Photoreceptor rescue

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Abstract

Photoreceptor cell death is the final, irreversible event in many blinding diseases including retinitis pigmentosa, age-related macular disease and retinal detachment. This paper examines the potential strategies for preventing photoreceptor cell death in the context of current understanding of the mechanisms of cell death. There is evidence to suggest that photoreceptor cells are inherently vulnerable, apoptosis is the final common pathway of photoreceptor cell loss, and other retinal cells play an important role in the survival of rods and cones. Furthermore, the rationale of using neurotrophic factors as therapeutic agents in retinal degeneration is discussed in detail. Photoreceptor rescue by manipulation of genes involved in apoptosis and some pharmacological agents is also described.

Key words Apoptosis, Gene manipulation, Neurotrophic factors, Photoreceptor, Retinal degeneration, Retinitis pigmentosa

The aim of this paper is to discuss potential strategies for preventing photoreceptor cell death in the context of current concepts of the mechanisms of cell death. As retinal transplantation and gene therapy are discussed elsewhere in this issue, growth factor therapy is covered in most detail.

Photoreceptor cell death is the final, irreversible event in many blinding diseases including retinitis pigmentosa, age-related macular disease and retinal detachment. Particularly in chronic, progressive disease, a modest reduction in the rate of photoreceptor cell loss might lead to a significant prolongation of useful vision, and over the last few years a number of developments have raised hopes that clinically effective treatments may become available during the next decade.

Inherent vulnerability of photoreceptor cells

The combination of several pieces of indirect evidence makes it likely that photoreceptor cells are inherently vulnerable. Firstly, it is remarkable how many different mutations and acquired insults lead to photoreceptor cell

death.¹ Secondly, there is a wave of cell death in post-mitotic photoreceptor cells when metabolic activity in the retina increases.² This, together with the high focal concentrations of mitochondria in photoreceptors and the very high blood supply to the choriocapillaris, suggests that there may be little reserve in the energy supply to photoreceptor cells.³ The complex inter-relationships between rods, cones, Müller cells and retinal pigment epithelium (RPE) add to the vulnerability in that failure of other cell types then leads to secondary photoreceptor cell loss. It seems possible, therefore, that photoreceptors live on a 'knife-edge' and that relatively mild insult can significantly increase the probability of cell death. One correlate of this is that the converse may also operate. That is, a therapy offering a relatively modest benefit to the cell may reduce the chances of cell death sufficiently to have an important clinical impact. This is especially likely to be the case in chronic, slowly progressive disorders such as retinitis pigmentosa.

Apoptosis in photoreceptor cells

Major advances have been made in our understanding of apoptosis over the last few years. Much of the detailed biochemistry of the final stages of the apoptotic cascade has been elucidated and apoptotic cell death (ACD) has been documented in a wide range of circumstances. In fact, it is now clear that ACD occurs in virtually every cell type, if not all cells, that have been studied appropriately. It is also clear that most pathological insults can provoke ACD if delivered at a dose below that expected to cause acute, necrotic cell death. Increasingly, there are reports of necrosis and apoptosis occurring side by side, and it is now clear that ACD is not a specific process. In line with this, apoptosis has been described in a wide variety of hereditary retinal degenerations,⁴⁻⁷ in light damage⁸ and following retinal detachment^{9,10} and in other types of retinal degeneration.¹¹

Substantially less is known about the specific upstream events leading to activation of the apoptotic cascade. However, recent observations demonstrating the importance of mitochondrial function and the high metabolic demands of rods make it likely that loss of

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N.H.V.C. is supported by a
MRC Clinical Training
Fellowship

calcium homeostasis, free radical damage and any other process leading to mitochondrial failure may be of significance. It remains unclear, however, why mutations in rhodopsin and other transduction proteins result in cell death.

The key role of retinal cell–cell interactions in photoreceptor cell survival

As discussed above, photoreceptors are in close anatomical and functional relationship with their neighbouring cells. The importance of the RPE is clear and perhaps most evident in the Royal College of Surgeons (RCS) rat, in which failure of phagocytosis of rod outer segments leads to marked outer retinal atrophy. Direct evidence for the significance of Müller cells is less abundant, although in a mouse with a mutation of AMOG (adhesion molecule of glia)¹² photoreceptor loss occurs secondary to glial failure. Finally, photoreceptors depend on each other for survival. Rod loss in diffuse retinitis pigmentosa (RP) is followed by cone loss, and in certain macular dystrophies the opposite holds true.

Raff focused attention on the importance of growth factors as intercellular mediators of cell survival.^{13,14} Most of his observations related primarily to cell populations in development, but it is clear that similar relationships exist in many adult tissues. Although there is little to suggest that any human photoreceptor cell degeneration is primarily due to growth factor failure, higher levels of growth factors have, in certain circumstances, the capacity to protect cells and much experimentation has addressed this mode of rescue in photoreceptors.

Cellular targets for photoreceptor rescue

Even though much remains to be learnt about the detailed pathogenesis of photoreceptor cell death, there are several potential cellular targets for rescue. At the cell membrane growth factor interactions with their receptors are important determinants of cell survival and these are discussed at length below. Ion homeostasis is also critical for all neuronal cells and a small number of studies have explored the capacity of calcium channel blockers to protect photoreceptors. Sustained increases in the levels of free calcium within cells lead to the activation of numerous potentially toxic enzyme systems and, importantly, can be toxic to mitochondria, leading to a vicious cycle of energy failure, free radical generation, further mitochondrial toxicity and loss of calcium homeostasis.

An important strategy for inherited degeneration is to replace non-functional proteins, although this may not be an effective approach in autosomal dominant disease. A more generic option would be to manipulate the machinery controlling apoptosis by, for instance, over-expressing genes such as *bcl-2* which protects from apoptosis, or reducing the expression of a gene such as *p53* which is known to be important in the execution of

the apoptotic programme in some cell–insult combinations.^{15,16} The appeal of interfering with the apoptotic pathway is that, given the ubiquity of apoptosis in chronic cell death, a single therapy might be effective for a very large number of conditions. Unfortunately, therapies operating so far downstream may be acting too late to do anything other than temporarily slow disease, and in early studies this appears to be the case.

Photoreceptor rescue, retinal injury and growth factors

An unexpected finding was observed during the early attempts at retinal transplantation for photoreceptor rescue. Focal injury to the retina appeared to protect photoreceptors from degeneration. This was clearly illustrated in the RCS rat, when mechanical injury produced by an injection of saline into the subretinal or into the vitreous, or even insertion of a needle without injection, led to protection of photoreceptors near the wound.^{17–19} This protection was not restricted to genetically determined retinal degeneration. Similar photoreceptor rescue by mechanical injury was observed in the light-induced retinal damage in the rat,²⁰ although interestingly this and related strategies are less effective in the mouse. Furthermore, this is not restricted to photoreceptor cells, as a similar self-protective mechanism appears to exist for retinal ganglion cells.²¹ These findings imply that the retina has a self-protective mechanism that can be activated to protect, at least partially, photoreceptors and ganglion cells from damage or death. Injury-induced photoreceptor rescue extends beyond the immediate vicinity of the lesion, suggesting that soluble factors are involved.^{17,18,22} As mechanical injury to the eye increases the expression of basic fibroblast growth factor (bFGF) and ciliary neurotrophic factor (CNTF) in the rat retina²³ it was logical to assume that these agents might be responsible for the protection and to explore the effects of growth factor injection on photoreceptor cell survival.

Neurotrophic factors

There are at least four families of neurotrophic factors that might be important in photoreceptor rescue: fibroblast growth factors such as bFGF, cytokine-associated neurotrophic factor such as CNTF, neurotrophins such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factors (IGF) such as IGF-I and IGF-II.

bFGF is a member of the fibroblast growth factor (FGF) family, a group of heparin-binding, single-chain polypeptides about 150–300 amino acids in length. They have a number of important roles in physiological and pathological processes as diverse as cell growth, differentiation, angiogenesis, tissue repair and transformation. At present, nine members in the FGF family are recognised. The first characterised members of the FGF family were acidic FGF (aFGF, FGF-1) and basic FGF (bFGF, FGF-2). They were purified as mitogens for

fibroblasts. The Int-2 (FGF-2), K-FGF (FGF-4), FGF-5, FGF-6, keratinocyte growth factor (KGF, FGF-7), androgen-induced growth factor (AIGF, FGF-8) and glial-activating factor (GAF, FGF-9) have subsequently been identified. These proteins share 30–70% amino acid sequence identity with each other.²⁴ In the eye, bFGF-labelled cells are present in the photoreceptor, inner nuclear and ganglion cell layers. It appears that bFGF is not only expressed by glial cells (Müller cells and astrocytes) but also by rod photoreceptors and subpopulations of cone bipolar cells and amacrine cells. It is, however, not expressed in cones.²⁵ The increase in bFGF expression after mechanical injury is generally rapid, but focal laser photocoagulation does not always increase bFGF expression.²⁶

CNTF was discovered and named for its actions on the parasympathetic neurons of the ciliary ganglia from chick embryos. A variety of actions has subsequently been identified. In its primary sequence, CNTF does not bear strong homology with any known protein. The tertiary structure of CNTF and its receptor are, however, similar to that of leukaemia inhibitory factor (LIF) and interleukin-6 (IL-6),²⁷ and hence it is often referred to as the cytokine associated neurotrophic factor. Human CNTF is a polypeptide with 200 amino acids and its gene has been mapped to chromosome 11q12.2.²⁸ It is synthesised by Schwann cells and there is a high concentration of CNTF in human optic nerve. It is, however, curious that CNTF is poorly secreted and usually appears to remain within its cell of synthesis. The mechanisms controlling CNTF synthesis and secretion are largely unknown, but it appears that the initial response to injury is the release of the CNTF within these cells followed by the upregulation of CNTF mRNA. This may explain the relatively slow rise of CNTF mRNA after mechanical injury.²⁷ Interestingly, bFGF release can lead to an increase in bFGF mRNA expression.²⁹

BDNF is a member of the neurotrophin family. This family binds to members of a group of transmembrane tyrosine kinase (Trk) receptors and other neurotrophins include nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5).³⁰ They have a wide range of cellular functions in the developing, mature and injured nervous system³⁰ and are known to be expressed by glial as well as neuronal cells. Although the function of the neurotrophins expressed by nerve cells has not been fully defined, the co-expression of some of these factors and their receptors in the same neurons suggests an autocrine or paracrine role.³⁰ At least *in vitro*, cortical neurons secrete BDNF in response to the activation of a voltage-sensitive calcium channel, which suggests that neuronal activity could influence neurotrophin expression. The expression of BDNF in the eye is not well characterised. However, the finding that the expression of BDNF mRNA was slightly reduced in the rat retina²³ and brain³¹ following mechanical injury was unexpected.

Insulin-like growth factors (IGF-I and IGF-II) are important for cell proliferation, differentiation and sustained survival of many tissues throughout the body

including the eye. IGF-I mRNA is expressed in retinal ganglion cells and endothelial cells of the choroid and ciliary processes. The IGF-I receptor mRNA has a more extensive distribution, which includes the retinal ganglion cell layer and inner nuclear layer.^{32,33} The IGF-I receptor gene is homologous to the *sevenless* gene in *Drosophila*, where it is essential for the differentiation of photoreceptor seven. There is also evidence to suggest that cone photoreceptors can produce IGF-I, which strongly influences the production of rod photoreceptors during development.³⁴ The exact role of these factors in the mature retina is less certain but, interestingly, scatter laser photocoagulation can increase the intravitreal levels of IGF-I in the miniature pig.²⁶

Growth factor photoreceptor rescue experiments to date

The first *in vivo* experimental success of the therapeutic use of growth factors in photoreceptor rescue was the use of bFGF in the RCS rat. Although the sham-injected eyes displayed localised photoreceptor rescue, there was significantly more survival in the eyes treated with bFGF, either intravitreally or subretinally.²² In the light-damaged model of retinal degeneration, a high degree of photoreceptor rescue was present with bFGF, BDNF, CNTF, midkine, interleukin-1 beta and aFGF. Lesser protection was seen with NT3, IGF-II and tumour necrosis factor alpha. NGF, epidermal growth factor, platelet-derived growth factor, insulin, IGF-1, heparin and laminin were without significant effect.^{20,35–38}

CNTF or its derivatives delay photoreceptor cell loss in retinal degeneration (*rd*) Q344ter mutant rhodopsin transgenic mice³⁹ and retinal dystrophy (*Rdy*) cats.⁴⁰ BDNF promotes outer segment formation in a feline model of retinal detachment.⁴¹ In most studies growth factors have been delivered by intraocular injection of the recombinant protein. CNTF has, however, also been successfully delivered by adenovirus gene therapy.⁴²

Growth factor rescue is not specific for photoreceptor cells. There are many examples within the brain and in ischaemia-induced retinal injury, intravitreal injections of bFGF, BDNF or CNTF 2 days before the ischaemia transiently protects retinal ganglion cells. Post-ischaemia injection of BDNF can prolong the protective effect. Furthermore, BDNF injection up to 3 days after the ischaemia alone can also protect the inner retina. The retrograde degeneration of the optic nerve after a crush lesion can also be prevented by BDNF and to a lesser extent by NT-3 or CNTF, but not by NGF, aFGF or bFGF.^{43,44}

There is increasing evidence to suggest a degree of specificity for different combinations of neurotrophic factors, cell type and mode of injury. For instance, CNTF protects oligodendrocytes from tumour necrosis factor (TNF)-mediated injury as well as serum-deprivation-induced apoptosis, but it does not protect fetal cortical neurons or glioma cell lines from the same insults.⁴⁵ Similarly, bFGF is effective in photoreceptor rescue in the RCS and light-damaged rats but as yet no effect has been demonstrated in the *rd* mouse. There is also an

impression that the retinal ganglion cells appear to be better protected by BDNF, whilst the photoreceptors appear to be better protected by bFGF and CNTF. Furthermore, neurotrophic factors have a synergistic effect in neuronal survival *in vitro*,^{46–51} and although this has not been demonstrated in an *in vivo* study of retinal degeneration it seems probable that a cocktail of growth factors may provide optimum rescue. Unfortunately, such a cocktail may also increase the likelihood of serious side-effects.

Potential side-effects of growth factor therapy

In our studies of photoreceptor cell rescue in the autosomal dominant *Rdy* cat⁴⁰ we have noticed two main problems. Firstly, all animals treated with monthly doses of a CNTF analogue developed posterior subcapsular cataracts. Secondly, especially in animals where therapy started early in life, retinal folds have formed. This finding has also been noted in other models and various studies are in progress to ascertain how to prevent this problem. Clearly in long-term clinical use there will be the possibility of generating neovascularisation or even proliferative vitreoretinopathy if subretinal injections are employed, and the more precisely the biological effect is targeted towards photoreceptors the better.

Photoreceptor rescue by manipulation of genes involved in apoptosis

Following the critical discovery of genes controlling developmental cell death in the nematode worm there has been a remarkably rapid increase in the understanding of several families of genes whose expression is associated with either promotion or inhibition of apoptosis.^{15,16,52,53} Part of the excitement associated with these discoveries was the possibility that by either enhancing the expression of 'survival' genes or inhibiting expression of 'death' genes it would be possible to rescue cells otherwise destined to die. The *bcl-2* gene family contains several different members, some of which promote cell survival and others of which hasten death. It appears as though the ratio of the two 'forces' in a single cell determines the likelihood of cell death. *Bcl-2* was the first member to be studied and it was shown to be a survival gene. Early results from experiments in which the *bcl-2* gene was over-expressed in transgenic animals were promising.⁵⁴ However, it is now clear that not all degeneration can be treated in this way,⁵⁵ or that protection is only temporary.⁵⁶ Perhaps this is not too surprising as *bcl-2* is likely to operate at a relatively downstream point and as the upstream, primary pathology develops the cell is likely to fail via other routes. The converse approach is to reduce expression of a gene required for apoptosis. *p53* is, in some circumstances, pro-apoptotic and in recent studies (unpublished observations) crossing mice with a targeted disruption of the *p53* gene that, as a result expressed no

functional protein, with the *rds* mouse led to delay, but again not prevention, of photoreceptor cell death by apoptosis.

Members of the immediate early gene family are more upstream targets that code for components of transcription factors. Immediate early genes increase their expression rapidly following a wide variety of 'insults' including light damage.⁵⁷ Expression of these genes is usually transient, but it is becoming clear that sustained expression is sometimes associated with nerve cell death. Interestingly, in a recent study, mice missing one of these genes, *c-fos*, were found to be more resistant to light damage than control animals expressing the protein.⁸ It will be interesting to see how sustained the rescue is in these animals.

Manipulation of apoptosis therefore provides a logical strategy for protecting photoreceptor cells, although sustained protection from pathological stimuli in adult animals may not be possible. Also, solving the problem of delivery of the genes to the cells will provide a major challenge to those developing gene therapy. Gene replacement, that is the expression of a previously malfunctioning mutant gene with a functional wild-type gene, and manipulation of the expression of apoptosis genes will require direct delivery to the appropriate retinal cell, typically the photoreceptor. This will present a major challenge. Where cellular targeting is less critical it may be possible to deliver genes in genetically engineered cells using a transplantation strategy.

Pharmacological photoreceptor cell rescue

Gene therapy and transplantation offer great potential in the future, but it may be that pharmacological intervention may be more practical, especially in the short to medium term. As mentioned above, ion channel blockers have been reported to have some benefit⁵⁸ and manipulation of dopaminergic pathways may also be of value.⁵⁹ It may also be possible to manipulate growth factor levels indirectly by pharmacological means. Recently, it has been shown that α -adrenergic drugs can increase retinal bFGF⁶⁰ and protect against light damage.

Conclusions

To date, despite enormous efforts, clinical success in the protection of central and peripheral neurons from genetic abnormalities or acquired insults has been limited. There is, however, now reason to believe that we are on the threshold of seeing effective, novel therapeutic strategies being implemented in the clinic.

References

1. Bird AC. Retinal photoreceptor dystrophies. *Am J Ophthalmol* 1995;119:543–62.
2. Maslim J, Stone J. Tissue oxygen is a factor in photoreceptor dystrophy: evidence from the RCS rat. *Invest Ophthalmol Vis Sci (Suppl)* 1996;37:686.

3. Cohen AI. The retina. In: Moses RA, Hart WM Jr, editors. Adler's physiology of the eye: clinical application. 8th ed. St Louis: CV Mosby, 1987:458–90.
4. Gregory CY, Bird AC. Cell loss in retinal dystrophies by apoptosis: death by informed consent! Br J Ophthalmol 1995;79:186–90.
5. Tso MO, Zhang C, Abler AS, *et al.* Apoptosis leads to photoreceptor degeneration in inherited retinal dystrophy of RCS rats. Invest Ophthalmol Vis Sci 1994;35:2693–9.
6. Portera-Cailliau C, Sung CH, Nathans J, Adler R. Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa. Proc Nat Acad Sci USA 1994;91:974–8.
7. Luthert PJ, Chong NHV, Barnett KC, Bird AC. An immunohistochemical study of the *Rdy* cat model of retinal dystrophy. Invest Ophthalmol Vis Sci (Suppl) 1997;38:1452.
8. Hafezi F, Steinbach JP, Marti A, *et al.* The absence of *c-fos* prevents light-induced apoptotic cell death of photoreceptors in retinal degeneration *in vivo*. Nature Med 1997;3:346–9.
9. Chang CJ, Lai WW, Edward DP, Tso MO. Apoptotic photoreceptor cell death after traumatic retinal detachment in humans. Arch Ophthalmol 1995;113:880–6.
10. Cook B, Lewis GP, Fisher SK, Adler R. Apoptotic photoreceptor degeneration in experimental retinal detachment. Invest Ophthalmol Vis Sci 1995;36:990–6.
11. Xu GZ, Li WW, Tso MO. Apoptosis in human retinal degenerations. Trans Am Ophthalmol Soc 1996;94:411–30.
12. Mølhagen M, Schachner M, Bartsch U. Apoptotic cell death of photoreceptor cells in mice deficient for the adhesion molecule on glia (AMOG, the beta 2-subunit of the Na,K-ATPase). J Neurocytol 1996;25:243–55.
13. Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD. Programmed cell death and the control of cell survival: lessons from the nervous system. Science 1993;262:695–700.
14. Raff MC. Social controls on cell survival and cell death. Nature 1992;356:397–400.
15. Kroemer G. The proto-oncogene *bcl-2* and its role in regulating apoptosis. Nature Med 1997;3:614–20.
16. Wyllie A. Clues in the p53 murder mystery. Nature 1997;389:237–8.
17. Alterio J, Courtois Y, Robelin J, Bechet D, Martelly I. Acidic and basic fibroblast growth factor mRNAs are expressed by skeletal muscle satellite cells. Biochem Biophys Res Commun 1990;166:1205–12.
18. Silverman MS, Hughes SE. Photoreceptor rescue in the RCS rat without pigment epithelium transplantation. Curr Eye Res 1990;9:183–91.
19. Li LX, Sheedlo HJ, Gaur V, Turner JE. Effects of macrophage and retinal pigment epithelial cell transplants on photoreceptor cell rescue in RCS rats. Curr Eye Res 1991;10:947–58.
20. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Basic fibroblast growth factor and local injury protect photoreceptors from light damage in the rat. J Neurosci 1992;12:3554–67.
21. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Nat Acad Sci USA 1994;91:1632–6.
22. Faktorovich EG, Steinberg RH, Yasumura D, Matthes MT, LaVail MM. Photoreceptor degeneration in inherited retinal dystrophy delayed by basic fibroblast growth factor. Nature 1990;347:83–6.
23. Wen R, Song Y, Cheng T, *et al.* Injury-induced upregulation of bFGF and CNTF mRNAs in the rat retina. J Neurosci 1995;15:7377–85.
24. Bernard O, Matthew P. Fibroblast growth factor (FGFs). In: Nicola NA, editor. Guidebook to cytokines and their receptors. Oxford: Oxford University Press, 1994:214–22.
25. Li ZY, Chang JH, Milam AH. A gradient of basic fibroblast growth factor in rod photoreceptors in the normal human retina. Vis Neurosci 1997;14:671–9.
26. Xiao M, Khaliq A, Moriarty P, McLeod D, Cranley J, Boulton M. The effect of scatter laser photocoagulation on intravitreal levels of growth factors in the miniature pig. Curr Eye Res 1996;15:923–31.
27. Richardson PM. Ciliary neurotrophic factor: a review. Pharmacol Ther 1994;63:187–98.
28. Lam A, Fuller F, Miller J, *et al.* Sequence and structural organization of the human gene encoding ciliary neurotrophic factor. Gene 1991;102:271–6.
29. Cao W, Wen R, Li F, Cheng T, Steinberg RH. Induction of basic fibroblast growth factor mRNA by basic fibroblast growth factor in Müller cells. Invest Ophthalmol Vis Sci 1997;38:1358–66.
30. Jelsma TN, Aguayo AJ. Trophic factors. Curr Opin Neurobiol 1994;4:717–25.
31. Ip NY, Wiegand SJ, Morse J, Rudge JS. Injury-induced regulation of ciliary neurotrophic factor mRNA in the adult rat brain. Eur J Neurosci 1993;5:25–33.
32. Burren CP, Berka JL, Batch JA. Localization studies of IGFBP-2 and IGFBP-5 in the anterior compartment of the eye. Curr Eye Res 1997;16:256–62.
33. Burren CP, Berka JL, Edmondson SR, Werther GA, Batch JA. Localization of mRNAs for insulin-like growth factor-I (IGF-I), IGF-I receptor, and IGF binding proteins in rat eye. Invest Ophthalmol Vis Sci 1996;37:1459–68.
34. Hoke KL, Fernald RD. Rod photoreceptor neurogenesis. Prog Retinal Eye Res 1997;16:31–49.
35. Unoki K, Ohba N, Arimura H, Muramatsu H, Muramatsu T. Rescue of photoreceptors from the damaging effects of constant light by midkine, a retinoic acid-responsive gene product. Invest Ophthalmol Vis Sci 1994;35:4063–8.
36. Masuda K, Watanabe I, Unoki K, Ohba N, Muramatsu T. Functional rescue of photoreceptors from the damaging effects of constant light by survival-promoting factors in the rat. Invest Ophthalmol Vis Sci 1995;36:2142–6.
37. LaVail MM, Unoki K, Yasumura D, Matthes MT, Yancopoulos GD, Steinberg RH. Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. Proc Nat Acad Sci USA 1992;89:11249–53.
38. LaVail MM, Faktorovich EG, Hepler JM, *et al.* Basic fibroblast growth factor protects photoreceptors from light-induced degeneration in albino rats. Ann NY Acad Sci 1991;638:341–7.
39. LaVail MM, Yasumura D, Matthes ME, Lau-Villacorta C, Sung CH, Steinberg RH. CNTF delays photoreceptor degeneration in *rd* and Q344ter mutant rhodopsin transgenic mice. Invest Ophthalmol Vis Sci (Suppl) 1996;37:2007.
40. Chong NHV, Waters L, Barnett KC, Bird AC, Luthert PJ. Axokine delays photoreceptor cell death in *Rdy* cats. Invest Ophthalmol Vis Sci (Suppl) 1997;38:1445.
41. Fisher SK, Guerin CJ, Linberg KA, Lewis GP. BDNF promotes outer segment regeneration in experimentally detached retina. Invest Ophthalmol Vis Sci (Suppl) 1995;36:963.
42. Cayouette M, Gravel C. Adenovirus-mediated gene transfer of ciliary neurotrophic factor can prevent photoreceptor degeneration in the retinal degeneration (*rd*) mouse. Hum Gene Ther 1997;8:423–30.
43. Weibel D, Kreutzberg GW, Schwab ME. Brain-derived neurotrophic factor (BDNF) prevents lesion-induced axonal die-back in young rat optic nerve. Brain Res 1995;679:249–54.
44. Mey J, Thanos S. Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats *in vivo*. Brain Res 1992;602:304–17.
45. DeSouza SD, Alinauskas KA, Antel JP. Ciliary neurotrophic factor selectively protects human oligodendrocytes from tumor necrosis factor-mediated injury. J Neurosci Res 1996;43:289–98.

46. Zurn AD, Winkel L, Menoud A, Djabali K, Aebischer P. Combined effects of GDNF, BDNF, and CNTF on motoneuron differentiation *in vitro*. *J Neurosci Res* 1996;44:133–41.
47. Mitsumoto H, Ikeda K, Klinkosz B, Cedarbaum JM, Wong V, Lindsay RM. Arrest of motor neuron disease in wobbler mice cotreated with CNTF and BDNF. *Science* 1994;265:1107–10.
48. Bianchi LM, Cohan CS. Effects of the neurotrophins and CNTF on developing statoacoustic neurons: Comparison with an otocyst-derived factor. *Dev Biol* 1993;159:353–65.
49. Hartnick CJ, Staecker H, Malgrange B, *et al.* Neurotrophic effects of BDNF and CNTF, alone and in combination, on postnatal day 5 rat acoustic ganglion neurons. *J Neurobiol* 1996;30:246–54.
50. Kato AC, Lindsay RM. Overlapping and additive effects of neurotrophins and CNTF on cultured human spinal cord neurons. *Exp Neurol* 1994;130:196–201.
51. Meyer-Franke A, Kaplan MR, Pfrieger FW, Barres BA. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron* 1995;15:805–19.
52. Hengartner MO, Horvitz HR. *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell* 1994;76:665–76.
53. Hengartner MO, Ellis RE, Horvitz HR. *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. *Nature* 1992;356:494–9.
54. Chen J, Flannery JC, LaVail MM, Steinberg RH, Xu J, Simon MI. *bcl-2* overexpression reduces apoptotic photoreceptor cell death in three different retinal degenerations. *Proc Nat Acad Sci USA* 1996;93:7042–7.
55. Joseph RM, Li T. Overexpression of *Bcl-2* or *Bcl-XL* transgenes and photoreceptor degeneration. *Invest Ophthalmol Vis Sci* 1996;37:2434–46.
56. Tsang SH, Chen J, Kjeldbye H, *et al.* Retarding photoreceptor degeneration in *Pdegtm1/Pdegtm1* mice by an apoptosis suppressor gene. *Invest Ophthalmol Vis Sci* 1997;38:943–50.
57. Rich KA, Zhan Y, Blanks JC. Aberrant expression of *c-fos* accompanies photoreceptor cell death in the *rd* mouse. *J Neurobiol* 1997;32:593–612.
58. Sahly I, Bar Nachum S, Suss Toby E, *et al.* Calcium channel blockers inhibit retinal degeneration in the retinal-degeneration-B mutant of *Drosophila*. *Proc Nat Acad Sci USA* 1992;89:435–9.
59. Bubenik GA, Purtill RA. The role of melatonin and dopamine in retinal physiology. *Can J Physiol Pharmacol* 1980;58:1457–62.
60. Wen R, Cheng T, Li Y, Cao W, Steinberg RH. Alpha 2-adrenergic agonists induce basic fibroblast growth factor expression in photoreceptors *in vivo* and ameliorate light damage. *J Neurosci* 1996;16:5986–92.