

Spontaneous Optic Neuropathy: Involvement of NF- κ B Activation by the Calcineurin Signal Cascade

Takuma Hayashi^{1*}, Tomoko Nakamura-Yanagidaira², Takao Hirano² and Toshinori Murata²

¹Department of Immunology and Infectious Disease, Shinshu University Graduate School of Medicine

²Department of Ophthalmology, Shinshu University Graduate School of Medicine

Abstract

Members of the nuclear factor-kappa B (NF- κ B)/Rel family, which act as transcriptional regulators, play important roles in neuronal cell death and survival. NF- κ Bp50 knockout (p50-KO) mice exhibit many of the features of human Normal Tension Glaucoma (NTG). The developmental mechanism of human NTG remains unclear, and a radical curative treatment has yet to be established. Therefore, the signal cascade that mediates spontaneous optic neuropathy in p50-KO mice, as a model of NTG, needs to be elucidated in more detail for the development of clinical therapies. In order to obtain a deeper understanding of NF- κ B-mediated death signaling, the effects of chemical reagents on spontaneous optic neuropathy have been examined histopathologically. Constitutively active cleaved forms of Calcineurin (CN), which have been reported to induce apoptosis, were detected in the retinas of p50-KO mice. Spontaneous retinal Ganglion Cell (RGC) death and degenerative changes to the optic nerve in p50-KO mice were both significantly reduced by the chronic administration of tacrolimus, a CN inhibitor. Experiments with cultured RGC cells supported the results of histological examinations on p50-KO mice, suggesting that CN activation leads to the activation of NF- κ B-induced Bax and caspase 3 and mediates spontaneous optic neuropathy in p50-KO mice. Previous studies demonstrated that the chronic administration of tacrolimus significantly reduced spontaneous optic neuropathy in p50-KO mice. A potential CN signal cascade spontaneously induces age-dependent RGC death and degenerative optic nerve changes in p50-KO mice. Novel research findings may provide new targets for therapeutic interventions in human NTG.

Keywords: NF- κ B; p50; Calcineurin; Glaucoma; NTG

***Corresponding Author:** Takuma Hayashi, Department of Immunology and Infectious Disease, Shinshu University Graduate School of Medicine 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan; Tel: +81-263-37-2611; E-mail: yoyoyo224@hotmail.com

Introduction

Glaucoma, one of the most common causes of visual impairment worldwide, is characterized by the apoptosis of Retinal Ganglion Cells (RGCs) [1]. Although increased Intraocular Pressure (IOP) has long been considered the primary cause of cell death, evidence from studies on Normal Tension Glaucoma (NTG) suggests that other factors are involved in the apoptosis of RGCs, which is induced by the potential neurotoxic role of glutamate, genetic background, and autoimmunity [2-4]. Although most anti-glaucomatous reagents are used to lower IOP, in some cases, the patient's condition deteriorates in spite of an IOP within the normal range. Therefore, it is considered necessary to identify factors that are independent of IOP to obtain a clearer understanding of the pathogenesis of glaucoma and guide efforts toward improved therapeutics.

Nuclear factor-kappa B (NF- κ B), which acts as a transcription factor, plays a key role in cell survival or the death signaling pathway, acute Central Nervous System (CNS) trauma, and chronic neurodegenerative disorders [5, 6]. The NF- κ B family, which is mainly composed of p50/p65 (RelA) heterodimers, is found in almost all animal cell types, and is involved in cellular responses to stimuli such as stress and cytokines [7]. In unstimulated cells, NF- κ B is sequestered to the cytoplasm by a family of inhibitors called I κ Bs. With the degradation of the I κ B inhibitor, NF- κ B is then free to enter the nucleus, in which it can

induce the expression of specific genes. Recent findings suggest that the binding site of the heterodimer p50/p65 (RelA) can also be occupied by the homodimer p50/p50, whereupon p50/p50 may function as a repressor to regulate the role of p50/p65 (RelA) as a transcription factor essential for neuronal responses [7]. The impaired regulation of NF- κ B has been linked to various diseases, such as cancer, inflammatory disorders, and autoimmune diseases, and has also been implicated in the processes of synaptic plasticity and memory [8]. In the CNS, activated p65 (RelA) has been suggested to participate in glutamate-induced neurotoxicity, N-methyl-D-aspartate (NMDA)-induced retinal neuronal cell death, retinal ischemia, and reperfusion injury [9-12]. However, the precise role of NF- κ B in cell death within the CNS remains controversial.

Excitotoxicity appears to be predominantly mediated in many types of neurons by signaling pathways, including Ca^{2+} influx through the NMDA receptor, a subtype of the glutamate receptor [13]. Therefore, NMDA antagonists, memantine, dizocilpine (MK-801), and Ca^{2+} channel blockers, such as flunarizine, verapamil, nicardipine, and lomerizine, may prevent retinal damage caused by NMDA [14-16]. Calcineurin (CN) is a eukaryotic Ca^{2+} - and calmodulin- dependent serine/threonine protein phosphatase that is highly expressed in the CNS and retina [17, 18]. The activation of CN has been shown to induce apoptosis in cultured neurons [19]. Tacrolimus, a CN inhibitor, exhibits neuroprotective effects in a wide range of models of acute apoptotic neuronal death in the CNS, such as trauma and stroke [20, 21]. In the retina, tacrolimus has been shown to confer neuroprotection on RGCs after optic nerve crush [22]. In addition, an increased IOP has been associated with the CN-mediated mitochondrial apoptotic pathway [23]. Furthermore, tacrolimus was found to inhibit glutamate-induced apoptosis in cultured RGCs [24]. Although the neuroprotective effects of these chemical reagents have been examined in transient studies employing glaucoma models, it has yet to be demonstrated

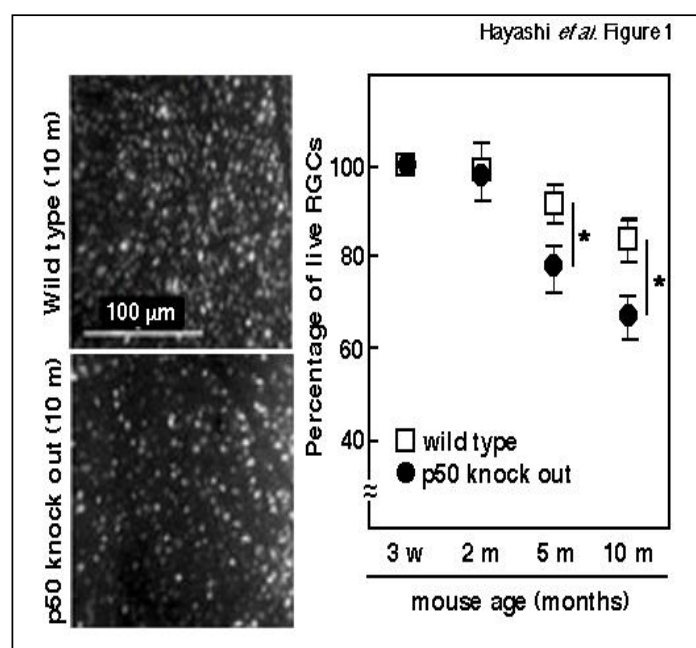
whether the chronic administration of chemical reagents to NTG-animal models has protective effects against neuropathy.

We previously reported that NF- κ Bp50 knockout (p50-KO) mice exhibited the spontaneous loss of RGCs independent of IOP, characteristic excavation of the optic nerve head, and degenerative changes to the optic nerve, with many features resembling human NTG [25]. However, the precise mechanism involved remains unknown. In a recent study, we attempted to identify the death signaling cascade in the initiation of spontaneous optic neuropathy in p50-KO mice as a model of NTG. Our findings demonstrated the neuroprotective effects of several chemical reagents, especially tacrolimus, against spontaneous RGC death in p50-KO mice. A defect in the p50/p50 homodimer, which functions as a repressor to regulate the role of p50/p65 (RelA), resulted in optic neuropathy via a CN signal cascade involving the impaired activation of NF- κ B in p50-KO mice. Research findings have provided a new insight into the role of p50 in the pathophysiology of optic neuropathy, with recent experiments involving p50-KO mice identifying CN signaling molecules including NF- κ B, suggesting targets for the development of therapeutic reagents. Research findings may provide new targets for therapeutic interventions in human NTG.

Age-dependent spontaneous RGC death due to apoptosis in NF- κ Bp50-KO mice

NF- κ B, which is expressed in RGCs as well as the CNS, plays important roles in processes such as synaptic activity and cell survival [6]. Morphometric evaluations indicated that, at 5 months, no significant difference existed in the thickness or construction of each retinal layer, especially the Inner Nuclear Layer (INL) and Outer Nuclear Layer (ONL). RGCs in p50-KO mice at age 3 weeks, 2 months, 5 months, and 10 months were counted using a retrograde labeling method, and their numbers at 5 months and 10 months of age (n=each 8~12 mice) were markedly lower than those in wild-type littermates (Figure 1).

Figure 1. Age-related retinal ganglion cell death in p50 knockout (KO) mice. Age-related retinal ganglion cell (RGC) death in p50 Knockout (KO) mice. Retrograde-labeled cells from 10 fields of identical size (230×150 μ m) in flat-mounted retinas were counted under a fluorescence microscope. The fields were located at approximately the same distance from the ora serata (500 μ m). Scale bar=100 μ m. The average number of RGCs per field was calculated for each retina and analyzed by a Bonferroni correction. Data are the mean $\pm$ SEM (each 8~12) (*P<0.01).



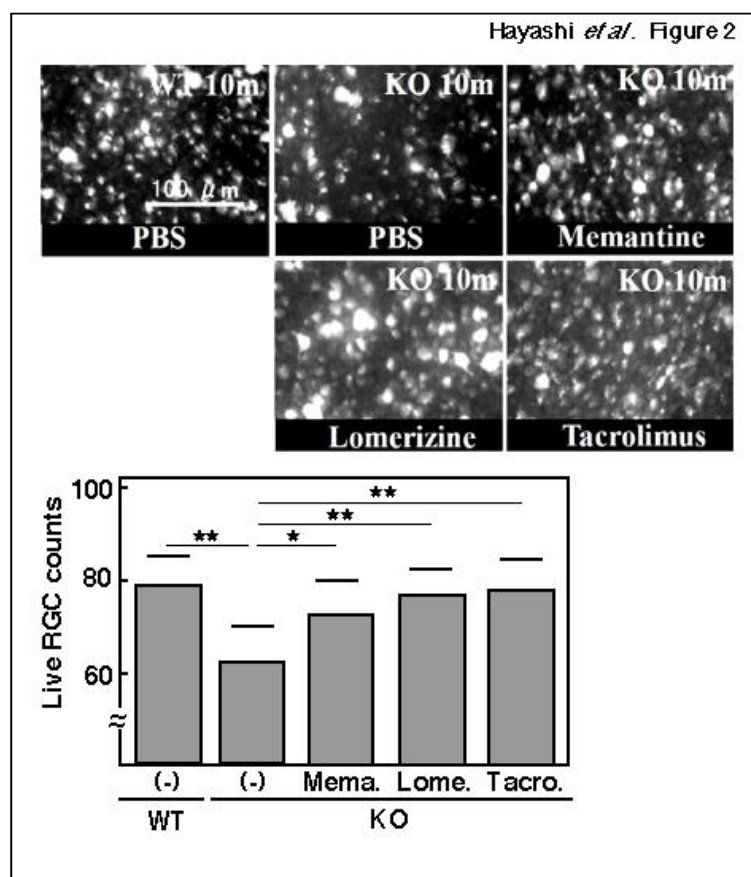
We examined p65 (RelA) expression at 2 months of age to determine whether p50-KO affected the activation of p65 (RelA) and RGC survival in the retina. p65 (RelA) was detected in the cytoplasm of RGCs of wild-type mice. Immunohistochemistry (IHC) studies clearly showed that p65 (RelA) spontaneously localized in the nucleus of RGCs in p50-KO mice under normal conditions; therefore, p50-KO in RGCs may induce the neuronal toxicity of p65 (RelA). Western Blot (W.B.) analyses strongly support IHC findings. Bax and Caspase 3, which play key roles in the promotion of apoptosis, have been shown to mediate stress-induced RGC death as well as glutamate-induced apoptosis in cultured neuronal cells [26-28]. Furthermore, Bax is required for the RGC death pathway in DBA/2J mice, which is an IOP-dependent glaucoma model. W.B. results showed that the expression of Bax in the retinas of p50-KO mice was slightly higher than that in wild-type mice, and activated forms of caspase 3 were detected in the

retinas of p50-KO mice. The constitutively active cleaved form of CN has been reported to induce apoptosis by elevating IOP in experimental glaucoma [27]. W.B. results revealed the presence of the full-length CN in all retinas examined, and that cleaved CN only occurred in retinas from p50-deficient mice.

Neuroprotective effects of the chronic administration of chemical reagents

In the present study, we investigated the age-related survival of RGCs in p50-KO mice, and whether the chronic administration of tacrolimus prevented the development of spontaneous optic neuropathy. We examined the neuroprotective effects of the chronic administration of chemical reagents on the development of spontaneous optic neuropathy. Tacrolimus, and additional reagents, memantine and lomerizine, which reportedly have neuroprotective effects, were intraperitoneally administered to p50-KO mice daily for 8 months, and the surviving RGCs were then counted using retrograde labeling. Age-related declines in RGC numbers for 10 months were significantly greater in p50-KO mice treated without the chronic administration than in wild-type mice: (Figure 2). RGC counts in p50-KO mice treated with chemical reagents were as follows: memantine: 69.1 $\pm$ 23.8 (n=9), lomerizine: 74.6 $\pm$ 19.4 (n=5), and tacrolimus: 75.4 $\pm$ 18.6 (n=5) (Figure 2). A statistical analysis of the cell counts of dye-filled RGCs in flat-mounted retinas revealed significant differences between the treatments with and without the chemical reagents, tacrolimus, memantine, and lomerizine (Figure 2). The appearance of TUNEL-positive RGCs in p50-KO mice was markedly decreased by the chronic administration of tacrolimus. These *in vivo* experiments provided strong evidence to support our hypothesis regarding the protective effects of the chronic administration of chemical reagents, especially tacrolimus, against age-dependent spontaneous RGC death.

Figure 2. Neuroprotection by various chemical reagents in p50 knockout (KO) mice: Photographs show a high-power view of retrograde-labeled retinal ganglion cells (RGCs) of flat mounts derived from p50 knockout (KO) and wild-type (WT) mice at 8 months of age. Scale bar=100 μ m. The bar graph shows RGC survival in 8-month-old KO and WT mice. Values represent the mean $\pm$ SD (n=each 5~11). Live RGC counts of KO mice were significantly decreased (**P<0.0001). However, the chronic administration of lomerizine or tacrolimus to KO mice for 6 months resulted in an increase in surviving RGC counts (**P<0.0001). The chronic administration of memantine to KO mice for 6 months mildly protected RGCs from neurotoxicity (*P=0.015). The number of surviving RGCs was counted using retrograde labeling methods. Data was analyzed by a Bonferroni correction.



The clear excavation of the optic nerve head was previously observed at 10 months of age in p50-KO mice [25]. Importantly, in the case of chemical reagent-treated mice, histological abnormalities in the optic nerve head were not detected in any mice examined (memantine: n=9, lomerizine: n=5, tacrolimus: n=5). As shown in the azan staining experiments, although an increase in collagenous fibers (blue staining) caused by axonal loss was clearly detected in the optic nerve sections of p50-KO mice, the chronic administration of tacrolimus markedly

suppressed collagenous fibers. An electron microscopic analysis of a cross-sectioned optic nerve revealed that expansion of the area of each axon at 8 months of age was greater in p50-KO mice than in age-matched wild-type mice, and was associated with a decrease in the number of axons. However, neither a decrease in the number of axons nor an increase in the area of connective tissue surrounding the axons was observed with the chronic administration of tacrolimus. However, the number of axons was significantly lower in p50-KO mice than in age-matched wild-type mice, but did not decrease in mice treated with tacrolimus. The chronic administration of chemical reagents, especially tacrolimus, effectively protected not only RGCs but also the optic nerve from spontaneous neuropathy. The amount of Bax in the RGCs of p50-KO mice was markedly decreased by the chronic administration of tacrolimus.

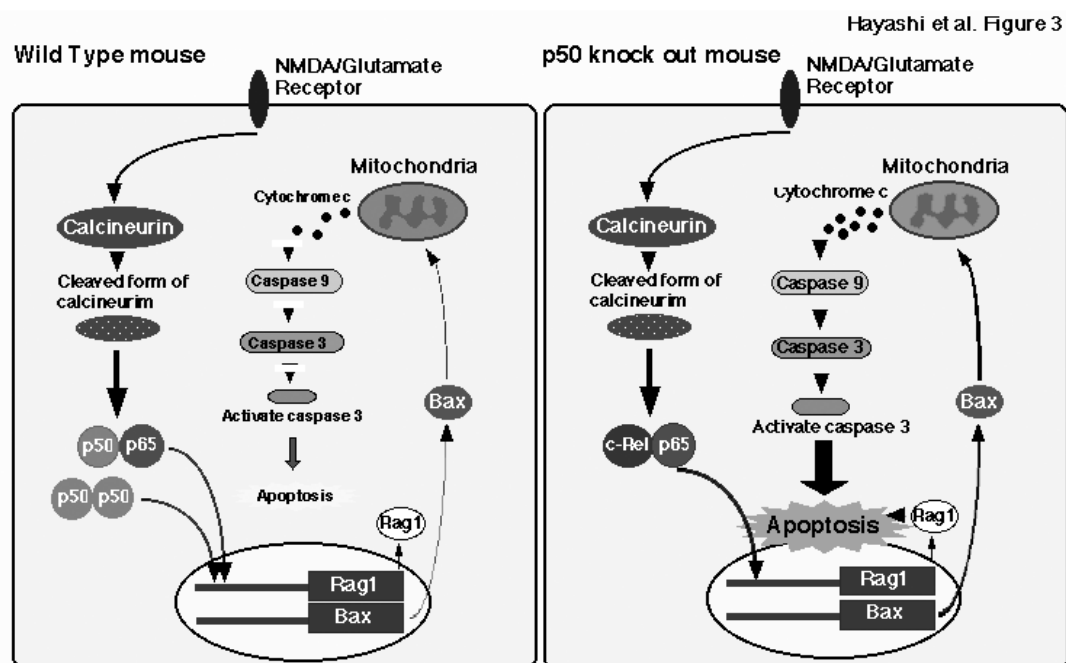
NTG shows chronic optic neuropathy and results in the loss of the visual field independently of IOP. Several population studies have suggested that NTG represents 20-90% of all primary open angle glaucoma cases, with percentages appearing to vary according to race [29]. Factors independent of IOP may contribute to disease progression, and recent studies showed that glaucoma was affected by multiple genetic and environmental factors [30,31]. Many NTG patients continue to have visual field loss in spite of a sufficient reduction in IOP. Therefore, it is important to understand the pathogenesis of NTG and establish methods of treatment.

Studies of postmortem brain tissues from patients with neurodegenerative disorders such as Alzheimer's or Parkinson's disease revealed increased NF- κ B activity closely associated with the neurodegenerative process. NF- κ B reportedly regulates apoptosis in response to stress in the nervous system, in addition to regulating apoptosis in a large variety of cells and tissues. Difficulties are associated with performing analyses using human materials; therefore, animal studies complement human studies and provide important insights into human NTG. Although glutamate transporter-deficient mice also exhibit spontaneous RGC death and optic nerve degeneration without an increase in IOP, they do not display the symptoms of chronic ailments observed in human NTG [32]. On the other hand, p50-KO

mice develop chronic spontaneous optic neuropathy, with many features resembling those of human NTG [25]. Since NF- κ B plays such a crucial role in optic neuropathy as well as the nervous system [33, 34], it is important to identify the signaling pathways leading to its activation in neurons. In our study, the constitutively active cleaved form of CN, which has been reported to induce apoptosis, was detected in the retina of p50-KO mice, and IHC experiments revealed that p65 (RelA) spontaneously translocated into the nucleolus of RGCs in p50-KO mice. Bax expression in the retina was higher in p50-KO mice than in wild-type mice, and activated caspase 3 was observed in p50-deficient mice. Examinations using the RGC cell line also demonstrated that the expression of p50 contributed greatly to the prevention of NMDA/glutamate-induced neurotoxicity and is required to protect RGCs and the optic nerve from spontaneous optic neuropathy (Figure 3). Therefore, p50 itself can form p50/p50 homodimers, which generally function as transcriptional

repressors [26, 35]. Our recent findings suggested that p50-KO activated CN-induced p65 (RelA), resulting in spontaneous RGC death. Since the stimulation of glutamate receptors results in membrane depolarization, which opens Ca^{2+} channels, leading to a rise in intracellular Ca^{2+} concentrations, Ca^{2+} may play an important role in NF- κ B activity during optic neuropathy. The activation of NF- κ B by various stimuli has already been shown to require Ca^{2+} for proper signal transduction [36, 37]. However, the exact process involved in transducing the Ca^{2+} signal in optic neuropathy has not yet been elucidated. To address this, we analyzed the neuroprotective effects of a Ca^{2+} blocker on RGCs in p50-KO mice. The protective effects of chemical reagents, which act on the Ca^{2+} -signaling pathway, against spontaneous optic nerve neuropathy was clearly demonstrated based on the chronic administration of reagents to p50-deficient mice as well as in transient experiments.

Figure 3. Putative biological function of NF- κ B in spontaneous optic neuropathy in p50 Knockout (KO) mice. A calcineurin (CaN) signal cascade involving the impaired activation of NF- κ B induced spontaneous Retinal Ganglion Cell (RGC) death. Recombination activating gene 1 (Rag1) reportedly plays a key role in spontaneous RGC death [40].



Recent research reports demonstrate that it is possible that the binding site of the heterodimer p50/p65 could also be occupied by the homodimer p50/p50, whereupon p50/p50 may function as a repressor to regulate p50/p65's role as a transcription factor essential for neuronal response. In p50 knock out cells, heterodimer c-Rel/p65 can markedly induce gene activation as transcription factor. Recent report demonstrated biological significance of Rag1 in optic neuropathy (ref.40).

CN, a critical component of several Ca^{2+} -dependent signaling pathways, controls transcriptional regulation, cell cycle progression, and cell survival. A recent study demonstrated a marked decrease in the level of NF- κ B constitutive activity when

CN was specifically inhibited, at both nuclear translocation and transactivation levels, suggesting the central importance of CN in NF- κ B activity in neurons [38]. Cleaved CN was detected in the retina of p50-KO mice, and the protective effect of tacrolimus

against optic neuropathy was then elucidated by transient experiments as well as chronic administration to p50-KO mice (Figure 3). Even though cultured RGCs were stimulated by glutamate, the pre-treatment with tacrolimus reduced activated caspase 3 and Bax expression in cultured RGCs. The amount of Bax in the RGCs and TUNEL-positive RGCs of p50-KO mice was markedly decreased by the chronic administration of tacrolimus. These findings suggest that a CN signal cascade involving the impaired activation of NF- κ B induced spontaneous RGC death (Figure 3).

Previous studies indicated the potential role of the immune system, i.e., autoantibodies against ocular antigens, in the pathogenesis of glaucoma [2]. p50-KO mice exhibited multiple immune response defects [7]. Our previous findings demonstrated the possible production of autoantibodies in the retinas of p50-KO mice [25]. Tacrolimus is an immunosuppressive reagent that is mainly used after allogenic organ transplant to reduce the activity of the patient's immune system, and, thus, lower the risk of organ rejection. Therefore, another possibility is that tacrolimus inhibits the immune system, especially autoimmunity. Further studies are needed to clarify the mechanism by which tacrolimus promotes RGC survival.

One important issue that remains to be resolved in neuronal physiology is how a signal that is released by neurotransmitters binding to their receptors at the synapse, is transduced into the nucleus to activate gene transcription. Despite the importance of NF- κ B to neuronal function and survival, little is known about the signaling pathways leading to its activation. In this study, we demonstrated that the expression of p50 was required for optic nerve cell survival, suggesting a connection between the loss of p50 expression and optic neuropathy. The mechanism by which CN-dependent pathways activate NF- κ B currently remains unknown; we utilized p50-KO mice as well as cultured RGCs to investigate the mechanisms by which the CN signal cascade stimulates NF- κ B-mediated neuronal cell death. Factors that act on the CN-dependent pathway may become targets in the clinical treatment of NTG. Clinical studies found a correlation between NTG and Migraine with Aura (MA): significant evidence of a link was found between the MA phenotype and the marker D4S1647 on 4q24, in which the p50-

coding gene is located [39]. There is currently no evidence to directly support a relationship between defective p50 expression and NTG. In conclusion, tacrolimus protected RGCs from chronic spontaneous optic neuropathy, and a CN signal cascade including the activation of NF- κ B induced chronic spontaneous optic neuropathy. Our results provide a new framework for understanding how cross-talk integrates various signals in a physiological context for the initiation of chronic optic neuropathy. These findings provide strong additional information regarding therapeutic targets for NTG.

Acknowledgments

We are grateful to Dr. Amer A. Beg (University of South Florida) for helpful discussions on our research findings. This study was supported in part by grants from the Ministry of Education, Culture, Science and Technology, and the Japan Foundation for the Prevention of Blindness.

References

1. Quigley HA. Neuronal death in glaucoma. *Prog Retin Eye Res.* 1999; 18: 39–57.
2. Tezel G, Wax MB. Glaucoma. *Chem Immunol Allergy* 2007; 92: 221-227.
3. Vorwerk CK, Lipton SA, Zurakowski D, Hyman BT, Sabel BA, Dreyer EB. Chronic low-dose glutamate is toxic to retinal ganglion cells. Toxicity blocked by memantine. *Invest Ophthalmol Vis Sci* 1996; 37: 1618-1624.
4. Votruba M. Molecular genetic basis of primary inherited optic neuropathies. *Eye* 2004; 18: 1126-1132.
5. Baeuerle PA, Baltimore D. I kappa B: a specific inhibitor of the NF-kappa B transcription factor. *Science* 1988; 242: 540-546.
6. Hayashi T, Faustman D. Essential role of human leukocyte antigen-encoded subunits in NF-kappaB activation and prevention of tumor necrosis factor-alpha-induced apoptosis. *J Biol Chem* 2000; 275: 5238-5247.
7. Sha WC, Liou HC, Tuomanen EI, Baltimore D. Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell* 1995; 80: 321-330.
8. Hoffman A, Lung TH, Baltimore D. Genetic analysis of NF-kappa B/Rel transcription factors defines functional specificities. *EMBO J* 2003; 22: 5530-5539.

9. Fan W, Cooper NG. Glutamate-induced NFκappaB activation in the retina. *Invest Ophthalmol Vis Sci* 2009; 50: 917-925.
10. Grilli M, Pizzi M, Memo M, Spano P. Neuroprotection by aspirin and sodium salicylate through blockade of NF-kappaB activation. *Science* 1996; 274: 1383-1385.
11. Nakai M, Qin ZH, Chen JF, Wang Y, Chase TN. Kainic acid-induced apoptosis in rat striatum is associated with nuclear factor-kappa B activation. *J Neurochem* 2000; 74: 647-658.
12. ChenYG, Zhang C, Chiang SK, Wu T, Tso MO. Increased nuclear factor- kappa B p65 immunoreactivity following retinal ischemia and reperfusion injury in mice. *J Neurosci Res* 2003; 72: 125-31.
13. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1988; 1: 623-34.
14. Lam TT, Abler AS, Kwong JM, Tso MO. N-Methyl-D-Aspartate (NMDA)-induced apoptosis in rat retina. *Invest Ophthalmol Vis Sci* 1999; 40:2391-7.
15. Lipton SA. Possible role for memantine in protecting retinal ganglion cells from glaucomatous damage. *Surv Ophthalmol* 2003; 48: s38-46.
16. Toriu N, Akaike A, Yasuyoshi H, Zhang S, Kashii S, Honda Y, Shimazawa M, Hara H. Lomerizine, a Ca²⁺ channel blocker, reduces glutamate-induced neurotoxicity and ischemia/reperfusion damage in rat retina. *Exp Eye Res* 2000; 70: 475-84.
17. Seitz DP, Pasha Mk, Singh B, Zhang S, Kashii S, Honda Y, Shimazawa M, Hara H. eye. *Invest Ophthalmol Vis Sci* 2002; 43: 15-21.
18. Nakazawa A, Usuda N, Matsui T, Hanai T, Matsushita S, Arai H, Sasaki H, Higuchi S. Localization of calcineurin in the mature and developing retina. *Histochem Cytochem* 2001; 49: 187-195.
19. Wu HY, Tomizawa K, Oda Y, Wei FY, Lu YF, Matsushita M, Li ST, Moriwaki A, Matsui H.. Critical role of calpain-mediated cleavage of calcineurin in excitotoxic neurodegeneration. *J Biol Chem* 2004; 279: 492-494.
20. Sharkey J, Butcher SP. Immunophilins mediate the neuroprotective effects of FK506 in focal cerebral ischaemia. *Nature* 1994; 371: 336-339.
21. Saxena K, Patro N, Patro I. FK506 protects following peripheral nerve injury via immunosuppression. *Cell Mol Neurobiol* 2007; 27:1049-57.
22. Freeman EE, Grosskreutz CL. The effects of FK506 on retinal ganglion cells after optic nerve crush. *Invest Ophthalmol Vis Sci* 2000; 41: 1111-115.
23. Huang W, Fileta JB, Dobberfuhr A, Filippopolous T, Guo Y, Kwon G, Grosskreutz CL. Calcineurin cleavage is triggered by elevated intraocular pressure, and calcineurin inhibition blocks retinal ganglion cell death in experimental glaucoma. *Proc Natl Acad Sci USA* 2005; 102: 12242-12247.
24. Kikuchi M, Kashii S, Mandai M, Yasuyoshi H, Honda Y, Kaneda K, Akaike A. Protective effects of FK506 against glutamate-induced neurotoxicity in retinal cell culture. *Invest Ophthalmol Vis Sci* 1998; 39: 1227-1232.
25. Takahashi Y, Katai N, Murata T, Taniguchi S, Hayashi T. Development of spontaneous optic neuropathy in NF-kappaBetap50-deficient mice; requirement for NF-kappa Beta p50 in ganglion cell survival. *Neuropathol Appl Neurobiol* 2007; 33: 692-705.
26. Iriyama T, Kamei Y, Kozuma S, Taketani Y. Bax-inhibiting peptide protects glutamate-induced cerebellar granule cell death by blocking Bax translocation. *Neurosci Lett* 2009; 451: 11-15.
27. Huang W, Fileta JB, Dobberfuhr A, Filippopolous T, Guo Y, Kwon G, Grosskreutz CL. Calcineurin cleavage is triggered by elevated intraocular pressure, and calcineurin inhibition blocks retinal ganglion cell death in experimental glaucoma. *Proc Natl Acad Sci USA* 2005; 102: 12242-12247.
28. Youle RJ, Strasser A. The BCL-2-protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 2008; 9: 47-59.
29. Bonomi L, Marchini G, Marraffa M, Bernardi P, De Franco I, Perfetti S, Varotto A, Tenna V. Prevalence of glaucoma and intraocular pressure distribution in a defined population. The Egna-Neumarkt Study. *Ophthalmology* 1998; 105: 209-215.
30. Osborne NN, Lascoratos G, Bron AJ, Chidlow G, Wood JP. A hypothesis to suggest that light is a risk factor in glaucoma and the mitochondrial optic neuropathies. *Br J Ophthalmol* 2006; 90:237-241.

31. Wiggs JL. Genetic etiologies of glaucoma. *Arch Ophthalmol* 2007; 125: 30-37.
32. Harada T, Harada C, Nakamura K, Quah HM, Okumura A, Namekata K, Saeki T, Aihara M, Yoshida H, Mitani A, Tanaka K.. The potential role of glutamate transporters in the pathogenesis of normal tension glaucoma. *J Clin Invest* 2007; 117: 1763-1770.
33. Hunot S, Brugg B, Ricard D, Michel PP, Muriel MP, Ruberg M, Faucheux BA, Agid Y, Hirsch EC. Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with Parkinson disease. *Proc Natl Acad Sci USA* 1997; 94: 7531-5536.
34. Kaltschmidt B, Uherek M, Volk B, Baeuerle PA, Kaltschmidt C. Transcription factor NF-kappaB is activated in primary neurons by amyloid beta peptides and in neurons surrounding early plaques from patients with Alzheimer disease. *Proc Natl Acad Sci USA* 1997; 94: 2642-2647.
35. Hara H, Morita T, Sukamoto T, Cutrer FM. Lomerizine(KB-2796), a new antimigraine drug. *CNS Drug* 1995; Rev. 1: 204-226.
36. Jefferson KK, Smith Jr MF, Bobak DA. Roles of intracellular calcium and NF-kappa B in the Clostridium difficile toxin A-induced up-regulation and secretion of IL-8 from human monocytes. *J Immunol* 1999; 163: 5183-51891.
37. Pahl HL, Baeuerle PA. Activation of NF-kappa B by ER stress requires both Ca²⁺ and reactive oxygen intermediates as messengers. *FEBS Lett* 1996; 392:129-136.
38. Lilienbaum A, Israel A. From calcium to NF-kappa B signaling pathways in neurons. *Mol Cell Biol* 2003; 23: 2680-2698.
39. Wessman M, Kallela M, Kaunisto MA, Marttila P, Sobel E, Hartiala J, Oswell G, Leal SM, Papp JC, Hämäläinen E, Broas P, Joslyn G, Hovatta I, Hiekkalinna T, Kaprio J, Ott J, Cantor RM, Zwart JA, Ilmavirta M, Havanka H, Färkkilä M, Peltonen L, Palotie A.. A susceptibility locus for migraine with aura, on chromosome 4q24. *Am J Hum Genet* 2002; 70: 652-662.
40. Hirano T, Murata T, Hayashi T. Physiological significance of Rag1 in neuronal death, especially optic neuropathy. *FEBS Journal* 2015; 282(1): 129-141.