Regulatory mechanisms of retinal ganglion cell death in normal tension glaucoma and potential therapies

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Abstract
Normal tension glaucoma (NTG) is a multifactorial optic neuropathy characterized by normal intraocular pressure, progressive retinal ganglion cell (RGC) death, and glaucomatous visual field loss. Recent studies have described the mechanisms underlying the pathogenesis of NTG. In addition to controlling intraocular pressure, neuroprotection and reduction of RGC degeneration may be beneficial therapies for NTG. In this review, we summarized the main regulatory mechanisms of RGC death in NTG, including autophagy, glutamate neurotoxicity, oxidative stress, neuroinflammation, immunity, and vasocostriction. Autophagy can be induced by retinal hypoxia and axonal damage. In this process, ischemia can cause mutations of optineurin and activate the nuclear factor-kappa B pathway. Glutamate neurotoxicity is induced by the over-stimulation of N-methyl-D-aspartate membrane receptors by glutamate, which occurs in RGCs and induces progressive glaucomatous optic neuropathy. Oxidative stress also participates in NTG-related glaucomatous optic neuropathy. It impairs the mitochondrial and DNA function of RGCs through the apoptosis signal-regulating kinase-JUN N-terminal kinase pathway. Moreover, it increases inflammation and the immune response of RGCs. Endothelin 1 causes endothelial dysfunction and impairment of ocular blood flow, promoting vasospasm and glaucomatous optic neuropathy, as a result of NTG. In conclusion, we discussed research progress on potential options for the protection of RGCs, including TANK binding kinase 1 inhibitors regulating autophagy, N-methyl-D-aspartate receptor antagonists inhibiting glutamate toxicity, ASK1 inhibitors regulating mitochondrial function, and antioxidants inhibiting oxidative stress. In NTG, RGC death is regulated by a network of mechanisms, while various potential targets protect RGCs. Collectively, these findings provide insight into the pathogenesis of NTG and potential therapeutic strategies.

Key Words: autophagy; endothelin 1; glutamate neurotoxicity; inhibitor; nerve regeneration; neuroinflammation; normal tension glaucoma; oxidative stress; retinal ganglion cell; vasocostruction

Introduction
Glaucoma is the second leading cause of blindness globally, and is expected to affect 118 million individuals by 2040 (Tham et al., 2014). Normal tension glaucoma (NTG) is a type of open-angle glaucoma with normal intraocular pressure (IOP) (i.e., ≤ 21 mmHg in 24 hours without medication) (Hirooka et al., 2021). NTG is a type of progressive glaucomatous optic neuropathy (GON) with higher prevalence in Asia versus other geographic regions (Esporcatte and Tavares, 2016). In NTG, optic disc excavation, retinal ganglion cell (RGC) death, and visual field defects are detected despite an IOP within the normal range (Cho and Kee, 2014). It has been reported that the IOP of patients with NTG often fluctuates between 15 and 20 mmHg (Kosior-Jarecka et al., 2016b). The body of RGCs is located in the retina, with their axons projecting to the brain nuclei through the optic nerve (Harada et al., 2020). The mechanism through which RGCs respond to injury has been investigated in various models (Daniel et al., 2018). The concept of neuroprotection against RGC death in glaucoma has been attracting considerable attention (Levin and Peeples, 2008; Francesca et al., 2015; Gossman et al., 2016).

A previous study reported that vascular factors are involved in the pathogenesis of NTG; moreover, oxidative stress, vasospasm, and endothelial dysfunction were identified as risk factors for the development of GON (Fan et al., 2015). Moreover, Trivi et al. (2019) summarized the vascular, mechanical, and genetic mechanisms involved in the pathogenesis of NTG. The prevention of RGC death and neuroprotection are important in NTG. Therefore, the objective of this review was to discuss the mechanisms involved in the regulation of RGC death, including related signaling pathways and transcription factors, as well as emphasize potential therapies for NTG.

Search Strategy
Studies on the mechanisms regulating RGC death in NTG, published from 1969 to 2022, were retrieved from the PubMed database. The search was conducted using the following terms: “autophagy” AND “RGC” OR “NTG” OR “normal tension glaucoma”; “glutamate” AND “RGC” OR “NTG” OR “normal tension glaucoma”; “oxidative stress” AND “RGC” OR “NTG” OR “normal tension glaucoma”; “neuroinflammation” AND “RGC” OR “NTG” OR “normal tension glaucoma”; “vasocostruction” AND “RGC” OR “NTG” OR “normal tension glaucoma”. The results of this search were further evaluated by screening the titles and abstracts of the articles.

Autophagy
Role of autophagy in RGCs
Autophagy, a highly conserved metabolic process, involves the degradation and recycling of cellular components (Klionsky, 2007). It participates in both cell death and survival (Maiese et al., 2012), which can be initiated by various stress signals, such as hypoxia, ionizing radiation, infections, and chemotherapeutic agents (Cadwell and Ken, 2016). Several autophagy-related phases are involved in this process: initiation/nucleation, elongation, maturation, fusion, and degradation (Fernández-Albarral et al., 2021). Initiation and nucleation are induced through nutrient starvation, during which an insulating membrane that engulfs damaged proteins and organelles is formed. Subsequently, the phagophore extends into a mature and closed autophagosome; these phases are termed elongation and maturation. Finally, in the fusion and degradation phases, the mature autophagosome fuses with a lysosome to form an autolysosome, followed by the degradation of its contents by proteases and lipases. The process of autophagy is regulated by numerous factors, and the normal cellular and tissue homeostasis depends on well-regulated autophagy (Cao et al., 2014). Previous studies have revealed that the autophagic capacity of cells plays a vital role in inflammatory and neurodegenerative diseases (Cuervo et al., 2005; Huang and Klionsky, 2007; Levine and Kroemer, 2008; Martinez et al., 2016; Jiang et al., 2022; Rickman et al., 2022). Therefore, the process of autophagy is closely related to the pathogenesis of several major ocular
Autophagy can be initiated by both retinal hypoxia and axonal damage (Sase et al., 2020; Li et al., 2021; Tang et al., 2021). In the dendrites of RGCs, the process of autophagy is launched to promote cellular protection, and autophagy may be an effective therapeutic method for inducing regeneration of the central nervous system (CNS) (Beckers et al., 2021; Mázala-de-Oliveira et al., 2022). Inhibition of autophagy induces the accumulation of apoptotic cells in the retinal neuroepithelium, where the proliferating neuroblasts differentiate into RGCs (Mellén et al., 2008). However, accelerating autophagy will aggravate the degradation of axons in traumatic injuries of RGCs (Figure 1).

Figure 1: Regulatory mechanisms of RGC death and potential therapeutic targets for NTG.

The main regulatory mechanisms of RGC death in NTG include autophagy, glutamate neurotoxicity, oxidative stress, neuroinflammation, immuno, and vasocostriction. Several related small molecule agents and clinical antioxidants are included. ASK: Apoptosis signal-regulating kinase; Dock3: dedicator of cytokines 3; EAAC1: excitatory amino acid carrier 1; ET-1: endothelin 1; GLAST: glutamate/aspartate transporter; H2O2: hydrogen peroxide; Hsp: heat shock protein; NF-kB: nuclear factor-kappa B; NMDA: N-methyl-D-aspartate; NTG: normal tension glaucoma; OBF: ocular blood flow; OPTN: optineurin; RGC: retinal ganglion cell; ROS: reactive oxygen species; TBK1: TANK-binding kinase 1.

Heterozygous mutations of optineurin (OPTN) have been reported in some familial and sporadic cases of NTG (Fuse et al., 2004; He et al., 2019). The GluSOSys (ESOK) mutation in OPCAN and Hispanic populations has been associated with the progression of NTG (Tin et al., 2005). However, the Meta891s (M98K) mutation was more commonly observed in Asians versus other populations (Alward et al., 2003). It has been verified that even transient expression of ESOK-mutated OPTN can induce apoptosis and death of RGC-5 cells (Chalasani et al., 2007). An initial study showed that homozygous OPTN knockout (KO) mice had reduced visual deterioration and preserved visual function, further supporting that suppression of OPTN may constitute a therapeutic strategy against glaucomatous neurodegeneration in NTG (Adi et al., 2021). The human OPTN gene codes for a 577-amino acid protein; it is organized into various domains, such as the nuclear factor-kappa B (NF-kB) essential molecule (NEMO)-like domain, ubiquitin-binding domain, leucine-zipper domain, and microtubule-associated protein 1 light chain 3 (Rezaie et al., 2002; Ying and Yue, 2012). As an autophagy receptor, OPTN plays an important role in autophagy, involving the degradation of ubiquitinated protein, mitochondrial damage, and bacterial ubiquitination (Korac et al., 2013; Wong and Holzbaur, 2014). Moreover, OPTN is a negative regulator of the NF-κB pathway, which is also linked to autophagy.

A close interaction between TANK (TRAF family member associated NF-κB activator)-binding kinase 1 (TBK1) and mutant ESOK OPTN protein, which protects mutant OPTN from dissolution, has been reported (Yuriko et al., 2013). TBK1 is a serine or threonine protein kinase, which is a non-canonical member of the IκB kinase (IKK) family and mediates the ability of TANK to initiate the NF-κB pathway (Pomerantz and Baltimore, 1999). TBK1 also participates in autophagy through phosphorylation of autophagy receptor proteins (Richer et al., 2016; Durand et al., 2018). In pedigrees with NTG, several research studies discovered copy number variations spanning the TBK1 gene, including duplications and deletions (Ritch et al., 2014; Awadalla et al., 2015; Kaurani et al., 2016). Particularly, mutations in both OPTN and TBK1 are closely related to the features of NTG cases (Finger et al., 2011). In a recent study, syntain 17, which has been implicated in autophagosome-lysosome fusion, was found to be a substrate for TBK1 phosphorylation. In summary, both syntain 17 and TBK1 play a vital role in the initiation of autophagy (Kumar et al., 2019).

Small molecules and therapeutic potential

TBK1 and IKK inhibitors have been developed and investigated in some cell-based studies and animal models (Table 1). Amlexanox, a specific small molecule inhibitor of TBK1 and IKK, was recently found to reduce cell proliferation, migration, and invasion by decreasing autophagy and the inhibition of melanoma-relevant proteins (Möller et al., 2020). An earlier clinical trial also demonstrated the glucose-controlling effect of amlexanox in patients (Oral et al., 2017). Eskiocak et al. (2017) found a TBK1/IKK inhibitor in the field of melanoma. In addition, several nonspecific inhibitors have also been studied in the xenograft environment. Research has shown that BX795 inhibits the growth of oral squamous cell xenograft (Bai et al., 2015). Another study showed that a dual TBK1/IKK inhibitor (compound 1) could enhance anti-programmed cell death 1 ligan 1 therapy in a xenograft model (Jenkins et al., 2018). Based on these discoveries in cancer and metabolic diseases, we should further investigate the potential neuroprotective and anti-inflammatory effects of TBK1/IKK inhibitors, particularly in NTG. Moreover, a novel technology termed proteolysis-targeting chimera (PROTAC) has become increasingly popular in recent years; this technology can be used to target a specific protein for degradation. Indeed, PROTAC has been used to decrease the selective degradation of TBK1 (Crew et al., 2017). Thomson et al. (2019) discovered a highly selective TBK1 inhibitor, termed GSK8612. This small molecule drug can be used to intercept the effect of TBK1 in the process of immunity, neuroinflammation, obesity, and cancer. Therefore, we investigated the functional roles of a TBK1 PROTAC and its therapeutic potential for NTG (Figure 1).

Table 1 | Summary of small molecule agents for the treatment of non-glaucoma disease

| Name | Target | Function | Disease/cell line | Publication |
|------|--------|---------|------------------|------------|
| Arunodic acid | s-100β | Activation of | Stroke | Tateishi et al., 2002 |
| BX795 | Induction of apoptosis | Squamous cell carcinoma | Bai et al., 2015 |
| Compound 1 (TBK1/IKK) | Enhancement of response to PD-1 blockade | Melanoma | Jenkins et al., 2018 |
| GSK8612 (TBK1) | Inhibition of IFNβ | TPH-1 | Thomson et al., 2019 |
| Amlexanox (TBK1/IKK) | Inhibition of autophagy | Melanoma | Möller et al., 2020 |

This table summarizes the targets, functions, disease models, and literature linked to small molecule agents which may possess protective activity for retinal ganglion cells and are considered potential therapeutic drugs for non-glaucoma disease. IFNβ: Interferon beta; IKK: IκB kinase; β-estradiol: estrogen; BX795: GSK8612 (TBK1 inhibitor) | TPH-1: human myeloid leukemia mononuclear cells.

Glutamate Toxicity

Molecular mechanism underlying glutamate toxicity

Glutamate plays an important role in GON. It is thought that glutamate and the related excitatory amino acids (EAAs) activate glutamate receptors, which is followed by mediation of excitatory synaptic transmission at photoreceptor/bipolar cell synapses and bipolar/ganglion cell synapses (Sucher et al., 1998). In RGCs, glutamate neurotoxicity is induced by the over-stimulation of N-methyl-D-aspartate (NMDA) membrane receptors by glutamate. In this process, intracellular Ca²⁺ influx is enhanced, which can cause organelle and DNA damage, including mitochondria and endoplasmic reticulum (Munemasa and Kitaoka, 2013). Glutamate receptor was previously regarded as a key regulatory mechanism for eliminating glutamate from the extracellular fluid of the retina (Danbolt, 2001). There are three transporters, namely glutamate transporter 1 (GLT-1), excitatory amino acid carrier 1 (EAAC1), and glutamate/aspartate transporter (GLAST) (Rauen et al., 2003). Notably, EAAC1- and GLAST-mice have shown progressive RGC loss and GON without elevation of IOP (Harada et al., 2007). To explore the relationship between mutation in the GLAST gene and susceptibility to glaucoma, Yanagisawa et al. (2020) sequenced the EAAT1 gene of patients with glaucoma. They identified four heterozygous mutations (A169G, E219D, T318A, and A329T), which caused amino acid substitutions in the EAAT1 protein. Furthermore, A169G and A329T mutations impaired glutamate uptake; however, E219D and T318A mutations did not have an effect on EAAT1. Moreover, GLAST-KO mice have been widely used to elucidate the mechanism underlying the development of NTG and provide potential therapeutic targets (Kimura et al., 2015; Dong et al., 2016; Sano et al., 2019).

Similarly, EAAC1-KO mice are suitable animal models for studying NTG with the characteristics of sporadic and age-dependent pathology (Harada et al., 2007). An earlier study has shown that apoptosis signal-regulating kinase 1 (ASK1) plays an important role in stress-induced apoptosis of RGCs in GLAST- KO mice, which can be beneficial in the treatment of NTG (Harada et al., 2010). Therefore, EAAT1/GLAST is responsible for the pathogenesis of NTG and a potential therapeutic target (Harada et al., 2020).

Potential therapeutic agents

As mentioned above, the over-stimulation of NMDA receptors led to neuronal cell death in the inner retina through the process of retinal excitotoxicity (Parsons et al., 1998). Using NMDA-induced retinal toxicity models, several studies have evaluated the effect of clinical drugs (Fang et al., 2010). Lai and Forster (1990) demonstrated that β-estradiol exerts a protective effect against NMDA-induced neurotoxicity. This finding suggests the potential protective effect of β-estradiol in the treatment of NMDA-related diseases, such as retinal ischemia and glaucoma. Another study utilizing the NMDA-induced retinal neurotoxicity model has shown a neuroprotective effect following the attenuation of delta9-tetrahydrocannabinol and cannabidiol, which is mediated by peroxynitrite (El-Remessy et al., 2003). It has been demonstrated that NMDA antagonists are effective in preventing neuronal degeneration in diseases (Kim et al., 2010; Chen et al., 2013; Dammak et al., 2021).
Alzheimer’s disease (Farlow, 2004). Researchers also focused on the effect of NMDA antagonists on RGCs (Takeda et al., 2018; Jiang et al., 2019). In Brown Norway rats, RGC degeneration, upstream changes in the optic nerve, actin cytoskeleton, and the associated deterioration in visual function mediated by NMDA receptors can be reversed by treatment with MK-801 (a NMDA receptor blocker) (Omokawa et al., 2014).

Owing to the characteristics of GLAST-deficient mice, drugs that are capable of increasing the levels of GLAST may be useful for neuroprotection. Arundic acid has some beneficial effects that are associated with the suppression of delayed extracellular glutamate accumulation in the cerebral artery occlusion model (Tateishi et al., 2002). Additionally, it has been demonstrated that adenosine receptor A2AR antagonists enhance the recovery of retinal function following an ischemic attack (Zhong et al., 2013). A recent study has shown the activating effect of SCH442416 (an A2AR antagonist), which increased glutamate uptake in Müller cells (Li et al., 2015). Furthermore, cyanin chloride (a type of anthocyanin) prevents hyperbaric pressure-induced death in Müller cells by increasing the levels of GLAST (Chen et al., 2018b). Together, the above findings suggest that some clinical drugs and receptor-related antagonists may be useful in the management of glaucoma. This evidence allows us to further investigate potential treatments for NTG (Figure 1, Tables 1 and 2).

### Table 1 | Summary of small molecule agents for the treatment of glaucoma

| Name               | Target           | Function                                      | Publication                        |
|--------------------|------------------|-----------------------------------------------|------------------------------------|
| SCH S8261          | A2AR             | Prevention of microglia morphology alterations and ROS production | Orgini et al., 1999; Aires et al., 2019 |
| THC                | Cannabinoid receptor CB1 | Protection of neuron cultures from glutamate-induced death | El-Remessy et al., 2003 |
| CBD                | Cannabinoid receptor CB1 | Protection of neuron cultures from glutamate-induced death | El-Remessy et al., 2003 |
| Dock3             | ASK1             | Prevention of oxidative stress-induced RGC death | Bessero and Clarke, 2010 |
| GBE                | Trehinitriose     | Protection of RGCs from apoptosis             | Baillargeon and Sonnet, 2010; Ghiso et al., 2013; Saccà et al., 2019 |
| CNQX               | KA receptors     | Suppression of RGC death                      | Omokawa et al., 2014 |
| VPA                | BDNF-TrkB        | Suppression of oxidative levels in RGCs        | Kimura et al., 2015 |
| CoQ10             | ATP              | Prevention of RGC death                      | Davis et al., 2017; Quaranta et al., 2019 |
| Chymo               | GLAST            | Protection of retinal Müller cells            | Chen et al., 2018b |
| Nicin             | NAD/NADP         | Higher nicin intake may be associated with a lower risk of developing glaucoma | Jung et al., 2018 |
| GSH                | Prevention of RGC degeneration and visual impairment | Sano et al., 2019 |
| ONL1204           | Fas receptor     | Abrogation of microglial activation and inhibition of the induction of multiple cytokines and chemokines | Krishnan et al., 2019 |
| AST                | ikB              | Inhibition of RGC degeneration                | Kikuchi et al., 2020 |
| Citonoe           | NO2              | Protection of neural tissues and visual function | van der Merwe et al., 2021 |
| β-Estradiol        | miR-320-3p       | Inhibition of inflammation                    | Xu et al., 2021 |
| miR-93            | ST3A            | Downregulation of retinal microglia-mediated neuroinflammation | Wang et al., 2021 |

The table summarizes targets, functions, and literature linked to small molecule agents which may possess protective activity for RGCs and are considered potential therapeutic drugs for normal tension glaucoma. A2AR: Adenosine receptor 2A; ASK1: apoptosis signal-regulating kinase 1; IL-18: interleukin-1β; TNF-α: tumor necrosis factor-alpha; TRPM8: transient receptor potential melastatin 8; ANF: atrial natriuretic factor; RGC: retinal ganglion cell; NMDA: N-methyl-D-aspartate; LTP: long-term potentiation; LTP: long-term potentiation; GABA:gamma-aminobutyric acid; CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione; NMDA: N-methyl-D-aspartate; GLAST: glutamate/aspartate transporter; GLAST: glutamate/aspartate transporter; GSH: glutathione; KA: kainic acid; NAC: N-acetylcysteine; NO2: nitrogen dioxide; ROS: reactive oxygen species; RGC: retinal ganglion cell; STAT3: signal transducer and activator of transcription 3; THCA: tetrahydrocannabinol; VA: valproic acid.

### Stress

**Molecular mechanism of oxidative stress in NTG**

Oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, during which oxidative processes are superior to antioxidative systems (Kimura et al., 2017). It has been recognized as one of the pathogenic factors of glaucoma. Notably, the plasma levels of glutathione (GSH) are decreased in primary open-angle glaucoma, including NTG (Park and Moon, 2012; Goyal et al., 2014). Oxidative stress is also involved in NTG-related ON (Trivi et al., 2019). The reduction in oxygen concentration leads to ROS formation, increasing the concentration of hydrogen peroxide (H2O2). This process further impairs electron flow and induces mitochondrial dysfunction. ROS formation induces oxidative damage to the mitochondria, and impairs cellular proteins and the DNA of RGCs as well as other factors (Chandra et al., 2013). The excessive production of antioxidant systems, leads to over-production of ROS and mitochondrial dysfunction in lamina cribrosa cells (McElnea et al., 2011). Moreover, a recent study revealed that oxidative stress was increased and blood flow was decreased in glaucomatous eyes compared to normal eyes (Noor et al., 2019). The reduction in oxygen concentration leads to ROS formation, increasing the concentration of hydrogen peroxide (H2O2). This process further impairs electron flow and induces mitochondrial dysfunction. ROS formation induces oxidative damage to the mitochondria, and impairs cellular proteins and the DNA of RGCs as well as other factors (Chandra et al., 2013). The excessive production of antioxidant systems, leads to over-production of ROS and mitochondrial dysfunction in lamina cribrosa cells (McElnea et al., 2011). Moreover, a recent study revealed that oxidative stress was increased and blood flow was decreased in glaucomatous eyes compared to normal eyes (Noor et al., 2019).
In glaucoma, the elevated IOP can trigger an innate immune response (Wei et al., 2019). Using an experimental model of glaucoma, several researchers observed increased microglia activity, cell density, and expression of the complement receptor of Clq in the retina and optic nerve, particularly prior to RGC and axonal loss (Howell et al., 2011; Trost et al., 2021). In contrast to the innate immune system, the adaptive immune responses involve T and B lymphocytes, require up to 7 days for activation, and are also featured in glaucoma (Li et al., 2019). Growing evidence shows that higher IOP, heat shock proteins (HSPs) are upregulated and significantly increased in human glaucomatous retinas (Soto and Howell, 2014). Chen et al. (2018a) reported that HSP-specific memory T cells are induced by commensal microorganisms and activated by host HSPs released in the retina, offering key evidence for the involvement of autoimmunity in glaucoma (Chen et al., 2018a). Moreover, elevated numbers of CD3+ CD8+ lymphocytes were observed in both NTG and primary open-angle glaucoma. Particularly, CD8+ HLA DR+ lymphocytes mediate NTG. Further studies on the immune response in NTG are warranted to elucidate the mechanism of neurodegeneration.

Microglial cells (immune cells residing in neural tissue) are responsible for synaptic maintenance and immune response to injury and inflammation (Colonna and Butovsky, 2017; Salter and Stevens, 2017). These cells act as neurovascular sensors and the first line of defense to injury in the CNS, including the brain and retina (Wei et al., 2019). Both adenosine 5’-triphosphate (ATP) release and microglial activation induce the mechanical stretch of astrocytes, leading to the elevation of nitric oxide and ET-1 in the extracellular matrix. Furthermore, the elevation of ATP (caused by mechanical stretch), ET-1, and related signaling pathways may improve the effectiveness of neuroprotective strategies (Silverman et al., 2016; Bosco et al., 2018; Reinehr et al., 2018). Therefore, the use of these molecules and their potential therapeutic targets in patients with NTG is warranted to assess the functional impact of these genetic polymorphisms.

The pathogenesis of NTG involves multiple factors. NTG is a type of glaucoma associated with progressive optic neuropathy in the absence of elevated IOP. Hence, therapeutic efforts should be focused on alleviating RGC death and protecting the optic nerve. The mechanisms involved in the regulation of RGC death are attracting considerable research attention. It has been demonstrated that several small molecules and their analogs are effective in animal models of glaucoma. Additional studies on these small molecules with neuroprotective properties are required for the discovery of potential therapeutic targets in patients with NTG.

**Conclusions**

In this review, we discuss and summarize the results of recent research on various mechanisms involved in the regulation of RGC death in NTG, and strategies for the inhibition of RGC death and protection of the optic nerve (Figure 2). Several small molecule agents related to autophagy, glutamate neurotoxicity, and neuroinflammation, as well as some clinical antioxidants inhibiting oxidative stress, have been identified. ET-1 and related signaling pathways are also analyzed to reveal novel therapeutic candidates for NTG.

**Research progress in neuroprotection**

NTG has been linked to several conditions, including nocturnal hypotension, inflammatory diseases, and alterations in C-reactive protein. Atalay et al. (2014) calculated that increased nitric oxide and platelet-to-lymphocyte ratios, C-reactive protein levels, and erythrocyte sedimentation rate in NTG. There were no significant differences in neurephillin-lymphocyte and platelet-to-lymphocyte ratios found, providing insight into the differential diagnosis of NTG. More interestingly, several researchers have discovered novel small molecule agents, which may control neuroinflammation and protect the optic nerve. Recent research has revealed that SCH 58261 (a potent and selective antagonist for human A2AR) prevents morphological alterations and apoptosis induction in microglial cells, providing its role in controlling retinal neuroinflammation (Ongini et al., 1999; Aires et al., 2019).

Moreover, in an inducible mouse model of glaucoma, a small peptide inhibitor of the C1q domain (ONL109) showed robust neuroprotection by abrogating the activation of microglial cells and inhibiting the induction of multiple cytokines and chemokines, components of the complement cascade, toll-like receptor pathway, and inflammasome pathway (Krishnan et al., 2013). Furthermore, studies have investigated the impact of endothelin (ET-1) on RGC and axonal loss. As the activation of microglial cells and attempted to identify potential targets for neuroprotective therapy. Wang et al. (2021) reported that the miR-93/92 signal transducer and activator of transcription 3 (miR-93/STAT3) pathway is related to the inhibition of death in microglial cells, promoting its role in controlling retinal neuroinflammation. Matrix-bound nanovesicles are a distinct class of extracellular vesicles localized specifically to the extracellular matrix. Studies have verified that these nanovesicles possess crucial functions in the rat model of ischemia-reperfusion injury (Pfeiffer et al., 2019).

**ET-1 and Vasoinconstriction**

Microcirculation is mainly regulated by vasoregulatory factors from endothelial cells, including nitric oxide and ET-1 (Haefliger et al., 2001). ET-1 mediates neurodegeneration in glaucoma (Syc-Mazurek et al., 2017; Ye and Meng, 2021). Marola et al. (2020) indicated that ET-1 can induce RGC death through the JUN-dependent pathway. ET-1 also activates c-Jun, and the DNA binding of c-Jun and CCAAT/enhancer-binding protein B (CEBPB) further indicates that ET-1 triggers c-Jun through endothelin receptors and c-Jun feedback elevates the expression of endothelin receptor (Wang et al., 2017). Further investigation is warranted to determine the upstream regulators and downstream targets of JUN signaling. This evidence may help to elucidate the mechanism underlying the regulation of RGC death by endothelin.

The limitations of the present review and several novel progresses in vision restoration should be acknowledged. Stark and Caprioli (2016) and Gu et al. (2021) focused on axon regeneration and reported that visual acuity may be reversed. A recent study showed that recticulum 3 (RTN3) can enhance neuroprotection and CNS axon regeneration, particularly RGC axon regeneration, thus improving visual function (Alhajlah et al., 2019).

In addition, Pfeiffer et al. (2019) described their early findings regarding structural neuroprotection and CNS axon regeneration and reported that visual acuity may be reversed. A recent study showed that recticulum 3 (RTN3) can enhance neuroprotection and CNS axon regeneration, particularly RGC axon regeneration, thus improving visual function (Alhajlah et al., 2019). In contrast to the primary progressive stage of visual field loss (Sugiyama et al., 1995), a recent study showed that the impact of ET-1 on RGC death and axonal loss (Howell et al., 2011; Trost et al., 2021). In addition, these studies are limited by the inclusion of one geographic region. Further studies are warranted to assess the functional impact of these genetic polymorphisms in a higher number of patients.

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