Tau Is Involved in Death of Retinal Ganglion Cells of Rats From Optic Nerve Crush

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PURPOSE. To determine whether tauopathies are associated with impaired autophagy and involved in the death of retinal ganglion cells (RGCs) of rats from an optic nerve crush (ONC).

METHODS. Short interfering RNA (siRNA) of the tau gene (si-Tau) or nontargeting siRNA (si-NC) was injected intravitreally 48 hours prior to ONC. The effects of silencing the tau gene on neuroprotection were determined by the number of Tuj-1-stained RGCs on days 7 and 14 after the ONC. Changes in the expressions of phosphorylated tau, P62, and LC3B were determined by immunobots and immunohistochemistry on day 7.

RESULTS. Autophagy was impaired in the retina on day 7 after the ONC as the P62 level increased by 3.1-fold from the sham control level with a reduction in the ratio LC3B2/LC3B1. There was a 2.1-fold increase of phosphorylated tau (ser 396) in the retina, and si-Tau depressed the increase by 1.3-fold ($n = 3$ each). The expressions of tau and P62 were well colocalized. They were observed in the somas of RGCs and retinal nerve fibers (RNFs), and these expressions were increased after the ONC. Pretreatment by si-Tau showed significant protection in the number of RGCs after the ONC. Specifically, the density of RGCs was $540 \pm 74.5$ cells/mm$^2$ on day 14 in the si-NC group, while the level was maintained at $1321 \pm 192$ cells/mm$^2$ in the si-Tau group ($n = 4$ each).

CONCLUSIONS. Silencing the tau gene is neuroprotective, and tauopathies may be involved in the death of RGCs after ONC. Impaired autophagy may be involved in ONC-induced tauopathies.

Keywords: tauopathy, P62, LC3, optic nerve crush, short interfering RNA (siRNA), autophagy

T

au is a protein that stabilizes and maintains the function of microtubules in the cells of the central nervous system (CNS) including the retinal ganglion cells (RGCs) and their axons. Studies using transgenic mice with a mutation of the human P301 tau gene have suggested that molecules of hyperphosphorylated human mutant tau are aggregated in the RGCs, which impairs axonal transport in the optic nerve. More recently, it has been shown that hyperphosphorylated and oligomeric tau species are accumulated in the retina of mice with experimental glaucoma, and an intravitreal injection of short interfering RNA (siRNA) against the tau gene decreased the abnormal tau levels leading to RGC protection. Thus, tauopathies that are caused by an accumulation of tau proteins are involved, at least in part, in the pathology of some optic nerve diseases.

Accumulation of misfolded or unfolded proteins is eliminated by the endoplasmic reticulum (ER) through a mechanism called ER-associated degradation. Another quality control of protein machinery is autophagy. Autophagy is a genetically programmed catabolic system that basically obtains energy sources by recycling metabolites during starvation. In addition, it has been shown that misfolded, aggregated proteins and defective organelles can be eliminated by autophagic degradation. Although the roles played by autophagy in the CNS are still not completely determined, this function is more important for neuronal cells that cannot decrease these detrimental substances through cell division. Abnormalities in the autophagy machinery are involved in several diseases in the CNS including Alzheimer’s and Parkinson’s diseases. Sequestosome 1/SQSTM1 (P62) is an adaptor protein that selectively carries ubiquitinated proteins to the autophagic machinery. Because genetic inactivation of P62 leads to accumulation of hyperphosphorylated tau and results in neurodegeneration, P62 plays a crucial role in preventing tauopathies through selective autophagy. However, it has not been determined whether tauopathies are involved in the death of RGCs after optic nerve injuries. In addition, the roles played by autophagic degradation of phosphorylated tau aggregates in the RGCs after optic nerve injuries have not been examined.

Thus, the purpose of this study was to determine whether autophagy is impaired in the RGCs after optic nerve injuries, and whether the accumulation of tau is involved in the death of RGCs. To accomplish this, we determined the changes in the expressions of phosphorylated tau and autophagy-related proteins, microtubule-associated protein 1 light chain 3 (LC3) and P62, by immunoblot and immunohistochemistry (IHC) in the retina of rats after optic nerve crush (ONC). In addition, we determined the detrimental roles of tau accumulation by blocking tau translation with an intravitreal injection of siRNA 48 hours prior to the ONC. The survival of RGCs was determined by IHC using neuron-specific class III $\beta$-tubulin (Tuj-1) staining on days 7 and 14 after the ONC.
Involvement of Tau in Death of RGC After Optic Nerve Injury

METHODS

Animals

Nine-week-old male Wistar rats were purchased from Japan SLC (Shizuoka, Japan) and housed in an air-conditioned room with a temperature of approximately 23°C and humidity of 60%. The room lights were set on a 12:12 light-dark cycle. All animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol was approved by the Committee of Animal Use and Care of the Osaka Medical College (No. 28024).

Chemicals

Unless noted otherwise, all chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Anesthesia and Euthanasia

All surgeries were performed under general anesthesia induced by an intraperitoneal injection of a mixture of medetomidine (0.75 mg), midazolam hydrochloride (4.0 mg), and butorphanol tartrate (5.0 mg/kg body weight). Rats were euthanized in a 13.8-L cage with wood-shaving bedding by exposure to CO₂ at a rate of 6 L/min.

Optic Nerve Crush

Animals were anesthetized as described, and an incision was made along the midline of the skull to expose the superior surface of the left eye. The superior rectus muscle was incised to expose the left optic nerve, and the left optic nerve was crushed 2 mm behind the eye with forceps for 10 seconds. Care was taken not to occlude the blood vessels and cause retinal ischemia. We confirmed that the retinal circulation was untouched in all animals.

Tau Gene Silencing With Short Interfering RNA

To determine the pathological roles of tau accumulation in the death of RGCs after ONC, the tau gene was silenced by an intravitreal injection of siRNA as has been described in detail by Chiasseu et al. 3 The siRNA for the microtubule-associated protein tau (MAPT (Shizuoka, Japan) and housed in an air-conditioned room with a temperature of approximately 23°C and humidity of 60%. The room lights were set on a 12:12 light-dark cycle. All animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol was approved by the Committee of Animal Use and Care of the Osaka Medical College (No. 28024).

To determine the pathological roles of tau accumulation in the death of RGCs after ONC, the tau gene was silenced by an intravitreal injection of siRNA as has been described in detail by Chiasseu et al. 3 The siRNA for the microtubule-associated protein tau (MAPT) gene was purchased from Dharmacon Horizon Discovery, Cambridge, UK), with the following sequences:

1. 5′-GCAUGUGACUACAGCUGCGA-3′;
2. 5′-AGUAGGGCCAGAUGCAGUAG-3′;
3. 5′-GAUAGAUGUCAGUGAAGAA-3′; and
4. 5′-GCAGGGAACAGGACGGA-3′ (SMART pool)

A nontargeting siRNA was used as a negative control (si-NC) with the following sequences:

1. 5′-UGGCUUUAUCAGGUCACUA-3′;
2. 5′-UGCUUUAUCAGGUGUGUGA-3′;
3. 5′-UGCUCUUAUCAGGUCAUGA-3′; and
4. 5′-UCUGCCUAUCAGGUGGA-3′

After penetrating the sclera with 27-gauge needle, siRNA (2 μg/μL; total volume, 5 μL) was injected into the vitreous chamber using a Hamilton syringe fitted with a 32-gauge needle under general anesthesia.

Immunohistochemistry

The results of earlier studies have shown that axotomy or crushing the optic nerve causes a reduction in the number of RGCs in a delayed fashion; the number of RGCs remains unchanged for 5 days after the ONC and then abruptly decreases to 50% on day 7 and to less than 10% on day 14. 11 Thus, the number of surviving RGCs was determined on days 7 and 14 after the ONC.

Rats were euthanized as described, and the retinas were carefully removed from the eyes according to the methods described in detail by Winkler. 12 The isolated retinas were fixed in 4% paraformaldehyde in PBS overnight. After washing in PBS and blocking in PBS containing 1% bovine serum and 0.5% Triton X-100, the retinas were incubated with Alexa 488-conjugated mouse monoclonal neuron-specific class III β-tubulin (TuJ-1, 1:500) antibody (Covance, Princeton, NJ, USA). TuJ-1 is a marker for RGCs. 13 The retinas were placed in the same medium overnight at 4°C and washed with PBS and coverslipped the next morning.

To determine the number of RGCs that were TuJ-1 positive, the stained flat mounts were photographed with a fluorescence microscope (BZ X700; Keyence, Osaka, Japan). Eight areas (0.48 × 0.48 mm) from the four quadrants of the retina at 1.0 and 1.5 mm from the margin of the optic disc were photographed. All of the TuJ-1-positive cells in an area of 0.2 × 0.2 mm at the center of each image were counted using the ImageJ program (http://imagej.nih.gov/ij/, provided in the public domain by the National Institutes of Health, Bethesda, MD, USA).

The mean density of the TuJ-1-positive cells/mm² was calculated, and the loss of RGCs was estimated by comparing the density in the retinas of animals receiving either siRNA for tau (si-Tau) or nontargeting siRNA (si-NC) 48 hours prior to the ONC to that of retinas from sham controls. The number of TuJ-1-positive cells was counted by one observer (KT) who was masked as to whether it was from an experimental or a sham animal.

In addition to the TuJ-1, retinas were incubated with Alexa 555-conjugated rabbit monoclonal antibody to tau (1:100; Abcam, Cambridge, UK), Alexa 594-conjugated rabbit polyclonal antibody to LC3B (1:100, Abcam), and Alexa 488-conjugated mouse monoclonal antibody to SQSTM1/p62 (1:50, Abcam) overnight at 4°C. To determine time-dependent changes in the expressions of tau, retinas were removed on days 1, 3, and 7 after the ONC. The retinas were photographed with a fluorescence microscope (BZ-X700) or a confocal laser microscope (TCS SP8; Leica, Wetzlar, Germany).

Protein Extraction

After euthanasia at the selected times, the retinas were isolated, and three retinas in each experimental group were pooled. They were homogenized in lysis buffer containing 1.0 mM phenylmethylsulfonfyl fluoride, 10 μM pepstatin A, 10 μM leupeptin, 10 μM aprotinin, 0.1% SDS, 1.0% Nonidet P-40, 5.0% sodium deoxycholate, 50 mM Tris-HCl (pH 7.6), and 150 mM NaCl. The suspension was centrifuged, and the supernatant was used to determine the total protein concentration by the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA).

Immunoblotting for Expression of Phosphorylated Tau, P62, and LC3B After Optic Nerve Crush

Immunoblotting was performed to determine the changes in the expressions of phosphorylated tau, P62, and LC3B.
phosphorylation of tau is known to occur at many sites, among which phosphorylation at ser 396 and 404 is one of the earliest events in Alzheimer’s disease and causes tau aggregates in the brain.14 Age-dependent increases of the phosphorylation at ser 396 and 404 is reported to occur in the murine retinas,3 and pseudo-phosphorylation at these sites increases the polymerization of tau.14 Thus, we used rabbit monoclonal antibody to phosphorlated tau at ser 396 (Abcam) for immunoblotting.

Samples were separated on a 7.5% SDS-polyacrylamide gel and transblotted onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% skim milk in TBS-T (pH 7.4, 0.1% Tween 20) followed by overnight incubation with a rabbit polyclonal anti-LC3B (Cell Signaling, Danvers, MA, USA), rabbit monoclonal anti-P62 (Abcam), rabbit monoclonal antiphosphorylated tau (ser 396, Abcam), and mouse monoclonal anti-total tau (A-10; Santa Cruz, Dallas, TX, USA) at 4°C. For internal control, α-tubulin (Merck Millipore, Darmstadt, Germany) was used. Immunoblots were done using pooled samples composed of three retinas in each group and assays were done in triplicate (n = 3).

The protein bands were made visible by horseradish peroxidase-conjugated appropriate secondary antibodies (Promega, Madison, WI, USA). The signals were intensified with an ECL plus Western blotting detection system (GE Healthcare, Buckinghamshire, UK). The densities of the bands of proteins were quantified with a luminescent image analyzer (LAS-3000; Fujifilm, Tokyo, Japan). The levels of expression of these proteins were quantified with the embedded software (Multi Gauge version 3.0) and standardized to the control level.

Statistical Analyses

The data are expressed as the means ± standard deviation (SD). Statistical analyses were done by 1-way analysis of variance (ANOVA), and if significant changes were detected, the Scheffé post hoc test was used. Student’s t-tests were used to determine if the difference between two groups was significant. The level of significance was set at P < 0.05.

RESULTS

Changes of P62 and LC3B After Optic Nerve Crush

P62 is an adaptor protein of selective autophagy, and its increase is an indication of impaired autophagy.15 Thus, we first determined time-dependent changes in the expression of P62 after the ONC. The levels of P62 (in fold units) on days 1, 3, 7, and 14 after the ONC (n = 3 each) were 1.56 ± 0.08-, 0.83 ± 0.09-, 1.44 ± 0.14-, and 1.44 ± 0.08-fold relative to the levels in the control rats, respectively. The increases of the P62 levels on days 1, 7, and 14 were significant relative to the control levels, but the differences among these levels were not significant (P > 0.05, 1-way ANOVA). The decrease on day 3 was not significant (P = 0.3, Scheffé). Thus, in subsequent experiments, we determined the P62 levels on day 7 after the ONC for the expression of other proteins by immunoblotting and IHC.

Representative proteins bands of LC3B and P62 are shown in Figure 1A. Their levels were quantified relative to the expression of α-tubulin as an internal control (Fig. 1B). On day 7 after the ONC, the level of LC3B1 was 1.59 ± 0.07-fold (P = 0.001, t-test) and that of LC3B2 was 0.93 ± 0.05-fold (P > 0.05) of the sham control (n = 3, each). The level of LC3B1 increased significantly but the levels of LC3B2 were not significantly different (P = 0.21, t-test). Thus, the ratio LC3B2/LC3B1 was significantly decreased from 10.1 ± 0.1% to 5.9 ± 0.4% after the ONC (P = 0.003, t-test). On the other hand, P62 was significantly increased to 3.1 ± 0.01-fold from the sham control (P < 0.001, t-test).

The changes in the expression of phosphorylated tau (ser 396) and total tau in the retina on day 7 after the ONC are shown in Figure 2. Representative protein bands are shown in Figure 2A, and the tau levels are quantified using level of α-tubulin as an internal control (Fig. 2B). There was a 2.1-fold increase of phosphorylated tau after the ONC in the si-NC group, and the intravitreal injection of si-Tau suppressed the increase to 1.3-fold. Similarly, there was a 2.8-fold increase in the total tau after the ONC in the si-NC group, and si-Tau suppressed the increase to 1.7-fold. These results suggested that the tau gene was silenced by the intravitreal injection of si-Tau 48 hours prior to the ONC. NOC alone caused comparable increases of phosphorylated and total tau in the si-NC group.

Changes in Expression of P62, LC3B, and Tau Proteins in RGCs After Optic Nerve Crush

Representative confocal images of flat-mounted retinas showing changes in the expression of Tuj-1 and LC3B in the RGCs are presented in Figure 3. The images were taken approximately 1.0 mm from the optic disc margin on day 7. The Tuj-1–stained RGCs are dense in the control (Fig. 3, sham), while the number of Tuj-1–positive cells was clearly reduced after the ONC on day 7 (Fig. 3, ONC). In the RGCs stained with...
Tuj-1 antibody, the nuclei were stained weakly (Supplementary Fig. S1), and very fine granules stained by LC3B were present chiefly in the somas of the RGCs in the sham control. Coarse LC3B granules or LC3B aggregates were seen in the dying RGCs that were poorly stained with the Tuj-1 antibody (Fig. 3, ONC; see legend).

Confocal images of flat-mounted retinas stained with antibodies against P62 and tau are shown in Figure 4. Fine granules stained with the P62 antibody can be seen in the somas of the RGCs (Fig. 4, sham). On day 7 after the ONC, the intensity of the P62 staining appeared to be increased. This finding is consistent with increased expression of retinal P62 by immunoblot (Fig. 1). In addition, P62 staining was irregularly distributed (Fig. 4, ONC). In some areas, the P62-stained regions were distributed in a spotty pattern (Fig. 4, ONC).

Tau was expressed in the somas of the RGCs in the sham control, and the retinal nerve fibers (RNFs) were also positively stained with tau (Fig. 4, sham). On day 7 after the ONC, intensities of tau appeared to be increased and the expression of tau was well colocalized with P62 (Fig. 4, ONC). The increased immunoreactivities to tau are consistent with the results from the immunoblots (Fig. 2).

**Time-Dependent Changes of Tau Expression in Retina by Immunohistochemistry**

Confocal images of flat-mounted retinas stained with P62 and tau antibodies (Fig. 5) indicated that there were time-dependent changes in the expression of these proteins. More specifically, the P62 granules were uniformly distributed in the somas of RGCs in the control retinas, and the P62 immunoreactivity 1 day after the ONC was nonuniform. On the third day, coarse P62 granules were present, and subsequently, a spotty pattern of P62 staining occurred in some regions as previously described.

In the control retinas, tau immunoreactivity was homogeneously distributed in the somas of RGCs and RNFs. After the ONC, tau staining in the RNFs became scattered on day 1 and intensified by day 3. On day 7, regions of tau staining were distributed in a spotty pattern colocalizing with P62 immunoreactivity. The immunoreactivity to tau appeared to intensify throughout the observation period.

**Effects of Tau Silencing on Death of RGCs After Optic Nerve Crush**

Representative confocal images of flat-mounted retinas demonstrating the effects of tau silencing on the survival of the RGCs on day 7 after the ONC are shown in Figure 6. The RGCs were double stained with Alexa 488–conjugated Tuj-1 and Alexa 555–conjugated tau. The number of Tuj-1–positive cells, most likely RGCs, was reduced after the ONC, while the immunoreactivities to tau were intensified in the somas of RGCs and RNFs (Fig. 6A, si-NC). Confocal images of the si-NC group are shown at higher magnification in Figure 6B (upper). The expression of tau appeared to be increased in the somas of dying RGCs that were poorly stained with Tuj-1 (Fig. 6B, upper). These findings suggest that the increase in the level of tau is associated with the loss of RGCs. Tau silencing by siRNA
The density of RGCs was maintained at significantly higher levels of each). A further reduction was observed on day 14 when the control (n = 4) on day 7 in the si-NC group (P < 0.01; Scheffe). A further reduction was observed on day 14 when the level was 540 ± 74.5 cells/mm² in the si-NC group (n = 4). The density of RGCs was maintained at significantly higher levels of 1540 ± 89.5 and 1321 ± 192 cells/mm² on days 7 and 14, respectively, in the si-Tau group (P < 0.01, Scheffe test; n = 4 each).

**DISCUSSION**

The results showed that phosphorylated tau was increased in the retina along with an increase of P62 after the ONC. In addition, the level of LC3B1 was increased while that of LC3B2 was unchanged, which led to a significant decrease in the ratio LC3B2/LC3B1. Immunohistochemistry revealed that the expressions of P62 and tau were well colocalized in the RGCs and RNFs. In addition, an intravitreal injection of si-Tau suppressed the death of the RGCs by the ONC.

Because silencing the tau gene led to a reduction of the phosphorylated and total tau levels in the retina and rescued RGCs after the ONC, we suggest that the accumulation of tau species may have damaging effects on the RGCs. Thus, tauopathy may be involved in the death of RGCs from the ONC. These results are in good agreement with studies of experimental glaucoma where increased intraocular pressure causes altered phosphorylation of tau leading to accumulation of tau oligomer in the RGCs and their dendrites.³

Tau plays a critical role in maintaining the microtubules through which axonal transport takes place.¹ Hyperphosphorylation of tau at specific sites is associated with the formation of tau oligomers, and their presence is negatively correlated with their affinity to the microtubules.¹⁷ Impairment of axonal transport is one of the mechanisms that explains the accumulation of tau in the RGCs and RNFs. It has also been shown that abnormal tau increases the propagation of neighboring neurons in a prion-like mechanism.¹⁸ Unlike glaucomatous insults, injuries of RGCs caused by ONC have a subsequent but hyperphosphorylation and aggregation of tau cause excitotoxicity of the RGCs.² These mechanisms may explain how depressing the increased tau levels in the retina rescues RGCs after the ONC.

The IHC analyses showed that the expression of tau was colocalized with P62, and these expressions were increased in the somas of RGCs and in the RNFs after the ONC. Because P62 binds to both ubiquitinated proteins and LC3, the P62 probably functions as an adaptor protein of selective autophagy.¹⁹ Thus, these findings indicate that the increased levels of tau were ubiquitinated and bound to P62 after the ONC and could not be degraded sufficiently through selective autophagy.

It is still controversial whether autophagy is beneficial or detrimental for the RGCs after optic nerve injuries. The ONC causes the entry of extracellular Ca²⁺ into the RGCs leading to a rapid rise in intracellular Ca²⁺, which can then trigger the activation of calpain²⁰ and autophagy-mediated axonal degeneration.²¹ Thus, the autophagy inhibitor, 3-methyl adenine (3MA), can significantly reduce axonal degeneration.²¹ The results of another study suggested that autophagy is activated in glaucomatous rats, and 3MA protected the RGCs from the glaucomatous insults.²² On the other hand, genetic ablation of Atg4 and Atg5 in mice decreased the viability of RGCs after optic nerve transection, and rapamycin, an autophagy inducer, protected the RGCs through the activation of autophagy.²³

LC3, a microtubule-associated light-chain protein, is contained in autophagosomes and can be an indicator of autophagic activities. Cytosolic LC3-1 is converted to lipid-modified LC3-2 that translocates to the autophagosome membranes.²⁴ Thus, the levels of LC3-2 are more associated with the autophagic activities than LC3-1.²⁵ Although the levels of LC3-2 are known to change in a time-dependent manner after optic nerve injuries,²⁶ our results showed that ONC did not cause significant changes in the LC3B-2 levels on day 7. However, the levels of LC3B-1 and P62 were increased. Because P62 is indispensable for autophagic degradation,¹⁹ the level of P62 must be correlated inversely with autophagic activities.²⁷ Although the LC3B-2 levels did not change significantly after the ONC, the increase of P62 suggests that autophagy was most likely impaired in the retina after the ONC.²⁸ A decrease in the ratio of LC3B1/LC3B2 in the retina after the ONC supports this idea.
P62 plays a crucial role in preventing tauopathy because genetic inactivation of P62 causes an accumulation of phosphorylated tau and neurodegeneration. There are some significant correlations in the time-dependent changes in the expressions of P62 and tau, namely, from uniform distribution to scattered and spotty distribution with time. These findings suggest that P62 may have affected the accumulation of tau. The results of studies using P301 tau mice have shown that the activation of autophagy by trehalose decreased the level of P62 and the accumulation of tau. These changes improved the neurologic deficits. Recently, therapeutic interventions against tauopathies have been investigated extensively, which included modulation of tau phosphorylation and inhibition of tau aggregation. Based on our observations that autophagy was impaired in the retina and tau was accumulated with P62 after the ONC, autophagy stimulation may have beneficial effects on the RGCs after optic nerve injuries.

There are several limitations to this study. One limitation was that we did not determine the changes in the expressions of hyperphosphorylated tau and tau oligomers in the retina. Although these tau species are more associated with tauopathies, no specific antibodies are commercially available for them. We did not examine fully the autophagic condition after ONC either. Although the level of P62 is inversely correlated with autophagic activities, an increase of P62 may also be associated with ER stress and impaired autophagic flux in the retina. Thus, increase of P62 along with tau accumulation may simply be correlative. However, our data are consistent with the roles of P62. Future studies using a P62 knockdown will be important to further explore the roles for P62 in degrading tau.

Another limitation was that we did not examine the possible mechanism(s) that enhances tau phosphorylation and accumulation in the retina after ONC. In this regard, we have previously shown that levels of phosphorylated mTOR (mammalian target of rapamycin) were increased in the retina on day 7 after ONC in rats. Mammalian TOR is a protein kinase that regulates protein synthesis and autophagy, and its

**Figure 5.** Representative confocal images of flat-mounted retinas double labeled with P62 and tau antibodies as well as the nuclear stain by 4',6-diamidino-2-phenylindole (DAPI). In the control retina, the somas of the RGC granules show uniformly distributed P62 immunopositive granules. The somas of RGCs and RNFs are homogenously stained with tau antibodies. One day after the optic nerve crush, P62 immunoreactivity in RGC somas became uneven (red arrows) while the intensity of tau staining in the RNFs appeared to increase in some areas (green arrows). On day 3, coarse P62 granules are present (arrowheads), and the staining intensity of P62 in the RGC somas is uneven. Seven days after optic nerve crush, P62 and tau staining were colocalized in a spotty pattern (arrows). Throughout the observation period, tau immunoreactivity appeared to increase. Bar: 50 μm; NC: negative control without exposure to primary antibodies.
activation also enhances tau phosphorylation and accumulation in the brain. Thus, activation of mTOR is one possible mechanism that could increase the phosphorylated tau after ONC.

From a therapeutic point of view, inhibition of mTOR by rapamycin may have beneficial roles on the RGCs through stimulation of autophagy. These issues need to be investigated in more detail.

In conclusion, tau and P62 are increased in the rat retina after ONC, and silencing the tau gene led to the survival of the RGCs. These findings indicate that tauopathies are involved in optic nerve injuries. Thus, therapeutic interventions targeting the tau gene may be promising strategies for some optic nerve injuries.

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