Role of Neurotrophin-4/5 in Neural Cell Death during Retinal Development and Ischemic Retinal Injury In Vivo

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PURPOSE. Neurotrophin (NT)-4/5 and brain-derived neurotrophic factor (BDNF) mediate cell survival through TrkB, a high-affinity tyrosine kinase receptor, and may prevent neural cell death in various pathologic conditions. This study was conducted to investigate the function of NT-4/5 in neural cell death during retinal development and ischemic retinal injury.

METHODS. Retinal development in wild-type, NT-4/5 knockout (KO), and NT-4/5:BDNF double-KO mice was histologically examined from postnatal day 0 (P0) to P90. Ischemic retinal injury was performed at P42, and NT-4/5 mRNA expression level and the extent of retinal cell death was quantitatively examined.

RESULTS. Real-time PCR analysis revealed increased NT-4/5 mRNA expression in the ischemic retina. In the NT-4/5 KO mouse, retinal development and structure were normal, but the strain was susceptible to ischemic injury on P42. In contrast, NT-4/5:BDNF double-KO mice showed delayed retinal development and died before P42.

CONCLUSIONS. These results suggest that NT-4/5, in combination with other trophic factors, is involved in the postnatal survival of retinal neurons during both development and degeneration. (*Invest Ophthalmol Vis Sci.* 2005;46:669–673) DOI:10.1167/iovs.04-0826

The growth factors in the neurotrophin family, which include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin (NT)-4/5, play important roles in the development of retina and visual system.[1–6] For example, limiting amounts of neurotrophins in the target area of the superior colliculus may control the apoptosis of retinal ganglion cells (RGCs) during the early postnatal period. In support of this hypothesis, NT-4/5 or BDNF injected into the superior colliculus promotes the survival of neonatal RGCs.[7,8] Control of cell survival by neurotrophins is mediated by two types of transmembrane glycoproteins: the trk tyrosine kinase receptors (TrkA, TrkB, and TrkC) and the neurotrophin receptor p75 (p75[NTR]).[1–3] Neurotrophins act in neural cell survival by activating trk tyrosine kinases, downstream of which a ras-dependent pathway leads to the activation of mitogen-activated protein (MAP) kinases.[9,10]

Neurotrophins are also involved in the survival of adult retinal neurons. For example, intraocular injection of NGF, BDNF, and NT-4/5 rescues RGCs after axotomy.[11–17] In addition, we recently found that neurotrophic factors released from retinal glia are involved in the process of photoreceptor degeneration.[18–20]

Another important pathologic condition that kills retinal neurons is ischemic injury. Ischemic injury is mainly associated with excessive concentrations of glutamate, which results in overactivation of glutamate receptors and initiates a cascade of events that leads to necrosis and/or apoptosis. Consistently, retinal neurons can be protected by glutamate receptor antagonists[21,22] and by selective inhibition of *N*-acetylated-α-linked acidic dipeptidase (NAALADase), which is an enzyme responsible for the hydrolysis of neuropeptide *N*-acetyl-aspartyl-glutamate to *N*-acetyl-aspartate and glutamate.[23] We also have demonstrated that glutamate transporters are crucial for the protection of retinal cells from ischemic injury.[24] A recent study in mice lacking both alleles for NT-4/5 or deficient in a single allele for BDNF has shown that expression of both NT-4/5 and BDNF, the high-affinity TrkB ligands, confers resistance to ischemic brain injury.[25] However, such a study has not yet been performed in the retina. In the present study, we examined the role of NT-4/5 and BDNF during retinal development and degeneration after ischemic injury in vivo.

MATERIALS AND METHODS

Animals

Experiments were performed in NT-4/5 KO[26] and NT-4/5:BDNF double-KO (NT-4/5:BDNF DKO)[27] mice, in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. For developmental studies, five NT-4/5 KO mice were killed on each of postnatal day (P)0, P5, P17, and P42 and five NT-4/5:BDNF DKO mice were killed on each of P0, P5, and P17. Ischemic injury was performed at P42.

Ischemic Retinal Injury

Ischemia was achieved, and the animals were treated essentially as previously described[23,24,28] Briefly, we instilled sterile saline into the anterior chamber of the left eye at 120 cm H_2O pressure for 20 or 40 minutes, whereas the right eye served as the nonischemic control. Seven days after reperfusion, animals were anesthetized with diethyl-ether and perfused transcardially with saline, followed by 4% parafor-maldehyde in 0.1 M phosphate buffer containing 0.5% picric acid at room temperature. The eyes were removed and postfixed overnight in the same fixative at 4°C and embedded in paraffin wax. The posterior part of the eyes was sectioned sagittally at 7 μm thickness through the optic nerve, mounted, and stained with hematoxylin and eosin. Ischemic damage was quantified in two ways.[23] First, the thickness of the inner retinal layer ([IRL], between the internal limiting membrane and the interface of the outer plexiform layer [OPL] and the outer nuclear layer [ONL]) were measured. Second, in the same sections, the number of neurons in the ganglion cell layer (GCL) was counted from one ora serrata through the optic nerve to the other ora serrata. The changes of the number of cells after ischemia were expressed in ratio compared with the nonischemic fellow eyes. Total RNA for PCR was prepared from whole retina 6 hours, 12 hours, 1 day, 3 days, and 7 days after ischemic injury.[28,29]

Quantitative Real-Time PCR Analysis

After RNA isolation and cDNA synthesis, the quality of the cDNA was controlled by PCR, using mouse-specific primers for glyceraldehyde-3-phosphate dehydrogenase (G3PDH; sense: 5'-ACC ACA GTC CAT GCC ATC AC-3'; antisense: 5'-TCC ACC ACC CTG TTG CTG TA-3'). Amplification of the expected PCR product (452 bp) was successful in all samples (data not shown).

Real-time RT-PCR (TaqMan PCR on the PRISM 7700 Sequence Detection System; Applied Biosystems, Inc. [ABI], Foster City, CA) was performed according to the manufacturer's protocol. The internal probe was labeled with the reporter dye FAM (6-carboxyfluorescein) at the 5' end and with the quencher dye TAMRA (6-carboxytetramethyl-rhodamine) at the 3' end. For NT-4/5, two primers (forward primer, 5'-GAGGCACTGGCTCTCAGAATG-3'; reverse primer, 5'-CGAATC-CAGCGCCAGC-3') and one probe (5'-FAM- CAGTCCTATGTGCGGGC-TAMRA-3') were used. For G3PDH, two primers (forward primer, 5'-TGCACCACCAACTGCTTAG-3'; reverse primer, 5'-GGATGCAGG-GATGATGTTC-3') and one probe (5'-VIC-CAGAAGACTGTGGATGGC-CCCTC-TAMRA-3') were used.

cDNA (50 ng) was reacted in a total volume of 20 μL with a master mix (TaqMan Universal PCR Master Mix; ABI). The final concentrations of probe and other primers were 200 and 400 nM, respectively. The conditions for PCR were as follows: 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The difference in the initial amount of total RNA between the samples was normalized in every assay by using the G3PDH gene expression as an internal standard. In each cDNA, the ratio of the copy number of NT-4/5 mRNA was divided by that of the G3PDH mRNA. This normalized value was used to determine the relative expression level of NT-4/5.

Immunoblot

Membrane extracts were prepared by homogenizing whole retina 24 hours after ischemic injury, in 8 to 10 volumes of ice-cold 50 mM Tris-HCl (pH 7.5) containing 150 mM NaCl. Three micrograms of the protein samples were electrophoresed on a 5% to 20% sodium dodecyl sulfate-polyacrylamide gradient gel and subsequently transferred to a filter (Immobilon-P; Millipore, Billerica, MA). The membrane was in-cubated with an anti-NT-4/5, affinity-purified polyclonal antibody (1 mg/mL; Santa Cruz Biotechnology, Santa Cruz, CA), or β-tubulin (1 mg/mL; Sigma-Aldrich, St. Louis, MO), and visualized with a chemilu-minescence Western blot kit (Roche Diagnostics, Mannheim, Ger-many).
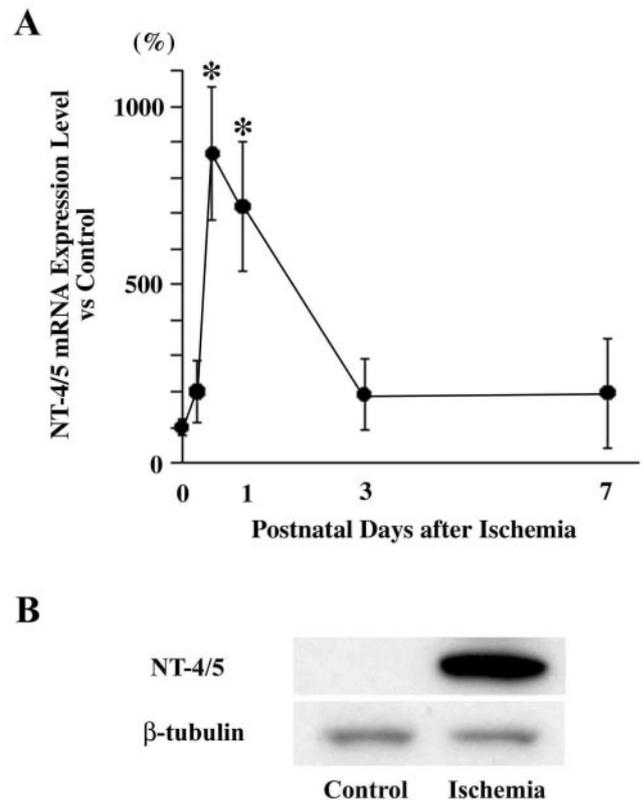

FIGURE 1. NT-4/5 expression after ischemic retinal injury. (A) Quan-titative analysis of NT-4/5 mRNA expression was performed 6 hours, 12 hours, 1 day, 3 days, and 7 days after ischemic retinal injury. Each data point represents the mean ± SEM of the values obtained from six independent experiments. *$P < 0.05$. (B) NT-4/5 protein expression was examined 1 day after ischemic injury by Western blot. Represen-tative data of three independent experiments are presented.

Quantification of Retinal Cell Apoptosis

Mouse retinas 1 or 3 days after ischemia was homogenized in 100 μL PBS containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and cen-trifuged at 15,000g for 10 minutes. A portion of supernatant was used to quantify protein concentration, and the rest was processed for assays. Retinal cell apoptosis was quantified with an apoptosis kit (Cell Death ELISA kit; Roche Diagnostics).

Statistics

Data are presented as the mean ± SEM, except as noted. When statistical analysis was performed, Student's t-test was used to estimate the significance of the results. Statistical significance was accepted at $P < 0.05$.

RESULTS

NT-4/5 Expression in the Ischemic Retina

Ischemic stress induces neural cell loss, mainly in the inner part of the retina.[23,24,28] To examine NT-4/5 mRNA expression levels in the ischemic retina, we performed quantitative real-time PCR analysis. As shown in Figure 1A, NT-4/5 mRNA expression increased significantly at 12 hours and 1 day after ischemic injury ($P < 0.05$) and returned to its normal level after 3 days. Consistently, compared with the internal standard (β-tubulin), NT-4/5 protein expression was upregulated 1 day after ischemic injury (Fig. 1B). These results suggest the pos-
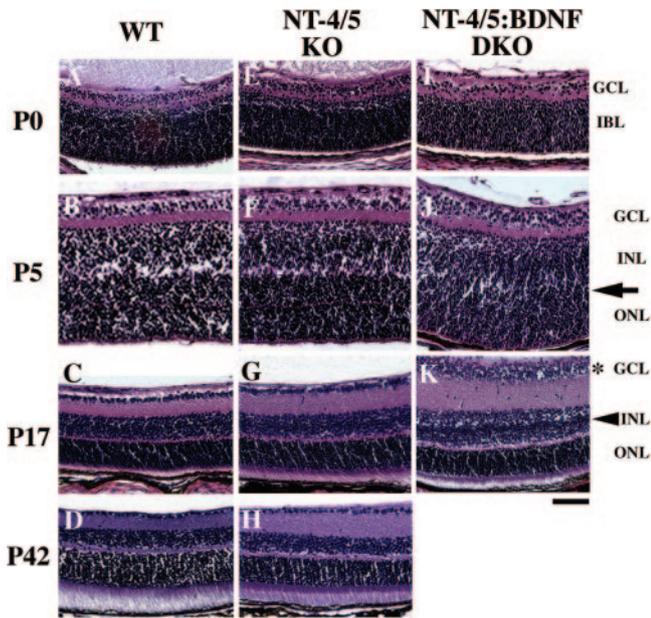
FIGURE 2. Retinal development in NT-4/5 and NT-4/5:BDNF DKO mice. Hematoxylin and eosin staining of retinal sections at P0 (**A, E, I**), P5 (**B, F, J**), P17 (**C, G, K**), and P42 (**D, H**) in wild-type (WT; **A–D**), NT-4/5 KO (**E–H**) and NT-4/5:BDNF DKO (NT-4/5:BDNF DKO; **I–K**). NT-4/5 KO and NT-4/5:BDNF DKO in P0, P5, and P17 are littermates. In NT-4/5:BDNF DKO retina, the border between the INL and the ONL was not clear at P5 (**J**, *arrow*), and the thickness of the GCL (**K**, *asterisk*) and the INL (**K**, *arrowhead*) was larger at P17. Retinal structure in NT-4/5 KO was normal at P42 (**H**). Scale bar, 50 μm.

sibility that NT-4/5 has some effect on retinal neurons during ischemic injury.

Normal Retinal Development and Structure in NT-4/5 KO Mice

We decided to use NT-4/5 KO mouse to determine the role of NT-4/5 in the ischemic retina. For this purpose, we first examined the retinal development and structure in NT-4/5 KO mouse. Figure 2 illustrates representative steps in the development and the maturation of the different layers of the retina. In the wild-type (WT) mouse retina, the structure of the GCL was clearly visible at postnatal day 0 (P0; Fig. 2A). The GCL was composed of multiple layers at P0 and P5 (Figs. 2A, 2B), but its thickness decreased and it became a monolayer by P17 (Fig. 2C). Other than the GCL, only the neuroblast layer (NBL), which was composed of poorly differentiated cells, was visible at P0 (Fig. 2A). The NBL was segregated in the inner nuclear layer (INL) and the ONL, which is composed of photoreceptor nuclei, by the OPL. The OPL started to form at P5 (Figs. 2B, 2F, 2J, arrow) and is visible as a gap between the INL and the ONL (Fig. 2C). This developmental sequence occurs in NT-4/5 KO mice with normal time course until P42 (Figs. 2E–H). Thus, the absence of NT-4/5 signaling does not seem to affect the differentiation and migration of retinal cells and their assembly into defined layers.

Effect of NT-4/5 on Ischemia-Induced Neural Cell Death

We next examined the effect of ischemic injury in WT and NT-4/5 KO mice. Histologic evaluation demonstrated a significant increase in ischemic damage in the NT-4/5 KO mice (Fig. 3C) compared with their WT littermates (Fig. 3B). The thickness of the IRL after 20 minutes of ischemia was 80% ± 7% ($n = 6$) in WT, but only 63% ± 8% ($n = 6$) in NT-4/5 KO mice

($P < 0.05$; Fig. 4A). Second, the percentage of surviving cells in the GCL was also decreased in NT-4/5 KO mice (54% ± 10%; $n = 6$; $P < 0.05$) compared with their WT littermates (74% ± 9%; $n = 6$; Fig. 4B). Similar results were observed after 40 minutes of ischemic injury (Figs. 4A, 4B). We also quantified retinal cell apoptosis 1 and 3 days after 20 minute ischemia. An ELISA demonstrated increased retinal cell apoptosis in NT-4/5 KO mice (125% ± 10% after 1 day and 121% ± 6% after 3 days) compared with WT littermates (100% ± 3% after 1 day and 100% ± 4% after 3 days; Fig. 4C). Thus, loss of endogenous NT-4/5 has a detrimental effect on inner retinal neurons during ischemic injury.

Retinal Development in NT-4/5:BDNF DKO Mice

Because NT-4/5 and BDNF mediate cell survival through the same high-affinity tyrosine kinase receptor, TrkB, we further tried to examine the ischemic damage in NT-4/5:BDNF DKO mice. However, we could not perform the experiments because most of the mice died within the first postnatal week.[27] In NT-4/5:BDNF DKO retina, OPL formation was not clear at P5 (Fig. 2J, arrow) and the GCL was still composed of multiple layers at P17 (Fig. 2K, asterisk). Although we could not examine NT-4/5:BDNF DKO retina beyond P18, the absence of both NT-4/5 and BDNF led to delayed development in the inner retina.

DISCUSSION

In the present study, NT-4/5 afforded neural cell protection against ischemic retinal injury in vivo. Ischemic retinal injury is now implicated in a number of pathologic states, such as retinal artery occlusion, glaucoma, and diabetic retinopathy.[21,24,28] Although long-term effects of NT-4/5 is limited in axotomized RGCs,[30] our present results suggest the possibility of management of ischemic injury with NT-4/5. Because other trophic factors, such as ciliary neurotrophic factor (CNTF) and glial cell line–derived neurotrophic factor (GDNF), also increase RGC survival and regeneration,[13,14,16,17,31,32] treatment by NT-4/5 in combination with these trophic factors may stimulate multiple cellular targets and activate separate mechanisms to prevent ischemia-induced neural cell death.

In addition, endogenous NT-4/5, in combination with BDNF, was required for normal retinal development. Retinal development is normal in NT-4/5 KO (Fig. 2E–H) and BDNF KO[33,34] (data not shown) mice. This may have occurred be-
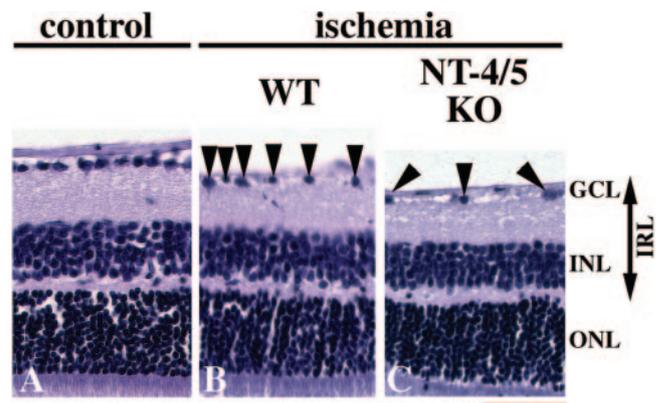

FIGURE 3. Ischemic retinal injury in NT-4/5 KO mice. Hematoxylin and eosin staining of retinal sections 7 days after 20 minutes transient ischemia-reperfusion (**B, C**) in wild-type (**B,** WT) and NT-4/5 KO (**C**) mice. Note the decreased number of cells in the GCL (*arrowheads*) and IRL thickness (*arrow*) in (**C**). Scale bar, 50 μm.
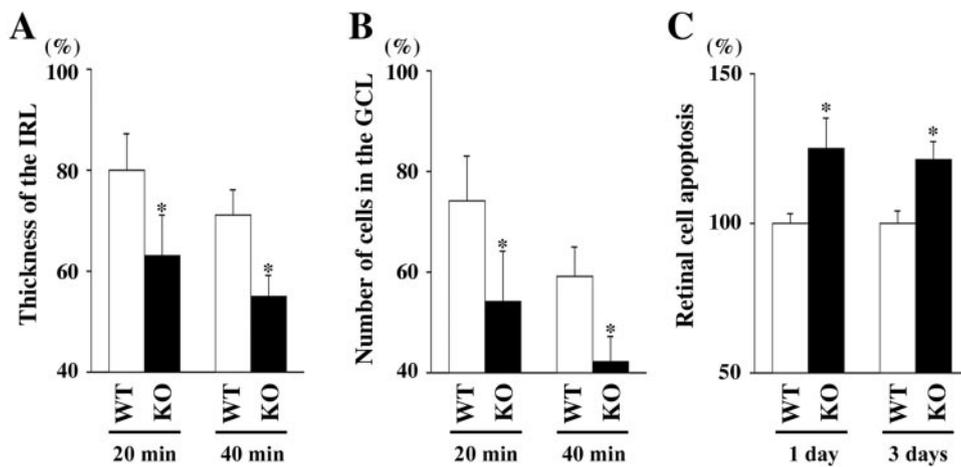
FIGURE 4. Quantitative analysis of ischemic retinal injury in NT-4/5 KO mice. Thickness of the IRL (**A**) and the number of cells in the GCL (**B**) after 20- and 40-minute ischemia as a percentage of the nonischemic fellow eyes. (**C**) Quantitative analysis of retinal cell apoptosis 1 day and 3 days after 20 minutes of ischemia. Results of six independent experiments are presented as the mean ± SEM. *$P < 0.05$.

cause of compensation by other trophic factors such as NT-3, because NT-3 is another possible ligand for TrkB, and its expression level is relatively high in the retina.[35–37] In contrast, NT-4/5:BDNF DKO mice showed unexpected delay in inner retinal development, and the GCL was still composed of multiple layers (Figs. 2I–K). The absence of NT-4/5 or BDNF compensated for each other, possibly with NT-3, but NT-3 alone may not be enough to compensate for the lack of two major TrkB ligands. In any event, these results suggest that NT-4/5 is involved in the postnatal survival of retinal neurons during both development and degeneration.

Our present findings may be of clinical relevance if NT-4/5 and its related molecules are to be used therapeutically in the treatment of human retinal diseases that otherwise lead to severe visual impairment. Recent studies have shown that neurotrophins may stimulate the glia–neuron pathway that indirectly leads to neural cell survival.[18–20,38] Because neurotrophin receptors are upregulated in Müller glial cells during retinal degeneration,[18–20,39–41] Müller cells may be another target for neuroprotection. In contrast, the mammalian TrkB locus undergoes alternative splicing to produce full-length and truncated receptors lacking the intracellular tyrosine kinase domain. Of note, truncated TrkB receptors may negatively influence neuron survival by interfering with the function of catalytic TrkB receptors.[42] Thus, overexpression of full-length TrkB or selective inhibition of truncated TrkB receptors may be effective during retinal degeneration. To find a better therapeutic method, further investigations on the precise role of NT-4/5 and its related molecules in other forms of retinal degeneration are necessary.

References

1. Parada LF, Tsoulfas P, Tessarollo L, et al. The Trk family of tyrosine kinases: receptors for NGF-related neurotrophins. *Cold Spring Harbor Symposia on Quantitative Biology*. 1992;57:43–51.
2. Barbacid M. The Trk family of neurotrophin receptors. *J Neurobiol*. 1994;25:1386–1403.
3. Kaplan DR, Stephens RM. Neurotrophin signal transduction by the Trk receptor. *J Neurobiol*. 1994;25:1404–1417.
4. Bothwell M. Functional interactions of neurotrophins and neurotrophin receptors. *Annu Rev Neurosci*. 1995;18:223–253.
5. von Bartheld CS. Neurotrophins in the developing and regenerating visual system. *Histol Histopathol*. 1998;13:437–459.
6. Ma L, Harada T, Harada C, et al. Neurotrophin-3 is required for appropriate establishment of thalamocortical connections. *Neuron*. 2002;36:623–634.
7. Cui Q, Harvey AR. At least two mechanisms are involved in the death of retinal ganglion cells following target ablation in neonatal rats. *J Neurosci*. 1995;15:8143–8155.
8. Ma YT, Hsieh T, Forbes ME, et al. BDNF injected into the superior colliculus reduces developmental retinal ganglion cell death. *J Neurosci*. 1998;18:2097–2107.
9. Greene LA, Kaplan DR. Early events in neurotrophin signaling via Trk and p75 receptors. *Curr Opin Neurobiol*. 1995;5:579–587.
10. Yoon SO, Casaccia-Bonnefil P, Carter B, et al. Competitive signaling between TrkA and p75 nerve growth factor receptors determines cell survival. *J Neurosci*. 1998;18:3273–3281.
11. Johnson JE, Barde YA, Schwab M, et al. Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. *J Neurosci*. 1986;6:3031–3038.
12. Carmignoto G, Maffei L, Candeo P, et al. Effect of NGF on the survival of rat retinal ganglion cells following optic nerve section. *J Neurosci*. 1989;9:1263–1272.
13. Mey J, Thanos S. Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. *Brain Res*. 1993;602:304–317.
14. Mansour-Robaey S, Clarke DB, Wang YC, et al. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. *Proc Natl Acad Sci USA*. 1994;91:1632–1636.
15. Sawai H, Clarke DB, Kittlerova P, et al. Brain-derived neurotrophic factor and neurotrophin-4/5 stimulate growth of axonal branches from regenerating retinal ganglion cells. *J Neurosci*. 1996;16:3887–3894.
16. Bosco A, Linden R. BDNF and NT-4 differentially modulate neurite outgrowth in developing retinal ganglion cells. *J Neurosci Res*. 1999;57:759–769.
17. Yan Q, Wang J, Matheson CR, et al. 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*. 1999;38:382–390.
18. Harada T, Harada C, Nakayama N, et al. Modification of glial-neuronal cell interactions prevents photoreceptor apoptosis during light-induced retinal degeneration. *Neuron*. 2000;26:533–541.
19. Harada T, Harada C, Kohsaka S, et al. Microglia-Müller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. *J Neurosci*. 2002;22:9228–9236.
20. Harada C, Harada T, Quah HMA, et al. Potential role of glial cell line-derived neurotrophic factor receptors in Müller glia cells during light-induced retinal degeneration. *Neuroscience*. 2003;122:229–235.
21. Lipton SA. Retinal ganglion cells, glaucoma and neuroprotection. *Prog Brain Res*. 2001;131:712–718.
22. Osborne NN, Melena J, Chidlow G, et al. 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*. 2001;85:1252–1259.
23. Harada C, Harada T, Slusher BS, et al. N-acetylated-α-linked-acidic dipeptidase inhibitor has a neuroprotective effect on mouse retinal

ganglion cells after pressure-induced ischemia. *Neurosci Lett*. 2000;292:134–136.

24. Harada T, Harada C, Watanabe M, et al. Functions of the two glutamate transporters GLAST and GLT-1 in the retina. *Proc Natl Acad Sci USA*. 1998;95:4663–4666.

25. Endres M, Fan G, Hirt L, et al. Ischemic brain damage in mice after selectively modifying BDNF or NT4 gene expression. *J Cereb Blood Flow Metab*. 2000;20:139–144.

26. Liebl DJ, Klesse LJ, Tessarollo L, et al. Loss of brain-derived neurotrophic factor-dependent neural crest-derived sensory neurons in neurotrophin-4 mutant mice. *Proc Natl Acad Sci USA*. 2000;97:2297–2302.

27. Liu X, Ernfors P, Wu H, et al. Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. *Nature*. 1995;375:238–241.

28. Harada T, Harada C, Wang YL, et al. Role of ubiquitin carboxy terminal hydrolase-L1 in neural cell apoptosis induced by ischemic retinal injury in vivo. *Am J Pathol*. 2004;164:59–64.

29. Harada T, Harada C, Sekiguchi M, et al. Light-induced retinal degeneration suppresses developmental progression of flip-to-flop alternative splicing in GluR1. *J Neurosci*. 1998;18:3336–3343.

30. Clarke DB, Bray GM, Aguayo AJ. Prolonged administration of NT-4/5 fails to rescue most axotomized retinal ganglion cells in adult rats. *Vision Res*. 1998;38:1517–1524.

31. Peterson WM, Wang Q, Tzekova R, et al. Ciliary neurotrophic factor and stress stimuli activate the Jak-STAT pathway in retinal neurons and glia. *J Neurosci*. 2000;20:4081–4090.

32. Koeberle PD, Ball AK. 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*. 2002;110:555–567.

33. Jones KR, Farinas I, Backus C, et al. Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell*. 1994;76:989–999.

34. Cellerino A, Carroll P, Thoenen H, et al. Reduced size of retinal ganglion cell axons and hypomyelination in mice lacking brain-derived neurotrophic factor. *Mol Cell Neurosci*. 1997;9:397–408.

35. Soppet D, Escandon E, Maragos J, et al. The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell*. 1991;65:895–903.

36. Hallböök F, Backstrom A, Kullander K, et al. Expression of neurotrophins and trk receptors in the avian retina. *J Comp Neurol*. 1996;364:664–676.

37. Bennett JL, Zeiler SR, Jones KR. Patterned expression of BDNF and NT-3 in the retina and anterior segment of the developing mammalian eye. *Invest Ophthalmol Vis Sci*. 1999;40:2996–3005.

38. Wexler EM, Berkovich O, Nawy, S. Role of the low-affinity NGF receptor (p75) in survival of retinal bipolar cells. *Vis Neurosci*. 1998;15:211–218.

39. Sarthy VP. Müller cells in retinal health and disease. *Arch Soc Esp Oftalmol*. 2000;75:367–368.

40. Wahlin KJ, Campochiaro PA, Zack DJ, et al. 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*. 2000;41:927–936.

41. Bringmann A, Reichenbach A. Role of Müller cells in retinal degenerations. *Front Biosci*. 2001;6:E72–E92.

42. Luikart BW, Nef S, Shipman T, et al. In vivo role of truncated trkb receptors during sensory ganglion neurogenesis. *Neuroscience*. 2003;117:847–858.