852):622–630
81. Olaisen B, Teisberg P, Gedde-Dahl T Jr (1982) The locus for apolipoprotein E (apoE) is linked to the complement component C3 (C3) locus on chromosome 19 in man. Hum Genet 62 (3):233–236
82. Souied EH, Benlian P, Amouyel P, Feingold J, Lagarde JP, Munnich A, Kaplan J, Coscas G, Soubrane G (1998) The epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration. Am J Ophthalmol 125(3):353–359
83. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9 (6):669–676
84. Kliffen M, Sharma HS, Mooy CM, Kerkvliet S, de Jong PT (1997) Increased expression of angiogenic growth factors in age-related maculopathy. Br J Ophthalmol 81(2):154–162
85. Wylegala E, Teper SJ (2007) VEGF in age-related macular degeneration. Part II. VEGF inhibitors use in age-related macular degeneration treatment. Klin Oczna 109(1–3):97–100
86. Kurihara T, Westenskow PD, Bravo S, Aguilar E, Friedlander M (2012) Targeted deletion of Vegfa in adult mice induces vision loss. J Clin Invest 122(11):4213–4217
87. Robinson GS, Ju M, Shih SC, Xu X, McMahon G, Caldwell RB, Smith LE (2001) Nonvascular role for VEGF: VEGFR-1, 2 activity is critical for neural retinal development. FASEB J Official Publ Federation Am Soc Exp Biol 15(7):1215–1217
88. Saint-Geniez M, Maharaj AS, Walshe TE, Tucker BA, Sekiyama E, Kurihara T, Darland DC, Young MJ, D'Amore PA (2008) Endogenous VEGF is required for visual function: evidence for a survival role on muller cells and photoreceptors. PLoS One 3(11):e3554

Part III
Neuroprotection for Other Retinal Diseases

Chapter 19
Neuroprotection for Retinal Detachment

Toshio Hisatomi

Abstract Recent data have provided important clues about the molecular mechanisms underlying retinal neurodegenerative diseases, including retinal detachment (RD). Photoreceptor cell death causes various types of cell death such as apoptosis, necrosis, autophagy, and necroptosis. Apoptosis is the major type of photoreceptor death in RD and is the most defined type in experimental and clinical settings. Most cell death in vertebrates proceeds via the mitochondrial pathway of apoptosis. Mitochondria contain proapoptotic factors such as cytochrome c and AIF in their intermembrane space. Furthermore, mitochondrial membrane permeabilization (MMP) is a critical event during apoptosis, representing the "point of no return" of the lethal process. Modern medicine is developing an increasing number of treatments for neurodegenerative disease, but no neuroprotective treatment has yet been established for RD. This chapter briefly reviews the mechanisms of cell death and neuroprotection for RD.

Keyword Apoptosis • Necroptosis • Necrosis • Photoreceptor • Retinal detachment

19.1 Introduction

Photoreceptor cell death is the ultimate cause of visual loss in various retinal disorders including retinal detachment (RD) and has been thought to occur mainly through apoptosis. Photoreceptor cells die when they are physically separated from the underlying retinal pigment epithelium (RPE) and choroidal vessels, which provide metabolic support to the outer layer of the retina. Retinal detachment

T. Hisatomi (✉)
Department of Ophthalmology, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka City 812-8582, Japan
e-mail: hisatomi@med.kyushu-u.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_19,

occurs in various retinal disorders, including age-related macular degeneration (AMD) [1], diabetic retinopathy [2], as well as rhegmatogenous, tractional, and exudative retinal detachment (RD) [3]. Although surgery is carried out to reattach the retina, only two-fifths of patients with rhegmatogenous RD involving the macula recover 20/40 or better vision [4]. Although various pathological changes occur in detached retina, studies on experimental models and human patient samples have shown that photoreceptor cell death is immediately induced as early as 12 h and peaks at around 2–3 days after RD [5]. Moreover, retinal imaging by optical coherence tomography has demonstrated that the microstructure of foveal photoreceptor cells is a critical factor predicting better visual function in patients who received successful RD repair [6]. These findings suggest that loss of photoreceptor cells may be an important cause of vision loss after RD. Therefore, identification of the mechanisms underlying photoreceptor cell death is critical to developing new treatment strategies for retinal disorders associated with RD. Apoptosis is the most characterized form of programmed cell death, where caspases play a central role for its induction. In experimental models of RD, dying photoreceptor cells exhibit the activation of caspases; however, there is a paradox that caspase inhibition alone does not provide a sufficient protection against photoreceptor cell loss, suggesting that there are other ways to die.

Therapeutic targeting of apoptosis may make more conceptual sense compared to necrosis, as apoptosis is a delayed event and an energy-dependent process. Mitochondria are considered as the central regulators of apoptotic cell death in vertebrates. In various paradigms of cell death, mitochondrial membrane permeabilization (MMP) delimits the frontier between life and death (Fig. 19.1). Mitochondria control the intrinsic pathway of apoptosis, in which MMP ignites the activation of caspases and other catabolic enzymes, and mitochondria participate in the extrinsic pathway of apoptosis, in which they amplify the self-destructive process [7–9]. Irrespective of its initiation at the inner or outer mitochondrial membrane, MMP culminates in the functional (dissipation of the mitochondrial membrane potential, shutdown of ATP synthesis, redox imbalance) and structural (reorganization of cristae, release of toxic intermembrane space proteins into the cytosol) collapse of mitochondria. MMP has a profound impact on cellular metabolism, activates caspase-dependent and caspase-independent executioner mechanisms, and finally results in the demise of the cell [10, 11]. The lethal consequences of MMP relate to the critical position occupied by the mitochondria in cellular bioenergetics and the release of proapoptotic proteins into the cytosol and the nucleus. Proapoptotic proteins liberated as a consequence of MMP include activators of the caspase cascade (e.g., cytochrome c) as well as caspase-independent death effectors (e.g., apoptosis-inducing factor (AIF) and endonuclease G) [12, 13]. Indeed, MMP is the main checkpoint of programmed cell death, and lethal pathways of signal transduction are often activated in neurodegenerative diseases. Hence, pharmacological agents that target mitochondria to subvert MMP are being evaluated as therapeutic approaches for treatment for neurodegenerative RD. Recent accumulating evidence also demonstrates that non-apoptotic forms of cell death, such as autophagic cell death and necrosis, are also regulated by specific

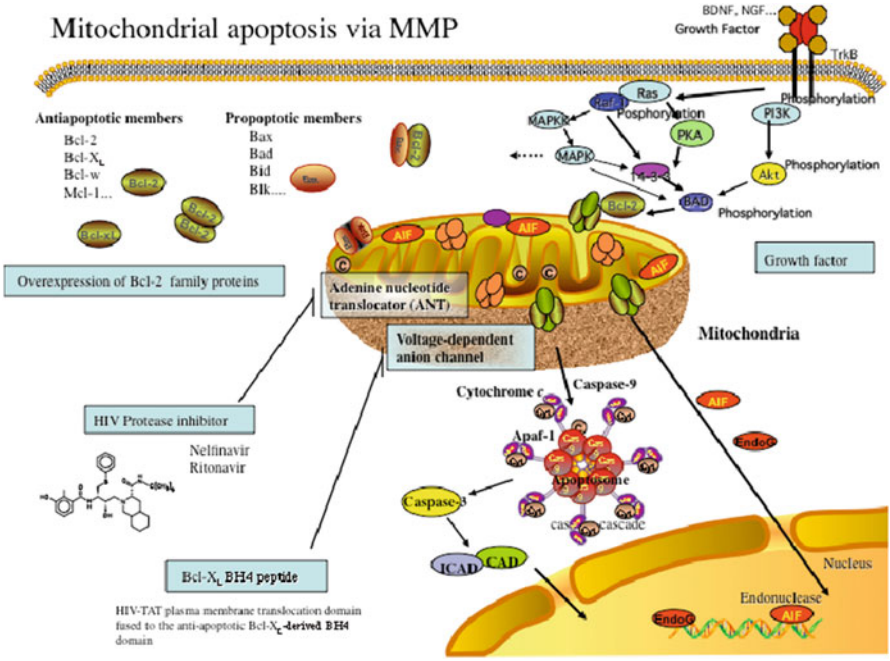


Fig. 19.1 Schematic drawing of mitochondrial apoptotic signaling via mitochondrial membrane permeabilization (MMP). MMP induces mitochondrial release of proapoptotic molecules such as cytochrome c and apoptosis-inducing factor (AIF)

molecular machinery, such as those mediated by autophagy-related proteins and receptor-interacting protein kinases, respectively. Here we summarize the current knowledge of cell death signaling and its roles in photoreceptor cell death after RD. A body of studies indicate that not only apoptotic but also autophagic and necrotic signaling are involved in photoreceptor cell death after RD. We summarized the important checkpoints of photoreceptor cell death and reviewed the current concepts on neuroprotection for RD.

19.2 Mechanism of Photoreceptor Apoptotic Cell Death

19.2.1 Mitochondrial Outer Membrane Permeabilization

MMP may originate at the outer membrane through at least two distinct mechanisms. These include the activation of proapoptotic proteins of the Bcl-2 family (e.g., Bax, Bak) to build up multimeric channels, allowing for the release of intermembrane space proteins [14], and formation of lipidic pores due to a direct

interaction between proapoptotic Bcl-2 family members (e.g., Bax, truncated Bid) and lipids contained in mitochondrial membranes [11, 15, 16]. Outer membrane permeabilization also occurs upon its physical rupture, be it induced accidentally or as part of a regulated mechanism originating at the inner membrane (the so-called mitochondrial permeability transition). Outer membrane permeabilization culminates in the release of proapoptotic intermembrane space proteins, which trigger the execution process of apoptotic cell death.

19.2.2 Mitochondrial Inner Membrane Permeabilization

Mitochondrial membrane permeabilization may also start at the inner membrane. In contrast to the outer membrane, the inner membrane from healthy cells is nearly impermeable to small solutes and ions. When inner membrane impermeability is lost, for instance following the opening of the so-called permeability transition pore complex (PTPC), solutes enter the mitochondrial matrix, accompanied by a net influx of water. The resulting osmotic imbalance provokes swelling of the matrix, followed by the rupture of both mitochondrial membranes. This process is known as mitochondrial permeability transition.

19.2.3 Importance of Caspase-Dependent Pathways on Neurodegenerative Disease

Cysteine aspartate-specific proteases or caspases are the central molecules involved in initiation and execution of apoptosis [17]. Caspases are processed through proteolytic cleavage at the sites containing aspartate residue. Mitochondria contain cytochrome c and caspase-9 in the intermembrane space, and these are liberated into cytoplasm after apoptotic insults. Cytochrome c forms apoptosome in the presence of ATP along with Apaf-1 and caspase-9, initiating the activation of caspase cascade [7, 8, 18]. The “forebrain over growth” (fog) mutation leads to an autosomal recessive neural tube closure defect due to the near-to-complete lack of Apaf-1 expression [19, 20]. However, a complete deficiency in Apaf-1 usually results in perinatal lethality, while fog/fog mice readily survive into adulthood [20, 21], allowing to assess the role of Apaf-1 and Apaf-1-dependent cytochrome c-mediated caspase activation in neuronal apoptosis. Apaf-1 deficiency reduced the RD-induced neuronal photoreceptor apoptosis, as assessed by TUNEL staining [22]. Therefore capsizes and related molecules offer opportunity to salvage the apoptotic cells by targeting them using anti-caspase therapy for neuroprotection.

19.2.4 Importance of AIF in Caspase-Independent Pathways on Neurodegenerative Disease

AIF is a 67 kDa flavoprotein in mammals and highly conserved among mammalian species (>95 % amino acid identity between mouse and human) and bears a highly significant homology with flavoprotein oxidoreductases from all eukaryotic and prokaryotic kingdoms in its C-terminal portion [23, 24]. AIF is normally confined to the mitochondrial intermembrane space; however, AIF translocates to the cytosol and to the nucleus after apoptotic insults (Fig. 19.1) [23–25]. Harlequin (Hq) mice exhibit an X chromosome-linked ataxia due to the progressive degeneration of terminally differentiated cerebellar neurons [26, 27]. The Hq mutation has been identified as a proviral insertion in the apoptosis-inducing factor (Aif) gene, also known as programmed cell death 8 (pdcd8), causing about 80 % reduction in AIF expression [27]. In contrast to Aif knockout mice (which die in utero) [28], Hq mice are born at normal Mendelian ratios and are healthy until the age of 3 months. After the apoptotic insult mediated by RD, Hq/Y retinas exhibited significantly less TUNEL+ apoptotic neural cells than wild-type controls and exhibited a reduced cell loss in the retina [22]. The similar neuroprotective effects were observed in brain ischemia in adult [29] and neonate [30]. Further research on AIF and caspase-independent pathways may reveal novel therapeutic target for neuroprotection.

19.3 Mechanism of Non-apoptotic Cell Death

Autophagy (Greek for "self-eating") is a process by which cell's own components such as macromolecules (e.g., proteins, lipids, and nucleic acids) and organelles (e.g., mitochondria) are degraded by the lysosome [31]. Macroautophagy (hereafter referred to autophagy) is the best-characterized autophagy pathway and involves the formation of autophagosomes and autolysosomes. Autophagosome is a double- or multi-membrane vacuole that sequesters cytoplasmic materials and fuses with lysosomes to form autolysosome, where its content is degraded. It is induced by nutrient starvation in order to provide recycled energy and eliminate damaged organelles [32]. From the genetic screening of autophagy-defective mutants in yeast, Tsukada, Ohsumi, and colleagues discovered a set of autophagy-related (Atg) genes, most of which have mammalian homologues [31, 33]. Although previous morphological studies identified accumulation of numerous autophagosomes/autolysosomes in dying cells, the roles of autophagy, either promoting or protecting against cell death, have been controversial. In specific circumstances, autophagy appears to mediate cell death via excessive self-degradation. Nonetheless, accumulating evidence has shown that autophagy is crucial for cell survival by regulating the turnover of intracellular contents in normal and most pathological conditions.

Although necrosis (Greek for "dead") was traditionally thought to be an uncontrolled process of cell death, it is now known to also have regulated components in certain instances. This regulated type of necrosis was discovered from the extensive studies of death receptor-induced cell death. Laster and colleagues observed that TNF-α caused not only apoptosis but also necrosis depending on cell types [34]. Intriguingly, Vercammen and colleagues demonstrated that, when death receptor-induced apoptosis is suppressed by the caspase inhibitor, the cells undergo an alternative necrotic cell death in murine L929 fibrosarcoma cells [35]. Twelve years later, Holler and colleagues identified that this death receptor-induced necrosis is mediated by the activation of receptor-interacting protein 1 (RIP1) [36]. Furthermore, three independent studies recently discovered that RIP3 is a crucial regulator of RIP1 kinase activation and subsequent necrosis. These advances in understanding the molecular basis of necrosis have revealed previously unrecognized roles of necrosis in health and various diseases including retinal degeneration [37, 38]. This RIP kinase-dependent regulated necrosis is termed as "necroptosis" or "programmed necrosis." However, in order to avoid confusion, we here express it descriptively as "RIP kinase-dependent necrosis" according to recent studies [37–39].

19.4 Inflammation and Cell Death

Cytokines and chemokines are released in response to tissue injury and mediate cell survival/death and inflammation. The vitreous fluid from patients with RD contains substantially higher levels of TNF-α, IL-1β, IL-6, IL-8, and MCP-1, compared to samples from patients without RD. Furthermore, Nakazawa and colleagues showed that the mRNA levels of TNF-α, IL-1β, and MCP-1 are upregulated as early as 1 h after experimental RD, suggesting that these cytokines/chemokines may actively contribute to the pathology of RD [40, 41]. Indeed, as described above, TNF-α induces photoreceptor cell death after RD, along with the activation of caspase-8 and RIP kinase. In addition, blockade of TNF-α suppresses the retinal infiltration of macrophages and microglial cells [40]. Therefore, not only pro-death but also proinflammatory signals of TNF-α could be involved in the photoreceptor loss after RD. In another study by Nakazawa and colleagues, genetic deficiency of *Mcp1* substantially reduces macrophage/microglia recruitment and attenuates photoreceptor cell loss after RD. In primary retinal mixed cultures, MCP-1 treatment induces photoreceptor cell loss; however, this cytotoxic effect is abolished by the removal of CD11b-positive macrophages/microglia from the retinal cultures [40]. These findings suggest that MCP-1 mediates photoreceptor cell death indirectly through activation and recruitment of macrophages/microglia after RD. Protection against RD-induced photoreceptor cell death is also obtained by genetic deletion of

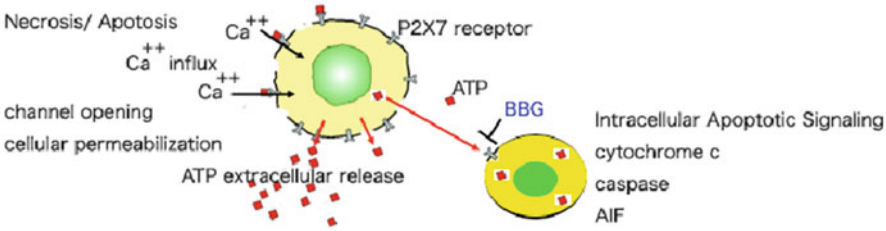


Fig. 19.2 Schematic drawing of ATP-induced cell death via P2X7 receptor. Necrotic and apoptotic dead cells release ATP into intracellular space, resulting in neighboring cell death. ATP and P2X7 receptor binding is blocked by competitive inhibitor of P2X7 receptor, Brilliant Blue G (BBG)

Cd11b/*Cd18*, an integrin critical for leukocyte recruitment, or the treatment with systemic steroids [40, 42], further confirming the pivotal role of inflammation in photoreceptor cell loss after RD. However, it should be noted that inflammation is not always detrimental, but also maintains tissue homeostasis by removing waste materials and dead cells.

Nucleic acids, proteins, and lipids released from dying or dead cells can initiate or modulate immune response, and these molecules are known as damage-associated molecular patterns (DAMPs). Among these, extracellular release of ATP is a key process to recruit macrophages to the site of injury (Fig. 19.2) [43]. ATP is secreted actively by exocytosis, anion channels, or transporters in an early phase of apoptosis or released passively via the loss of membrane integrity. ATP activates inflammasome through its action on P2X7 or mediates cell death via the formation of P2X7-dependent pore and caspase activation. Notomi and colleagues showed that intraocular injection of ATP induces photoreceptor cell death through the activation of P2X7 in mice and primary retinal cultures [44, 45]. In addition, extracellular ATP is substantially increased in the vitreous of AMD patients associated with subretinal hemorrhage [45]. High-mobility group box 1 (HMGB1), a nuclear protein that regulates transcription, has a divergent extracellular function as DAMP. HMGB1 is passively released from necrotic cells, but not from apoptotic cells. Arimura and colleagues showed that the extracellular release of HMGB1 occurs in human patients and experimental models of RD [46], suggesting that HMGB1 released from dying or dead photoreceptor cells may modulate retinal inflammatory response and degeneration after RD. Besides HMGB1, multiple proteins and nucleic acids such as S100, HSP, histones, DNA, and mRNA augment cytokine production and modulate tissue injury when they are released into the extracellular space. Investigating the roles of these DAMPs in retinal degeneration will lead to better understanding of the pathogenesis of the diseases and identification of novel therapeutic targets.

19.5 Neuroprotection Against Photoreceptor Cell Death for RD

19.5.1 MMP and Mitochondrial Release of Proapoptotic Factors

Mitochondrial membrane permeabilization (MMP) is a critical event during apoptosis, representing the "point of no return" of the lethal process. Cytochrome c is released from the mitochondria upon MMP and binds to cytosolic apoptotic protease-activating factor-1 (Apaf-1) to induce its dimerization and a conformational change [47]. Apaf-1 then oligomerizes into apoptosomes that recruit and activate caspase-9 followed by serial activation of caspase-3 and other apoptosis-execution molecules [48, 49]. However, MMP may cause cell death even if caspases are inhibited [50] and a broad caspase inhibitor, Z-VAD-fmk, fails to inhibit neuronal apoptosis [5, 51]. AIF is a caspase-independent apoptogenic factor and is normally confined to the mitochondrial intermembrane space [23]. During apoptosis, AIF translocates to the cytosol and then to the nucleus where it triggers peripheral chromatin condensation and interacts with cyclophilin A to generate a DNAse complex that is responsible for the so-called "large-scale" DNA degradation to fragments of approximately 50 kbp [23, 52]. AIF translocation has been reported for mammalian neural cells in numerous cases, for instance for photoreceptors upon retinal detachment (RD) [5], dopaminergic neurons in models of Parkinson's disease [53] including phenylpyridinium toxicity [54], and photoreceptor cells in retinitis pigmentosa [55]. Pharmacological targeting of MMP inhibition may limit the release of these proapoptotic intermembrane space proteins into the cytosol (Fig. 19.1).

19.5.2 Growth Factor-Dependent Survival of Neuronal Cells

An increasing number of growth factors and nerve growth factor has been shown to support cell survival in various apoptotic insult in neuronal cells [56]. These include nerve growth factor family (NGF, BDNF, etc.), basic fibroblast growth factor (bFGF), and vascular permeability factor (VEGF). BDNF and bFGF have shown substantial neuroprotective effects in neuronal apoptosis of retinal detachment [5], brain ischemia, and Parkinson's disease. These growth factors activated their counter receptors (i.e., NGF receptors) and stimulate cellular intrinsic pathways (i.e., activation of PI3K/Akt signaling pathways and mitogen-activated protein kinase (MAPK) pathways which leads to the upregulation of protein expression) [57]. Recently pigment epithelium-derived factor (PEDF) has also been reported to be neuroprotective in retinal degeneration in retinitis pigmentosa. While the

receptor of PEDF remains unclear, lentivirus-mediated retinal gene transfer of PEDF inhibited MMP as examined by mitochondrial potential, inhibiting subsequent AIF translocation from mitochondria to nucleus in neural cells [51].

19.5.3 Overexpression of Bcl-2 Family Proteins for Inhibiting MMP for Neuroprotection

Bcl-2 family proteins are essential regulators of apoptosis and over 30 members have been discovered that share homology in Bcl-2 homology regions (BH1 to BH4) [18, 58]. These can be grouped into Bcl-2-like survival factors (antiapoptotic) [59, 60] and Bcl-2-like death factors (proapoptotic) [59, 61, 62]. The Bcl-2-like survival factors possess BH1–BH4 domains that mediate their prosurvival function and gate the release of apoptotic proteins by maintaining the MMP. The proapoptotic members of Bcl-2 family protein promote MMP via perturbation of mitochondrial membrane integrity and the interactions with other proapoptotic and antiapoptotic factors and hetero-dimerize with MMP-related proteins such as adenine nucleotide translocator (ANT) and voltage-dependent anion channel (VDAC). The overexpression of antiapoptotic members of Bcl-2 family protein has been reported to be neuroprotective [18]. Bcl-2 overexpression protects against neuron loss within the ischemic model, inhibiting cytochrome c accumulation and caspase-3 activation [63]. Moreover Bcl-2 transfection via herpes simplex virus blocks AIF translocation from mitochondria to nucleus [64]. The ratio of antiapoptotic and proapoptotic proteins may determine the fate of neurons by antagonizing and competing each other [15, 65].

19.5.4 A Cell-Permeable Peptide Corresponding to the BH-4 Domain of Bcl-XL Inhibits Neuronal Apoptosis via Blocking MMP

The recent reports indicate that (at least) two post-mitochondrial cell death pathways participate in neuronal apoptosis, namely, caspase-independent apoptosis (e.g., AIF and endonuclease G) and cytochrome c-dependent caspase activation in the apoptosome. Both AIF and the apoptosome are activated as a result of MMP. Recently, a cell-permeable MMP-inhibitory recombinant fusion protein, HIV-TAT BH4, composed of the HIV-TAT plasma membrane translocation domain and the antiapoptotic Bcl-XL-derived BH4 domain, has been reported to inhibit neuronal apoptosis [22, 66–68]. Cell-penetrating peptide constructs such as HIV-1 TAT basic domain and related peptides have been developed to deliver bioactive peptides into cells [67–73]. Rapid and receptor-independent uptake of TAT-conjugated peptides has been demonstrated to occur in vitro and in vivo [67]. Intraperitoneal

injection of HIV-TAT protein led to its distribution into neuronal cells (in vivo), as this was seen after addition of HIV-TAT BH4 to primary neuronal cell cultures (in vitro) [22]. These fusion proteins resulted in robust protein transduction in neurons and inhibited caspase-3 activation in ischemic neurons [70], AIF translocation in neonatal brain damage [68], and AIF and cytochrome c translocation in retinal cells [22]. The Bcl-2 family proteins constitute a major life-or-death decisive point that can be manipulated to target the neuroprotective therapy.

19.5.5 A Novel Mechanism of HIV Protease Inhibitors for Neuroprotection Through Inhibition of Mitochondrial Apoptosis

HIV protease inhibitors (PIs) have originally been designed to block the formation of HIV viral proteins by viral proteases and are currently administered to millions of patients with HIV worldwide [74]. Recently, it has been reported that PIs do not only inhibit virus replication but also suppress CD4+ T lymphocyte apoptosis at concentrations similar to those that are achieved in the plasma of PI-treated patients [75]. In several cases, HIV-infected individuals recovered normal levels of circulating CD4+ T cells upon PI treatment although the therapy had no effects on the viral titers, suggesting that PIs might inhibit apoptosis of CD4+ T cells in vivo, independently from their effect on HIV replication [74, 76, 77]. Several groups investigated the mechanisms by which PIs inhibit apoptosis. Altered transcriptional regulation of regulatory proteins [78] and direct inhibition of caspase-1 [79] or calpain [80] have been reported. However, these proposed mechanisms may not explain the ability of PIs to block cell death induced by a wide range of apoptotic insults [75] and are not compatible with other studies reporting poor effects of PIs on effector caspases or the net synthesis of apoptosis regulators [81]. Recently, PIs were shown to inhibit the MMP-dependent release of cytochrome c [81, 82] via direct binding to and inhibition of the adenine nucleotide translocator, a protein from the inner mitochondrial membrane that can form pores and mediate MMP [83]. However the protective effects of PIs contrast with the observation that pharmacological caspase inhibitors largely fail to inhibit cell death [84]. PIs may simultaneously block caspase-dependent (e.g., activation of caspase-9 and caspase-3) and caspase-independent cell death pathways (e.g., AIF translocation) via blocking MMP, presumably inhibiting the adenine nucleotide translocator [22]. In our studies, PI had substantial antiapoptotic and neuroprotective effect on retinal photoreceptors after RD, in a non-virus-associated disease with excessive apoptosis. Paradoxically, PIs may also induce apoptosis, particularly of transformed cells, when used at higher doses [78, 85]. Further studies may provide the mechanistic insight of PIs effect on apoptosis and increased application for neuroprotection.

19.5.6 P2X7 Receptor (P2RX7) Blockade by Brilliant Blue G (BBG)

Recently, adenosine-5′-triphosphate (ATP) has been discovered as a major extracellular messenger that can contribute to lethal signaling [86]. Neuronal cells release intracellular ATP to extracellular space under stress or cell death. Extracellular ATP can act on purinergic receptors, which are classified into two classes, the ionotropic, ligand-gated P2X receptors and the metabotropic, G protein-coupled P2Y receptors [87]. Among the seven subtypes of mammalian P2X receptors, the P2X7 receptor (P2RX7) differs from other P2X receptor subtypes by its long cytoplasmic, carboxy-terminal tail (240 amino acids) and mediates cellular signals that can trigger cell death. In the retina, P2RX7 is expressed in both inner and outer retinal neurons, including retinal ganglion cells [88] and photoreceptors [89]. P2RX7 has physiological functions as a neurotransmitter receptor in the retina [89], while photoreceptors have been shown to undergo apoptosis by excessive ATP [44, 45, 90]. Recently, it has been shown that photoreceptor apoptosis involves P2RX7 activation with caspase-8 and caspase-9 cleavage and mitochondrio-nuclear translocation of AIF [44]. Moreover, photoreceptor apoptosis can be attenuated by Brilliant Blue G, a pharmacological P2RX7 antagonist, by blocking ATP acting on P2RX7 (Fig. 19.2) [45]. Indeed, BBG administration can confer neuroprotective effects in several models of Alzheimer's disease, Parkinson's disease, and spinal cord injury [37–39] as well as in the retina [35, 40, 41]. BBG is also known as an adjuvant approved for intraoperative use in ocular surgery. In chromovitrectomy, BBG is introduced to improve the visualization of intraocular tissues for specific procedures, such as internal limiting membrane (ILM) peeling during vitrectomy [42, 43]. Various retinal diseases could be linked to important elevations of extracellular ATP, accelerating neuronal cell death and irreversible tissue damage. Furthermore, any type of photoreceptor cell death could release ATP, making positive feed forward loop to worsen the surrounding tissue damage [45]. P2RX7 antagonists including BBG may have a potential neuroprotective therapeutic effect in RD and other retinal diseases with excessive extracellular ATP by blocking cell death loop.

19.5.7 A Novel Neuroprotection for Necrosis

Although apoptosis is a predominant form cell death after RD, previous morphological analysis described the presence of necrosis in RD-induced photoreceptor cell death [91]. However, necrosis was not considered as a therapeutic target for a long period, because of the general concept that necrosis is an uncontrolled process of cell death. During photoreceptor cell death after RD, death ligands such as TNF-α and Fas-L, which mediate not only apoptosis but also necrosis, are upregulated and contribute to photoreceptor cell loss [40, 92]. However, caspase

inhibition by the pan-caspase inhibitor Z-VAD is not sufficient to prevent photoreceptor cell loss after RD [5]. Given the emerging roles of RIP kinase-dependent necrosis especially in conditions where caspase pathway is inhibited, we hypothesized that RIP kinase may act as an alternative pathway of photoreceptor cell death after RD. Trichonas and colleagues demonstrated that, while RIP3 is barely detectable in the normal retina, its expression increases over tenfold in the retina after RD [41]. Because the expression levels of RIP3 have been shown to correlate with necrotic responses in various cell lines, the increased RIP3 may sensitize cells to undergo necrosis in these pathological conditions. Furthermore, our morphological analysis using TEM showed that treatment with Z-VAD decreases apoptosis but substantially increases necrotic cell death of photoreceptors [38]. These necrotic changes after caspase inhibition are rescued by additional Nec-1 treatment or Rip3 deficiency. These findings clearly demonstrate that RIP kinase-dependent necrosis is an essential pathway for photoreceptor cell death after RD, which acts in concert with caspase-dependent apoptosis [38]. These findings suggest that RIP kinase is critical in medicating necrosis in RD and that targeting RIP kinase by itself or in combination with apoptosis inhibitors may be a potential strategy for treatment of retinal disorders.

19.6 Conclusions

MMP is a pivotal event in the pathogenesis of acute and chronic neurodegenerative disorders. Thus various neurodegenerative disorders that involve apoptosis could be amenable to drug- and gene-based therapies that target MMP. The existence of multiple cell death pathways with both overlapping and cross-talking molecular mechanisms may explain the observation that inhibition of one such pathway may enhance alternative ones. These information suggest that neuroprotection should optimally be directed at multiple and/or comprehensive targets. The therapeutic concept to target mitochondria, especially MMP, to block the release of multiple proapoptotic proteins and protect energy metabolism is substantially valuable for neuroprotection. A combination therapy with different approach targeting apoptosis, autophagy, necrosis, or inflammation may have additive effects for further application for neuroprotection. Photoreceptor cell death is the ultimate cause of vision loss in RD and other retinal disorders. The regulatory roles of mitochondria in each cell death mechanism and the interaction between cell death mechanisms during retinal degeneration warrant further investigation. We believe that further studies of the complex molecular mechanisms underlying photoreceptor cell death will lead to better understanding of the pathophysiology of RD and developing novel therapeutics for preventing vision deficits after RD.

References

1. Dunaief JL, Dentchev T, Ying GS, Milam AH (2002) The role of apoptosis in age-related macular degeneration. Arch Ophthalmol 120(11):1435–1442
2. Barber AJ, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW (1998) Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. J Clin Invest 102(4):783–791
3. Cook B, Lewis GP, Fisher SK, Adler R (1995) Apoptotic photoreceptor degeneration in experimental retinal detachment. Invest Ophthalmol Vis Sci 36(6):990–996
4. Campo RV, Sipperley JO, Sneed SR, Park DW, Dugel PU, Jacobsen J, Flindall RJ (1999) Pars plana vitrectomy without scleral buckle for pseudophakic retinal detachments. Ophthalmology 106(9):1811–1815, discussion 1816
5. Hisatomi T, Sakamoto T, Murata T, Yamanaka I, Oshima Y, Hata Y, Ishibashi T, Inomata H, Susin SA, Kroemer G (2001) Relocalization of apoptosis-inducing factor in photoreceptor apoptosis induced by retinal detachment in vivo. Am J Pathol 158(4):1271–1278
6. Schocket LS, Witkin AJ, Fujimoto JG, Ko TH, Schuman JS, Rogers AH, Baumal C, Reichel E, Duker JS (2006) Ultrahigh-resolution optical coherence tomography in patients with decreased visual acuity after retinal detachment repair. Ophthalmology 113(4):666–672
7. Kroemer G, Zamzami N, Susin SA (1997) Mitochondrial control of apoptosis. Immunol Today 18(1):44–51
8. Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281(5381):1309–1312
9. Hengartner MO (2000) The biochemistry of apoptosis. Nature 407(6805):770–776
10. Ferri KF, Kroemer G (2001) Mitochondria–the suicide organelles. Bioessays 23(2):111–115
11. Galluzzi L, Vitale I, Kepp O, Seror C, Hangen E, Perfettini JL, Modjtahedi N, Kroemer G (2008) Methods to dissect mitochondrial membrane permeabilization in the course of apoptosis. Methods Enzymol 442:355–374
12. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G (2006) Mechanisms of cytochrome c release from mitochondria. Cell Death Differ 13(9):1423–1433
13. Li LY, Luo X, Wang X (2001) Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 412(6842):95–99
14. Zamzami N, Kroemer G (2001) The mitochondrion in apoptosis: how Pandora's box opens. Nat Rev Mol Cell Biol 2(1):67–71
15. Green DR, Kroemer G (2004) The pathophysiology of mitochondrial cell death. Science 305 (5684):626–629
16. Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. Physiol Rev 87(1):99–163
17. Riedl SJ, Shi Y (2004) Molecular mechanisms of caspase regulation during apoptosis. Nat Rev Mol Cell Biol 5(11):897–907
18. Mehta SL, Manhas N, Raghubir R (2007) Molecular targets in cerebral ischemia for developing novel therapeutics. Brain Res Rev 54(1):34–66
19. Harris BS, Franz T, Ullrich S, Cook S, Bronson RT, Davisson MT (1997) Forebrain overgrowth (fog): a new mutation in the mouse affecting neural tube development. Teratology 55 (4):231–240
20. Honarpour N, Gilbert SL, Lahn BT, Wang X, Herz J (2001) Apaf-1 deficiency and neural tube closure defects are found in fog mice. Proc Natl Acad Sci U S A 98(17):9683–9687
21. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R, Penninger JM, Mak TW (1998) Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 94(6):739–750
22. Hisatomi T, Nakazawa T, Noda K, Almulki L, Miyahara S, Nakao S, Ito Y, She H, Kohno R, Michaud N, Ishibashi T, Hafezi-Moghadam A, Badley AD, Kroemer G, Miller JW (2008) HIV protease inhibitors provide neuroprotection through inhibition of mitochondrial apoptosis in mice. J Clin Invest 118(6):2025–2038

23. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397(6718):441–446
24. Lorenzo HK, Susin SA, Penninger J, Kroemer G (1999) Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. Cell Death Differ 6 (6):516–524
25. Daugas E, Susin SA, Zamzami N, Ferri KF, Irinopoulou T, Larochette N, Prevost MC, Leber B, Andrews D, Penninger J, Kroemer G (2000) Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis. FASEB J 14(5):729–739
26. Barber BR (1971) Research news. Mouse News Lett 45:34–35
27. Klein JA, Longo-Guess CM, Rossmann MP, Seburn KL, Hurd RE, Frankel WN, Bronson RT, Ackerman SL (2002) The harlequin mouse mutation downregulates apoptosis-inducing factor. Nature 419(6905):367–374
28. Joza N, Oudit GY, Brown D, Benit P, Kassiri Z, Vahsen N, Benoit L, Patel MM, Nowikovsky K, Vassault A, Backx PH, Wada T, Kroemer G, Rustin P, Penninger JM (2005) Muscle-specific loss of apoptosis-inducing factor leads to mitochondrial dysfunction, skeletal muscle atrophy, and dilated cardiomyopathy. Mol Cell Biol 25(23):10261–10272
29. Culmsee C, Zhu C, Landshamer S, Becattini B, Wagner E, Pellecchia M, Blomgren K, Plesnila N (2005) Apoptosis-inducing factor triggered by poly(ADP-ribose) polymerase and Bid mediates neuronal cell death after oxygen–glucose deprivation and focal cerebral ischemia. J Neurosci 25(44):10262–10272
30. Zhu C, Wang X, Huang Z, Qiu L, Xu F, Vahsen N, Nilsson M, Eriksson PS, Hagberg H, Culmsee C, Plesnila N, Kroemer G, Blomgren K (2007) Apoptosis-inducing factor is a major contributor to neuronal loss induced by neonatal cerebral hypoxia-ischemia. Cell Death Differ 14(4):775–784, Epub 2006 Oct 2013
31. Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, Ohsumi Y (1998) A protein conjugation system essential for autophagy. Nature 395 (6700):395–398
32. Schworer CM, Mortimore GE (1979) Glucagon-induced autophagy and proteolysis in rat liver: mediation by selective deprivation of intracellular amino acids. Proc Natl Acad Sci USA 76 (7):3169–3173
33. Tsukada M, Ohsumi Y (1993) Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett 333(1–2):169–174
34. Laster SM, Wood JG, Gooding LR (1988) Tumor necrosis factor can induce both apoptotic and necrotic forms of cell lysis. J Immunol 141(8):2629–2634
35. Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W, Grooten J, Fiers W, Vandenabeele P (1998) Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. J Exp Med 187(9):1477–1485
36. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J (2000) Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 1(6):489–495
37. Murakami Y, Matsumoto H, Roh M, Suzuki J, Hisatomi T, Ikeda Y, Miller JW, Vavvas DG (2012) Receptor interacting protein kinase mediates necrotic cone but not rod cell death in a mouse model of inherited degeneration. Proc Natl Acad Sci U S A 109(36):14598–14603
38. Murakami Y, Notomi S, Hisatomi T, Nakazawa T, Ishibashi T, Miller JW, Vavvas DG (2013) Photoreceptor cell death and rescue in retinal detachment and degenerations. Prog Retin Eye Res 37:114–140
39. Green DR, Oberst A, Dillon CP, Weinlich R, Salvesen GS (2011) RIPK-dependent necrosis and its regulation by caspases: a mystery in five acts. Mol Cell 44(1):9–16
40. Nakazawa T, Hisatomi T, Nakazawa C, Noda K, Maruyama K, She H, Matsubara A, Miyahara S, Nakao S, Yin Y, Benowitz L, Hafezi-Moghadam A, Miller JW (2007) Monocyte

chemoattractant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. Proc Natl Acad Sci U S A 104(7):2425–2430
41. Trichonas G, Murakami Y, Thanos A, Morizane Y, Kayama M, Debouck CM, Hisatomi T, Miller JW, Vavvas DG (2010) Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis. Proc Natl Acad Sci U S A 107(50):21695–21700
42. Nakazawa T, Kayama M, Ryu M, Kunikata H, Watanabe R, Yasuda M, Kinugawa J, Vavvas D, Miller JW (2011) Tumor necrosis factor-alpha mediates photoreceptor death in a rodent model of retinal detachment. Invest Ophthalmol Vis Sci 52(3):1384–1391
43. Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8 (6):752–758
44. Notomi S, Hisatomi T, Kanemaru T, Takeda A, Ikeda Y, Enaida H, Kroemer G, Ishibashi T (2011) Critical involvement of extracellular ATP acting on P2RX7 purinergic receptors in photoreceptor cell death. Am J Pathol 179(6):2798–2809
45. Notomi S, Hisatomi T, Murakami Y, Terasaki H, Sonoda S, Asato R, Takeda A, Ikeda Y, Enaida H, Sakamoto T, Ishibashi T (2013) Dynamic increase in extracellular ATP accelerates photoreceptor cell apoptosis via ligation of P2RX7 in subretinal hemorrhage. PLoS One 8(1): e53338
46. Arimura N, Ki-i Y, Hashiguchi T, Kawahara K, Biswas KK, Nakamura M, Sonoda Y, Yamakiri K, Okubo A, Sakamoto T, Maruyama I (2009) Intraocular expression and release of high-mobility group box 1 protein in retinal detachment. Lab Invest 89(3):278–289
47. Bao Q, Riedl SJ, Shi Y (2005) Structure of Apaf-1 in the auto-inhibited form: a critical role for ADP. Cell Cycle 4(8):1001–1003
48. Bao Q, Shi Y (2007) Apoptosome: a platform for the activation of initiator caspases. Cell Death Differ 14(1):56–65
49. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW (2002) Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. Mol Cell 9(2):423–432
50. Bouchier-Hayes L, Lartigue L, Newmeyer DD (2005) Mitochondria: pharmacological manipulation of cell death. J Clin Invest 115(10):2640–2647
51. Murakami Y, Ikeda Y, Yonemitsu Y, Onimaru M, Nakagawa K, Kohno R, Miyazaki M, Hisatomi T, Nakamura M, Yabe T, Hasegawa M, Ishibashi T, Sueishi K (2008) Inhibition of nuclear translocation of apoptosis-inducing factor is an essential mechanism of the neuroprotective activity of pigment epithelium-derived factor in a rat model of retinal degeneration. Am J Pathol 173(5):1326–1338
52. Cande C, Vahsen N, Kouranti I, Schmitt E, Daugas E, Spahr C, Luban J, Kroemer RT, Giordanetto F, Garrido C, Penninger JM, Kroemer G (2004) AIF and cyclophilin A cooperate in apoptosis-associated chromatinolysis. Oncogene 23(8):1514–1521
53. Wang H, Shimoji M, Yu SW, Dawson TM, Dawson VL (2003) Apoptosis inducing factor and PARP-mediated injury in the MPTP mouse model of Parkinson's disease. Ann N Y Acad Sci 991:132–139
54. Chu CT, Zhu JH, Cao G, Signore A, Wang S, Chen J (2005) Apoptosis inducing factor mediates caspase-independent 1-methyl-4-phenylpyridinium toxicity in dopaminergic cells. J Neurochem 94(6):1685–1695
55. Sanges D, Comitato A, Tammaro R, Marigo V (2006) Apoptosis in retinal degeneration involves cross-talk between apoptosis-inducing factor (AIF) and caspase-12 and is blocked by calpain inhibitors. Proc Natl Acad Sci U S A 103(46):17366–17371
56. Chaum E (2003) Retinal neuroprotection by growth factors: a mechanistic perspective. J Cell Biochem 88(1):57–75
57. Bogaerts V, Theuns J, van Broeckhoven C (2008) Genetic findings in Parkinson's disease and translation into treatment: a leading role for mitochondria? Genes Brain Behav 7(2):129–151

58. Borner C (2003) The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. Mol Immunol 39(11):615–647
59. Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G, Thompson CB (1993) Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74(4):597–608
60. Gibson L, Holmgreen SP, Huang DC, Bernard O, Copeland NG, Jenkins NA, Sutherland GR, Baker E, Adams JM, Cory S (1996) Bcl-w, a novel member of the bcl-2 family, promotes cell survival. Oncogene 13(4):665–675
61. Oltvai ZN, Milliman CL, Korsmeyer SJ (1993) Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74(4):609–619
62. Inohara N, Ding L, Chen S, Nunez G (1997) Harakiri, a novel regulator of cell death, encodes a protein that activates apoptosis and interacts selectively with survival-promoting proteins Bcl-2 and Bcl-X(L). EMBO J 16(7):1686–1694
63. Zhao H, Yenari MA, Cheng D, Sapolsky RM, Steinberg GK (2003) Bcl-2 overexpression protects against neuron loss within the ischemic margin following experimental stroke and inhibits cytochrome c translocation and caspase-3 activity. J Neurochem 85(4):1026–1036
64. Zhao H, Yenari MA, Cheng D, Barreto-Chang OL, Sapolsky RM, Steinberg GK (2004) Bcl-2 transfection via herpes simplex virus blocks apoptosis-inducing factor translocation after focal ischemia in the rat. J Cereb Blood Flow Metab 24(6):681–692
65. Kroemer G, Reed JC (2000) Mitochondrial control of cell death. Nat Med 6(5):513–519
66. Asoh S, Ohsawa I, Mori T, Katsura K, Hiraide T, Katayama Y, Kimura M, Ozaki D, Yamagata K, Ohta S (2002) Protection against ischemic brain injury by protein therapeutics. Proc Natl Acad Sci U S A 99(26):17107–17112
67. Dietz GP, Kilic E, Bahr M (2002) Inhibition of neuronal apoptosis in vitro and in vivo using TAT-mediated protein transduction. Mol Cell Neurosci 21(1):29–37
68. Yin W, Cao G, Johnnides MJ, Signore AP, Luo Y, Hickey RW, Chen J (2006) TAT-mediated delivery of Bcl-xL protein is neuroprotective against neonatal hypoxic-ischemic brain injury via inhibition of caspases and AIF. Neurobiol Dis 21(2):358–371
69. Shimizu S, Konishi A, Kodama T, Tsujimoto Y (2000) BH4 domain of antiapoptotic Bcl-2 family members closes voltage-dependent anion channel and inhibits apoptotic mitochondrial changes and cell death. Proc Natl Acad Sci U S A 97(7):3100–3105
70. Cao G, Pei W, Ge H, Liang Q, Luo Y, Sharp FR, Lu A, Ran R, Graham SH, Chen J (2002) In vivo delivery of a Bcl-xL fusion protein containing the TAT protein transduction domain protects against ischemic brain injury and neuronal apoptosis. J Neurosci 22(13):5423–5431
71. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF (1999) In vivo protein transduction: delivery of a biologically active protein into the mouse. Science 285(5433):1569–1572
72. Sugioka R, Shimizu S, Funatsu T, Tamagawa H, Sawa Y, Kawakami T, Tsujimoto Y (2003) BH4-domain peptide from Bcl-xL exerts anti-apoptotic activity in vivo. Oncogene 22 (52):8432–8440
73. Hotchkiss RS, McConnell KW, Bullok K, Davis CG, Chang KC, Schwulst SJ, Dunne JC, Dietz GP, Bahr M, McDunn JE, Karl IE, Wagner TH, Cobb JP, Coopersmith CM, Piwnica-Worms D (2006) TAT-BH4 and TAT-Bcl-xL peptides protect against sepsis-induced lymphocyte apoptosis in vivo. J Immunol 176(9):5471–5477
74. Staszewski S, Morales-Ramirez J, Tashima KT, Rachlis A, Skiest D, Stanford J, Stryker R, Johnson P, Labriola DF, Farina D, Manion DJ, Ruiz NM, Study 006 Team (1999) Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. N Engl J Med 341(25):1865–1873
75. Phenix BN, Cooper C, Owen C, Badley AD (2002) Modulation of apoptosis by HIV protease inhibitors. Apoptosis 7(4):295–312
76. Deeks SG, Grant RM (1999) Sustained CD4 responses after virological failure of protease inhibitor-containing therapy. Antivir Ther 4(Suppl 3):7–11
77. Badley AD (2005) In vitro and in vivo effects of HIV protease inhibitors on apoptosis. Cell Death Differ 12(Suppl 1):924–931

78. Estaquier J, Lelievre JD, Petit F, Brunner T, Moutouh-De Parseval L, Richman DD, Ameisen JC, Corbeil J (2002) Effects of antiretroviral drugs on human immunodeficiency virus type 1-induced CD4(+) T-cell death. J Virol 76(12):5966–5973
79. Sloand EM, Kumar PN, Kim S, Chaudhuri A, Weichold FF, Young NS (1999) Human immunodeficiency virus type 1 protease inhibitor modulates activation of peripheral blood CD4(+) T cells and decreases their susceptibility to apoptosis in vitro and in vivo. Blood 94 (3):1021–1027
80. Ghibelli L, Mengoni F, Lichtner M, Coppola S, De Nicola M, Bergamaschi A, Mastroianni C, Vullo V (2003) Anti-apoptotic effect of HIV protease inhibitors via direct inhibition of calpain. Biochem Pharmacol 66(8):1505–1512
81. Phenix BN, Lum JJ, Nie Z, Sanchez-Dardon J, Badley AD (2001) Antiapoptotic mechanism of HIV protease inhibitors: preventing mitochondrial transmembrane potential loss. Blood 98 (4):1078–1085
82. Matarrese P, Gambardella L, Cassone A, Vella S, Cauda R, Malorni W (2003) Mitochondrial membrane hyperpolarization hijacks activated T lymphocytes toward the apoptotic-prone phenotype: homeostatic mechanisms of HIV protease inhibitors. J Immunol 170 (12):6006–6015
83. Weaver JG, Tarze A, Moffat TC, Lebras M, Deniaud A, Brenner C, Bren GD, Morin MY, Phenix BN, Dong L, Jiang SX, Sim VL, Zurakowski B, Lallier J, Hardin H, Wettstein P, van Heeswijk RP, Douen A, Kroemer RT, Hou ST, Bennett SA, Lynch DH, Kroemer G, Badley AD (2005) Inhibition of adenine nucleotide translocator pore function and protection against apoptosis in vivo by an HIV protease inhibitor. J Clin Invest 115(7):1828–1838
84. Green DR, Kroemer G (2005) Pharmacological manipulation of cell death: clinical applications in sight? J Clin Invest 115(10):2610–2617
85. Gaedicke S, Firat-Geier E, Constantiniu O, Lucchiari-Hartz M, Freudenberg M, Galanos C, Niedermann G (2002) Antitumor effect of the human immunodeficiency virus protease inhibitor ritonavir: induction of tumor-cell apoptosis associated with perturbation of proteasomal proteolysis. Cancer Res 62(23):6901–6908
86. Ellis HM, Horvitz HR (1986) Genetic control of programmed cell death in the nematode C. elegans. Cell 44(6):817–829
87. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR (1993) The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell 75 (4):641–652
88. Dubyak GR, el-Moatassim C (1993) Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. Am J Physiol 265(3 Pt 1):C577–C606
89. Abbracchio MP, Burnstock G (1994) Purinoceptors: are there families of P2X and P2Y purinoceptors? Pharmacol Ther 64(3):445–475
90. Ferrari D, Los M, Bauer MK, Vandenabeele P, Wesselborg S, Schulze-Osthoff K (1999) P2Z purinoreceptor ligation induces activation of caspases with distinct roles in apoptotic and necrotic alterations of cell death. FEBS Lett 447(1):71–75
91. Erickson PA, Fisher SK, Anderson DH, Stern WH, Borgula GA (1983) Retinal detachment in the cat: the outer nuclear and outer plexiform layers. Invest Ophthalmol Vis Sci 24(7):927–942
92. Zacks DN, Boehlke C, Richards AL, Zheng QD (2007) Role of the Fas-signaling pathway in photoreceptor neuroprotection. Arch Ophthalmol 125(10):1389–1395

Chapter 20
Neuroinflammation

Kazuichi Maruyama

Abstract Ocular inflammation is known to be accompanied by retinal cell impairment. In uveitis, there are numerous causes of the retinal cell damage. However, secondary intraocular hypertension and direct inflammation caused by the uveitis are major concerns, as both of these can affect retinal cell survival. This is especially the case in sarcoidosis and Vogt–Koyanagi–Harada disease, which are the most common types of uveitis seen clinically in Japan. In addition, Behçet's disease and acute retinal necrosis are also known to have a poor prognosis when patients are unable to receive precise treatments at specific time points during the clinical progress of the disease.

Keywords Posner–Schlossman Syndrome • Secondary glaucoma • Steroid-induced glaucoma • Trabeculitis • Uveitis

20.1 Uveitis-Induced Retinal Cell Damage

In Japan, uveitis has been shown to consist of several idiopathic diseases, including sarcoidosis, Behçet's disease, and Vogt–Koyanagi–Harada disease (VKH) disease. Moreover, it has been suggested that infectious diseases such as viral, fungal, and bacterial infections should also be included as causes of uveitis. When trying to determine the etiology of uveitis, it is important to collect detailed medical histories and perform thorough physical examinations. Observation of the course of the disease may be one of the best ways to diagnose uveitis. However, diagnoses made without clinical examinations or evaluations of laboratory data from patient

K. Maruyama, M.D., Ph.D. (✉)
Department of Ophthalmology, Tohoku University Graduate School of Medicine,
1-1 Seiryo-machi Aoba-ku, Sendai, Miyagi 980-8574, Japan
e-mail: maruyama-k@oph.med.tohoku.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_20,

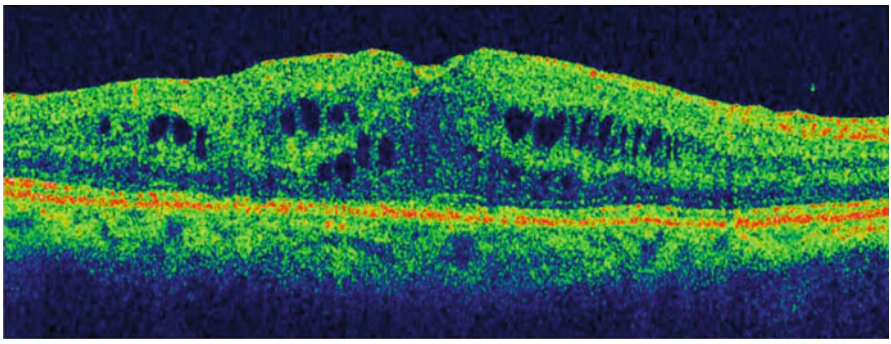


Fig. 20.1 Chronic inflammation caused cystoid macular edema in a sarcoidosis patient

specimens may lead to the misdiagnosis of many cases. In fact, the number of idiopathic uveitis cases has now increased to about 30 % of all uveitis patients in the Japanese population [1].

20.1.1 Sarcoidosis

Sarcoidosis is an idiopathic inflammatory disorder characterized by noncaseating epithelioid cell granulomas in multiple tissues and organs [2, 3]. While this disease most commonly affects the lung, other tissues (such as the heart, central nervous system, or spleen) may also be affected [4]. Although the exact cause of sarcoidosis is currently unknown, studies have linked the disease with genetic, environmental, or age-related heterogeneity [5, 6]. Even though endogenous and pathogenic bacterial species have been detected in sarcoid lesions, their association with disease etiology remains unclear [7]. It has been surmised that genetic predispositions, compounded by other factors, may trigger granulomatous responses to endogenous or infectious microbes in sarcoidosis [6, 8]. It has been further reported that 30–60 % of patients with sarcoidosis suffer from ocular involvement [9–12]. While bilateral anterior and/or posterior uveitis is most common, the conjunctiva, the lacrimal gland, and the orbit of the eye can also be affected. The clinical presentation of sarcoid uveitis is characteristically marked by iris nodules, mutton-fat keratic precipitates, and tent-shaped peripheral anterior synechia in the anterior segment of the eye. Phlebitis and vitreitis, resulting in snowball-like vitreous opacity, are the common posterior segment findings. Chronic uveitis can result in the formation of an epiretinal membrane and cystoid macular edema (Fig. 20.1), leading to severe visual impairment [13]. Thus, in order to prevent retinal damage, an early adequate diagnosis and treatment are needed.

Uveitis is commonly treated with topical corticosteroids, but in cases accompanied by the formation of an epiretinal membrane or longstanding vitreous opacity, systemic corticosteroid or surgical intervention may be necessary. To diagnose

sarcoidosis uveitis, an examination method using flow cytometry analysis was developed for analyzing vitreous specimens. One of the unique immunological features of vitreous lymphocytosis in ocular sarcoidosis that has been reported is a characteristically high CD4/CD8 ratio. Diagnoses of ocular sarcoidosis based on a CD4/CD8 ratio of vitreous-infiltrating lymphocytes greater than 3.5 have been shown to have a sensitivity of 100 % and a specificity of 96.3 %, both of which are remarkably high values. Costabel et al. reported that a CD4/CD8 ratio greater than 3.5 in lymphocytes obtained from BAL fluid had a sensitivity of 53 % and a specificity of 94 % [14]. Thus, Kojima et al. proposed that a high CD4/CD8 ratio of lymphocytes obtained from the vitreous has a high diagnostic value and is comparable to that of a high CD4/CD8 ratio in BAL fluid lymphocyte [15]. The primary therapy used to treat sarcoidosis uveitis is the administration of corticosteroids [16, 17].

The primary goal of these sarcoidosis uveitis treatments is to prevent the progression. To that end, systemic corticosteroid and surgical removal of the vitreous opacity, which includes inflammatory cells and high amounts of cytokine [18], are useful treatments. Sarcoidosis uveitis is often responsive to corticosteroid, with the use of a high initial dose followed by slow tapering shown to be effective in managing most patients. Therefore, administration of this precise treatment without hesitation should be used to prevent chronic inflammation. In all patients with ocular manifestation of sarcoidosis, the treatment goal is to control the inflammatory disease and prevent permanent visual impairment changes such as photoreceptor damage caused by chronic cystoid macular edema.

20.1.2 VKH Disease

VKH is also one of the most diagnosed types of uveitis in the Japanese population. This systemic disorder involves several organs, including the eyes, ears, skin, and meninges. VKH is a bilateral ocular condition. Although the initial activity observed in VKH is minimal, the conditions associated with the subsequent inflammation are quite severe. Bilateral shallowing of the anterior chamber due to swelling of the ciliary body is sometimes noted during clinical examinations. This secondary angle-closure glaucoma develops into intraocular hypertension due to blockage of the aqueous humor outflow. Glaucoma that is associated with VKH can be difficult to control. A study by Forster et al. previously reported that almost 40 % of VKH patients required surgical and medical therapy for their secondary glaucoma [19]. For the posterior segment in VKH disease, optic nerve swelling and nonrhegmatogenous serous retinal detachment are observed (Fig. 20.2). Furthermore, several fundus changes are also observed during the disease process (Fig. 20.3). In severe VKH, choroidal neovascularization (CNV) is found in the subretinal space and optic nerve area. Moreover, CNV has been found to strongly affect visual acuity. A previous study that examined VKH reported finding extramacular disorder, presumably in an area of reactive proliferation of the retinal pigment epithelium and with perturbation of Bruch's

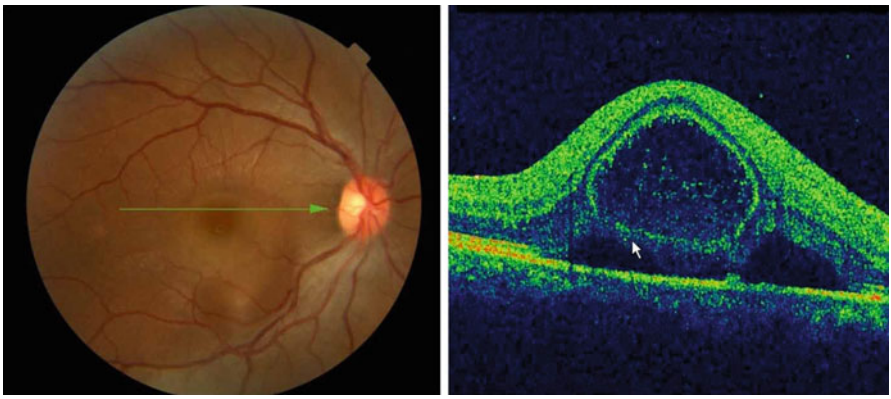


Fig. 20.2 Serous detachment with fibrin formation in a VKH patient. *Green arrow* indicated the scan line of OCT. *White arrow* indicated the fibrin formation in retinal layer during acute clinical course

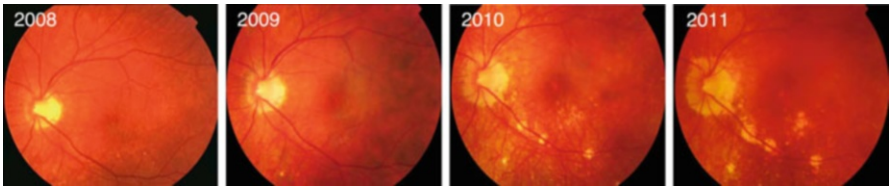


Fig. 20.3 Time course for the posterior segment in a VKH patient with recurrence

membrane [20]. Keino et al. additionally reported that the sunset glow appearance was a process of VKH that was more common in eyes with chronic inflammation [21]. Chronic inflammation in VKH is commonly observed in the anterior segment. The persistent inflammation at the anterior segment induces several dysfunctions of the aqueous flow system, such as severe posterior and anterior synechia. Due to the extended high ocular pressure, dysfunction of the aqueous flow at the anterior segment leads to the development of secondary glaucoma. In order to prevent disease progression in VKH, accurate diagnoses need to be made, followed by specific treatments using high concentrations of steroids or immunosuppressive drugs such as cyclosporine.

20.1.3 Behçet's Disease

Recently, Behçet's disease has become a less commonly observed type of uveitis in the Japanese population due to improvement of the hygienic status over the past few decades. Even so, Behçet's disease should still be considered one of the most

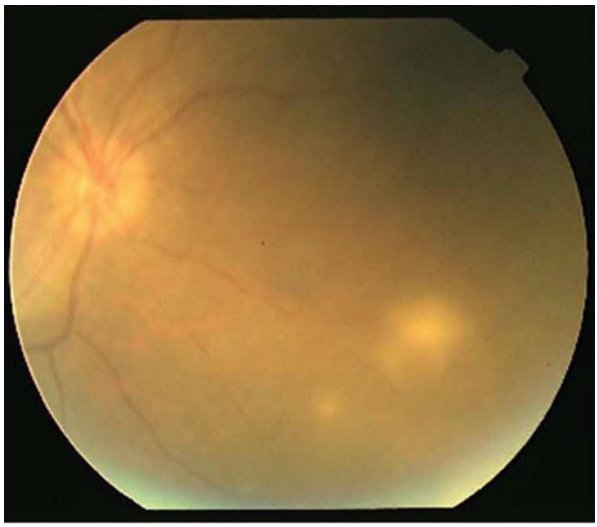


Fig. 20.4 Acute severe attack of Behçet's disease at the posterior segment

important diseases that need to be treated in order to prevent blindness. Behçet's disease is a multisystem disorder in which there are recurrent severe intraocular inflammatory episodes along with a high incident of oral and mucosal ulcerations. Ocular manifestations especially have serious implications for the patient, as the disease can affect both the anterior and posterior segments. In retinal lesions, the disease directly affects the visual acuity by inducing irreversible alterations of the retinal cells due to multiple types of severe vasculitis (vaso-occlusive and neovascularization) with retinal ischemia and hemorrhage (Fig. 20.4). Necrotizing arteriolitis and phlebitis with thromboses have also been reported with Behçet's disease [22]. The neurologic lesions include perivascular cuffing with mononuclear cells, focal necrosis, demyelinization, and gliosis. Inflammatory cytokines such as IFN-gamma, interleukin 6 and interleukin 8, and tissue necrotizing factor (TNF)-alpha have been reported to be much higher in the aqueous humor and vitreous specimens of Behçet's disease [23]. In addition, iris neovascularization can occur and lead to the induction of secondary glaucoma.

For ophthalmologists, Behçet's disease treatments have proven to be difficult to perform, especially in younger male patients. This is because symptoms in young men are more severe and have a higher recurrence as compared to that seen in older men or female patients. Indeed, in younger patients, macular retinal cells in the posterior segment are especially destroyed due to multiple severe attacks that cause vascular damage (Fig.20.5). Treatments that have been used in an attempt to suppress these acute attacks include steroids, immunosuppressive drugs (cyclosporine, tacrolimus), interferon alpha, and colchicine. However, these drugs have not been able to improve the visual acuity loss in young male patients who have had severe and recurrent attacks.

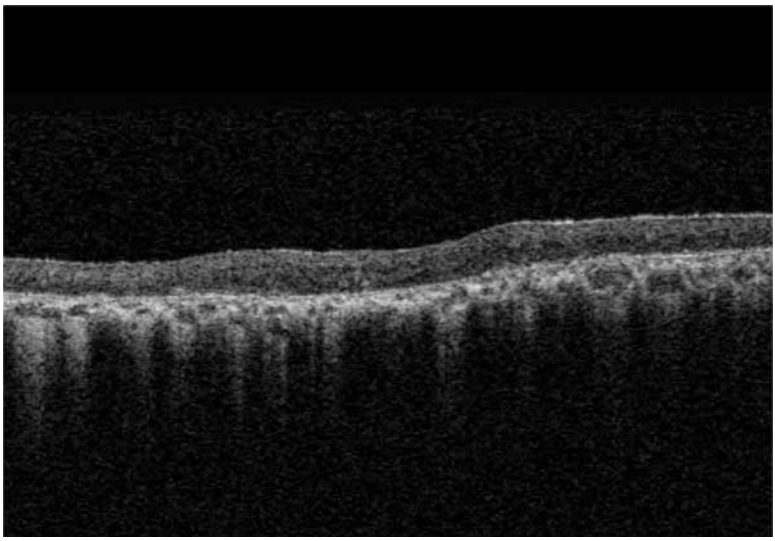


Fig. 20.5 Terminal retinal change at the posterior segment; thinning of the neural retina occurred and the retinal layer was destroyed

An evaluation of evidence-based studies found 21 randomized studies in which only azathioprine and ciclosporin were effective for treating ocular symptoms [24, 25]. Nussenblatt et al. reported that combined ciclosporin and prednisone therapy suppressed the disorder and permitted use of lower doses of both medications [26]. Recently, a novel drug has been found and used in suppressing attacks of Behçet's disease. Infliximab is a chimeric monoclonal antibody that is directed against TNF-alpha, in addition to being able to cause secretion of cells such as macrophages. This antibody has been reported to be effective in treating Behçet's disease [27]. However, issues concerning the induction of autoantibodies against infliximab during the therapy still need to be clarified before this drug can be used as a routine treatment. Even so, infliximab may become a beneficial treatment for Behçet's disease in the near future.

20.1.4 Acute Retinal Necrosis (ARN)

ARN is a type of infected uveitis in which severe and aggressive retinal damage occurs during the clinical course. Almost all patients who suffer from ARN develop severe visual acuity loss due to ischemic retinitis, multiple rhegmatogenous retinal detachment, viral infiltration of the nerve, or optic nerve distention. VZV infection is especially more severe than the herpes simplex viral (HSV) infection (Figs. 20.6 and 20.7). Patients with VZV and ARN have optic neuropathy, aggressive retinal

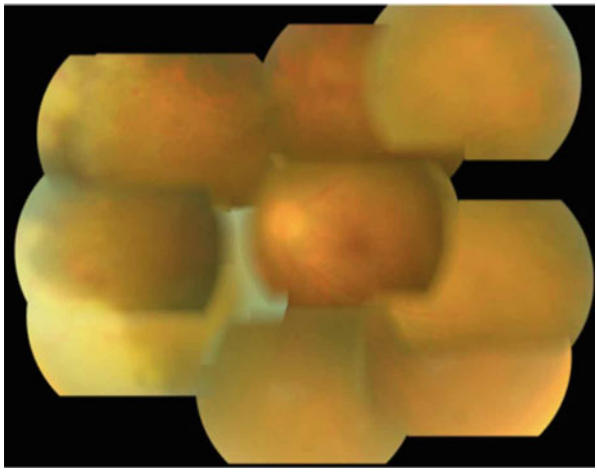


Fig. 20.6 Induction of ARN caused by HSV-2

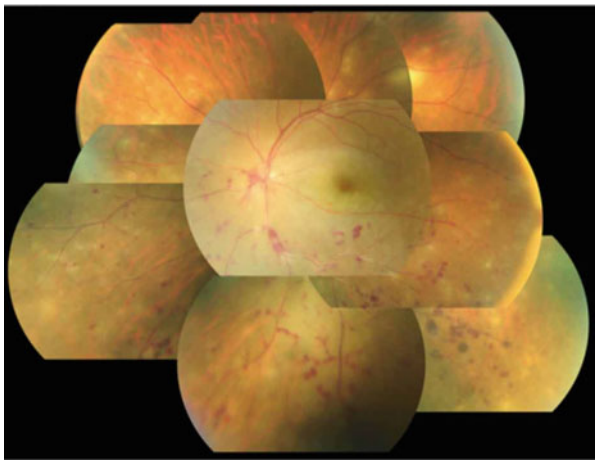


Fig. 20.7 Induction of ARN caused by VZV

necrosis, and vascular occlusion. Once the retinal infected lesion starts in the midperiphery, it then expands to the posterior segment (Fig. 20.8). Cytopathology of the vitreous section of ARN demonstrated that there were a number of infiltrated cells, especially T cells. However, the T-cell population in the vitreous specimens was different from that observed in other types of uveitis such as sarcoidosis. It has also been reported that more CD8 T cells infiltrate into the vitreous in ARN [15]. In addition, explosive inflammatory cytokines have been shown to exist in the vitreous in ARN [18]. Thus, these cytokines may be involved in the development of ARN.

ARN was initially believed to be an autoimmune disease [28]. However, Culbertson et al. examined electron microscopic pictures of human enucleated eyes and reported that the herpes virus existed in the retinal lesion [29]. Recently, Ganatra et al. and Sugita et al. further demonstrated that viral DNA such as VZV

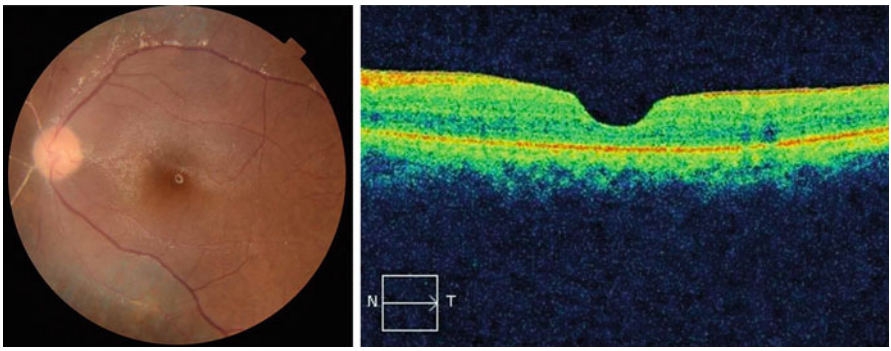


Fig. 20.8 White vessel at the posterior segment of ARN (postoperatively after filling with silicon oil); IS/OS line was not seen in the macular area

and HSV type 1 or 2 could be detected in vitreous or aqueous humor samples by the polymerase chain reaction (PCR) method [30–32]. The executive committee of the American Uveitis Society subsequently published a set of a standard diagnostic criteria for the ARN syndrome [33].

To suppress viral activity and the severe inflammation that accompanies a viral infection, it is important to diagnose ARN at its initial stage so that it can be quickly treated with the correct concentration of drugs such as aciclovir and steroid. When patients do not receive a suitable treatment for ARN, retinal cells are almost certainly destroyed by retinal vascular occlusion, multiple rhegmatogenous retinal detachment, viral infiltration of the nerve, or optic nerve distention. As a result, the visual acuity will quickly decrease. In such cases, surgical treatment is warranted to repair the retinal detachment. If posterior vitreous detachment occurs during the clinical course, this can lead to multiple retina tears and the start of retinal detachment. While other effects have also been reported to occur after surgical treatment, such as a reduction of the viral amount in the vitreous cavity, there has yet to be any well-controlled study data that support the effectiveness of these therapeutic interventions.

20.2 Secondary Intraocular Hypertension

Secondary intraocular hypertension is induced by aqueous outflow disturbance that involves a mechanical or functional disorder. Mechanical disorders are due to anatomical issues such as anterior or posterior synechia (Figs. 20.9 and 20.10) or deposits at the trabecular meshwork (induced by steroids) [34]. In contrast, functional disorders, such as trabeculitis, do not have any anatomical component associated with ocular inflammation (Fig. 20.11). However, viral infections can cause induction of inflammation at the trabecular meshwork that leads to decreases in the aqueous humor outflow [35, 36]. As a result, intraocular pressure (IOP)

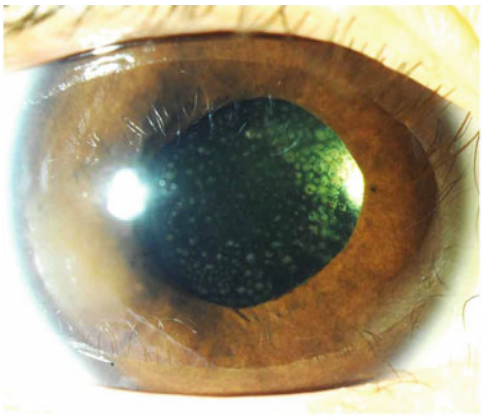


Fig. 20.9 Iris bombé and anterior synechia in a sarcoidosis patient

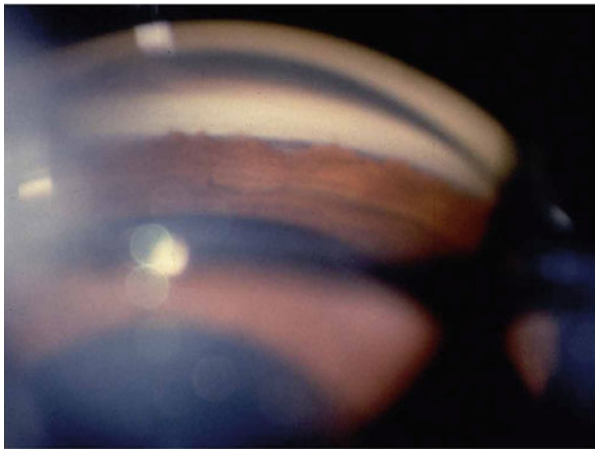


Fig. 20.10 Peripheral anterior synechia in a sarcoidosis patient

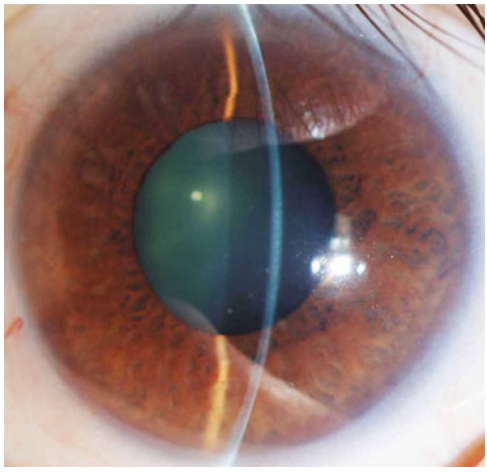


Fig. 20.11 White precipitate at the corneal endothelial layer and iris degeneration in a PSS patient

increases, which leads to damage of the retinal ganglion cells, especially at the optic nerve disc. To prevent these retinal cell damages, the most important factor in the treatment is an early and precise diagnosis. However, a diagnosis can be especially difficult to make when trying to distinguish between steroid-induced ocular hypertension and ocular hypertension caused by trabeculitis. This is an important problem, as the treatments for these two types of disease are completely different. Trabeculitis is an inflammatory-induced ocular hypertension, and thus treatments require the use of steroids. Moreover, as compared to the diagnosis of trabeculitis, it is even more difficult when trying to diagnose non-inflamed conditions in the anterior chamber. Therefore, clinicians need to be very careful when diagnosing these diseases in order to avoid excess administration of steroids and unnecessary surgical procedures in these patients.

20.2.1 Steroid-Induced Glaucoma

In steroid-induced glaucoma, the IOP is elevated primarily due to increased outflow resistance of the aqueous humor. Increased responsiveness to steroids may be facilitated by the upregulation of the glucocorticoid receptors that are found on the trabecular meshwork cells [37]. In cultured human trabecular meshwork cells, glucocorticoids increased the expression of extracellular matrix proteins, such as fibronectin, glycosaminoglycans, and elastin [38]. It has also been reported that steroids suppress the phagocytic activity, which has been shown to lead to increased deposition of extracellular matrix at the trabecular meshwork [39–41]. In some cases, patients have noticed a visual field defect and a downregulation of their visual acuity after adverse changes in their ocular status. When treating these patients with steroids, the best way to discover the development of steroid-induced ocular hypertension is to check the IOP at each of their monthly clinical examinations.

20.2.2 Trabeculitis

Obstruction of the trabecular meshwork in uveitis patients occurs due to disruption of the blood and aqueous barrier. When inflammation damages or destroys this barrier, many cells such as neutrophils and red blood cells enter into the trabecular meshwork. As a result, there is obstruction of the aqueous outflow. Another possible cause of the trabecular pathway obstruction may be related to the dysfunction of endothelial cells within Schlemm's canal. This canal is essentially an endothelium-lined tube that resembles that of a lymphatic vessel. The inside of

the canal is nearest to the aqueous humor. Dysfunction of Schlemm's canal may be caused by the chronic inflammation of the uveitis or due to a viral infection caused by cytomegalovirus, herpes simplex (HSV) type 1, or the varicella-zoster virus (VZV) [36, 42]. As a result, this leads to an elevation of the IOP due to the aqueous humor obstruction.

20.2.3 Posner–Schlossman Syndrome

One of the characteristics of Posner–Schlossman Syndrome (PSS) is a sudden and repetitive elevation of the IOP. Although originally it was not believed that PSS attacks caused permanent damage to the eye, later reports have questioned the benign nature of this syndrome [43, 44]. The aim of PSS treatment is to control both the inflammation and the elevated IOP. The favored initial treatment involves a combined regimen of anti-inflammatory and anti-glaucoma eye drops. To normalize the IOP when the eye is inflamed, it is important to use a topical or oral steroid to control the inflammation. Moreover, Chee et al. reported that some of PSS patients are infected by cytomegalovirus and then the patients need precise antiviral treatment [45]. In some cases, however, patients are not responsive to steroid treatment, and it becomes necessary to perform filtration surgery in order to prevent visual field loss due to the elevated IOP. It has been further reported that trabeculectomy or filtration surgery has been effective in some of these cases [46].

20.3 Summary

Controlling inflammation is perhaps the most important action for preventing the continuing damage of the retinal cells that arises secondarily from uveitis or ocular inflamed conditions. In some cases, clinical examinations are able to detect the presence of secondary ocular hypertension, such as steroid-induced ocular hypertension or PSS, in patients prior to any severe visual function loss. In order to determine the correct patient treatment, these changes need to be detected as early as possible. In contrast, it has been previously reported that inflammatory cytokines such as TNF-alpha play a role in preventing retinal cell death in a retinal detachment animal model [47]. Although high inflammatory conditions normally occur in uveitis, retinal cells have been shown to be able to survive without any uveitis symptoms such as vascular occlusion or direct attachment of pathogenic factors (virus, bacterial, etc.). Thus, the material presented in this review suggests that preventing adverse events via the use of precise diagnoses and specific treatments may be the best way to rescue retinal cells from death or damage.

References

1. Ohguro N, Sonoda KH, Takeuchi M, Matsumura M, Mochizuki M (2012) The 2009 prospective multi-center epidemiologic survey of uveitis in Japan. Jpn J Ophthalmol 56:432–435
2. Newman LS, Rose CS, Maier LA (1997) Sarcoidosis. N Engl J Med 336:1224–1234
3. Hunninghake GW, Crystal RG (1981) Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. N Engl J Med 305:429–434
4. (1999) Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med 160: 736–755 http://www.ncbi.nlm.nih.gov/pubmed/?term=Joint+Statement+of+the+American+Thoracic+Society+(ATS)%2C
5. Hiraga Y (1994) An epidemiological study of clustering of sarcoidosis cases. Nippon Rinsho 52:1438–1442
6. Hosoda Y, Hiraga Y, Odaka M et al (1976) A cooperative study of sarcoidosis in Asia and Africa: analytic epidemiology. Ann N Y Acad Sci 278:355–367
7. Ishige I, Usui Y, Takemura T, Eishi Y (1999) Quantitative PCR of mycobacterial and propionibacterial DNA in lymph nodes of Japanese patients with sarcoidosis. Lancet 354:120–123
8. McGrath DS, Goh N, Foley PJ, du Bois RM (2001) Sarcoidosis: genes and microbes–soil or seed? Sarcoidosis Vasc Diffuse Lung Dis 18:149–164
9. Lynch JP 3rd, Sharma OP, Baughman RP (1998) Extrapulmonary sarcoidosis. Semin Respir Infect 13:229–254
10. Crick RP, Hoyle C, Smellie H (1961) The Eyes in Sarcoidosis. Br J Ophthalmol 45:461–481
11. Ohara K, Okubo A, Sasaki H, Kamata K (1992) Intraocular manifestations of systemic sarcoidosis. Jpn J Ophthalmol 36:452–457
12. Jabs DA, Johns CJ (1986) Ocular involvement in chronic sarcoidosis. Am J Ophthalmol 102:297–301
13. Bonfioli AA, Orefice F (2005) Sarcoidosis. Semin Ophthalmol 20:177–182
14. Costabel U, Zaiss AW, Guzman J (1992) Sensitivity and specificity of BAL findings in sarcoidosis. Sarcoidosis 9(Suppl 1):211–214
15. Kojima K, Maruyama K, Inaba T et al (2012) The CD4/CD8 ratio in vitreous fluid is of high diagnostic value in sarcoidosis. Ophthalmology 119:2386–2392
16. Siltzbach LE, James DG, Neville E et al (1974) Course and prognosis of sarcoidosis around the world. Am J Med 57:847–852
17. Palestine AG, Nussenblatt RB, Chan CC (1986) Treatment of intraocular complications of sarcoidosis. Ann N Y Acad Sci 465:564–574
18. Nagata K, Maruyama K, Uno K et al (2012) Simultaneous analysis of multiple cytokines in the vitreous of patients with sarcoid uveitis. Invest Ophthalmol Vis Sci 53:3827–3833
19. Forster DJ, Rao NA, Hill RA, Nguyen QH, Baerveldt G (1993) Incidence and management of glaucoma in Vogt-Koyanagi-Harada syndrome. Ophthalmology 100:613–618
20. Nowilaty SR, Bouhaimed M (2006) Photodynamic therapy for subfoveal choroidal neovascularisation in Vogt-Koyanagi-Harada disease. Br J Ophthalmol 90:982–986
21. Keino H, Goto H, Usui M (2002) Sunset glow fundus in Vogt-Koyanagi-Harada disease with or without chronic ocular inflammation. Graefes Arch Clin Exp Ophthalmol 240:878–882
22. Mullaney J, Collum LM (1985) Ocular vasculitis in Behcet's disease. A pathological and immunohistochemical study. Int Ophthalmol 7:183–191
23. Ahn JK, Yu HG, Chung H, Park YG (2006) Intraocular cytokine environment in active Behcet uveitis. Am J Ophthalmol 142:429–434
24. Masuda K, Nakajima A, Urayama A, Nakae K, Kogure M, Inaba G (1989) Double-masked trial of cyclosporin versus colchicine and long-term open study of cyclosporin in Behcet's disease. Lancet 1:1093–1096

25. Yazici H, Pazarli H, Barnes CG et al (1990) A controlled trial of azathioprine in Behcet's syndrome. N Engl J Med 322:281–285
26. Whitcup SM, Salvo EC Jr, Nussenblatt RB (1994) Combined cyclosporine and corticosteroid therapy for sight-threatening uveitis in Behcet's disease. Am J Ophthalmol 118:39–45
27. Okada AA, Goto H, Ohno S, Mochizuki M (2012) Multicenter study of infliximab for refractory uveoretinitis in Behcet disease. Arch Ophthalmol 130:592–598
28. Topilow HW, Nussbaum JJ, Freeman HM, Dickersin GR, Szyfelbein W (1982) Bilateral acute retinal necrosis. Clinical and ultrastructural study. Arch Ophthalmol 100:1901–1908
29. Culbertson WW, Blumenkranz MS, Haines H, Gass DM, Mitchell KB, Norton EW (1982) The acute retinal necrosis syndrome. Part 2: Histopathology and etiology. Ophthalmology 89:1317–1325
30. Ganatra JB, Chandler D, Santos C, Kuppermann B, Margolis TP (2000) Viral causes of the acute retinal necrosis syndrome. Am J Ophthalmol 129:166–172
31. Sugita S, Iwanaga Y, Kawaguchi T et al (2008) Detection of herpesvirus genome by multiplex polymerase chain reaction (PCR) and real-time PCR in ocular fluids of patients with acute retinal necrosis. Nihon Ganka Gakkai Zasshi 112:30–38
32. Sugita S, Shimizu N, Watanabe K et al (2008) Use of multiplex PCR and real-time PCR to detect human herpes virus genome in ocular fluids of patients with uveitis. Br J Ophthalmol 92:928–932
33. Holland GN (1994) Standard diagnostic criteria for the acute retinal necrosis syndrome. Executive Committee of the American Uveitis Society. Am J Ophthalmol 117:663–667
34. Tawara A, Tou N, Kubota T, Harada Y, Yokota K (2008) Immunohistochemical evaluation of the extracellular matrix in trabecular meshwork in steroid-induced glaucoma. Graefes Arch Clin Exp Ophthalmol 246:1021–1028
35. Takahashi T, Ohtani S, Miyata K, Miyata N, Shirato S, Mochizuki M (2002) A clinical evaluation of uveitis-associated secondary glaucoma. Jpn J Ophthalmol 46:556–562
36. Miserocchi E, Waheed NK, Dios E et al (2002) Visual outcome in herpes simplex virus and varicella zoster virus uveitis: a clinical evaluation and comparison. Ophthalmology 109:1532–1537
37. Zhang X, Clark AF, Yorio T (2008) FK506-binding protein 51 regulates nuclear transport of the glucocorticoid receptor beta and glucocorticoid responsiveness. Invest Ophthalmol Vis Sci 49:1037–1047
38. Steely HT, Browder SL, Julian MB, Miggans ST, Wilson KL, Clark AF (1992) The effects of dexamethasone on fibronectin expression in cultured human trabecular meshwork cells. Invest Ophthalmol Vis Sci 33:2242–2250
39. Benedikt O, Roll P (1979) The trabecular meshwork of a non-glaucomatous eye with the exfoliation syndrome. Electronmicroscopic study. Virchows Arch A Pathol Anat Histol 384:347–355
40. Rohen JW, Linner E, Witmer R (1973) Electron microscopic studies on the trabecular meshwork in two cases of corticosteroid-glaucoma. Exp Eye Res 17:19–31
41. Roll P, Benedikt O (1979) Electronmicroscopic studies of the trabecular meshwork in corticosteroid glaucoma. Klin Monbl Augenheilkd 174:421–428
42. van Boxtel LA, van der Lelij A, van der Meer J, Los LI (2007) Cytomegalovirus as a cause of anterior uveitis in immunocompetent patients. Ophthalmology 114:1358–1362
43. Kass MA, Becker B, Kolker AE (1973) Glaucomatocyclitic crisis and primary open-angle glaucoma. Am J Ophthalmol 75:668–673
44. Jap A, Sivakumar M, Chee SP (2001) Is Posner Schlossman syndrome benign? Ophthalmology 108:913–918
45. Chee SP, Bacsal K, Jap A, Se-Thoe SY, Cheng CL, Tan BH (2008) Clinical features of cytomegalovirus anterior uveitis in immunocompetent patients. Am J Ophthalmol 145:834–840
46. Dinakaran S, Kayarkar V (2002) Trabeculectomy in the management of Posner-Schlossman syndrome. Ophthalmic Surg Lasers 33:321–322
47. Nakazawa T, Kayama M, Ryu M et al (2011) Tumor necrosis factor-alpha mediates photoreceptor death in a rodent model of retinal detachment. Invest Ophthalmol Vis Sci 52:1384–1391

Chapter 21
Optic Neuritis

Xiaoli Guo

Abstract Optic neuritis (ON) is inflammation of the optic nerve and is the most common type of optic neuropathy. There is a strong association between ON and multiple sclerosis (MS), an acute inflammatory demyelinating syndrome of the central nervous system (CNS). A large number of etiological factors have been identified for ON including genetic susceptibility, smoking, exposure to the Epstein–Barr virus (EBV), and low exposure to sunlight. And the pathology of ON continues to be clarified with the advances in medicine and clinical peripheral devices. Since ON can cause severe visual loss, it draws much attention to detect the disease in early stage and finding a treatment that will restore visual function. In this chapter, the latest progress regarding the noninvasive detection methods for ON is summarized and current understanding of mechanisms underlying ON is reviewed, with references to MS. Lastly, existing drugs and chemicals that were developed for other therapeutic purposes are discussed as potential treatment strategies for ON.

Keywords ASK1 • EAE • Glial innate immunity • Multiple sclerosis • Optic neuritis

21.1 Introduction

Optic neuritis (ON) is inflammation of the optic nerve and is the most common type of optic neuropathy. Optic neuritis typically affects young adults ranging from 18 to 45 years of age, with a strong female predominance. The annual incidence is approximately 5/100,000, with a total prevalence estimated to be 115/100,000. Patients usually present with an acute reduction of visual acuity, orbital pain

X. Guo (✉)
Visual Research Project, Tokyo Metropolitan Institute of Medical Science,
2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan
e-mail: guo-xl@igakuken.or.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_21,

exacerbated by eye movements, dyschromatopsia, and an afferent papillary defect, with or without swelling of the optic nerve head. Substantial progress has been made recently in understanding the pathogenesis of ON and also in detection methods that allow better diagnosis and disease management. In this chapter, the first part reviews the current knowledge of ON followed by a summary of noninvasive detection methods, such as optical coherence tomography (OCT) and multifocal electroretinography (mfERG), and their use in monitoring ON. The last part discusses treatment strategies for ON using drugs and chemicals that are developed for treating other diseases, with the emphasis on modulation of glial innate immunity.

21.2 Clinical Features of Optic Neuritis

21.2.1 Etiology of Optic Neuritis and Multiple Sclerosis

A large number of etiological factors have been identified for ON including genetic susceptibility, smoking, exposure to the Epstein–Barr virus (EBV) [1], and low exposure to sunlight (presumed to be mediated through vitamin D deficiency) [2]. There is a strong association between ON and multiple sclerosis (MS), an acute inflammatory demyelinating syndrome of the central nervous system (CNS), in which ON is the initial presentation in approximately 20 % of MS cases and 30–70 % of MS patients develop ON during the course of their disease [3, 4]. It is reported that the cumulative probability of developing MS within 15 years after onset of ON is 50 % and strongly relates to the presence of lesions on a baseline non-contrast-enhanced magnetic resonance imaging (MRI) of the brain [5]. In this study, 25 % of patients with idiopathic ON and normal baseline brain MRI developed MS during follow-up compared with 72 % of patients with 1 or more lesions. After 10 years, the risk of developing MS was very low for patients without baseline lesions but remained substantial for those with lesions. Therefore, a close follow-up is recommended for ON patients who present factors associated with risk for MS. Early immunomodulatory treatment may be individually considered as it can delay progression into MS and reduce the rate of new lesion development [5, 6].

21.2.2 Clinical Examination and Treatment for Optic Neuritis

The clinical features of the patients specify the type of examination required. Generally complete ophthalmic, neurological, and systemic examinations should be performed for the diagnosis of ON [7, 8]. Among them, neurological examinations including orbital and brain MRI are performed with or without gadolinium

(Gd) preferably within 2 weeks after the onset of symptoms [9]. Systemic examinations including cerebrospinal fluid analysis consist of determination of total protein, albumin, IgG, IgA, IgM, glucose, lactate, cell count, microbiological/virological analysis, and oligoclonal bands. Current treatments of ON include intravenous steroid medications (cortisone medications such as prednisone [Deltasone, Orasone, Prednicen-M, Liquid Pred] and methylprednisolone [Solu-Medrol]) as shown in the Optic Neuritis Treatment Trial (ONTT) and immunomodulator or immunosuppressive therapy to reduce the incidence of future attacks [10]. Since ON can cause severe visual loss, it draws much attention to finding a treatment that will restore visual function.

21.2.3 Neuromyelitis Optica

Neuromyelitis optica (NMO), also known as Devic's disease, is an acute inflammatory demyelinating disease mainly involving the optic nerve and spinal cord. Optic neuritis in NMO and MS are nearly identical in their initial presentation. However, there are clear differences in the sites and pathology of lesions and in their clinical course and response to therapies. NMO primarily affects the optic nerve and spinal cord, causing blindness and paralysis, with relatively little brain pathology. The prevalence of NMO is ~2–4 per 100,000 individuals and ~6–8 times more prevalent in women than in men [11]. The incidence of NMO is greater in Asians and Blacks than in Caucasians. The median age of onset is 39 years, and 80–90 % of NMO patients have relapsing ON and myelitis rather than a monophasic course. Demyelinating NMO is more violent and devastating than MS; hence, its correct diagnosis is very important. In more than 85 % of patients with NMO, attack recurs in the form of ON, transverse myelitis (TM), or both, resulting in around 50 % of cases in paralysis or blindness within 5 years. In some cases, patients with TM in the cervical spine experience respiratory failure and even death. The majority of NMO patients are seropositive for autoantibodies (NMO-IgG) against extracellular epitope(s) on aquaporin-4 (AQP4) [12], a plasma membrane water channel expressed in astrocytes throughout the CNS [13]. Though AQP4-IgG was initially thought to be a serum marker of NMO, perhaps related to astrocyte damage, there is now strong evidence that AQP4-IgG is pathogenic in NMO. AQP4-IgG binding to AQP4 on astrocytes is thought to cause complement-dependent cytotoxicity, leading to leukocyte infiltration, cytokine release, and blood–brain barrier breakdown. These initial events lead to oligodendrocyte death, myelin loss and neuron death, and consequent clinical neurological deficit. NMO is particularly amenable to treatment by plasmapheresis, presumably due to removal of serum AQP4 antibodies [14]. Previous studies and case series have found that plasmapheresis is effective in suppressing acute attacks in 50–89 % of patients with NMO [15, 16]. Other NMO therapies include immunosuppression and B-cell depletion [17].

21.3 Noninvasive Detection Methods for Optic Neuritis

21.3.1 Optical Coherence Tomography

Optical coherence tomography (OCT) is originally developed for monitoring retinal diseases. The increased resolution of spectral domain OCT allows automatic identification of all retinal layers and measurement of the macular thickness and volume [18]. In addition, OCT allows direct visualization and measurement of the optic nerve head topography and of the retinal nerve fiber layer (RNFL) thickness with micron-scale resolution [19]. Quantification of the RNFL thickness by OCT provides an indirect measure of axonal and neuronal loss in the anterior visual pathways. It is particularly useful in glaucoma and ON, in which the anterior visual pathways are commonly affected. The RNFL thickness has been attracting much interest as a biomarker for optic neuropathies and for numerous neurological disorders such as MS [20]. The RNFL and macular thicknesses measured using OCT were significantly thinner in eyes of MS patients with or without previous history of ON compared to controls [21], and their degree of thinning was greater in NMO eyes [21, 22]. Since OCT is noninvasive, easy to perform, and highly reproducible, it can be used as a practical tool for visualizing axonal loss in ON patients and/or as an endpoint in clinical trials.

21.3.2 Multifocal Electroretinography

Conventional electroretinograms (ERGs) have been useful for assessing changes in retinal function. The ERG is noninvasive, allows repeated assessment, and can be performed in a relatively short period. Clinical ERGs recorded in response to a full-field flash provide a quantitative assessment of global retinal function but do not provide local response information. Focal ERGs permit assessment of localized retinal activity but require detailed procedures to minimize the effects of stray light and require considerable time and effort to obtain responses from multiple retinal regions [23, 24]. In 1991, Sutter et al. [25] developed a technique using nonlinear systems analysis to extract local responses from a continuous ERG. This approach, termed multifocal electroretinography (mfERG), permits simultaneous assessment of local responses from a large number of retinal areas. Because it can provide topographical analysis of retinal function in a relatively short period, this technique has already been used in a variety of clinical and preclinical studies of human vision [26, 27]. In brief, the mfERG technique involves the presentation of multiple local flashes in a predefined order (m-sequence) and subsequent extraction of the corresponding ERGs from the field potential by computation of the cross correlation between the stimulus m-sequence and the response cycle. While the first-order mfERG reflects the function of the outer retina, the second-order mfERG is a sensitive indicator of inner retinal dysfunction [28] that cannot be detected by

conventional ERG; it is the latter that makes it useful for ON. RGCs contribute to the human mfERG response, and the second-order kernel is impaired in glaucoma patients [29]. The mfERG technique was also developed to assess retinal function in the small-eyed animals such as rat and mice [30]. mfERGs can be recorded using a Visual-Evoked Response Imaging System (VERIS), which allows reliable assessment of visual function in animal models representing glaucoma [31] and ON [32].

21.4 Glial Innate Immunity in Optic Neuritis

21.4.1 Innate Immunity

The vertebrate immune response can be divided into innate and acquired immunity. Innate immunity is a multicomponent system. The first line of defense includes cellular barriers, such as the skin, the mucosal surfaces, and the gastrointestinal and respiratory tracts. The second line of defense is composed of the innate immune cells, mostly of myeloid origin, including dendritic cells, macrophages, monocytes in the periphery, and microglia cells in the CNS. Innate immunity also includes non-myeloid cells such as thymic epithelial cells in the periphery and astrocytes in the CNS. Compared with acquired immunity, far less attention has been directed toward innate immunity as it has been regarded as a relatively nonspecific system. However, recent studies have shown that the innate immune system has a greater degree of specificity than previously thought and that it is highly developed in its ability to discriminate between self and foreign pathogens [33]. This discrimination relies, to a great extent, on a family of evolutionarily conserved receptors, known as the Toll-like receptors (TLRs), which have a crucial role in early host defense against invading pathogens [33, 34].

The TLRs are type I integral membrane glycoproteins and they are expressed on microglia, astrocytes, neutrophils, dendritic cells, and macrophages in the CNS and peripheral nervous system. So far, 11 members of the TLR family have been identified in mammals. Upon activation, TLRs trigger a cascade of intracellular signaling pathways, leading to the induction of inflammatory and innate immune responses, which in turn regulate adaptive immune responses [35]. Increasing evidence has supported the idea that TLRs also recognize endogenous molecules that are released from damaged tissues, thereby regulating inflammatory responses and subsequent tissue repair. These findings imply that TLRs on glial cells may also be involved in the inflammatory response to tissue damage in the nervous system.

21.4.2 Function of Glial Cells in Optic Neuritis

21.4.2.1 Inhibition of Glial Cell Activation Ameliorates Neuroinflammation

Recent studies have shown the importance of the local environment, especially the activation of glial cells during neuroinflammation [36, 37]. Astrocytes are the most abundant cells in the CNS, accounting for nearly half of the brain's cells in humans and approximately one-third in mice. Astrocytes, as part of the innate immune system, express a large array of TLRs, and its activation has been associated with human demyelinating disorders such as MS [38], NMO [39, 40], and animal models (including experimental autoimmune encephalomyelitis (EAE) [41, 42], and cuprizone-induced demyelination [43]). Astrocytes secrete chemokines that attract both peripheral immune cells to the inflamed CNS (e.g., T cells, monocytes, and dendritic cells) and resident CNS cells (microglia, NG2) to the lesions sites. The glial scar, a physical barrier found around demyelinated lesions in MS and EAE, is primarily composed of interwoven astrocytic processes held together by tight junctions [44]. Furthermore, astrocytes express factors, such as proteoglycan, that inhibit the maturation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes and even prevent axonal growth by different ways [45]. On the other hand, a predominant group of inflammatory cells are highly activated phagocytic macrophages and microglia. The chronic presence of activated macrophage/microglia is likely undesirable for several reasons. First, a strong correlation is observed between macrophage/microglia activity and both acute axonal injury [46] and loss of oligodendrocytes [47]. Second, medium conditioned by microglia kills oligodendrocytes in culture [48]; in MS lesions, microglia in the process of stripping myelin can be found [49]. Third, there is persistent activation of microglia in relapsing–remitting EAE even after CD4+ number wanes [50]. Finally, reducing macrophage/microglia activity with clodronate liposomes or genetic deletion alleviates disease activity in EAE [51].

Some efforts have been made to modulate the activity of glial cells using pharmacological tools, which successfully ameliorated ON in the animal model of MS. MW01-5-188WH is an orally bioavailable and brain-penetrating small molecule compound that prevents glial cell activation [52], which significantly reduces the clinical score of myelin oligodendrocyte glycoprotein (MOG)-induced EAE mice [53]. Recently, the phosphodiesterase inhibitor dipyridamole has been shown to reduce clinical and histological outcomes of EAE by inhibition of microglia activity [54]. Current ON and MS therapies do not target microglial activity to any significant extent. However, one candidate drug, minocycline, which has microglia-inactivating and other immunomodulatory properties [55], is currently in a phase III trial for early MS [56]. Although further studies are required, glial cells might be a promising therapeutic strategy for the treatment of ON.

21.4.2.2 TLR-ASK1-p38 Pathway in Glial Cells Regulates the Severity of Optic Neuritis

Advances in elucidating the downstream pathways of TLRs identified apoptosis signal-regulating kinase 1 (ASK1), which is one of the members of mitogen-activated protein kinase (MAPK) kinase kinase that plays a role in the c-Jun N-terminal kinase (JNK) and p38 MAPK pathways [57]. ASK1 is activated by various cytotoxic stressors as well as receptor-mediated inflammatory signals, such as lipopolysaccharide (LPS) and tumor necrosis factor (TNF), and mediates diverse biological signals leading to cell death, differentiation, and senescence [58]. ASK1 is an evolutionarily conserved signaling intermediate for innate immunity [59]. In mammals, TLRs activate p38, JNK, and NF-κB cascades, leading to the induction of many key cytokine genes [60]. Among them, ASK1 specifically mediates LPS-induced TLR4 signaling to p38 through a reactive oxygen species (ROS)-dependent pathway in dendritic cells and splenocytes [59]. This finding provided a unique link between cellular stress responses and innate immunity. On the other hand, several lines of evidence suggest that ASK1 plays key roles in human diseases that are closely related to dysfunction of cellular responses to oxidative stress and endoplasmic reticulum (ER) stressors [61].

During ON, TLR expression is upregulated in activated astrocytes and microglia, and TLR signaling can synergize with ASK1-p38 signaling in the release of key chemokines (MCP-1, MIP-1α, RANTES, IP-10, etc.) from astrocytes to cause autoimmune inflammation. Genetic deletion or pharmacological inhibition of ASK1 attenuates ON and improved visual impairment detected by mfERG [32] (Fig. 21.1). In addition, TLR-ASK1-p38 signaling in microglial cells seems to modulate the progress of demyelination by altering the release of proinflammatory components such as TNF-α and iNOS, indicating that the TLR-ASK1-p38 pathway in glial cells might be a new therapeutic strategy for ON.

21.5 Therapy with Existing Drugs or Chemicals

Currently, the number of drugs that effectively mitigate MS is increasing. Although these drugs are also effective in ON, they show limited efficacy. While they can ameliorate the early inflammatory phase of demyelinating processes in MS, they do not inhibit glial cell activation, protect neurons, or restore myelin [62]. On the other hand, developing a brand-new drug takes an enormous amount of time, money, and effort, mainly because of bottlenecks in the therapeutic development process. Delays and barriers can mean that translation of a promising molecule into an approved drug often takes more than 13 years [63]. It is crucial to advance strategies to reduce this time frame, decrease costs, and improve success rates. Drug rescue and repurposing are two such strategies. “Drug rescue” refers to research using small molecules and biologics that were previously used in studies

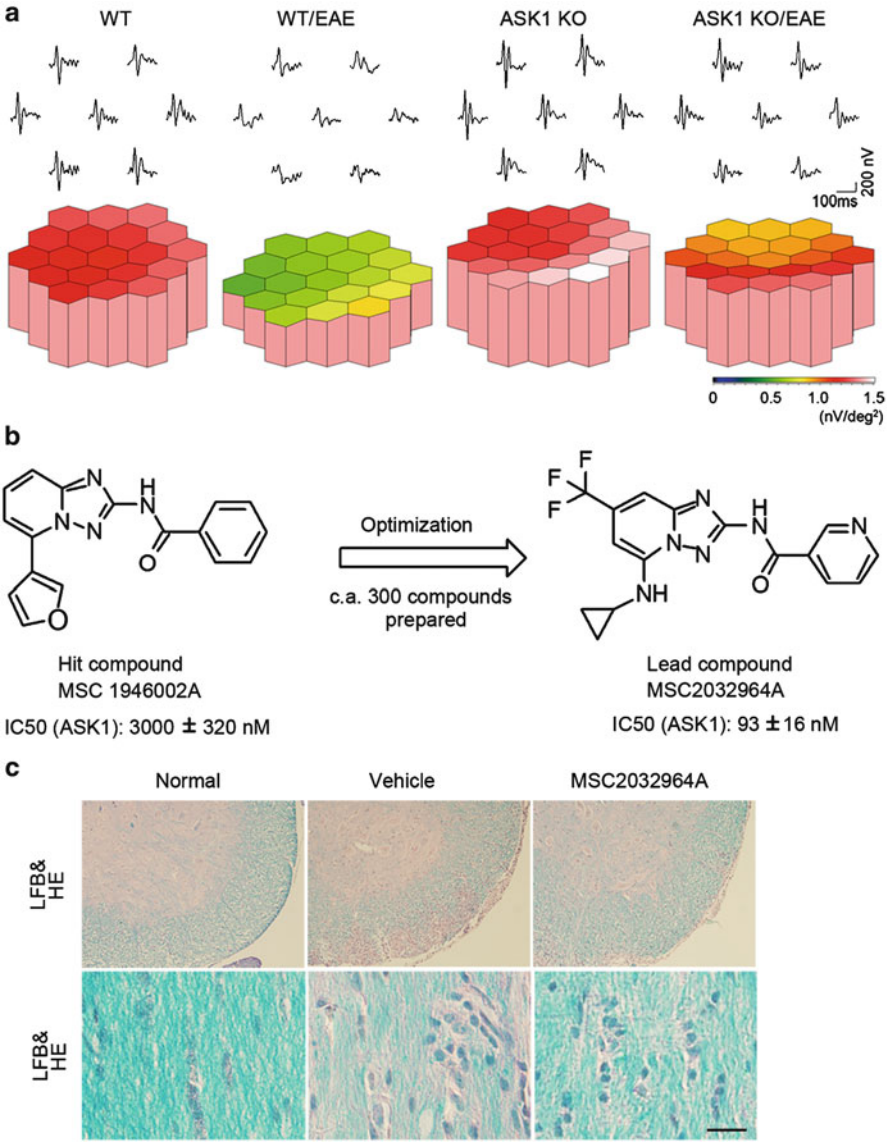


Fig. 21.1 Genetic deletion or pharmacological inhibition of ASK1 attenuates optic neuritis and improves visual impairment. (**a**) Multifocal electroretinogram evaluation of visual function in EAE mice. *Upper panels*: visual responses recorded from seven different areas in the retina. *Lower panels*: the amplitude variation across the arrays presented in three-dimensional plots. The higher the amplitude, the better the visual function. (**b**) Optimization of ASK1 inhibitors. Medicinal chemistry optimization led to the identification of the potent ASK1 inhibitor MSC2032964A. (**c**) Representative histology of the spinal cords (*upper panels*) and optic nerves (*lower panels*) of EAE mice treated with vehicle or MSC2032964A. Luxol fast blue (LFB) and hematoxylin and eosin (HE) staining revealed a protective effect of MSC2032964A on myelin sheath. Modified from Guo et al. [32]

but not further developed. “Drug repurposing” generally refers to reexamination of small molecules or biologics that are approved to treat one disease or condition to see if it is safe and effective for treating other diseases. In recent years, an increasing number of pharmaceutical development programs have been focusing on the development of new therapeutics based on existing agents. In this section, some efforts on “drug repurposing” for ON are summarized.

21.5.1 Neuroprotective Agents and Antioxidants

The role of ROS, such as superoxide and H_2O_2, in the pathogenesis of ON and MS has been emerging [64]. Several antioxidants such as tempamine and thymoquinone have been shown to reduce the severity of EAE [65, 66], and suppression of mitochondrial oxidative stress by SOD2 gene delivery provided long-term neuroprotection in experimental ON [67].

Geranylgeranylacetone (GGA) is an acyclic isoprenoid compound and has been used clinically for the treatment of gastric ulcers with an extremely low toxicity. GGA rapidly upregulates the expression of heat shock proteins in variety of tissues [68] and alleviates polyglutamine-mediated motor neuron disease [69]. Since GGA has been shown to exert a protective effect on retinal ganglion cells [70], this drug may be available for neuroprotection in ON.

Spermidine is a naturally occurring polyamine essential for life [71]. Increased synthesis of spermidine promoted optic nerve regeneration in vivo [72]. Moreover, spermidine was reported to play key roles in mediating protection against oxidative damage caused by H_2O_2 in cultured mouse fibroblasts [73]. Eisenberg et al. [74] demonstrated that administration of spermidine extended the lifespan of yeast, flies, worms, and human immune cells by upregulating the lysosomal/vacuolar degradation pathway, referred to as autophagy, which leads to enhanced resistance to oxidative stress and decreased cell death.

Studies on EAE mice demonstrated that GGA or spermidine treatment prevented neural cell death in the ganglion cell layer and reduced the extents of demyelination in the optic nerve, which resulted in significant improvement of visual function [75, 76]. Moreover, spermidine administration attenuated clinical scores of EAE mice and reduced the extents of demyelination in the spinal cords through its antioxidative effect (Fig. 21.2). Heat shock protein 72 is a negative regulator of ASK1, which is activated in response to various stimuli including ROSs [77], and GGA induces this stress protein in various retinal cell types. These results suggest that antioxidants in combination with inhibitors of ROSs’ downstream signals, such as ASK1 inhibitors, might prove effective for the treatment of ON.

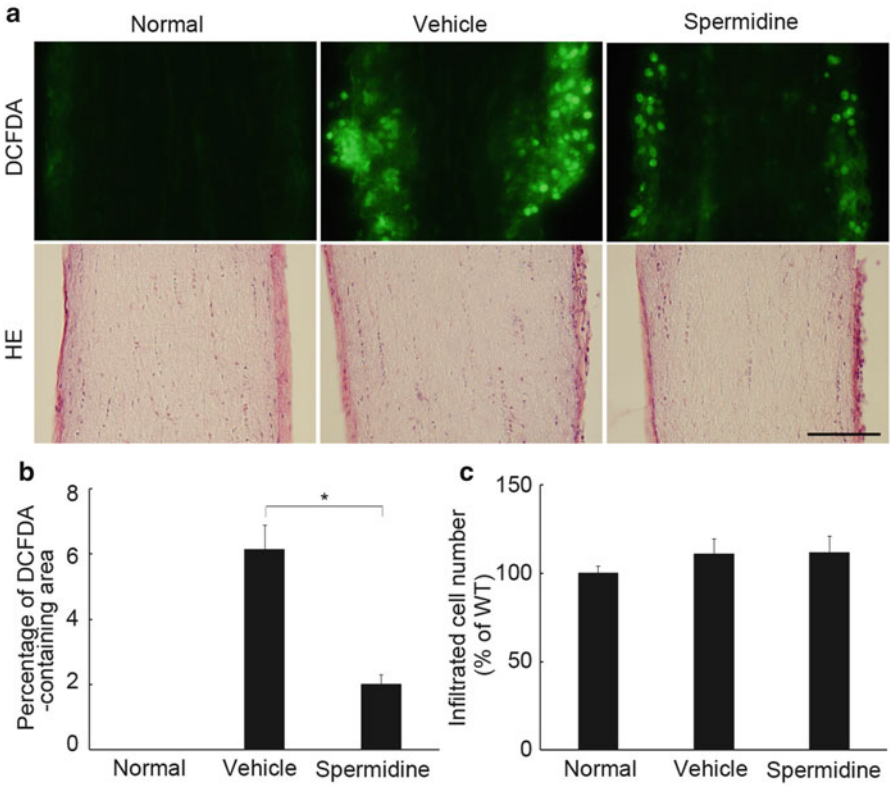


Fig. 21.2 Anti-oxidative effects of spermidine on EAE mice. (**a**) Hydrogen peroxide detection in the optic nerve using the probe 2′–7′ dichlorofluorescein diacetate (DCFDA). (**b**) Quantification of DCFDA-stained areas. (**c**) Quantitative analysis of cell infiltrates detected by HE staining. Since the extent of inflammation was not ameliorated, antioxidant effects of spermidine may be more dominant than its antiinflammatory effects. Modified from Guo et al. [76]

21.5.2 Myelin Repair

Remission in MS is largely dependent on migration of OPCs to sites of injury and subsequent differentiation to mature cells capable of repair [78, 79]. Studies evaluating the presence and relative densities of OPCs at sites of chronically demyelinated lesions in MS indicate that it is not a failure of repopulation or migration of OPCs but rather inhibition of OPC differentiation at sites of injury that contributes to disease progression [80]. Hence, the identification of small molecules that selectively induce differentiation of OPCs at sites of demyelinated lesions and thereby enhance remyelination would have a considerable impact on the development of new effective treatments for MS [81]. Through a three-stage screening, Deshmukh et al. [82] identified the drug benztropine that may finally raise the hope of myelin repair. Benztropine is well established as an approved

treatment for Parkinson's disease [83] and readily crosses the blood–brain barrier. Benztropine was reevaluated as it was found to particularly enhance myelin basic protein (MBP) production in searching process for agents that would induce differentiation of oligodendrocytes. Moreover, benztropine enhanced axon myelination when added to a co-culture of oligodendrocyte progenitors and neurons and promoted remyelination in two animal models of demyelination: EAE (immune-mediated) and cuprizone-induced demyelination (chemically induced) models. Another potential target may be Olig1, a basic helix–loop–helix (bHLH) transcription factor expressed in mature oligodendrocytes and their progenitor cells in the developing CNS. Targeted disruption of Olig1 indicated that Olig1 plays important roles during development and maturation of oligodendrocytes [84]. Following induction of EAE, mice that lack Olig1 expression showed reduced number of degenerating axons in the optic nerve and their visual function was better preserved [85], suggesting that interrupting Olig1 signaling pathways with small molecules may provide a novel therapeutic approach for ON.

21.6 Conclusion

Optic neuritis is the initial presentation in approximately 20 % of MS cases, which makes early detection of the disease important. Progresses in noninvasive detection methods will not only facilitate diagnosis of the disease, but also advance the evaluation of therapies. Moreover, research into glial innate immunity regulation, neuroprotection agents, and myelin restoration might open the door to new avenues for the treatment of ON.

References

1. Handel AE, Williamson AJ, Disanto G et al (2010) An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. PLoS One. doi:10.1371/journal.pone.0012496
2. Islam T, Gauderman WJ, Cozen W et al (2007) Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. Neurology 69:381–388
3. Miller D, Barkhof F, Montalban X et al (2005) Clinically isolated syndromes suggestive of multiple sclerosis, part I: natural history, pathogenesis, diagnosis, and prognosis. Lancet Neurol 4:281–288
4. Frohman EM, Fujimoto JG, Frohman TC et al (2008) Optical coherence tomography: a window into the mechanisms of multiple sclerosis. Nat Clin Pract Neurol 4:664–675
5. Optic Neuritis Study Group (2008) Multiple sclerosis risk after optic neuritis: final optic neuritis treatment trial follow-up. Arch Neurol 65:727–732. doi:10.1001/archneur.65.6.727
6. Marques IB, Matias F, Silva ED et al (2013) Risk of multiple sclerosis after optic neuritis in patients with normal baseline brain MRI. J Clin Neurosci. doi:10.1016/j.jocn.2013.06.013

7. Mehrotra A, Jaffery N, Saxena R et al (2007) Comparative evaluation of megadose methylprednisolone with dexamethasone for treatment of primary typical optic neuritis. Indian J Ophthalmol 55:355–359
8. Menon V, Saxena R, Misra R et al (2011) Management of optic neuritis. Indian J Ophthalmol 59:117–122. doi:10.4103/0301-4738.77020
9. Balcer LJ (2006) Clinical practice. Optic neuritis. N Engl J Med 354:1273–1280
10. Tullman MJ, Lublin FD, Miller AE (2002) Immunotherapy of multiple sclerosis-current practice and future directions. J Rehabil Res Dev 39:273–285
11. Wingerchuk DM, Lennon VA, Lucchinetti CF et al (2007) The spectrum of neuromyelitis optica. Lancet Neurol 6:805–815
12. Lennon VA, Kryzer TJ, Pittock SJ et al (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 202:473–477
13. Nielsen S, Nagelhus EA, Amiry-Moghaddam M et al (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 17:171–180
14. Ratelade J, Zhang H, Saadoun S et al (2012) Neuromyelitis optica IgG and natural killer cells produce NMO lesions in mice without myelin loss. Acta Neuropathol 123:861–872. doi:10.1007/s00401-012-0986-4
15. Watanabe S, Nakashima I, Misu T et al (2007) Therapeutic efficacy of plasma exchange in NMO-IgG-positive patients with neuromyelitis optica. Mult Scler 13:128–132
16. Wang KC, Wang SJ, Lee CL et al (2011) The rescue effect of plasma exchange for neuromyelitis optica. J Clin Neurosci 18:43–46. doi:10.1016/j.jocn.2010.05.030
17. Cree BA, Lamb S, Morgan K et al (2005) An open label study of the effects of rituximab in neuromyelitis optica. Neurology 64:1270–1272
18. Garvin MK, Abràmoff MD, Wu X et al (2009) Automated 3-D intraretinal layer segmentation of macular spectral-domain optical coherence tomography images. IEEE Trans Med Imaging 28:1436–1447. doi:10.1109/TMI.2009.2016958
19. Sakata LM, Deleon-Ortega J, Sakata V et al (2009) Optical coherence tomography of the retina and optic nerve – a review. Clin Experiment Ophthalmol 37:90–99. doi:10.1111/j.1442-9071.2009.02015.x
20. Frohman EM, Fujimoto JG, Frohman TC et al (2008) Optical coherence tomography: a window into the mechanisms of multiple sclerosis. Nat Clin Pract Neurol 4:664–675. doi:10.1038/ncpneuro0950
21. Monteiro ML, Fernandes DB, Apóstolos-Pereira SL et al (2012) Quantification of retinal neural loss in patients with neuromyelitis optica and multiple sclerosis with or without optic neuritis using Fourier-domain optical coherence tomography. Invest Ophthalmol Vis Sci 53:3959–3966. doi:10.1167/iovs.11-9324
22. Schneider E, Zimmermann H, Oberwahrenbrock T et al (2013) Optical coherence tomography reveals distinct patterns of retinal damage in neuromyelitis optica and multiple sclerosis. PLoS One 8:e66151
23. Sandberg MA, Ariel M (1977) A hand-held two channel stimulator ophthalmoscope. Arch Ophthalmol 95:1881–1882
24. Jones R, King–Smith PE, Loffing DH et al (1986) Stray light contributions to the focal electroretinogram (ERG). Clin Vis Sci 1:153–160
25. Sutter EE (1991) The fast m-transform: a fast computation of cross-correlations with binary m-sequences. Society Ind Appl Math 20:686–694
26. Sutter EE, Tran D (1992) The field topography of ERG components in man, I: the photopic luminance response. Vision Res 32:433–446
27. Hood DC, Seiple W, Holopigian K et al (1997) A comparison of the components of the multifocal and full-field ERGs. Vis Neurosci 14:533–544
28. Hasegawa S, Ohshima A, Hayakawa Y et al (2001) Multifocal electroretinograms in patients with branch retinal artery occlusion. Invest Ophthalmol Vis Sci 42:298–304

29. Sutter EE, Bearse MA Jr (1999) The optic nerve head component of the human ERG. Vision Res 39:419–436
30. Ball SL, Petry HM (2000) Noninvasive assessment of retinal function in rats using multifocal electroretinography. Invest Ophthalmol Vis Sci 41:610–617
31. Harada T, Harada C, Nakamura K et al (2007) The potential role of glutamate transporters in the pathogenesis of normal tension glaucoma. J Clin Invest 117:1763–1770
32. Guo X, Harada C, Namekata K et al (2010) Regulation of the severity of neuroinflammation and demyelination by TLR-ASK1-p38 pathway. EMBO Mol Med 2:504–515. doi:10.1002/emmm.201000103
33. Janeway CA Jr, Medzhitov R (2002) Innate immune recognition. Annu Rev Immunol 20:197–216
34. Beutler B, Rietschel ET (2003) Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol 3:169–176
35. Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2:675–680
36. Ponomarev ED, Shriver LP, Maresz K et al (2005) Microglial cell activation and proliferation precedes the onset of CNS autoimmunity. J Neurosci Res 81:374–389
37. Tanuma N, Sakuma H, Sasaki A et al (2006) Chemokine expression by astrocytes plays a role in microglia/macrophage activation and subsequent neurodegeneration in secondary progressive multiple sclerosis. Acta Neuropathol 112:195–204
38. Nair A, Frederick TJ, Miller SD (2008) Astrocytes in multiple sclerosis: a product of their environment. Cell Mol Life Sci 65:2702–2720. doi:10.1007/s00018-008-8059-5
39. Fujihara K (2011) Neuromyelitis optica and astrocytic damage in its pathogenesis. J Neurol Sci 306:183–187. doi:10.1016/j.jns.2011.02.018
40. Hinson SR, Romero MF, Popescu BF et al (2012) Molecular outcomes of neuromyelitis optica (NMO)-IgG binding to aquaporin-4 in astrocytes. Proc Natl Acad Sci U S A 109:1245–1250. doi:10.1073/pnas.1109980108
41. Farez MF, Quintana FJ, Gandhi R et al (2009) Toll-like receptor 2 and poly(ADP-ribose) polymerase 1 promote central nervous system neuroinflammation in progressive EAE. Nat Immunol 10:958–964. doi:10.1038/ni.1775
42. Kang Z, Altuntas CZ, Gulen MF et al (2010) Astrocyte-restricted ablation of interleukin-17-induced Act1-mediated signaling ameliorates autoimmune encephalomyelitis. Immunity 32:414–425. doi:10.1016/j.immuni.2010.03.004
43. Skripuletz T, Bussmann JH, Gudi V et al (2010) Cerebellar cortical demyelination in the murine cuprizone model. Brain Pathol 20:301–312. doi:10.1111/j.1750-3639.2009.00271.x
44. Goritz C, Dias DO, Tomilin N et al (2011) A pericyte origin of spinal cord scar tissue. Science 333:238–242. doi:10.1126/science.1203165
45. Silver J, Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci 5:146–156
46. Kuhlmann T, Lingfeld G, Bitsch A et al (2002) Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 125:2202–2212
47. Lucchinetti C, Brück W, Parisi J et al (1999) A quantitative analysis of oligodendrocytes in multiple sclerosis lesions: a study of 113 cases. Brain 122:2279–2295
48. Merrill JE, Zimmerman RP (1991) Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TGFβ. Glia 4:327–331
49. Peterson JW, Bö L, Mörk S et al (2002) VCAM-1-positive microglia target oligodendrocytes at the border of multiple sclerosis lesions. J Neuropathol Exp Neurol 61:539–546
50. Rasmussen S, Wang Y, Kivisäkk P et al (2007) Persistent activation of microglia is associated with neuronal dysfunction of callosal projecting pathways and multiple sclerosis-like lesions in relapsing–remitting experimental autoimmune encephalomyelitis. Brain 130:2816–2829
51. Heppner FL, Greter M, Marino D et al (2005) Experimental autoimmune encephalomyelitis repressed by microglial paralysis. Nat Med 11:146–152

52. Ralay Ranaivo H, Craft JM, Hu W et al (2006) Glia as a therapeutic target: selective suppression of human amyloid-β-induced upregulation of brain proinflammatory cytokine production attenuates neurodegeneration. J Neurosci 26:662–670
53. Guo X, Nakamura K, Kohyama K et al (2007) Inhibition of glial cell activation ameliorates the severity of experimental autoimmune encephalomyelitis. Neurosci Res 59:457–466
54. Sloka S, Metz LM, Hader W et al (2013) Reduction of microglial activity in a model of multiple sclerosis by dipyridamole. J Neuroinflammation 10:89. doi:10.1186/1742-2094-10-89
55. Yong VW, Wells J, Giuliani F et al (2004) The promise of minocycline in neurology. Lancet Neurol 3:744–751
56. Metz LM, Li D, Traboulsee A, GA/Minocycline Study Investigators et al (2009) Glatiramer acetate in combination with minocycline in patients with relapsing–remitting multiple sclerosis: results of a Canadian, multicenter, double-blind, placebo-controlled trial. Mult Scler 15:1183–1194. doi:10.1177/1352458509106779
57. Ichijo H, Nishida E, Irie K et al (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science 275:90–94
58. Chiang E, Dang O, Anderson K et al (2006) Cutting edge: apoptosis-regulating signal kinase 1 is required for reactive oxygen species-mediated activation of IFN regulatory factor 3 by lipopolysaccharide. J Immunol 176:5720–5724
59. Matsuzawa A, Saegusa K, Noguchi T et al (2005) ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity. Nat Immunol 6:587–592
60. Akira S, Takeda K (2004) Toll-like receptor signalling. Nat Rev Immunol 4:499–511
61. Harada C, Nakamura K, Namekata K et al (2006) Role of apoptosis signal-regulating kinase 1 in stress-induced neural cell apoptosis in vivo. Am J Pathol 168:261–269
62. Steinman L (2004) Immune therapy for autoimmune diseases. Science 305:212–216
63. Paul SM, Mytelka DS, Dunwiddie CT et al (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov 9:203–214. doi:10.1038/nrd3078
64. Gilgun-Sherki Y, Melamed E, Offen D (2004) The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. J Neurol 251:261–268
65. Kizelsztein P, Ovadia H, Garbuzenko O et al (2009) Pegylated nanoliposomes remote-loaded with the antioxidant tempamine ameliorate experimental autoimmune encephalomyelitis. J Neuroimmunol 213:20–25. doi:10.1016/j.jneuroim.2009.05.019
66. Mohamed A, Waris HM, Ramadan H et al (2009) Amelioration of chronic relapsing experimental autoimmune encephalomyelitis (cr-eae) using thymoquinone. Biomed Sci Instrum 45:274–279
67. Qi X, Lewin AS, Sun L et al (2007) Suppression of mitochondrial oxidative stress provides long-term neuroprotection in experimental optic neuritis. Invest Ophthalmol Vis Sci 48:681–691
68. Ooie T, Takahashi N, Saikawa T et al (2001) Single oral dose of geranylgeranylacetone induces heatshock protein 72 and renders protection against ischemia/reperfusion injury in rat heart. Circulation 104:1837–1843
69. Katsuno M, Sang C, Adachi H et al (2005) Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci USA 102:16801–16806
70. Harada C, Nakamura K, Guo X et al (2007) Neuroprotective effect of geranylgeranylacetone against ischemia-induced retinal injury. Mol Vis 13:1601–1607
71. Igarashi K, Kashiwagi K (2010) Modulation of cellular function by polyamines. Int J Biochem Cell Biol 42:39–51. doi:10.1016/j.biocel.2009.07.009
72. Deng K, He H, Qiu J et al (2009) Increased synthesis of spermidine as a result of upregulation of arginase I promotes axonal regeneration in culture and in vivo. J Neurosci 29:9545–9552. doi:10.1523/JNEUROSCI.1175-09.2009

73. Rider JE, Hacker A, Mackintosh CA et al (2007) Spermine and spermidine mediate protection against oxidative damage caused by hydrogen peroxide. Amino Acids 33:231–240
74. Eisenberg T, Knauer H, Schauer A et al (2009) Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 11:1305–1314. doi:10.1038/ncb233
75. Guo X, Harada C, Namekata K et al (2009) Effect of geranylgeranylacetone on optic neuritis in experimental autoimmune encephalomyelitis. Neurosci Lett 462:281–285. doi:10.1016/j.neulet.2009.07.028
76. Guo X, Harada C, Namekata K et al (2011) Spermidine alleviates severity of murine experimental autoimmune encephalomyelitis. Invest Ophthalmol Vis Sci 52:2696–2703. doi:10.1167/iovs.10-6015
77. Park HS, Cho SG, Kim CK et al (2002) Heat shock protein hsp72 is a negative regulator of apoptosis signal-regulating kinase 1. Mol Cell Biol 22:7721–7730
78. Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9:839–855. doi:10.1038/nrn2480
79. Kremer D, Aktas O, Hartung HP et al (2011) The complex world of oligodendroglial differentiation inhibitors. Ann Neurol 69:602–618. doi:10.1002/ana.22415
80. Chang A, Tourtellotte WW, Rudick R et al (2002) Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 346:165–173
81. Kuhlmann T, Miron V, Cui Q et al (2008) Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 131:1749–1758. doi:10.1093/brain/awn096
82. Deshmukh VA, Tardif V, Lyssiotis CA et al (2013) A regenerative approach to the treatment of multiple sclerosis. Nature 502:327–332. doi:10.1038/nature12647
83. Burgyone K, Aduri K, Ananth J et al (2004) The use of antiparkinsonian agents in the management of drug-induced extrapyramidal symptoms. Curr Pharm Des 10:2239–2248
84. Arnett HA, Fancy SP, Alberta JA et al (2004) bHLH transcription factor Olig1 is required to repair demyelinated lesions in the CNS. Science 306:2111–2115
85. Guo X, Harada C, Namekata K et al (2010) Delayed onset of experimental autoimmune encephalomyelitis in Olig1 deficient mice. PLoS One 9:e13083. doi:10.1371/journal.pone.0013083

Chapter 22
Neuroprotection by Endothelial Progenitor Cells for Retinal Degeneration

Shinichi Fukuda

Abstract Retinitis pigmentosa is an inherited eye disorder that leads to profound vision loss and is characterized by retinal neuron degeneration, fundus pigment changes, optic disc atrophy, and vasculature attenuation. Endothelial progenitor cells (EPCs), which contribute to angiogenesis, are categorized into two subpopulations according to their aldehyde dehydrogenase (ALDH) activity. Injection of EPCs with low ALDH activity (Alde-Low) revealed a greater ability for neuroprotection and vasoprotection compared with injection of EPCs with high ALDH activity in a mouse model of retinitis pigmentosa. However, EPCs do not possess direct neuroprotective or vasoprotective ability and must recruit other neuroprotective and vasoprotective cells. Alde-Low EPCs selectively recruited neuroprotective and vasoprotective F4/80+/Ly6c+ monocyte-derived macrophages from bone marrow through CCL2 secretion. F4/80+/Ly6c+ monocyte-derived macrophages from bone marrow were immature and revealed characteristics that differed from those of retinal residual microglia. CCR2 (the receptor for CCL2), neurotrophic factors, and anti-inflammatory mediators were highly expressed in migrated F4/80+/Ly6c+ monocyte-derived macrophages when compared with retinal residual microglia. These new findings suggest that novel therapies using EPCs may delay the progression of retinal degeneration.

Keywords Aldehyde dehydrogenase activity • Endothelial progenitor cells • Macrophage • Retinal degeneration

S. Fukuda (✉)
Department of Ophthalmology, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575, Japan
e-mail: caesar.shihtzu@gmail.com

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_22, © Springer Japan 2014

22.1 Cell Therapy for Retinal Degeneration

22.1.1 Retinal Degeneration

Retinitis pigmentosa is a group of inherited eye disorders that lead to night blindness during adolescence, peripheral visual field loss during young adulthood, and central vision impairment during later life that typically progresses to complete blindness [1]. Moreover, arteriolar attenuation, fundus pigment alteration, and optic disc atrophy are characteristic of retinitis pigmentosa. In the animal model of retinitis pigmentosa, there are similar characteristics with human such as degeneration of outer nuclear layer, vascular attenuation, and fundus pigmentation (Fig. 22.1).

Visual symptoms of retinitis pigmentosa result from the gradual loss of rod and cone cells, the two photoreceptor cell types of the retina. Rod cells mediate achromatic vision and are almost entirely responsible for night vision. Cone cells are responsible for color vision and thus function best in daylight. In patients with retinitis pigmentosa, the outer nuclear layer of the retina is degenerated, which is where most rod and cone cells are present. Retinal function measured with an electroretinogram generally shows decreased rod and cone response amplitudes, along with delays in photoreceptor cell activity several years before symptoms of night blindness, visual field scotomas, or decreased visual acuity appear. The inner nuclear layer of the retina comprises amacrine, bipolar, and horizontal cell neurons. The ganglion cell layer is fairly well preserved in retinitis pigmentosa; however, many of these cells also degenerate during the later stages of the disease.

Over 100 genes responsible for retinitis pigmentosa have been identified, most of which encode photoreceptor-specific proteins [2]. The incidence of retinitis

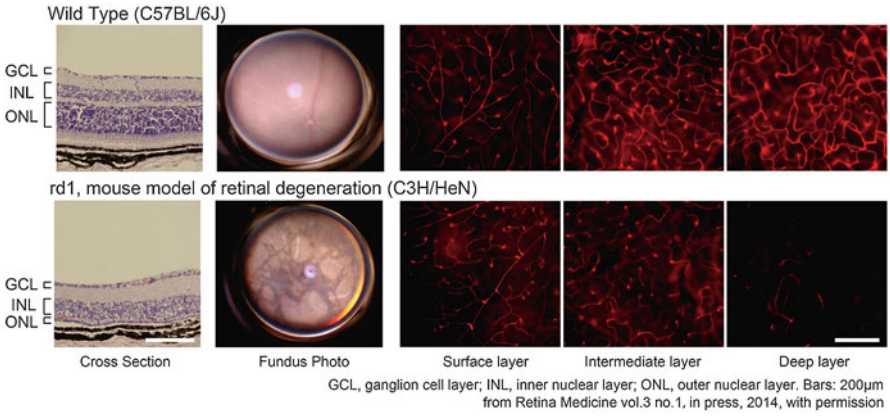


Fig. 22.1 Retinal neural cell, fundus photograph, and vasculature changes in WT mice (C57BL/6, *top panels*) or rd1 mice (C3H/HeN, *bottom panels*)

pigmentosa is one in 3,500; approximately 30–40 % cases are autosomal-dominant, 50–60 % are autosomal-recessive, and 5–15 % are attributed to an X-linked trait.

At present, no cure exists for retinitis pigmentosa. Studies have demonstrated that patients taking vitamins A and E can show slower declines in electroretinogram amplitudes compared with those not taking such supplements; however, these reductions have been small in magnitude. Another nutritional treatment for retinitis pigmentosa patients includes docosahexaenoic acid (DHA). DHA is an omega-3 fatty acid that is present in high concentrations in oily fishes such as salmon, tuna, and mackerel. In addition, photoreceptor cell membranes that contain rhodopsin and cone opsins contain very high concentrations of DHA; therefore, DHA appears to be important for photoreceptor cell function. Despite widespread efforts to better understand and treat retinitis pigmentosa, no existing treatment can effectively slow or reverse the progression of this disease [2, 3]. Moreover, mechanisms underlying photoreceptor cell apoptosis remain unclear. Gene therapy [3, 4] and neurotrophic factor therapies [5, 6] have been explored in recent years, and a wide range of cell populations that include retinal and neural progenitor cells, retinal cells from young postnatal mice, embryonic stem (ES) cells, mesenchymal stromal cells, and hematopoietic stem cells have been used in intravitreal injection or subretinal transplantation experiments [7–12].

22.1.2 ES Cell and Induced Pluripotent Stem (iPS) Cell Therapy

ES cells originate from the inner cell mass of blastocyst stage embryos. They can remain in an undifferentiated state for indefinite time but retain their ability to differentiate into derivatives of all three germ layers [13]. ES cells have been investigated to treat several degenerative diseases, including retinitis pigmentosa; however, the use of human ES cells in clinical applications faces ethical concerns because their use involves human embryos. Adult somatic cells can be reprogrammed by forcing expression of a defined set of transcription factors and returned to the pluripotency state present in the early embryo. By expressing just the three transcription factors Oct4, Sox2, and Klf4, somatic cells can be reprogrammed into iPS cells [14]. iPS cells are functionally equal to ES cells because they express ES cell markers. Properties of ES and iPS cells make them an attractive donor source for cell therapies in tissues damaged by disease or injury [15]. The replacement of damaged cells with transplanted donor cells represents a hopeful approach for regeneration therapy. It was recently been reported that human ES and iPS cells can differentiate into retinal progenitors, retinal pigment epithelium cells, and photoreceptors [16]. Therefore, retinal degeneration may be managed with transplantation of photoreceptor precursors or ES cell-derived progenitors that can form synaptic connections to the host retina and thereby improve visual function [8]. It takes quite a long time to realize organ transplantation from ES cells or iPS cells, so it needs to consider delay progression of disease.

22.1.3 Mesenchymal Stem Cell (MSC) Therapy

Another new approach for targeting degenerative diseases has been developed using stem cells that deliver therapeutic genes and their products. The neuroprotective ability of neural stem cells derived from ES cells has been well studied and clinically applied [10]. However, the isolation and use of neural stem cells are limited by ethical concerns. MSCs have been proposed as an alternative source for therapeutic stem cells. MSCs are ideal for therapeutic applications because they are self-renewing cells that can differentiate into osteoblasts, chondroblasts, fibroblasts, or adipocytes when exposed to appropriate cell culture conditions [17]. Moreover, MSCs can differentiate into nonmesenchymal cells (i.e., neurons), endothelial cells, skin cells, or hepatocytes. Previous studies have reported that MSCs may be therapeutic in bone and cardiovascular repair, lung fibrosis treatment, and spinal cord injury [18, 19]. Furthermore, MSCs secrete neurotrophic factors that promote neuron survival, including brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), and nerve growth factor (NGF) [20]. The conditioned medium of bone marrow-derived MSC cultures promotes proliferation of photoreceptor cells cultured in vitro and inhibits cell death, which is presumably mediated by neurotrophic factors secreted by MSCs. Bone marrow-derived MSC transplantation in vivo can slow retinal cell degeneration in RCS rats, which has been histologically and functionally demonstrated by a reduction in photoreceptor cell loss with electroretinogram activity [7]. Bone marrow-derived MSCs are considered a potentially useful cell therapy for retinitis pigmentosa [7]. MSCs would be advantageous because they allow autogenic transplantation. In fact, MSCs can be isolated from adult tissues, including bone, fat, skeletal muscle, synovium, and dental pulp, as well as fetal tissues, including umbilical cord blood, placenta, amniotic membranes and fluid, and Wharton's jelly. Though umbilical cord blood-derived MSCs can be harvested without imposing risks on the donor, the number of umbilical cord blood-derived MSCs available to therapy is extremely low [21]. Adipose tissue-derived MSCs may provide an alternative source for therapeutic stem cells because of their facile isolation and extensive self-renewal capacity. In addition, adipose tissue-derived MSCs can be cultured for several months with low levels of senescence and retain their potential for differentiating into various cell types [22]. Human adipose-derived stem cell-conditioned medium inhibited photoreceptor cell degeneration and retinal dysfunction from light-induced damage. Several factors secreted by human adipose-derived stem cells such as the tissue inhibitor of metalloproteinase-1 (TIMP-1) and the secreted protein acidic and rich in cysteine (SPARC) protected against light-induced retinal damage caused both in vitro and in vivo. Therefore, human adipose-derived stem cells have a therapeutic potential in retinal degenerative diseases through their secreted factors [23].

Recently, functional MSCs and endothelial progenitor cells (EPCs) identified and isolated from human umbilical cord blood [21, 24] were reported to be effective for clinical application [25]. Intravitreally injected umbilical cord blood-derived

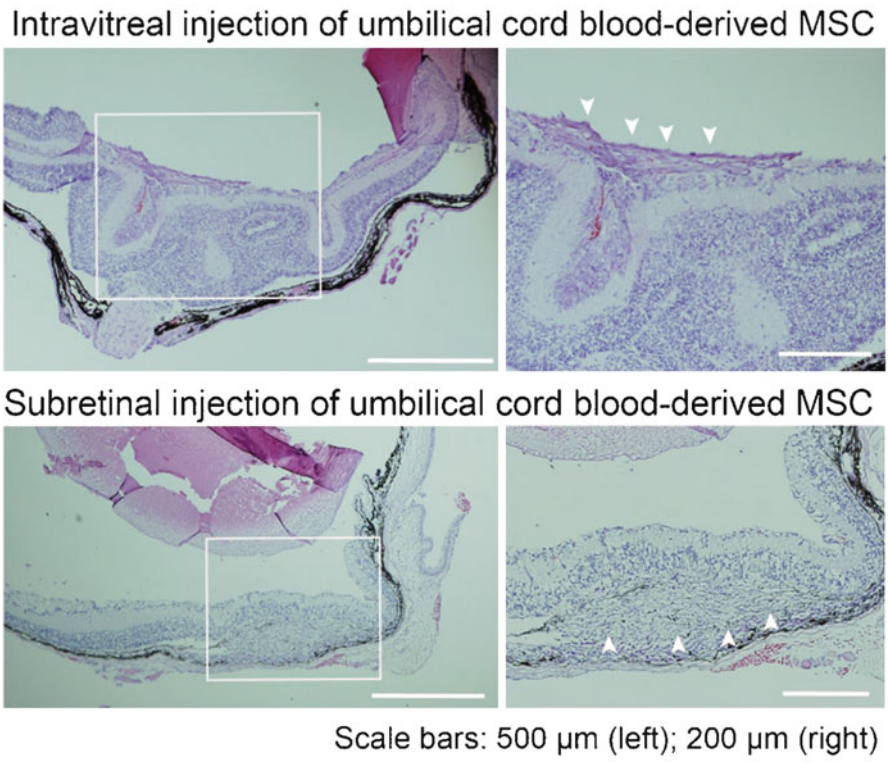


Fig. 22.2 Retinal cross sections of eyes injected with umbilical cord blood-derived MSC intravitreally (*top*) and subretinally (*bottom*)

MSCs fractured the retinal layer structure, resulting in the appearance of a fibrous membrane and resulted in retinal detachment and hemorrhage. In addition, subretinally injected umbilical cord blood-derived MSCs ruptured the retinal layer structure and caused neural damage rather than neuroprotection, which contradicted findings of the previous study (Fig. 22.2) [7]; however, injected EPCs did not produce such complications. A different origin of MSCs and performing experiments in an animal model may have affected the results. Interestingly, another study reported that umbilical cord blood-derived and adipose tissue-derived MSCs possessed opposite characteristics; on one hand, umbilical cord blood-derived MSCs inhibited brain tumor growth and induced apoptosis, whereas on the other hand, adipose tissue-derived MSCs promoted brain tumor growth [26]. Increased mRNA expression levels of angiogenic factors, including vascular endothelial growth factor (VEGF), angiopoietin 1 (Ang1), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and stromal-derived factor-1 (SDF-1/CXCL12), were observed in adipose tissue-derived MSCs. Further, opposite effects of adipose tissue-derived MSCs and umbilical cord blood-derived MSCs on tumor growth clearly demonstrate that such differences

must be considered for safety reasons when choosing a stem cell source for clinical applications. Similarly, caution may be important when using cell therapy for retinitis pigmentosa. MSC-specific cell-surface markers are yet to be determined, but MSCs are reported to be positive for cell-surface markers CD105, CD73, HLA-ABC, CD29, CD44, CD71, CD90, CD106, CD120a, and CD129 but negative for CD45, CD14, CD31, and CD34 markers. Although MSCs derived from different sources have similar characteristics, they differ in their gene expression profile and physiology.

22.1.4 Bone Marrow-Derived Hematopoietic Stem Cells

Bone marrow contains several stem cell populations such as hematopoietic stem cells that give rise to all other blood cells. Hematopoietic stem cells can be categorized as lineage-positive or lineage-negative subpopulations according to their potential for differentiation into formed elements of the blood. The lineage-negative population contains a variety of progenitor cells, including those capable of becoming vascular endothelial cells. These EPCs migrate from the bone marrow in response to a variety of signaling molecules and can target sites of angiogenesis. Lineage-negative hematopoietic stem cells injected directly into the eye can target activated astrocytes and participate in the normal developmental angiogenesis in neonatal mice or in injury-induced neovascularization [27]. Stabilization of the vasculature with lineage-negative hematopoietic stem cells, which integrate into degenerating retinal vessels, has demonstrated neuroprotective effects [9]. However, hematopoietic stem cells are a heterogeneous population, and therefore, it may be difficult to comprehend which cell types are most effective.

22.2 EPC Therapy for Retinal Degeneration

22.2.1 Characteristics of EPCs

EPCs that circulate in the blood and differentiate into vascular endothelial cells were originally identified as a population of stem cells in human peripheral blood and characterized by their expression of CD34, VEGFR-2, and CD133 markers [28–30]. Because these molecules are also expressed in hematopoietic stem cell populations, hematopoietic contamination of EPCs should be expected. Moreover, a recent study reported that the so-called EPCs included several cell subpopulations, including $CD34^+$ $AC133^+$ KDR^+ cells and endothelial colony-forming cells. Endothelial colony-forming cells, which possess a hierarchy of EPCs, express endothelial cell-surface antigens but not hematopoietic cell-surface antigens. In addition, they can be identified in clonal plating conditions and revealed robust

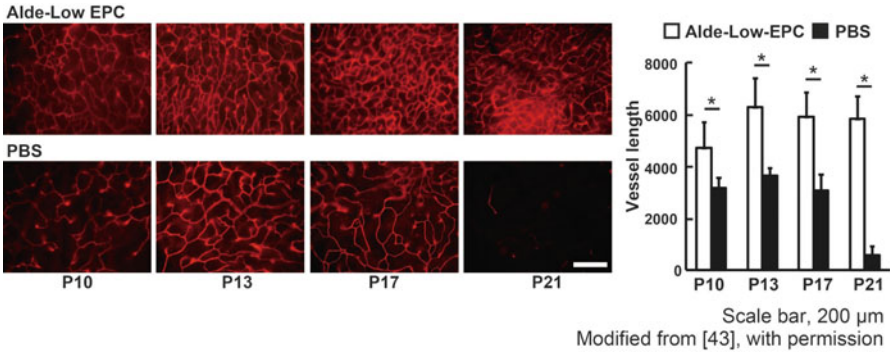


Fig. 22.3 Vasoprotective effect of Alde-Low EPC

proliferative potential and vessel-forming activity [31, 32]. EPCs have now been isolated from other sources, including peripheral blood, umbilical cord blood, and bone marrow [33–35]. EPCs have been recently used to treat cardiovascular disease and severe limb ischemia [36]. However, clinical applications for EPC transfusion are limited because of the small number of cells available [36]. Umbilical cord blood has an advantage over peripheral blood and bone marrow as a source of EPCs because it is more accessible and contains a higher number of EPCs [37].

22.2.2 EPCs and Neuroprotection

Therapeutic effects of EPCs on central and peripheral nervous systems were recently investigated [38–40]. Nevertheless, it remains unclear whether an increased blood flow through revascularization improves neural injury, or whether EPCs have neuroprotective or neurotrophic effects. EPCs attenuated brain ischemic injury in an animal model [38, 39]. SDF-1 was upregulated in the ischemic brain and induced EPC migration through the CXC chemokine receptor 4 (CXCR4), which is expressed in a majority of EPCs [38]. In diabetic neuropathy, dual angiogenic and neurotrophic effects of EPCs were confirmed by a higher proliferation of Schwann cells and endothelial cells cultured in hypoxic EPCs-conditioned media [40]. In both cases, stroke and diabetic neuropathy involved hypoxic environments.

In retinitis pigmentosa, retinal vascular apoptosis is speculated to be followed by decreased metabolic demands because of retinal degeneration. The exact relationship between the retinal neuron apoptosis and vascular attenuation is not well understood, and the intraretinal oxygen environment of retinitis pigmentosa is still a matter of controversy. Few reports have demonstrated that oxygen levels in retinal tissue increased with retinal vessel narrowing because of autoregulation [41, 42]. Further, a therapeutic effect of functional EPCs in the retinitis pigmentosa mouse model was recently reported (Figs. 22.3 and 22.4) [43]. Functional EPCs

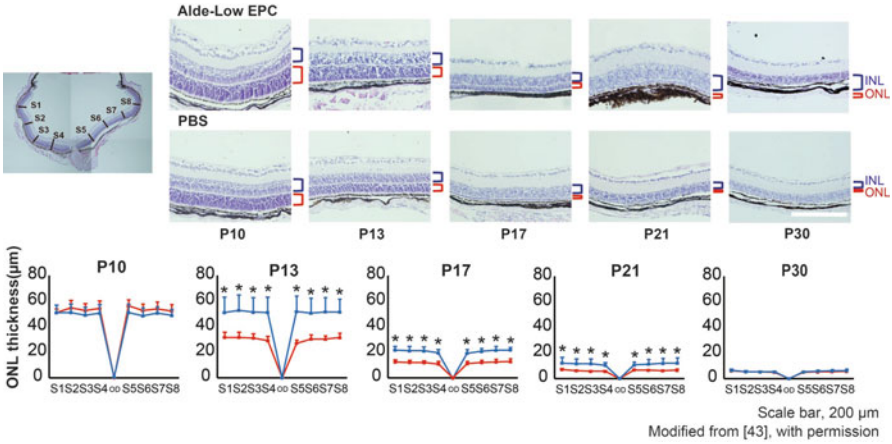


Fig. 22.4 Neuroprotective effect of Alde-Low EPC

demonstrate a strong hypoxic response through upregulation of hypoxia-inducible factor proteins, VEGF, and CXCR4 [24]. However, the hypoxic response of EPCs may not contribute toward the management of retinitis pigmentosa. Alde-Low EPCs have considerable migratory activity because of CXCR4 upregulation under 1 % oxygen concentrations [24]. Nonetheless, CXCR4 downregulation in Alde-Low EPCs did not decrease their neuroprotective and vasoprotective abilities. Moreover, EPCs were directly injected into the eye; therefore, no long distance migration was required.

22.2.3 Functional EPCs

EPCs have been used in clinical practice for several ischemic diseases such as cardiac angina and severe limb ischemia [28–30, 33, 35–37]. To date, clinical applications for EPC transfusion are limited because of the small number of available cells [36]. In a recent study, ALDH activity was reported to be an effective marker of functional EPCs [24]. Characteristically, functional EPCs have high proliferative and migratory capabilities and exhibit a significant capacity to regenerate ischemic tissue [24]. It was demonstrated that EPCs with low ALDH expression (Alde-Low EPCs) levels migrated to an ischemic region and decreased the necrotic area in a mouse model of skin flap ischemia after making an incision on the dorsal surface [24]. In a rat model of acute cerebral infarction, Alde-Low EPCs accumulated and migrated into the infarcted site and subsequently decreased the infarcted volume [25]. SDF-1 and CXCR4 signaling pathways contribute to the migration of Alde-Low EPCs. CXCR4 is a 7-transmembrane spanning G-protein-coupled receptor that is expressed in different types of stem cells, progenitor cells,

and mature endothelial cells. The chemokine SDF-1, a proinflammatory cytokine involved in cell trafficking and adhesion, exclusively binds to its cognate receptor CXCR4 [44]. Previous studies have shown that the SDF-1/CXCR4 signaling pathway regulates angiogenesis and vasculogenesis during development through the mobilization, migration, and differentiation of EPCs [45]. SDF-1 strongly expressed in the ischemic area. CXCR4 expression significantly increased in Alde-Low EPCs (functional EPCs) when compared with Alde-High (low-functioning EPCs) or control cells under hypoxic conditions, whereas Alde-Low EPCs have a higher migratory activity in vitro compared with Alde-High EPCs [24].

As mentioned above, ALDH activity is an effective marker for functional EPCs because Alde-Low EPCs are more functional compared with Alde-High EPCs [24]. Recent studies have discussed the concept of what constitutes EPCs based on their proliferative potential [32]. A hierarchy of EPCs has become increasingly complex and confusing, and the definition of EPC has been controversial. Therefore, isolation methods of EPCs are variable among investigators [32]. For future clinical use of EPCs, efficient isolation of highly proliferative EPCs will be important to the generation of a reliable and safe cell-based therapy. Highly proliferative EPCs based on their ALDH activity were identified and referred to as "Alde-Low EPCs." The frequency of Alde-Low EPCs in the umbilical cord blood was greater than 35 % by flow cytometry analysis [24, 28].

22.2.4 Recruitment of Neuroprotective Macrophages by EPCs

There is no definitive evidence that clarifies whether EPCs possess direct neuroprotective ability. Presumably, EPCs have no direct neuroprotective ability. Nevertheless, the increase of blood flow resulting from revascularization ability of EPCs improves neural injury. EPCs recruit neuroprotective cells (Fig. 22.5) [43]. It has been reported that transplanted EPCs not only recruited monocytes/macrophages and promoted neovascularization but also accelerated dermal wound healing [24, 36, 46, 47]. Alde-Low EPCs recruited monocyte-derived macrophages through CCL2 secretion and rescued vessel and photoreceptor cells in a mouse model of retinal degeneration (Figs. 22.3, 22.4, and 22.5) [43]. CCL2 is a small cytokine belonging to the CC chemokine family. CCL2, which is also known as monocyte chemoattractant protein 1, recruits leukocytes such as macrophages or microglia during retinal inflammation, injury, or chronic disease [48–52]. A previous report indicated that EPCs released large amounts of CCL2 [53]. In vitro neuroprotective functions of CCL2 have been recently reported [54, 55]; however, no in vivo neuroprotective effect has been described—only CCL2 recombinant injection into mouse model of retinitis pigmentosa.

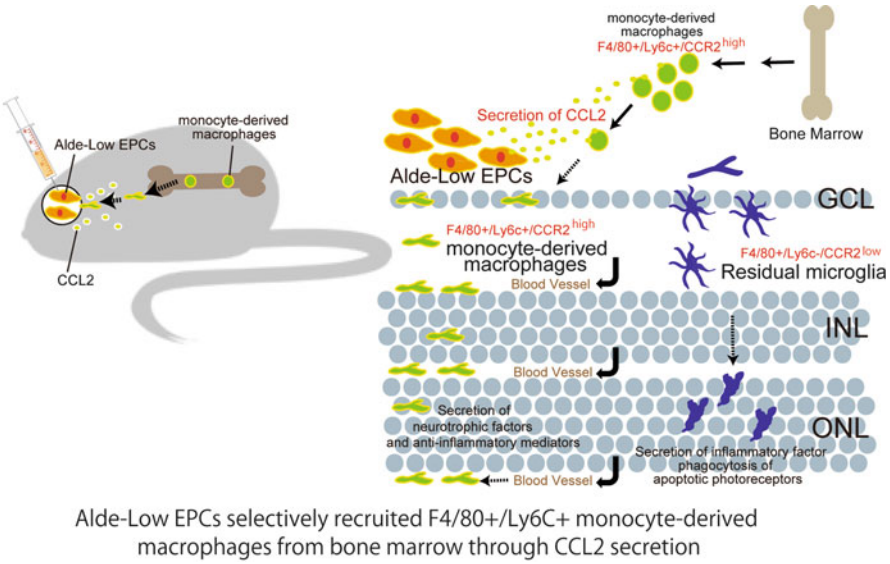


Fig. 22.5 Schematic summarizing the migrated monocyte-derived macrophages (F4/80^{+}/Ly6c^{+}/CCR2^{high} cells) recruited by Alde-Low EPC through CCL2-expressed neurotrophic factors and anti-inflammatory mediators. On the other hand, residual microglia (F4/80^{+}/Ly6c^{-}/CCR2^{low} cells) expressed TNF-α and was associated with phagocytosis of apoptotic photoreceptors

It is interesting that neuroprotective and vasoprotective effects of Alde-Low EPC injections were diminished by clodronate liposomes, which have a powerful depletion effect on macrophages or microglia [56]. Liposomes are artificially prepared lipid vesicles that contain concentric phospholipid bilayers with entrapping aqueous compartments. They can be used to encapsulate strongly hydrophilic molecules dissolved in aqueous solutions, including clodronate, a nontoxic bisphosphonate. Following liposome injection, macrophages ingested and digested liposomes, which was followed by intracellular release and accumulation of clodronate. At a certain intracellular concentration, clodronate induces macrophage apoptosis. Therefore, clodronate liposome decreased the recruitment of bone marrow-derived macrophages into the degenerating retina and did not influence the number of tissue-resident microglia [57]. This finding indicates that both neuroprotection and vasoprotection were not directly caused by Alde-Low EPCs or CCL2, yet they were affected by numerous migrated monocyte-derived macrophages (Fig. 22.5). Neuroprotective and vasoprotective monocyte-derived macrophages in the peripheral blood were present in extremely low percentages. Intriguingly, Alde-Low EPCs efficiently induced monocyte-derived macrophages from peripheral blood to neurodegenerative and vasodegenerative regions (Fig. 22.5) [43]. Mononuclear cells in the peripheral blood were isolated after intravitreal Alde-Low EPC injection, and flow cytometric analysis revealed a higher frequency of F4/80+/Ly6c+ cells in the peripheral blood of Alde-Low EPC-injected mice than in that of PBS-injected control mice. This suggests that

Alde-Low EPCs induce migration of F4/80+/Ly6c+ cells in the peripheral blood from the bone marrow. In another study, F4/80+/Ly6c+ cells in the peripheral blood were isolated and injected into the subretinal space of rd1 mice, and the number of rescued cells was analyzed. F4/80+/Ly6c+ cells revealed neuroprotective effects, but these effects were localized to the injected site. Interestingly, injected monocyte-derived macrophages remained at the rescued region and expressed IL-10 and IGF-1. Monocyte-derived macrophages in peripheral blood contributed to neuroprotective and vasoprotective effects.

Are these migrated neuroprotective macrophages mediated by Alde-Low EPCs similar to residual macrophages or microglia in the retina? Residual macrophages or microglia play a significant pathogenic role in inflammatory diseases [48–51] and retinitis pigmentosa [52]. However, previous reports have assessed residual macrophages or microglia rather than circulating monocytes or macrophages from bone marrow. Circulating monocytes or macrophages are classified into two main subsets of monocytes or macrophages: Ly-6c^{+}/CX$_3$CR1^{low}/CCR2^{+} cells that infiltrate injured tissues and Ly-6c^{-}/CX$_3$CR1^{high}/CCR2^{-} cells that primarily contribute to tissue-resident macrophages [58]. CX$_3$CR1 is expressed in the mononuclear myeloid lineage, regardless of the origin (resident or infiltrating), and is highly expressed in microglia in the central nervous system and the retina [52, 59]. Recently, CX$_3$CR1 and its ligand fractalkine (CX$_3$CL1) signaling recruited few subsets of macrophages and related neuroprotection and anti-inflammation [60, 61]. However, previous studies suggest that CX$_3$CL1/CX$_3$CR1 signaling did not play a significant role during the degenerative process of rd1 mice and in neuroprotection and vasoprotection by Alde-Low EPCs [43, 52]. CX$_3$CL1 expression did not change during retinal degeneration in rd1 mice [52]. Moreover, no significant differences were observed in CX$_3$CR1 expression between migrated monocyte-derived macrophages (F4/80^{+}/Ly6c^{+} cells) and residual microglia (F4/80^{+}/Ly6c^{-} cells) [43]. C-C motif receptor 2 (CCR2) is a receptor for CCL2, and CCL2/CCR2 is important for recruiting Ly6c^{+} monocyte-derived macrophages from bone marrow [43]. A deficiency in CCL2/CCR2 can impair Ly6c^{+} monocyte entry into the blood and consequently, into the inflammatory tissue [62, 63]. Moreover, CCL2/CCR2 signaling was crucial for upregulation of IGF-1 mRNA expression of macrophages. It was recently reported that migrated monocyte-derived macrophages (F4/80^{+}/Ly6c^{+} cells) revealed increased IGF-1 expression, one of the endogenous neurotrophic factors, than residual microglia (F4/80^{+}/Ly6c^{-} cells) [43]. Ly6c is expressed in monocytes in the bone marrow and shortly after their migration into the circulation. This indicates that Ly6c^{+} cells are immature macrophages. Impaired recruitment of CCR2^{+}/Ly6c^{high}/CD11c^{+} monocytes has been implicated as a mechanism underlying poor recovery in spinal cord injury models. Injection of Ly6c^{high} monocytes into the circulation enhanced recovery after spinal cord injury. These monocytes expressed interleukin 10 and therefore played an anti-inflammatory role [64].

Another study reported that monocyte-derived macrophages (F4/80^{+}Ly6c^{+}CD11b^{+}CX3CR1-GFP^{int}) infiltrated the retina of mice with glutamate intoxication and increased retinal ganglion cell survival and retinal progenitor cell renewal [60]. A crucial element in the pathophysiology of all forms of

glaucoma is the death of retinal ganglion cells, which is also a hallmark of several other retinal neuropathies. Interestingly, inhibition of this infiltration by anti-CCR2 neutralization antibody resulted in a decreased survival of retinal ganglion cells and decreased the number of proliferating retinal progenitor cells in the ciliary body. Otherwise, enhancing the circulating monocyte pool led to an increased retinal ganglion cell survival. Ablation of the endogenous monocyte pool decreased the number of transcripts encoding anti-inflammatory mediators such as TGF-β1/2 and interleukin 10 but increased those for proinflammatory mediators. Infiltrating monocyte-derived macrophages revealed anti-inflammatory and neuroprotective effects and downregulated the accumulation of other immune cells [60].

In Alde-Low EPCs injection into retinal degeneration models, migrated monocyte-derived macrophages, as compared with residual microglia, revealed increased expression of anti-inflammatory mediators such as TGF-β1 and interleukin 10 but decreased expression of proinflammatory factors such as tumor necrosis factor-α [43]. In retinitis pigmentosa, activated residual microglia migrate into deeper retinal layers and express tumor necrosis factor-α before the onset of photoreceptor cell death [52]. These properties suggested that migrated monocyte-derived cells could be regarded as inflammation-resolving macrophages. Intriguingly, there was a paradox with respect to retinal detachment. CCL2 concentration was significantly higher in patients with retinal detachment [65, 66], and CCL2 also correlated with retinal detachment-induced photoreceptor apoptosis [51]. Retinal detachment is toxic to photoreceptor cells and induces their apoptosis in wild-type mice [51]. However, experimentally induced retinal detachment preserved photoreceptors and the surrounding retinal vasculature by decreasing apoptosis of photoreceptors in rd1 mice. This decrease in apoptosis resulted from the activation of retinal progenitor cells [67]. However, the exact cytokines that were upregulated, neurotrophic factors, or migrated inflammation-resolving monocyte-derived macrophages that counteract photoreceptor degeneration remain unclear.

Human umbilical vein endothelial cells (HUVEC) are derived from endothelium veins of the umbilical cord. HUVEC and HUVEC with CCL2 overexpression failed to recruit monocyte-derived macrophages and consequently, failed to induce neuroprotection and vasoprotection in a retinitis pigmentosa mice model [43]. The presence of EPCs may be important for recruiting monocyte-derived macrophages from bone marrow. Recruitment of monocyte-derived macrophages may not only be supported by CCL2 but also by other factors secreted by EPCs. EPCs are thought to release multiple synergistic, therapeutic, and angiogenic factors, growth factors, and chemokines compared with HUVEC [28–33]. In vitro, the proliferative activity of HUVECs plateaued within a short time period, and the confluent cell number was much smaller compared with those of EPCs [24]. These fundamental differences between EPCs and HUVEC may contribute to recruitment of monocyte-derived macrophages after their transplantation into the retinitis pigmentosa model.

Aldehyde dehydrogenase is an enzyme responsible for oxidizing intercellular aldehydes [68] that plays an important role in ethanol, vitamin A [69, 70], and cyclophosphamide metabolism [71]. A recent report demonstrated that

hematopoietic and neural stem cells are enriched in cells with high ALDH activity [72–74]. MSCs of human bone marrow and human umbilical cord blood with high ALDH activity revealed better proliferation and multipotential differentiation abilities [21, 75]. However, human Alde-Low EPCs isolated from umbilical cord blood revealed highly proliferative and migratory abilities and displayed a greater ability to regenerate ischemic tissue than did Alde-High EPCs [24]. Human adipose-derived adult stem cells with high ALDH activity did not increase the chondrogenic differentiation potential in comparison with unsorted cells by ALDH activity [76]. ALDH activity was downregulated in MSCs when compared to terminally differentiated fibroblast [77]. These reports suggested that the relationship between ALDH activities and cell functions of progenitor cells or stem cells may vary according to the cell type and culture methods.

Cell transplantation therapy may be a general approach for managing photoreceptor cell loss in which degenerating photoreceptor cells may be replaced by transplanted cells. However, the exact relationship between the survival rate of transplanted cells and host nutrient vessels were not investigated in retinal tissues. More advanced retinitis pigmentosa is associated with severe arteriolar attenuation. Prior injection of Alde-Low EPCs rescued retinal vascular attenuation and increased the survival rate of the graft in transplantation of cells from postnatal mice retina. The resident retinal vascular system may be an important factor for graft survival rate, or prior injection of EPCs may contribute to an improvement in the recipient retinal environment, for example, through anti-inflammatory effects.

Therapies using EPCs and migrated monocyte-derived macrophages can be extremely advantageous and are potentially applicable for all genetic types of retinal degeneration. These therapies are particularly promising in human retinitis pigmentosa because this disease shows a number of mutations. The neural and vascular protection induced by Alde-Low EPCs and migrated microglia represents a promising new therapy for delaying not only the progression of retinitis pigmentosa but also the evolution of other neurodegenerative diseases in the central nervous system.

Acknowledgments This work was supported by a Research Grant from the Study Group on Chorioretinal Degeneration and Optic Atrophy, the Ministry of Health, Labor and Welfare, Japan.

References

1. Hartong DT, Berson EL, Dryja TP (2006) Retinitis pigmentosa. Lancet 368:1795–1809
2. Humphries P, Kenna P, Farrar GJ (1992) On the molecular genetics of retinitis pigmentosa. Science 256:804–808
3. Bramall AN, Wright AF, Jacobson SG, McInnes RR (2010) The genomic, biochemical, and cellular responses of the retina in inherited photoreceptor degenerations and prospects for the treatment of these disorders. Annu Rev Neurosci 33:441–472
4. Cideciyan AV (2010) Leber congenital amaurosis due to RPE65 mutations and its treatment with gene therapy. Prog Retin Eye Res 29:398–427

5. Frasson M, Picaud S, Leveillard T, Simonutti M, Mohand-Said S, Dreyfus H, Hicks D, Sabel J (1999) Glial cell line-derived neurotrophic factor induces histologic and functional protection of rod photoreceptors in the rd/rd mouse. Invest Ophthalmol Vis Sci 40:2724–2734
6. LaVail MM, Yasumura D, Matthes MT, Lau-Villacorta C, Unoki K, Sung CH, Steinberg RH (1998) Protection of mouse photoreceptors by survival factors in retinal degenerations. Invest Ophthalmol Vis Sci 39:592–602
7. Inoue Y, Iriyama A, Ueno S, Takahashi H, Kondo M, Tamaki Y, Araie M, Yanagi Y (2007) Subretinal transplantation of bone marrow mesenchymal stem cells delays retinal degeneration in the RCS rat model of retinal degeneration. Exp Eye Res 85:234–241
8. MacLaren RE, Pearson RA, MacNeil A, Douglas RH, Salt TE, Akimoto M, Swaroop A, Sowden JC, Ali RR (2006) Retinal repair by transplantation of photoreceptor precursors. Nature 444:203–207
9. Otani A, Dorrell MI, Kinder K, Moreno SK, Nusinowitz S, Banin E, Heckenlively J, Friedlander M (2004) Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. J Clin Invest 114:765–774
10. Meyer JS, Katz ML, Maruniak JA, Kirk MD (2006) Embryonic stem cell-derived neural progenitors incorporate into degenerating retina and enhance survival of host photoreceptors. Stem Cells 24:274–283
11. Bartsch U, Oriyakhel W, Kenna PF, Linke S, Richard G, Petrowitz B, Humphries P, Farrar GJ, Ader M (2008) Retinal cells integrate into the outer nuclear layer and differentiate into mature photoreceptors after subretinal transplantation into adult mice. Exp Eye Res 86:691–700
12. Yang P, Seiler MJ, Aramant RB, Whittemore SR (2002) Differential lineage restriction of rat retinal progenitor cells in vitro and in vivo. J Neurosci Res 69:466–476
13. Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154–156
14. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
15. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 318:1920–1923
16. Osakada F, Jin ZB, Hirami Y, Ikeda H, Danjyo T, Watanabe K, Sasai Y, Takahashi M (2009) In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. J Cell Sci 122:3169–3179
17. Caplan AI (1991) Mesenchymal stem cells. J Orthop Res 9:641–650
18. Cancedda R, Bianchi G, Derubeis A, Quarto R (2003) Cell therapy for bone disease: a review of current status. Stem Cells 21:610–619
19. Laflamme MA, Murry CE (2005) Regenerating the heart. Nat Biotechnol 23:845–856
20. Chen Q, Long Y, Yuan X, Zou L, Sun J, Chen S, Perez-Polo JR, Yang K (2005) Protective effects of bone marrow stromal cell transplantation in injured rodent brain: synthesis of neurotrophic factors. J Neurosci Res 80:611–619
21. Nagano M, Kimura K, Yamashita T, Ohneda K, Nozawa D, Hamada H, Yoshikawa H, Ochiai N, Ohneda O (2010) Hypoxia responsive mesenchymal stem cells derived from human umbilical cord blood are effective for bone repair. Stem Cells Dev 19:1195–1210
22. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24:1294–1301
23. Sugitani S, Tsuruma K, Ohno Y, Kuse Y, Yamauchi M, Egashira Y, Yoshimura S, Shimazawa M, Iwama T, Hara H (2013) The potential neuroprotective effect of human adipose stem cells conditioned medium against light-induced retinal damage. Exp Eye Res 116C:254–264
24. Nagano M, Yamashita T, Hamada H, Ohneda K, Kimura K, Nakagawa T, Shibuya M, Yoshikawa H, Ohneda O (2007) Identification of functional endothelial progenitor cells suitable for the treatment of ischemic tissue using human umbilical cord blood. Blood 110:151–160

25. Nakamura K, Tsurushima H, Marushima A, Nagano M, Yamashita T, Suzuki K, Ohneda O, Matsumura A (2012) A subpopulation of endothelial progenitor cells with low aldehyde dehydrogenase activity attenuates acute ischemic brain injury in rats. Biochem Biophys Res Commun 418:87–92
26. Akimoto K, Kimura K, Nagano M, Takano S, To'a Salazar G, Yamashita T, Ohneda O (2013) Umbilical cord blood-derived mesenchymal stem cells inhibit, but adipose tissue-derived mesenchymal stem cells promote, glioblastoma multiforme proliferation. Stem Cells Dev 22:1370–1386
27. Otani A, Kinder K, Ewalt K, Otero FJ, Schimmel P, Friedlander M (2002) Bone marrow-derived stem cells target retinal astrocytes and can promote or inhibit retinal angiogenesis. Nat Med 8:1004–1010
28. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964–967
29. Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N et al (2000) In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood 95:3106–3112
30. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA et al (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 95:952–958
31. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA (2007) Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood 109:1801–1809
32. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K, Pollok K, Ferkowicz MJ, Gilley D, Yoder MC (2004) Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 104:2752–2760
33. Shi Q, Rafii S, Wu MH, Wijelath ES, Yu C, Ishida A, Fujita Y, Kothari S, Mohle R, Sauvage LR et al (1998) Evidence for circulating bone marrow-derived endothelial cells. Blood 92:362–367
34. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL (2001) Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. Br J Haematol 115:186–194
35. Murohara T, Ikeda H, Duan J, Shintani S, Sasaki K, Eguchi H, Onitsuka I, Matsui K, Imaizumi T (2000) Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest 105:1527–1536
36. Rafii S, Lyden D (2003) Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med 9:702–712
37. Hristov M, Erl W, Weber PC (2003) Endothelial progenitor cells: mobilization, differentiation, and homing. Arterioscler Thromb Vasc Biol 23:1185–1189
38. Fan Y, Shen F, Frenzel T, Zhu W, Ye J, Liu J, Chen Y, Su H, Young WL, Yang GY (2010) Endothelial progenitor cell transplantation improves long-term stroke outcome in mice. Ann Neurol 67:488–497
39. Ohta T, Kikuta K, Imamura H, Takagi Y, Nishimura M, Arakawa Y, Hashimoto N, Nozaki K (2006) Administration of ex vivo-expanded bone marrow-derived endothelial progenitor cells attenuates focal cerebral ischemia-reperfusion injury in rats. Neurosurgery 59:679–686
40. Jeong JO, Kim MO, Kim H, Lee MY, Kim SW, Ii M, Lee JU, Lee J, Choi YJ, Cho HJ et al (2009) Dual angiogenic and neurotrophic effects of bone marrow-derived endothelial progenitor cells on diabetic neuropathy. Circulation 119:699–708
41. Yu DY, Cringle SJ, Su EN, Yu PK (2000) Intraretinal oxygen levels before and after photoreceptor loss in the RCS rat. Invest Ophthalmol Vis Sci 41:3999–4006
42. Yu DY, Cringle S, Valter K, Walsh N, Lee D, Stone J (2004) Photoreceptor death, trophic factor expression, retinal oxygen status, and photoreceptor function in the P23H rat. Invest Ophthalmol Vis Sci 45:2013–2019

43. Fukuda S, Nagano M, Yamashita T et al (2013) Functional endothelial progenitor cells selectively recruit neurovascular protective monocyte-derived F4/80/Ly6c macrophages in a mouse model of retinal degeneration. Stem Cells 31:2149–2161
44. Bagri A, Gurney T, He X, Zou YR, Littman DR, Tessier-Lavigne M, Pleasure SJ (2002) The chemokine SDF1 regulates migration of dentate granule cells. Development 129:4249–4260
45. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, Kikutani H, Kishimoto T (1996) Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature 382:635–638
46. van der Strate BW, Popa ER, Schipper M, Brouwer LA, Hendriks M, Harmsen MC, van Luyn MJ (2007) Circulating human CD34+ progenitor cells modulate neovascularization and inflammation in a nude mouse model. J Mol Cell Cardiol 42:1086–1097
47. Suh W, Kim KL, Kim JM, Shin IS, Lee YS, Lee JY, Jang HS, Lee JS, Byun J, Choi JH et al (2005) Transplantation of endothelial progenitor cells accelerates dermal wound healing with increased recruitment of monocytes/macrophages and neovascularization. Stem Cells 23:1571–1578
48. Chen L, Wu W, Dentchev T, Zeng Y, Wang J, Tsui I, Tobias JW, Bennett J, Baldwin D, Dunaief JL (2004) Light damage induced changes in mouse retinal gene expression. Exp Eye Res 79:239–247
49. Meleth AD, Agron E, Chan CC, Reed GF, Arora K, Byrnes G, Csaky KG, Ferris FL 3rd, Chew EY (2005) Serum inflammatory markers in diabetic retinopathy. Invest Ophthalmol Vis Sci 46:4295–4301
50. Jo N, Wu GS, Rao NA (2003) Upregulation of chemokine expression in the retinal vasculature in ischemia-reperfusion injury. Invest Ophthalmol Vis Sci 44:4054–4060
51. Nakazawa T, Hisatomi T, Nakazawa C, Noda K, Maruyama K, She H, Matsubara A, Miyahara S, Nakao S, Yin Y et al (2007) Monocyte chemoattractant protein 1 mediates retinal detachment-induced photoreceptor apoptosis. Proc Natl Acad Sci U S A 104:2425–2430
52. Zeng HY, Zhu XA, Zhang C, Yang LP, Wu LM, Tso MO (2005) Identification of sequential events and factors associated with microglial activation, migration, and cytotoxicity in retinal degeneration in rd mice. Invest Ophthalmol Vis Sci 46:2992–2999
53. Zhang Y, Ingram DA, Murphy MP, Saadatzadeh MR, Mead LE, Prater DN, Rehman J (2009) Release of proinflammatory mediators and expression of proinflammatory adhesion molecules by endothelial progenitor cells. Am J Physiol Heart Circ Physiol 296:H1675–H1682
54. Madrigal JL, Leza JC, Polak P, Kalinin S, Feinstein DL (2009) Astrocyte-derived MCP-1 mediates neuroprotective effects of noradrenaline. J Neurosci 29:263–267
55. Eugenin EA, D'Aversa TG, Lopez L, Calderon TM, Berman JW (2003) MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. J Neurochem 85:1299–1311
56. van Rooijen N, Sanders A, van den Berg TK (1996) Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine. J Immunol Methods 193:93–99
57. Sasahara M, Otani A, Oishi A, Kojima H, Yodoi Y, Kameda T, Nakamura H, Yoshimura N (2008) Activation of bone marrow-derived microglia promotes photoreceptor survival in inherited retinal degeneration. Am J Pathol 172:1693–1703
58. Geissmann F, Jung S, Littman DR (2003) Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 19:71–82
59. Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN, Adhikari S, Thompson DA et al (1998) Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 95:10896–10901
60. London A, Itskovich E, Benhar I, Kalchenko V, Mack M, Jung S, Schwartz M (2011) Neuroprotection and progenitor cell renewal in the injured adult murine retina requires healing monocyte-derived macrophages. J Exp Med 208:23–39

61. Donnelly DJ, Longbrake EE, Shawler TM, Kigerl KA, Lai W, Tovar CA, Ransohoff RM, Popovich PG (2011) Deficient CX3CR1 signaling promotes recovery after mouse spinal cord injury by limiting the recruitment and activation of Ly6Clo/iNOS+ macrophages. J Neurosci 31:9910–9922
62. Lu H, Huang D, Saederup N, Charo IF, Ransohoff RM, Zhou L (2011) Macrophages recruited via CCR2 produce insulin-like growth factor-1 to repair acute skeletal muscle injury. FASEB J 25:358–369
63. Lu H, Huang D, Ransohoff RM, Zhou L (2011) Acute skeletal muscle injury: CCL2 expression by both monocytes and injured muscle is required for repair. FASEB J 25:3344–3355
64. Shechter R, London A, Varol C, Raposo C, Cusimano M, Yovel G, Rolls A, Mack M, Pluchino S, Martino G et al (2009) Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med 6: e1000113
65. Abu el-Asrar AM, Van Damme J, Put W, Veckeneer M, Dralands L, Billiau A, Missotten L (1997) Monocyte chemotactic protein-1 in proliferative vitreoretinal disorders. Am J Ophthalmol 123:599–606
66. Capeans C, De Rojas MV, Lojo S, Salorio MS (1998) C-C chemokines in the vitreous of patients with proliferative vitreoretinopathy and proliferative diabetic retinopathy. Retina 18:546–550
67. Kaneko H, Nishiguchi KM, Nakamura M, Kachi S, Terasaki H (2008) Retardation of photoreceptor degeneration in the detached retina of rd1 mouse. Invest Ophthalmol Vis Sci 49:781–787
68. Storms RW, Trujillo AP, Springer JB, Shah L, Colvin OM, Ludeman SM, Smith C (1999) Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. Proc Natl Acad Sci U S A 96:9118–9123
69. Russo JE, Hilton J (1988) Characterization of cytosolic aldehyde dehydrogenase from cyclophosphamide resistant L1210 cells. Cancer Res 48:2963–2968
70. Labrecque J, Bhat PV, Lacroix A (1993) Purification and partial characterization of a rat kidney aldehyde dehydrogenase that oxidizes retinal to retinoic acid. Biochem Cell Biol 71:85–89
71. Russo JE, Hilton J, Colvin OM (1989) The role of aldehyde dehydrogenase isozymes in cellular resistance to the alkylating agent cyclophosphamide. Prog Clin Biol Res 290:65–79
72. Armstrong L, Stojkovic M, Dimmick I, Ahmad S, Stojkovic P, Hole N, Lako M (2004) Phenotypic characterization of murine primitive hematopoietic progenitor cells isolated on basis of aldehyde dehydrogenase activity. Stem Cells 22:1142–1151
73. Hess DA, Meyerrose TE, Wirthlin L, Craft TP, Herrbrich PE, Creer MH, Nolta JA (2004) Functional characterization of highly purified human hematopoietic repopulating cells isolated according to aldehyde dehydrogenase activity. Blood 104:1648–1655
74. Corti S, Locatelli F, Papadimitriou D, Donadoni C, Salani S, Del Bo R, Strazzer S, Bresolin N, Comi GP (2006) Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity. Stem Cells 24:975–985
75. Gentry T, Foster S, Winstead L, Deibert E, Fiordalisi M, Balber A (2007) Simultaneous isolation of human BM hematopoietic, endothelial and mesenchymal progenitor cells by flow sorting based on aldehyde dehydrogenase activity: implications for cell therapy. Cytotherapy 9:259–274
76. Estes BT, Wu AW, Storms RW, Guilak F (2006) Extended passaging, but not aldehyde dehydrogenase activity, increases the chondrogenic potential of human adipose-derived adult stem cells. J Cell Physiol 209:987–995
77. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W et al (2005) Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 33:1402–1416

Chapter 23
Optic Nerve Regeneration

Kazuhiko Namekata

Abstract In the adult central nervous system (CNS), axon regeneration of damaged neurons is very difficult as the intrinsic regeneration capacity of neurons is suppressed by environmental conditions after birth. Like other mammalian CNS neurons, retinal ganglion cells (RGCs) are unable to regenerate after optic nerve injury and thus, once they are damaged it can cause irreversible visual loss. There are a number of reasons for the failure of axon regeneration. One such reason is the presence of myelin-associated axon growth inhibitors such as Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp). These molecules create an environment that restricts axon regeneration. On the other hand, there are molecules that promote axon regeneration, such as trophic factors and inflammation-related factors. Recent studies revealed that CNS neurons, including RGCs, can regenerate if the environment surrounding the damaged neurons is suitable for regrowth. This condition may be achieved by application of mixed trophic factors and proinflammatory molecules that promote axon regeneration and/or by suppression of axon growth-inhibition signaling, such as RhoA/ROCK signaling. In this review, recent discoveries on molecular mechanisms underlying optic nerve regeneration are discussed.

Keywords ASK1 • Axon regeneration • Dock3 • Oncomodulin • PTEN

23.1 Introduction

Axon regeneration of injured adult mammalian central nervous system (CNS) tissues is very limited as the intrinsic capacity of neurons to regenerate is suppressed by environmental conditions after birth. Therefore, disease or injury

K. Namekata (✉)
Visual Research Project, Tokyo Metropolitan Institute of Medical Science,
2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan
e-mail: namekata-kz@igakuken.or.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_23,

that causes destruction or damage to axons and neuronal networks typically results in permanent functional deficits of the CNS. It is known that molecules that are major suppressors to regenerative axon growth and sprouting are secreted following injury to the adult CNS tissues. On the other hand, there are molecules that can promote neuronal regeneration, such as growth-promoting extracellular matrix molecules, cell adhesion molecules, neurotrophic factors, and inflammation molecules. Recent studies showed that both locally available growth regulatory molecules and intracellular signaling molecules greatly influence injury-induced neuronal growth. Various degrees of growth and sprouting of injured CNS neurons have been achieved by lowering inhibitory cues, by increasing environmental growth-promoting cues, or by activation of cell growth programs. Injured axons receiving combination of these molecules, so as to activate growth-promoting signaling and attenuate growth-inhibitory signaling, will be able to regenerate effectively and advances in this field will achieve full functional recovery of adult CNS neurons after injury in the near future.

23.2 Growth-Inhibitory Factors for Axon Regeneration

In the adult mammalian CNS, axon regeneration is restricted by a number of environmental and intracellular growth-inhibition mechanisms. These mechanisms hinder axon regeneration, but they are essential for stabilization of intricate networks of neuronal connectivity. In response to injury to the CNS, a spontaneous repair process is activated, but it is not sufficient for complete recovery, leading to neurological deficits. Therefore, a detailed understanding of the inhibitory mechanisms in the CNS repair process and finding strategies to overcome the inhibition would be useful both in biological and clinical contexts. In this section, myelin-associated inhibitor molecules and RhoA signaling are discussed followed by a brief summary of the role of phosphatase and tensin homologue (PTEN), which is one of the powerful suppressors of the intrinsic regenerative ability of the mature neuron.

23.2.1 Myelin-Associated Inhibitor Molecules

The most well-characterized myelin-associated inhibitor molecules are Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp). Nogo, also known as Reticulon-4, is an axonal growth inhibitor in the CNS. To date, three isoforms, namely, Nogo-A, Nogo-B, and Nogo-C, have been identified [1–3]. Nogo-A is the longer variant and the principal isoform in the CNS, suggesting that this isoform may play a major role in restricting CNS regeneration [4, 5]. Nogo-A expression is mainly found in oligodendrocytes in the postnatal CNS [4, 5].

However, Nogo-A is also expressed in the periaxonal sheath and synapses of neurons [4]. The carboxyl terminus of Nogo-A shares homology with Nogo-B and Nogo-C and contains the hydrophilic 66-amino acid segment (Nogo-66) between two hydrophobic domains [2], which exhibits high-affinity binding to its neuronal receptor, the Nogo receptor (NgR). Besides this homologous carboxyl terminal region, Nogo-A possesses domains that also potently inhibits neurite outgrowth in vitro through biochemically distinct mechanisms from NgR signaling [1–3, 6, 7].

MAG, a member of the immunoglobulin superfamily, is expressed at the cell surface and possesses five extracellular immunoglobulin-like domains, a transmembrane domain, and two alternatively spliced C-terminal tails [8]. Recombinant MAG inhibits neurite outgrowth in vitro, and this inhibition is suppressed by immunodepletion of MAG. Although the growth-inhibitory nature of MAG is well established, MAG knockout mice do not show enhanced regrowth of injured corticospinal or optic nerve axons [9]. Interestingly, MAG is known to show contrasting effects on neurite outgrowth depending on the developmental stages: MAG promotes neurite outgrowth of many types of embryonic and neonatal neurons [10], whereas it inhibits neurite outgrowth from a range of primary neurons at mature stages [11].

OMgp, a member of the leucine-rich repeat protein family, is another one of the molecules that inhibits axonal outgrowth [4]. OMgp is localized to the cell surface and is expressed in myelin of oligodendrocytes [12]. OMgp is also highly expressed in many types of neurons in the mature CNS [13, 14]. Although OMgp-deficient mice do not promote axonal outgrowth in axotomized corticospinal tissues, increased sprouting has been reported [15].

These three molecules, Nogo, MAG, and OMgp, impair axon regeneration via a common pathway; they all activate the neuronal glycosylphosphatidylinositol (GPI)-anchored receptor NgR [16–18]. There are three subtypes of NgR: NgR1, NgR2, and NgR3. Although single genetic deletion of NgR1 failed to reduce the inhibitory effects on neurite outgrowth, triple deletion of NgR1, NgR2, and NgR3 in mice promoted optic nerve regeneration after axotomy [19, 20]. The NgR is expressed in microglia and macrophages at injury sites within the CNS and peripheral nervous system (PNS) [21, 22]. In neurons, activation of NgR signaling induces growth cone collapse through the Rho-GTPase pathway by association with two signal-transducing binding partners, p75 (the low-affinity neurotrophin receptor, p75NTR) and LINGO-1 (leucine-rich repeat and immunoglobulin domain-containing Nogo receptor-interacting protein) [16–18, 23]. Moreover, TROY, a tumor necrosis factor (TNF) receptor family member that is widely expressed in postnatal and adult neurons, has been identified to act as a substitute for p75 in the NgR1/p75/LINGO-1 receptor complex and respond to the myelin-associated inhibitor molecules [24, 25]. In addition, another functional receptor for Nogo, MAG, and OMgp has been identified as the paired immunoglobulin-like receptor B (PirB) [26, 27]. PirB is expressed in adult CNS and it inhibits regeneration signaling after injury [6, 7].

23.2.2 RhoA Signaling

Rho-GTPases (RhoA, Rac1, and Cdc42) reorganize cytoskeleton by regulation of actin filament assembly and disassembly through interaction with a series of cytosolic enzymes. They play a major role in actin polymerization, branching and depolymerization, and directing actin-myosin-dependent contractility to control the retrograde transport of F-actin within the growth cones. Activated RhoA induces actinomyosin contractility and stress fiber formation, resulting in inhibition of growth cone extension. In contrast, activation of the other Rho-GTPases Rac1 and Cdc42 is associated with extension of filopodia and lamellipodia, leading to cell migration. In general, it is established that RhoA is the negative regulator and Rac1 and Cdc42 are the positive regulators of Rho-GTPases-associated neurite outgrowth and extension of growth cones [28]. Accumulated evidence indicates that RhoA activation is one of the central events in NgR signaling-mediated inhibition of axon regeneration. Activated RhoA stimulates Rho-kinase (ROCK), which is a major RhoA effector, and suppresses actin dynamics by activating LIM kinase and inactivating cofilin in the growth cone, causing inhibition of axon regeneration. In contrast, inactivation of RhoA by the ADP-ribosyltransferase C3, a bacterial protein that efficiently and irreversibly inactivates RhoA, has been shown to suppress the inhibitory effects of myelin-associated inhibitor molecules and recover axonal outgrowth in retinal neurons [29]. Application of C3 at the injury site of the optic nerve or intravitreal injection of a cell-permeable version of C3 leads to RGC axons extending beyond the lesion site and growing into the distal nerve segment [29, 30]. Furthermore, viral expression of C3 in RGCs enables axons to regenerate following optic nerve injury and further enhances the extent of axon regeneration after lens injury [31]. Similarly treatment of RGCs with specific inhibitors for ROCK overcomes myelin and chondroitin sulfate proteoglycans (CSPG) inhibition in vitro and allows axons to regenerate beyond the lesion site of the optic nerve in vivo [32–34]. Therefore, suppression of the RhoA/ROCK signaling pathway might be a more effective strategy for axon regeneration than directly neutralizing the myelin-associated inhibitor molecules.

23.2.3 PTEN

The phosphatase and tensin homologue (PTEN) is a tumor suppressor gene and is a well-known negative regulator of cell growth and migration in most cell types. PTEN dephosphorylates phosphatidylinositol 3,4,5-triphosphate (PIP3) and inhibits the phosphoinositide 3-kinase (PI3K) signaling pathway [35, 36]. PTEN deficiency leads to neuronal hypertrophy and defects in cell migration, dendrite arborization, and myelination in the nervous system [35, 36]. In the retinal tissue, PTEN has also been shown to be a critical regulator during development. Accordingly, conditional knockout of PTEN causes RGC, horizontal cell, and amacrine

cell hypertrophy and expansion of the inner plexiform layer [36]. In addition, PTEN also plays an important role, as a major downstream effector of the E3 ligase NEDD4 signaling pathway, in regulating RGC terminal arborization [37]. Recently, it has been revealed that PTEN plays a major role in inhibition of optic nerve regeneration following optic nerve injury [38]. Studies on mice with deletion of PTEN demonstrate a striking regeneration capacity of the adult optic nerve, and it synergistically promotes robust axon regeneration with conditions that also stimulate regeneration, such as deletion of the suppressor of cytokine signaling (SOCS) 3 gene [39] and inflammation; the latter will be discussed in more detail later.

23.3 Promoting Axon Regeneration

Some factors can overcome the inhibitory signaling and exert robust axon regeneration by increasing growth-promoting signaling. Application of growth regulatory molecules, such as trophic factors, induces suppression of cell death and enhances neuronal growth in damaged neurons. In addition, some intracellular signaling and cytoskeletal remodeling play major roles in injury-induced neuronal growth and regeneration. In this section, various approaches for increasing axon regeneration are discussed.

23.3.1 Trophic Factors

Optic nerve regeneration requires survival of RGCs and thus, neuroprotection is one of the most important aspects of axon regeneration. The classical neurotrophins are a family of diffusible trophic proteins in the CNS that mediate several cellular responses, such as proliferation, differentiation, axon growth, as well as dendrite and synapse formation [40]. Members of the nerve growth factor (NGF) that belong to the family of neurotrophins (brain-derived neurotrophic factor, BDNF; neurotrophin-3, NT-3; neurotrophin-4/5, NT-4/5) protect retinal cells in animal models of retinal degeneration [41–44]. Neurotrophins regulate cell survival through the three tropomyosin-related kinase (Trk) receptors TrkA, TrkB, and TrkC, and stimulation of these receptors activates Ras-dependent mitogen-activated protein (MAP) kinases [45–47]. In addition, neurotrophins activate p75NTR, which is also involved in NgR signaling, and induce apoptosis; in fact this mechanism by neurotrophins appears to be essential for developmentally regulated cell death in both peripheral and central neurons [48–51]. Mature RGCs express all three Trk receptors, but significant neuroprotection of axotomized RGCs has been reported only with the TrkB receptor ligands BDNF and NT4/5 [52–54]. In fact, BDNF is one of the most effective survival agents for axotomized RGCs [54, 55]. In addition, RGCs express several receptors of other trophic factors such as the fibroblast growth factor receptor (FGFR1), glial cell-derived neurotrophic factor

(GDNF) family receptor α1 (Ret/GFRα1), and hepatocyte growth factor receptor (HGFR), suggesting that these receptor activations may also play an important role in neuroprotection and neuroregeneration. Accordingly intravitreal application of FGF2 and GDNF reportedly increases the survival of mature RGCs following optic nerve injury [56–58]. For most neurotrophic factors, it is not clear whether their beneficial effects act directly on RGCs or indirectly via activation of other retinal cells such as Müller cells, which then release additional factors that promote protection of RGCs. There seems to be an intricate cross talk of multiple signaling in the mammalian visual system, and the combinatorial treatment with trophic factors might be useful for axon regeneration.

23.3.2 Inflammation

Inflammation is the key event of host defense responses to injury, tissue ischemia, autoimmune reactions, or infectious agents. In the CNS, inflammation responses are very complex and depending on the stimulation, neuroinflammation exhibits neuroprotective or neurotoxic effects or even a mixture of the two [59–61]. The direct induction of an inflammatory response in the eye shows dramatic positive effects on RGC axon regeneration [55, 59, 62, 63].

23.3.2.1 Macrophages

Puncture of the ocular lens induces a robust regenerative response in mature axotomized RGCs [64, 65]. As a result, axotomy-induced cell death is markedly delayed, and most of these neurons regenerate lengthy axons into the axon growth supportive environment of a peripheral nerve graft. Moreover, lens injury also enables RGCs to regenerate axons into the inhibitory environment of either a transected/resutured nerve or a crushed optic nerve [59]. Thus, lens injury induces neuroprotection and axon growth promotion and to some degree suppresses the effects of inhibitory molecules that create unsuitable environment for neuroregeneration. The lens-injury-induced ability to regrow axons is not a simple consequence of its neuroprotective effect on RGCs. This is because RGCs that have been pretreated in vivo by lens injury show, in contrast to untreated controls, spontaneous axon growth with higher growth rate on a growth-permissive substrate [66]. In addition, an earlier study reported that introducing activated macrophages into the rat optic nerve enhanced the survival or regeneration of RGC axons [67], and lens injury was associated with an influx of activated macrophages into the eye [65].

The transfer of peripherally activated macrophages into the visual system was previously reported to enhance axon regeneration [68, 69]. The lens injury induces macrophage infiltration into the vitreous and macrophage-derived inflammatory factors can enhance the regenerative capacity of RGC axons, in which the effect of macrophage-mediated optic nerve regeneration is most effective within 3 days of

axotomy [59]. After injection of zymosan, a yeast cell wall preparation, into the retina, macrophages are subsequently activated and infiltrate into the retina. Zymosan can increase both survival of axotomized RGCs and axon regeneration [55, 59, 63, 65]. Earlier studies demonstrated a great increase in the RGC survival rate by a vehicle injection into the eye [54] or by implanting fragments of pre-injured peripheral nerve into the vitreous [70, 71]. These effects are probably due to activated macrophages induced by injury. In addition to zymosan, another macrophage activator oxidized galectin-1 can promote axon regeneration in RGCs after axotomy [72].

23.3.2.2 Oncomodulin

Oncomodulin, a small Ca^{2+}-binding protein, has been identified as one of the principal macrophage-derived molecules to mediate axon regeneration and plays a key role in stimulating RGCs to regenerate their axons following intravitreal inflammation [73]. It was shown that oncomodulin is secreted by inflammatory cells, such as macrophages, and binds to an unknown receptor on RGCs. In the presence of mannose and forskolin, oncomodulin in culture elicited more extensive axon outgrowth than any other well-established growth factors, such as BDNF, ciliary neurotrophic factor (CNTF), and GDNF, and as much outgrowth as macrophage-conditioned media. In addition, when oncomodulin was immune-depleted from macrophage-conditioned media, activity of axon outgrowth was decreased. Most strikingly, in the presence of an agent to elevate intracellular cyclic adenosine monophosphate (cAMP), oncomodulin stimulated extensive axon regeneration in the mature optic nerve in vivo, and this effect was diminished by the peptide antagonist inactivating the putative oncomodulin receptor or the anti-oncomodulin antibody that neutralizes oncomodulin [74]. Thus, oncomodulin is a novel, potent growth-promoting factor for optic nerve regeneration. Most recently, it has been shown that neutrophils, as well as macrophages, infiltrate and secrete oncomodulin after axotomy. In mice, great numbers of neutrophils enter into the vitreous chamber within 12 h of inducing inflammation by zymosan and produce high levels of oncomodulin [75]. Because infiltration of neutrophils is the first responder of the innate immune system, this event might play an essential role in oncomodulin-mediated axon regeneration in RGCs after axotomy.

Recent studies demonstrated that PTEN is an important regulator of axon regeneration and PTEN deficiency successfully induces robust long-distance axon regeneration in mice following optic nerve injury [38]. The zymosan and cAMP application combined with PTEN gene deletion enabled optic nerve fibers to regrow to its full length to innervate visual brain areas such as the dorsal lateral geniculate nucleus and superior colliculus [76] (Fig. 23.1). Partial recovery of visually guided behaviors, assessed by tests such as the visual cliff test, optomotor response test, circadian photoentrainment test, and pupillary light reflex test, suggests the formation of functional synapses at visual centers of the brain. These studies shed light upon possible targets and strategies to repair the optic nerve following traumatic or glaucomatous injury.

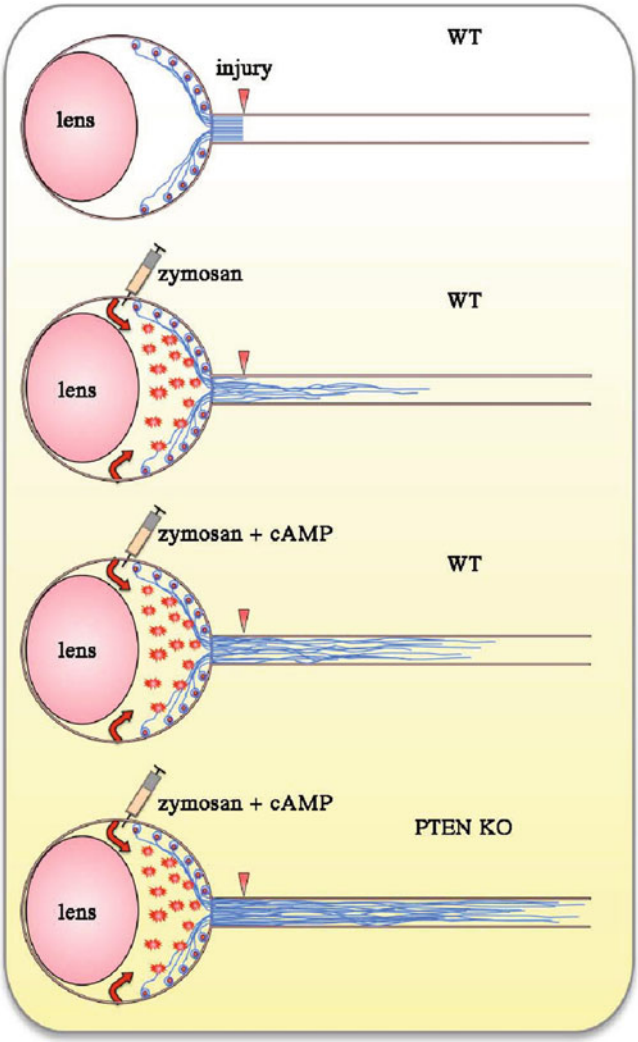


Fig. 23.1 Optic nerve regeneration. Intraocular injection of zymosan and cAMP induces infiltration of activated macrophage into vitreous and promotes axon regeneration of injured optic nerve. In PTEN-deficient mice, the combined treatment induces robust optic nerve regeneration

23.3.3 DLK and ASK1

Dual leucine zipper kinase (DLK) is a mitogen-activated protein kinase (MAPK) kinase kinase and is localized in several areas of the developing nervous system, such as the brain, spinal cord, and sensory ganglia [77]. In response to oxidative

stress and deletion of trophic factors, activation of DLK-dependent signaling cascades leads to rapid neuron degeneration during development [78]. Conversely, DLK deletion protects several classes of neurons from apoptosis in mouse embryos [78, 79]. The primary response of DLK signaling is neural cell death, although DLK can activate both proapoptotic and regenerative programs in response to optic nerve injury [80]. A role of DLK in promoting axon regeneration in diverse model systems has emerged. In this regard, the DLK homologues DLK-1 (*Caenorhabditis elegans*) and Wallenda (*Drosophila*) have been shown to regulate axon regeneration after injury [81–83], and in mammals, the DLK-JNK signaling pathway has been reported to regulate regenerative responses to axonal injury in the retina [80, 84].

In addition to DLK, apoptosis signal-regulating kinase 1 (ASK1), an evolutionarily conserved MAPK kinase kinase, is also associated with RGC death after optic nerve injury. ASK1 relays its apoptotic signals to the stress activated MAPK family members, p38 and c-Jun N-terminal kinase [85]. p38 is activated by environmental stress such as nitric oxide, as well as by proinflammatory cytokines like TNF and interleukin-1. In addition, axotomy of the optic nerve or intraocular injection of *N*-methyl-D-aspartate (NMDA) activates p38, which leads to neural cell apoptosis. The interruption of the ASK1-p38 pathway by deletion of ASK1 gene or by a p38 inhibitor prevents RGC death caused by optic nerve injury [86]. Strikingly, administration of a p38 inhibitor even after optic nerve injury was effective as a treatment, suggesting strongly of a possibility that p38 is a potential therapeutic target for optic neuropathy due to traumatic optic nerve injury, optic neuritis, ischemia, and glaucoma [87–90].

23.3.4 Dock3

Actin filament degradation induces immobility and/or collapse of the growth cone pathways and thus, it is important to induce rearrangement of actin filaments in order to promote axon elongation. In addition to actin filaments, microtubule filaments are also crucially involved in axon regeneration. One feature of injured CNS axons is that their microtubules depolymerize at the axon stump. Thus, these microtubules lose their protrusive activity, which propels axon growth and growth cone formation. In contrast, axonal lesions in the PNS retain bundled microtubules and provide support for growing axons.

Actin rearrangement can be induced by activation of Rac1, a member of the Rho family of small G proteins. Dock3, a guanine exchange factor (GEF) that is exclusively expressed in the CNS, induces Rac1 activation and membrane ruffling [91]. Overexpression of Dock3 promotes optic nerve regeneration after axotomy by binding to WAVE, a key regulator of actin assembly, and by reorganizing actin filaments at the growth cone [92]. Interestingly, Dock3 is a downstream effector of

BDNF-TrkB signaling, in which Dock3 forms a Fyn-Dock3-WAVE complex at the plasma membrane upon BDNF stimulation [92]. Thus, translocation of Dock3 induces Rac1 activation and recruits WAVE to the plasma membrane, leading to enhanced actin rearrangement at the growth cone. In addition to this TrkB-Fyn signaling pathway, BDNF-induced RhoG activation also recruits Dock3 to the plasma membrane by forming a RhoG-Elmo-Dock3 complex, resulting in increased neurite outgrowth [93]. Therefore, Dock3-induced axon extension requires translocation of Dock3 to the plasma membrane in both TrkB-Fyn and TrkB-RhoG pathways. These observations suggest that the effects of BDNF on axon regeneration can be explained, at least partly, by Dock3-induced Rac1 activation and actin rearrangement via multiple pathways.

Furthermore, Dock3 binds to and inactivates glycogen synthase kinase-3β (GSK-3β) and enhances BDNF-dependent axonal outgrowth [94]. In recent years, GSK-3β emerged as a master regulator of microtubule dynamics in growth cones; it phosphorylates several substrates including collapsin response mediator proteins (CRMPs), adenomatous polyposis coli (APC), and cytoplasmic linker-associated proteins [95–97]. Inactive GSK-3β allows the interaction of CRMP-2 and APC to tubulin filaments and thus promotes microtubule polymerization and induces axonal outgrowth, suggesting that Dock3-mediated GSK-3β inactivation also regulates the formation and elongation of axons via microtubule dynamics.

The observations that Dock3 signaling is involved in both actin- and microtubule-based axon elongations suggest a significant role of Dock3 in axon regeneration (Fig. 23.2). Indeed, Dock3 null mice exhibit axon degeneration and sensorimotor impairments [98]. Dock3 was initially identified as a binding protein of presenilin, which is associated with γ-secretase activity in β-amyloid precursor protein (APP) processing [99]. Dock3 overexpression induces suppression of Aβ secretion by promoting APP degradation [100]. Since Dock3 was accumulated in neurofibrillary tangles and decreased in soluble fraction of brain extract from Alzheimer's disease patient compared to age-matched controls [99, 101], it was hypothesized that the decreased level of Dock3 may accelerate the pathogenesis of Alzheimer's disease via axon degeneration.

23.4 Conclusions and Future Directions

Recent studies revealed that injured CNS neurons possess regrowth activity, like PNS neurons, provided that they are exposed to environmentally suitable growth conditions. Investigation into optic nerve regeneration offers critical information for developing novel therapies for optic nerve disorders, such as glaucoma, optic neuritis, and traumatic optic neuropathies by traffic accidents. Accumulated

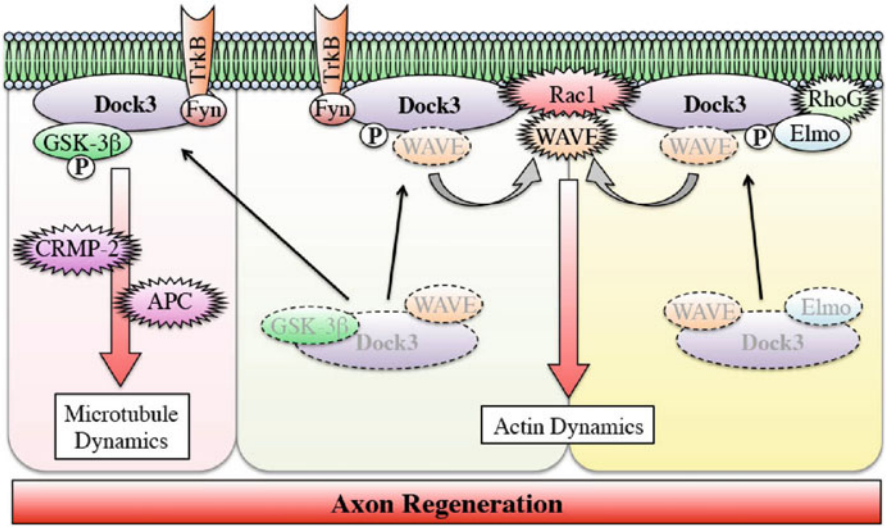


Fig. 23.2 The role of Dock3 in axonal regeneration. Dock3 recruits GSK-3β to the plasma membrane by BDNF-TrkB signaling, where GSK-3β is phosphorylated and inactivated. Inactivation of GSK-3β results in an increased amount of the nonphosphorylated active form of CRMP2 and APC, leading to stimulation of microtubule dynamics (*left* signal pathway). In addition, Dock3 also recruits WAVE to the plasma membrane, which allows Dock3 to activate Rac1 at the plasma membrane. Activated Rac1 and WAVE are dissociated from phosphorylated Dock3 and stimulate actin reorganization (*middle* signal pathway). In addition to TrkB signaling, Dock3-Elmo complex is recruited to the plasma membrane by forming a ternary complex with activated RhoG. Here, Dock3 is phosphorylated and thus Rac1 is efficiently activated, leading to further stimulation of actin dynamics (*right* signal pathway)

evidence indicates that combinatorial treatment is more effective for axon regeneration than manipulating a single factor. Therefore, it is important to discover the best combination of multiple factors that allow not just axon regeneration but also functional recovery.

References

1. Chen MS, Huber AB, van der Haar ME et al (2000) Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403:434–439
2. GrandPre T, Nakamura F, Vartanian T et al (2000) Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 403:439–444
3. Prinjha R, Moore SE, Vinson M et al (2000) Inhibitor of neurite outgrowth in humans. Nature 403:383–384

4. Wang KC, Koprivica V, Kim JA et al (2002) Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 417:941–944
5. Huber AB, Weinmann O, Brosamle C et al (2002) Patterns of Nogo mRNA and protein expression in the developing and adult rat and after CNS lesions. J Neurosci 22:3553–3567
6. Fournier AE, GrandPre T, Strittmatter SM (2001) Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 409:341–346
7. Oertle T, Schwab ME (2003) Nogo and its paRTNers. Trends Cell Biol 13:187–194
8. Lai C, Watson JB, Bloom FE et al (1987) Neural protein 1B236/myelin-associated glycoprotein (MAG) defines a subgroup of the immunoglobulin superfamily. Immunol Rev 100:129–151
9. Bartsch U, Bandtlow CE, Schnell L et al (1995) Lack of evidence that myelin-associated glycoprotein is a major inhibitor of axonal regeneration in the CNS. Neuron 15:1375–1381
10. Johnson PW, Abramow-Newerly W, Seilheimer B et al (1989) Recombinant myelin-associated glycoprotein confers neural adhesion and neurite outgrowth function. Neuron 3:377–385
11. DeBellard ME, Tang S, Mukhopadhyay G et al (1996) Myelin-associated glycoprotein inhibits axonal regeneration from a variety of neurons via interaction with a sialoglycoprotein. Mol Cell Neurosci 7:89–101
12. Mikol DD, Stefansson K (1988) A phosphatidylinositol-linked peanut agglutinin-binding glycoprotein in central nervous system myelin and on oligodendrocytes. J Cell Biol 106:1273–1279
13. Habib AA, Marton LS, Allwardt B et al (1998) Expression of the oligodendrocyte-myelin glycoprotein by neurons in the mouse central nervous system. J Neurochem 70:1704–1711
14. Lee JK, Case LC, Chan AF et al (2009) Generation of an OMgp allelic series in mice. Genesis 47:751–756
15. Ji B, Case LC, Liu K et al (2008) Assessment of functional recovery and axonal sprouting in oligodendrocyte-myelin glycoprotein (OMgp) null mice after spinal cord injury. Mol Cell Neurosci 39:258–267
16. Domeniconi M, Cao Z, Spencer T et al (2002) Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. Neuron 35:283–290
17. Liu BP, Fournier A, GrandPre T et al (2002) Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. Science 297:1190–1193
18. Wang KC, Kim JA, Sivasankaran R et al (2002) P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. Nature 420:74–78
19. Zheng B, Atwal J, Ho C et al (2005) Genetic deletion of the Nogo receptor does not reduce neurite inhibition in vitro or promote corticospinal tract regeneration in vivo. Proc Natl Acad Sci U S A 102:1205–1210
20. Dickendesher TL, Baldwin KT, Mironova YA et al (2012) NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. Nat Neurosci 15:703–712
21. David S, Fry EJ, Lopez-Vales R (2008) Novel roles for Nogo receptor in inflammation and disease. Trends Neurosci 31:221–226
22. Fry EJ, Ho C, David S (2007) A role for Nogo receptor in macrophage clearance from injured peripheral nerve. Neuron 53:649–662
23. Mi S, Lee X, Shao Z et al (2004) LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci 7:221–228
24. Park JB, Yiu G, Kaneko S et al (2005) A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. Neuron 45:345–351
25. Shao Z, Browning JL, Lee X et al (2005) TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. Neuron 45:353–359
26. Atwal JK, Pinkston-Gosse J, Syken J et al (2008) PirB is a functional receptor for myelin inhibitors of axonal regeneration. Science 322:967–970

27. Cai D, Shen Y, De Bellard M et al (1999) Prior exposure to neurotrophins blocks inhibition of axonal regeneration by MAG and myelin via a cAMP-dependent mechanism. Neuron 22:89–101
28. Hall A, Lalli G (2010) Rho and Ras GTPases in axon growth, guidance, and branching. Cold Spring Harb Perspect Biol 2:a001818
29. Lehmann M, Fournier A, Selles-Navarro I et al (1999) Inactivation of Rho signaling pathway promotes CNS axon regeneration. J Neurosci 19:7537–7547
30. Bertrand J, Winton MJ, Rodriguez-Hernandez N et al (2005) Application of Rho antagonist to neuronal cell bodies promotes neurite growth in compartmented cultures and regeneration of retinal ganglion cell axons in the optic nerve of adult rats. J Neurosci 25:1113–1121
31. Fischer D, Petkova V, Thanos S et al (2004) Switching mature retinal ganglion cells to a robust growth state in vivo: gene expression and synergy with RhoA inactivation. J Neurosci 24:8726–8740
32. Lingor P, Teusch N, Schwarz K et al (2007) Inhibition of Rho kinase (ROCK) increases neurite outgrowth on chondroitin sulphate proteoglycan in vitro and axonal regeneration in the adult optic nerve in vivo. J Neurochem 103:181–189
33. Lingor P, Tonges L, Pieper N et al (2008) ROCK inhibition and CNTF interact on intrinsic signalling pathways and differentially regulate survival and regeneration in retinal ganglion cells. Brain 131:250–263
34. Ahmed Z, Berry M, Logan A (2009) ROCK inhibition promotes adult retinal ganglion cell neurite outgrowth only in the presence of growth promoting factors. Mol Cell Neurosci 42:128–133
35. Marino S, Krimpenfort P, Leung C et al (2002) PTEN is essential for cell migration but not for fate determination and tumourigenesis in the cerebellum. Development 129:3513–3522
36. Cantrup R, Dixit R, Palmesino E et al (2012) Cell-type specific roles for PTEN in establishing a functional retinal architecture. PLoS One 7:e32795
37. Drinjakovic J, Jung H, Campbell DS et al (2010) E3 ligase Nedd4 promotes axon branching by downregulating PTEN. Neuron 65:341–357
38. Park KK, Liu K, Hu Y et al (2008) Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 322:963–966
39. Sun F, Park KK, Belin S et al (2011) Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. Nature 480:372–375
40. Ebadi M, Bashir RM, Heidrick ML et al (1997) Neurotrophins and their receptors in nerve injury and repair. Neurochem Int 30:347–374
41. LaVail MM, Unoki K, Yasumura D et al (1992) Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. Proc Natl Acad Sci U S A 89:11249–11253
42. Harada T, Harada C, Kohsaka S et al (2002) Microglia-Müller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. J Neurosci 22:9228–9236
43. Harada C, Harada T, Quah HM et al (2005) Role of neurotrophin-4/5 in neural cell death during retinal development and ischemic retinal injury in vivo. Invest Ophthalmol Vis Sci 46:669–673
44. Harada C, Guo X, Namekata K et al (2011) Glia- and neuron-specific functions of TrkB signalling during retinal degeneration and regeneration. Nat Commun 2:189
45. Parada LF, Tsoulfas P, Tessarollo L et al (1992) The Trk family of tyrosine kinases: receptors for NGF-related neurotrophins. Cold Spring Harb Symp Quant Biol 57:43–51
46. Greene LA, Kaplan DR (1995) Early events in neurotrophin signalling via Trk and p75 receptors. Curr Opin Neurobiol 5:579–587
47. Harada T, Harada C, Nakayama N et al (2000) Modification of glial-neuronal cell interactions prevents photoreceptor apoptosis during light-induced retinal degeneration. Neuron 26:533–541

48. Bredesen DE, Rabizadeh S (1997) p75NTR and apoptosis: Trk-dependent and Trk-independent effects. Trends Neurosci 20:287–290
49. Nakamura K, Namekata K, Harada C et al (2007) Intracellular sortilin expression pattern regulates proNGF-induced naturally occurring cell death during development. Cell Death Differ 14:1552–1554
50. Harada C, Harada T, Nakamura K et al (2006) Effect of p75NTR on the regulation of naturally occurring cell death and retinal ganglion cell number in the mouse eye. Dev Biol 290:57–65
51. Harada T, Harada C, Parada LF (2007) Molecular regulation of visual system development: more than meets the eye. Genes Dev 21:367–378
52. Mey J, Thanos S (1993) Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. Brain Res 602:304–317
53. Cohen A, Bray GM, Aguayo AJ (1994) Neurotrophin-4/5 (NT-4/5) increases adult rat retinal ganglion cell survival and neurite outgrowth in vitro. J Neurobiol 25:953–959
54. Mansour-Robaey S, Clarke DB, Wang YC et al (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A 91:1632–1636
55. Pernet V, Di Polo A (2006) Synergistic action of brain-derived neurotrophic factor and lens injury promotes retinal ganglion cell survival, but leads to optic nerve dystrophy in vivo. Brain 129:1014–1026
56. Sievers J, Hausmann B, Unsicker K et al (1987) Fibroblast growth factors promote the survival of adult rat retinal ganglion cells after transection of the optic nerve. Neurosci Lett 76:157–162
57. Koeberle PD, Ball AK (1998) Effects of GDNF on retinal ganglion cell survival following axotomy. Vision Res 38:1505–1515
58. Koeberle PD, Ball AK (2002) Neurturin enhances the survival of axotomized retinal ganglion cells in vivo: combined effects with glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor. Neuroscience 110:555–567
59. Yin Y, Cui Q, Li Y et al (2003) Macrophage-derived factors stimulate optic nerve regeneration. J Neurosci 23:2284–2293
60. Correale J, Villa A (2004) The neuroprotective role of inflammation in nervous system injuries. J Neurol 251:1304–1316
61. Butovsky O, Ziv Y, Schwartz A et al (2006) Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cell Neurosci 31:149–160
62. Leroy K, Yilmaz Z, Brion JP (2007) Increased level of active GSK-3beta in Alzheimer's disease and accumulation in argyrophilic grains and in neurones at different stages of neurofibrillary degeneration. Neuropathol Appl Neurobiol 33:43–55
63. Lorber B, Berry M, Logan A (2005) Lens injury stimulates adult mouse retinal ganglion cell axon regeneration via both macrophage- and lens-derived factors. Eur J Neurosci 21:2029–2034
64. Fischer D, Pavlidis M, Thanos S (2000) Cataractogenic lens injury prevents traumatic ganglion cell death and promotes axonal regeneration both in vivo and in culture. Invest Ophthalmol Vis Sci 41:3943–3954
65. Leon S, Yin Y, Nguyen J et al (2000) Lens injury stimulates axon regeneration in the mature rat optic nerve. J Neurosci 20:4615–4626
66. Fischer D, He Z, Benowitz LI (2004) Counteracting the Nogo receptor enhances optic nerve regeneration if retinal ganglion cells are in an active growth state. J Neurosci 24:1646–1651
67. Lazarov-Spiegler O, Solomon AS, Schwartz M (1998) Peripheral nerve-stimulated macrophages simulate a peripheral nerve-like regenerative response in rat transected optic nerve. Glia 24:329–337
68. Lazarov-Spiegler O, Solomon AS, Zeev-Brann AB et al (1996) Transplantation of activated macrophages overcomes central nervous system regrowth failure. FASEB J 10:1296–1302

69. Rapalino O, Lazarov-Spiegler O, Agranov E et al (1998) Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. Nat Med 4:814–821
70. Berry MJ 2nd, Brivanlou IH, Jordan TA et al (1999) Anticipation of moving stimuli by the retina. Nature 398:334–338
71. Berry M, Carlile J, Hunter A (1996) Peripheral nerve explants grafted into the vitreous body of the eye promote the regeneration of retinal ganglion cell axons severed in the optic nerve. J Neurocytol 25:147–170
72. Okada T, Ichikawa M, Tokita Y et al (2005) Intravitreal macrophage activation enables cat retinal ganglion cells to regenerate injured axons into the mature optic nerve. Exp Neurol 196:153–163
73. Yin Y, Henzl MT, Lorber B et al (2006) Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. Nat Neurosci 9:843–852
74. Yin Y, Cui Q, Gilbert HY et al (2009) Oncomodulin links inflammation to optic nerve regeneration. Proc Natl Acad Sci U S A 106:19587–19592
75. Kurimoto T, Yin Y, Habboub G et al (2013) Neutrophils express oncomodulin and promote optic nerve regeneration. J Neurosci 33:14816–14824
76. de Lima S, Koriyama Y, Kurimoto T et al (2012) Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. Proc Natl Acad Sci U S A 109:9149–9154
77. Hirai S, de Cui F, Miyata T et al (2006) The c-Jun N-terminal kinase activator dual leucine zipper kinase regulates axon growth and neuronal migration in the developing cerebral cortex. J Neurosci 26:11992–12002
78. Ghosh AS, Wang B, Pozniak CD et al (2011) DLK induces developmental neuronal degeneration via selective regulation of proapoptotic JNK activity. J Cell Biol 194:751–764
79. Itoh A, Horiuchi M, Wakayama K et al (2011) ZPK/DLK, a mitogen-activated protein kinase kinase kinase, is a critical mediator of programmed cell death of motoneurons. J Neurosci 31:7223–7228
80. Watkins TA, Wang B, Huntwork-Rodriguez S et al (2013) DLK initiates a transcriptional program that couples apoptotic and regenerative responses to axonal injury. Proc Natl Acad Sci U S A 110:4039–4044
81. Hammarlund M, Nix P, Hauth L et al (2009) Axon regeneration requires a conserved MAP kinase pathway. Science 323:802–806
82. Yan D, Wu Z, Chisholm AD et al (2009) The DLK-1 kinase promotes mRNA stability and local translation in *C. elegans* synapses and axon regeneration. Cell 138:1005–1018
83. Xiong X, Wang X, Ewanek R et al (2010) Protein turnover of the Wallenda/DLK kinase regulates a retrograde response to axonal injury. J Cell Biol 191:211–223
84. Shin JE, Cho Y, Beirowski B et al (2012) Dual leucine zipper kinase is required for retrograde injury signaling and axonal regeneration. Neuron 74:1015–1022
85. Matsuzawa A, Saegusa K, Noguchi T et al (2005) ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity. Nat Immunol 6:587–592
86. Katome T, Namekata K, Guo X et al (2013) Inhibition of ASK1-p38 pathway prevents neural cell death following optic nerve injury. Cell Death Differ 20:270–280
87. Kikuchi M, Tenneti L, Lipton SA (2000) Role of p38 mitogen-activated protein kinase in axotomy-induced apoptosis of rat retinal ganglion cells. J Neurosci 20:5037–5044
88. Harada C, Nakamura K, Namekata K et al (2006) Role of apoptosis signal-regulating kinase 1 in stress-induced neural cell apoptosis in vivo. Am J Pathol 168:261–269
89. Harada C, Namekata K, Guo X et al (2010) ASK1 deficiency attenuates neural cell death in GLAST-deficient mice, a model of normal tension glaucoma. Cell Death Differ 17:1751–1759
90. Guo X, Harada C, Namekata K et al (2010) Regulation of the severity of neuroinflammation and demyelination by TLR-ASK1-p38 pathway. EMBO Mol Med 2:504–515

91. Namekata K, Enokido Y, Iwasawa K et al (2004) MOCA induces membrane spreading by activating Rac1. J Biol Chem 279:14331–14337
92. Namekata K, Harada C, Taya C et al (2010) Dock3 induces axonal outgrowth by stimulating membrane recruitment of the WAVE complex. Proc Natl Acad Sci U S A 107:7586–7591
93. Namekata K, Watanabe H, Guo X et al (2012) Dock3 regulates BDNF-TrkB signaling for neurite outgrowth by forming a ternary complex with Elmo and RhoG. Genes Cells 17:688–697
94. Namekata K, Harada C, Guo X et al (2012) Dock3 stimulates axonal outgrowth via GSK-3beta-mediated microtubule assembly. J Neurosci 32:264–274
95. Yoshimura T, Kawano Y, Arimura N et al (2005) GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. Cell 120:137–149
96. Kim WY, Zhou FQ, Zhou J et al (2006) Essential roles for GSK-3s and GSK-3-primed substrates in neurotrophin-induced and hippocampal axon growth. Neuron 52:981–996
97. Kumar P, Lyle KS, Gierke S et al (2009) GSK3beta phosphorylation modulates CLASP-microtubule association and lamella microtubule attachment. J Cell Biol 184:895–908
98. Chen Q, Peto CA, Shelton GD et al (2009) Loss of modifier of cell adhesion reveals a pathway leading to axonal degeneration. J Neurosci 29:118–130
99. Kashiwa A, Yoshida H, Lee S et al (2000) Isolation and characterization of novel presenilin binding protein. J Neurochem 75:109–116
100. Chen Q, Kimura H, Schubert D (2002) A novel mechanism for the regulation of amyloid precursor protein metabolism. J Cell Biol 158:79–89
101. Chen Q, Yoshida H, Schubert D et al (2001) Presenilin binding protein is associated with neurofibrillary alterations in Alzheimer's disease and stimulates tau phosphorylation. Am J Pathol 159:1597–1602