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Glial cells as a promising therapeutic target of glaucoma: beyond the IOP

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Glial cells, a type of non-neuronal cell found in the central nervous system (CNS), play a critical role in maintaining homeostasis and regulating CNS functions. Recent advancements in technology have paved the way for new therapeutic strategies in the fight against glaucoma. While intraocular pressure (IOP) is the most well-known modifiable risk factor, a significant number of glaucoma patients have normal IOP levels. Because glaucoma is a complex, multifactorial disease influenced by various factors that contribute to its onset and progression, it is imperative that we consider factors beyond IOP to effectively prevent or slow down the disease's advancement. In the realm of CNS neurodegenerative diseases, glial cells have emerged as key players due to their pivotal roles in initiating and hastening disease progression. The inhibition of dysregulated glial function holds the potential to protect neurons and restore brain function. Consequently, glial cells represent an enticing therapeutic candidate for glaucoma, even though the majority of glaucoma research has historically concentrated solely on retinal ganglion cells (RGCs). In addition to the neuroprotection of RGCs, the proper regulation of glial cell function can also facilitate structural and functional recovery in the retina. In this review, we offer an overview of recent advancements in understanding the non-cell-autonomous mechanisms underlying the pathogenesis of glaucoma. Furthermore, state-of-the-art technologies have opened up possibilities for regenerating the optic nerve, which was previously believed to be incapable of regeneration. We will also delve into the potential roles of glial cells in the regeneration of the optic nerve and the restoration of visual function.

KEYWORDS

adeno-associated virus, astrocytes, cell transplantation, glaucoma, intraocular pressure, microglia, Müller cells

Abbreviations: CNS, central nervous system; ECM, extracellular matrix; GCL, ganglion cell layer; IL, interleukin; IOP, intraocular pressure; IPL, inner plexiform layer; LC, lamina cribrosa; NMDA, *N*-methyl-D-aspartate; RGC, retinal ganglion cell; OL, oligodendrocyte; ONC, optic nerve crush; ONH, optic nerve head; OPC, oligodendrocyte precursor cell; PR, photoreceptor; scRNA-seq, single-cell RNA sequence.

1 Introduction

Neurons in the mammalian central nervous system (CNS) have long been perceived as incapable of regeneration in the adult tissue (1). Similarly, retinal neurons have historically been considered non-regenerative, leading to the perception that blindness resulting from retinal neurodegenerative diseases and optic neuropathies is untreatable. Consequently, visual impairment in glaucoma, a leading global cause of blindness, has traditionally been thought of as irreversible. However, decades of extensive research have revealed that there is potential for restoring visual function even after the onset of ocular neurodegenerative diseases. Given that retinal ganglion cells (RGCs), responsible for transmitting visual information to the brain, are selectively damaged in glaucoma, therapeutic efforts have primarily focused on cell-autonomous mechanisms. These strategies have achieved significant success in preventing RGC death in glaucoma model animals and regenerating the optic nerve following optic nerve injuries. However, achieving a complete recovery of visual function remains a formidable challenge. To attain this goal, non-cell-autonomous mechanisms must also be considered, as numerous extrinsic factors play a role in regulating RGC degeneration and optic nerve regrowth. In this review, we delve into the pathogenic mechanisms of glaucoma and explore potential molecular targets for the restoration of visual function. We particularly focus on glial cells, a type of non-neuronal cell within the nervous system, as potential sources of these extrinsic factors.

2 Glaucoma

Glaucoma, progressive optic neuropathy, is the leading cause of blindness worldwide that affects more than 70 million people (2, 3). Despite the multifaceted nature of the disease, with numerous risk factors influencing its onset and progression (4), elevated intraocular pressure (IOP) is the most well-known and modifiable factor (5, 6). The scourge of blindness in glaucoma finds its genesis in the grievous impairment suffered by the optic nerve and RGCs. RGC degeneration is a hallmark of glaucoma (7), while the damage at the optic nerve head (ONH) — the part where RGC axons coalesce to form the optic nerve (8) — precedes the onset of visual field loss in glaucoma (9). Moreover, dendritic and synaptic degeneration in RGCs are also initial events that play a pivotal role in the progression of the disease (10, 11). Given the intrinsic limitations associated with the regenerative potential of both RGCs and optic nerves, extensive research endeavors have concentrated on the dual objectives of averting RGC demise and forestalling optic nerve degeneration, with the ultimate aim of reinstating visual function. A growing body of evidence has suggested that the regenerative capacity of the ocular tissue can be modifiable by various factors including the intracellular signaling molecules, extracellular factors, and environmental conditions. While interventions targeting cell-autonomous mechanisms have yielded substantial strides in optic nerve regeneration, the realization of comprehensive functional recovery remains a formidable challenge.

3 Heterogeneity in glial cells in the ocular tissue

Glial cells, non-neuronal cell types in the nervous system, are not confined to the brain or spinal cord but also exist in the ocular tissue, such as the retina and optic nerve. In the retina, three types of glial cells exist: astrocytes, Müller cells, and microglia (Figure 1A). Astrocytes localize at the innermost surface of the retina and closely attach to blood vessels with their processes. Müller cells are the retina-specific astrocyte-lineage cells which are characterized by a vertical stalk spanning through the retina. Müller cells are limited to the retina, while astrocytes are highly enriched in the ONH and optic nerve (ON). Oligodendrocytes (OLs) and their precursors (oligodendrocyte precursor cells, OPCs) are present in the ON as illustrated in Figure 1B. RGC axon remain unmyelinated in most mammalian retina, with myelination commencing behind the myelination-transition zone located behind the globe. The myelination of the optic nerve facilitates the rapid transduction of visual information from the retina to the brain. Microglia, resident immune cells in the nervous tissues, including the retina, also contribute to the heterogeneity. Prior investigations have indicated that dysregulations in Müller cells and astrocytes can lead to RGC degeneration and visual dysfunction in the absence of elevated IOP (12, 13), underscoring their pivotal roles in the pathogenesis of normal tension glaucoma (NTG). Glial cells exhibit a remarkable degree of phenotypic plasticity, demonstrating either neurotoxic or neuroprotective attributes (14, 15). While it is widely acknowledged that reactive glial cells are frequently associated with neurotoxic functions, the appropriate regulation of glial cells has the potential to mitigate neuronal damage in a variety of neurodegenerative disease and CNS injury models (16–19). For instance, microglia-derived IL-1α, TNF, and C1q can induce the transformation of astrocytes into a neurotoxic phenotype, leading to damage to RGCs (14). Blockades, such as those preventing the formation of neurotoxic astrocytes, have been shown to protect RGCs in glaucoma model mice (20). On the other hand, microglia-derived TNFα, IL-1β, and IL-6 can induce astrocytes become reactive and neuroprotective (15). The neuroprotective effects of reactive astrocytes depend on STAT3 activity. Blocking the STAT3 signal in the astrocytes exacerbates RGC damage and visual impairment in the glaucoma model (21), emphasizing the crucial role of reactive astrocytes in protecting RGCs in glaucoma. Moreover, astrocytes and microglia tend to alter their phenotype in association with the disease state, exhibiting a relatively neuroprotective phenotype during the initial stages of neurodegenerative diseases in the brain (22, 23). In the case of the DBA/2J mouse, an inherited glaucoma model, reactive astrocytes have been found to confer protective effects upon RGCs during the early stages (23). Understanding these mechanisms and the precisely modulating glial cells represent an appealing avenue for neuroprotection. Alongside the phenotypic changes of glial cells, recent advancements in single-cell RNA sequencing (scRNA-seq) have unveiled heterogeneity among glial cells, revealing distinct subclusters that exhibit neuroprotective functions even under pathological conditions in the brain (24). While the most recent

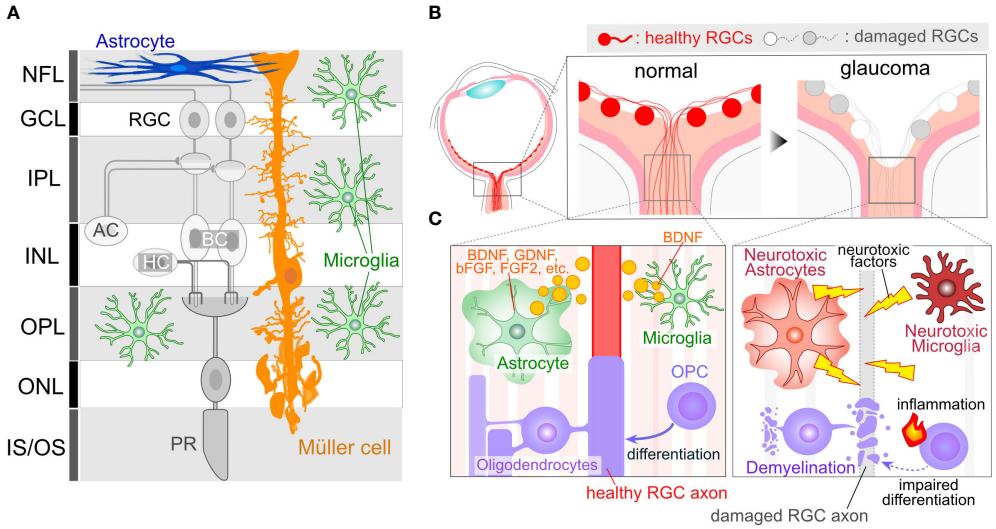

FIGURE 1
Glial Cells in Ocular Tissues. **(A)** Retinal Structure and Cellular Components: The retina comprises several neural layers. In the ganglion cell layer (GCL), one can find the cell bodies of RGCs. Additionally, some displaced amacrine cell (AC) somas are also localized within the GCL. RGC axons extend through the nerve fiber layer (NFL) and converge to form the optic nerve at the optic nerve head (ONH). Within the inner plexiform layer (IPL), RGC dendrites interact with axons from ACs or bipolar cells (BCs), forming synapses. Cell bodies for Acs and BCs reside in the inner nuclear layer (INL). Toward the outer part of the INL, horizontal cell (HC) bodies are present. In the outer plexiform layer (OPL), synapses formed by BCs, HCs, and photoreceptors (PRs) can be observed. PR cell bodies are located in the outer nuclear layer (ONL). PRs receive support from the retinal pigment epithelium, situated on the outer side of the PR inner/outer segments. Astrocytes are primarily found in the innermost retinal layer. Microglia are distributed across several retinal layers, including NFL/GCL, IPL, and OPL. Müller cells, which are retina-specific astrocyte-lineage cells, span vertically throughout the entire retinal thickness, with their cell bodies located in the INL, extending fine processes toward the synapses. **(B)** ONH Cupping in Glaucoma: ONH cupping represents well-characterized structural changes in the eyes of human glaucoma patients. These structural alterations may result in deformation and damage to RGC axons. Importantly, this change occurs in association with glial activations, suggesting that glial cells may contribute to the enlargement of cupping. **(C)** RGC axons in the ONH are unmyelinated and are directly enveloped by astrocytes and microglia. The ON is myelinated behind the optic nerve lamina region by OLs. OPCs also exist in the ON. Glial cells in normal conditions provide support for RGC axon integrity, including the production of neuroprotective factors (e.g. BDNF, GDNF, bFGF, and FGF2 from astrocytes and BDNF from microglia). However, in glaucoma, glial cells may undergo phenotypic changes, transitioning to neurotoxic states, which can lead to damage to RGC axons.

scRNA-sec data from the human and mouse retinas have successfully detected several subclasses of astrocytes, Müller cells, and microglia (25–29), these data are derived from human diseases or mouse models rather than specifically from glaucoma. Furthermore, in many cases of scRNA-seq data, ocular astrocytes and microglia are found as only a minor population and single cluster (13). Obtaining their subclusters in the scRNA-seq data requires cell isolation from at least several retinae (30). Given that scRNA-seq data from brain neurodegenerative disease model animals have detected disease-associated subclusters of glial cells (i.e. disease-associated microglia or astrocytes) that dynamically affect disease progression (31, 32), ocular glial cells in the human glaucoma patients or animal models would likely exhibit similar subclusters. Technical advancements in this field will enable us to uncover glaucoma-associated subclusters of glial cells and their role in glaucoma.

4 The Roles of glial cells in the enlargement of the ONH cupping

RGCs position their cell bodies within the ganglion cell layer (GCL), with their dendrites extending into the inner plexiform layer (IPL) (Figure 1A). GC axons converge to form the optic nerve at the ONH. The optic nerve exits the eye via the lamina cribrosa (LC), a mesh-like structure through which RGC axons pass. In human patients diagnosed with glaucoma, structural alterations in the LC lead to an enlargement of the ONH cupping (Figure 1B), a characteristic feature observable through ophthalmoscopy. Given that RGC axons passing through the LC are subject to deformation and damage due to ONH cupping, it becomes imperative to elucidate the cellular and molecular mechanisms underlying the pathogenesis of glaucoma. Rodents, frequently employed as experimental or genetic models for glaucoma, have traditionally been believed not to possess an LC structure that is rich in collagen, as is the case in humans (33). Instead, the equivalent region in rodents, known as the glial lamina, is highly enriched in astrocytes expressing glial fibrillary acidic protein (GFAP) (33). Given that the human LC also consists of astrocytes, and considering the close proximity of these astrocytes to RGC axons, any changes in their function are likely to exert a significant influence on the optic nerve (Figure 1C). In addition, the LC contains microglia, which become reactive and accumulate in response to optic nerve injuries (34). In humans, the LC is enriched in collagen, a major component of the extracellular matrix (ECM) (9). The enlargement of the ONH cupping is induced by ECM remodeling, a process initiated by degradation and production of ECM. Matrix metalloproteinases (MMPs), highly expressed in astrocytes and microglia, plays a role

in ECM degradation (35, 36). Single nucleotide polymorphisms (SNPs) in the *MMP9* gene are associated with a higher risk of primary open-angle glaucoma (POAG) and NTG (37). Additionally, the production of ECM is crucial for tissue remodeling. Mutations or SNPs in ECM genes such as thrombospondin1 (*THBS1*) or fibronectin (*FNDC3B*) are linked to the risk of glaucoma (38, 39), and both are produced by astrocytes (40–42). Furthermore, since ONH cupping can be observed in patients irrespective of their IOP levels, including POAG and NTG, it is plausible that tissue changes and the pathogenesis of glaucoma are more closely linked to glial dysfunction than to elevated IOP.

5 The role of glial cells in promoting axonal regeneration within the ONH

Axonal injury occurring at the ONH stands as one of the pivotal events in the initiation and progression of glaucomatous pathology (Figure 2A). The optic nerve crush (ONC) model has been established as a well-recognized experimental paradigm for the assessment of axonal regeneration. Earlier investigations have unequivocally illustrated that axonal regeneration can be augmented through the grafting of peripheral nerves (43–45). This underscores the critical role played by extracellular factors and/or the microenvironment in regulating the regenerative capacity of RGC axons. In the context of spinal cord injury (SCI), the resurgence of axonal growth is contingent upon the presence of reactive astrocytes, while scar-forming astrocytes express molecules conducive to axonal growth, such as laminin (46). The effects are further potentiated by neurotrophic factors, which elicit a robust resurgence of axonal growth through the astrocytic scar and across lesion cores, exceeding control conditions by more than a hundredfold (47).

Microglia also harbor the potential to support axonal growth. The transplantation of immature microglia has been shown to significantly enhance the recuperative process and foster axon regeneration following SCI (48). These microglial cells manifest the expression of various ECM proteins, notably including fibronectin and thrombospondin (Figure 2B). Furthermore, they exhibit the presence of endopeptidase inhibitors, which serve as crucial regulators in the resolution of inflammation. The ECM-mediated facilitation of axonal regrowth is also instigated by astrocytes (46). Moreover, microglia have been observed to elicit

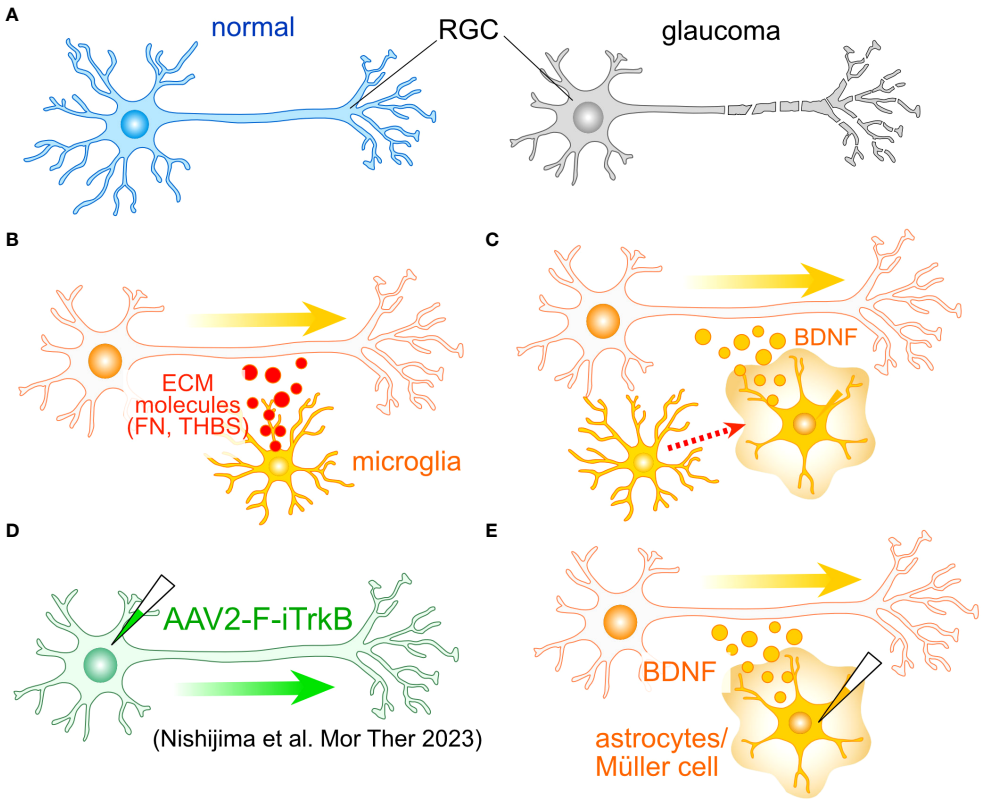

FIGURE 2
Potential roles of glial cells in the regeneration of RGC axon. **(A)** One of the most critical aspects of glaucoma is the damage to RGC axons. To identify potential molecular targets for axonal regeneration, researchers often employ the optic nerve crush (ONC) model. **(B)** Tissue regenerative microglia. Microglia involved in tissue regeneration express extracellular matrix (ECM) molecules like fibronectin (FN) and thrombospondin (THBS), potentially facilitating the regeneration of RGC axons. **(C)** Microglia-induced BDNF Expression: Microglia can induce the expression of BDNF in Müller cells, which may also accelerate axon regeneration. **(D)** An example of cell-autonomous enhancement of RGC axon regeneration. The induction of the farnesylated intracellular domain of TrkB (F-iTrkB) leads to a remarkable enhancement of axonal regeneration following ONC. **(E)** AAV-mediated expression of BDNF in astrocytes and Müller cells may stimulate axon regeneration and provide protection to RGCs, respectively.

the expression of brain-derived neurotrophic factor (BDNF) in Müller cells (49) (Figure 2C). Concurrent administration of neurotrophic factors alongside glia-mediated support has been demonstrated to engender a robust resurgence of axon growth following SCI (47). Consequently, the amalgamation of intrinsic mechanisms with glia-mediated support holds the promise of inducing a synergistic and remarkable rejuvenation of RGC axons.

6 The role of Müller cells in the protection of RGCs

Notably, neurotrophic factor signaling in reactive astrocytes has been documented to exert a protective influence on RGCs during the early stages of glaucoma (23). Among the neurotrophic factors, BDNF and its receptor TrkB are postulated to be pivotal in upholding the integrity of RGCs in glaucoma (50, 51). Müller cells emerge as the primary source of neurotrophic factors, their induction is triggered by various stimuli and insults (52–57). The sustained expression of BDNF in Müller cells has been demonstrated to confer protection upon RGCs following optic nerve injury (58, 59). Recent research has spotlighted the adeno-associated virus (AAV)-mediated enhancement of TrkB signaling in RGCs, leading to both cryoprotection against glaucoma and a vigorous resurgence of RGC axons (60) (Figure 2D). Collectively, these findings posit that Müller cell-derived neurotrophic factors, with particular emphasis on BDNF, hold paramount importance in protecting RGCs against glaucoma. Additionally, both astrocytes and Müller cells emerge as promising candidates for promoting the regeneration of RGC axons (Figure 2E).

7 Synapse disassembly in the context of glaucoma

Glaucoma has traditionally been regarded as an optic neuropathy that results in optic nerve damage and RGC degeneration. RGC dendrites receive inputs from bipolar and amacrine cells, establishing synaptic connections in the IPL (Figure 1A). Visual information from photoreceptors is relayed to RGCs, then transmitted via the optic nerve to visual centers in the brain. Dendritic atrophy and synapse loss in RGCs can lead to visual deficits. It is well-established that RGCs undergo age-related dendritic atrophy preceding the degeneration of their cell bodies (61) (Figure 3A). Dendritic atrophy and synapse loss in RGCs represent shared structural characteristics observed in animal models of glaucoma and post-mortem human retinas (10, 11, 61–67). An accumulating body of evidence suggests that dendritic atrophy in RGCs and synapse loss within the IPL constitute early indicators of glaucomatous pathology (67–71), alongside optic nerve and RGC soma degeneration. Although achieving selective control over dendritic/synaptic atrophy poses a challenge, several studies have demonstrated that inhibiting atrophy is associated with the protection of RGC soma (11, 64, 72). Prevention of dendritic atrophy has been realized through various approaches, including blockade of the complement pathway (11), intravitreal injection of chondroitinase ABC (66) or BDNF (72). In glaucoma, synapses within the IPL are marked by complement C1q (70), and blocking the complement pathway has been shown to confer protection to RGCs (11, 73). Resident microglia eliminate the synapses with C1q and its downstream C3 (70). These extracellular signals and molecules appear to be promising targets for glaucoma treatment. Among them, ANX007, an anti-C1q monoclonal antibody, is currently undergoing clinical trials for the treatment of glaucoma (74).

8 The involvement of glial cells in synaptic maintenance

Glial cells play crucial roles in the regulation of synapses, both under normal physiological conditions and in pathological contexts. For instance, glial cells serve as important regulators during the critical period, which is a developmental stage characterized by

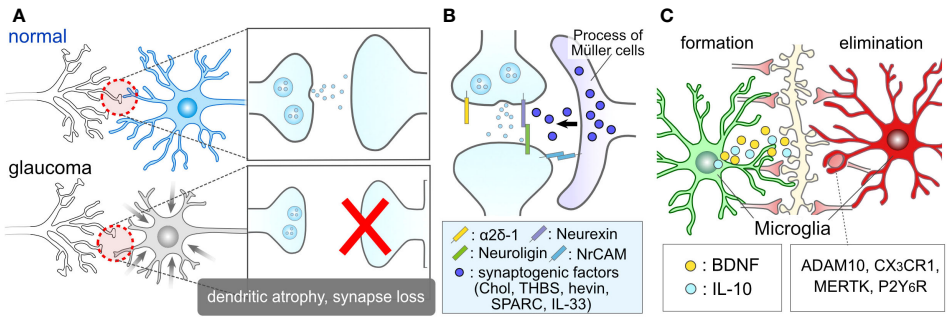

FIGURE 3
Glial Roles in Synapse Elimination and Formation. **(A)** Synaptic damage in glaucoma. Glaucoma results in the damage of RGC dendrites and retinal synapses. **(B)** Müller cell association with retinal synapses. The processes of Müller cells closely interact with retinal synapses. They might release synaptogenic factors, including cholesterol (Chol), thrombospondin (THBS), hevin, SPARC, interleukin-33 (IL-33), and regulate RGC synapses. **(C)** Microglial involvement in synapse formation and elimination. Microglia are recognized for their role in inducing synapse formation through contact and the release of factors such as BDNF and IL-10. Moreover, microglia are well-documented contributors to synapse elimination through various molecules, including ADAM10, CX3CR1, MERTK, and $P2Y_6R$.

heightened synaptic plasticity within the nervous system (75, 76). Astrocytes contribute to the formation of synapses through contact-mediated signaling (77, 78) and the production of synaptogenic factors, such as cholesterol (79), thrombospondin (80), hevin, SPARC (81), IL-33 (82) and neuronal adhesion molecule (83) (Figure 3B). While these factors were initially identified in brain astrocytes, they should also be expressed by ocular astrocytes and Müller cells, as evidenced by recent single-cell RNA sequencing data in the human retina, which shows high expression of these genes in astrocytes and Müller cells (84). Microglia also play a role in synaptogenesis through direct contact with synapses (85–87) and the secretion of molecules like interleukin 10 (IL-10) and BDNF (88–90) (Figure 3C). Müller cells serve as a source of BDNF in the retina and express or produce BDNF under various conditions and stimuli (49, 54, 57, 91, 92). The BDNF signal plays a regulatory role in the formation of dendrites in RGCs (93). Given that the fine processes of Müller cells intimately associate with RGC soma (94, 95), dendrites (96), and axons (97), BDNF derived from Müller cells is likely to have a significant impact on the regulation of RGC dendrites.

Glial cells also play a pivotal role in synapse elimination. Microglia, recognized as professional phagocytes, contribute to synapse elimination through various molecular mechanisms, including complement, ADAM10, CX3CR1, MERTK, and $P2Y_6$ receptors (70, 98–103) (Figure 3C). Microglia-mediated synapse elimination serves as a crucial regulator in both the formation and maintenance of physiological neural circuits, as well as the disruption of pathological neural circuits. Dysfunctions in purinergic signaling, such as $P2Y_6$ receptors, have been implicated in the pathogenesis of glaucoma (104–106). Additionally, astrocytes, considered non-professional phagocytes, also participate in synapse pruning through various factors like MEGF10 and MERTK (107–110). In the absence of microglia, astrocytes adopt phagocytic capabilities via TAM receptors (111). Beyond the individual responses of these cells, astrocytes and microglia coordinate their phagocytic functions (112). Moreover, bidirectional communication between them dynamically governs their functions and exerts an influence on synaptic and neuronal conditions (14, 15, 113, 114). These findings underscore the close relationship between glial conditions and synaptic conditions, highlighting glial cells as promising therapeutic targets in the context of glaucoma.

9 Oligodendrocyte dysfunction or loss in the context of glaucoma

Glial cells surrounding the optic nerve, such as OLs and OPC, may also play an important role in the pathogenesis of glaucoma. Myelin, formed by OLs, accelerates signal transduction through axons and provides essential energetic support. Deletion of the gene encoding myelin basic protein (MBP), highly expressed in OLs, leads to axonal swelling and degeneration (115). OLs exhibit persistent turn over, continuously replenished by newly differentiated cells from OPCs. Blocking OL turnover results in reduced myelination and axonal damage (116), highlighting the indispensability of OLs for axon homeostasis and functions.

Traditionally, glaucoma is not categorized as a demyelinating disease, however, emerging evidence suggests dysfunction and potential loss of OLs in glaucoma. DBA/2J mouse model demonstrates OL loss (117). A recent human study has indicated increased radial diffusivity within the optic radiations, serving as a surrogate marker for myelin damage (118). The study also observed a delay in the conduction of multifocal visual evoked potential, indicative of slowed conduction associated with myelin loss. In both the optic nerve injury (118) and glaucoma (119) animal models, OL loss and demyelination precede RGC damage. Maintaining OPC differentiation and myelination involves cell-autonomous mechanisms, such as thyroid hormone (120). Hypothyroidism is suggested as a risk factor for glaucoma (121–123), supporting the idea that impaired OL function contributes to the pathogenesis of glaucoma.

Additionally, non-cell-autonomous mechanisms may play a role. In the DBA/2J mouse, microglia in the myelinated region express and up-regulate the expression of Galectin-3/Mac-2, a phagocytosis-related gene (117), suggesting the involvement of microglia in myelin phagocytosis and the demyelination process. OPCs also express key phagocytotic genes and engage in axon pruning during the developing stage of mouse cortex (124), though the pathological consequence of OPC phagocytotic function in glaucoma remains unclear. OPCs may contribute to neuroinflammation and demyelination via low-density lipoprotein-related receptor 1 (LRP1) (125).

Another potential mechanism involves astrocyte-mediated cholesterol support (126). In the experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), astrocytes show down-regulated cholesterol synthesis and increased immune responses. Furthermore, phagocytosis by astrocytes may contribute to the demyelination (127, 128). Beyond demyelination, altered energetic support by OLs may be a crucial factor in glaucoma. In human patients with glaucoma, OL mitochondria are small (129). In DBA/2J mouse, monocarboxylate transporter 1 (MCT1), a lactate transporter, is down-regulated in OLs (130), suggesting reduced energetic support by OLs in glaucoma.

Preserving or restoring myelin could be a promising target for glaucoma treatment. Since cholesterol synthesis is promoted during remyelination (131), expediting cholesterol synthesis in astrocytes and/or oligodendrocytes may prove beneficial for glaucoma. Activation of astrocytic ABCA1 stimulates cholesterol synthesis (126) and supports oligodendrocyte survival and myelination (132). Astrocyte-derived CXCL1 also promotes remyelination by stimulating CXCR2 in OLs (133). A ketogenic diet might present an appealing approach to enhance energy availability by reversing the decline in MCT1 (130). Considering that neuronal activity boosts myelination (134, 135), visual stimulation could also be an attractive method for restoring RGC axons and visual function (136).

10 Tools for regulating glial cells and their potential role in glaucoma treatment

As mentioned earlier, glial cells have the capacity to influence the synapses, axons, and soma of RGCs, whether in a degenerative or regenerative manner. Beyond their neuroprotective capabilities, they hold significant potential for stimulating the regeneration of ocular structures and functions. In this section, we discuss various tools for controlling glial cell functions and their potential application in future glaucoma treatments. A summary of the advantages and disadvantages of each technique is shown in Table 1.

10.1 Adeno-associated virus targeting glia for gene therapy in glaucoma

The genetic approach stands as a potent method for addressing neurodegenerative diseases, including ocular conditions (137). In addition to gene therapy aimed at neurons, targeting non-neuronal cells could also prove effective in treating glaucoma. Previously approved gene therapies have operated through non-cell-autonomous mechanisms. An exemplar is Luxturna™ (voretigene neparvovec-rzyl), the inaugural gene therapy approved for treating patients afflicted with inherited retinal dystrophy, a rare genetic disorder affecting the retina (138, 139). In this disease, blindness arises from photoreceptor (PR) degeneration, yet Luxturna™ targets the retinal pigment epithelium (RPE). The restoration of RPE functions provides support and protection to PRs. Such non-cell-autonomous mechanisms could similarly be applied to glaucoma. For cell type-specific gene therapy using AAV, specific promoters tailored for each cell type are employed. The glial fibrillary acidic protein (GFAP) promoter is a key promoter for astrocytes, and the gfaABC$_1$D promoter, exhibiting nearly 100% specificity with 2-fold greater activity (140), is now widely adopted for precise astrocyte-specific gene manipulation via AAV. For achieving Müller cell specificity, there have been developments in engineering AAV capsids. Capsid variants derived from AAV6, such as ShH10 and ShH10Y, demonstrate efficient gene expression in Müller cells upon intravitreal injection (141–143). Moreover, the retinaldehyde-binding protein 1 (RLBP1) promoter has been successful in inducing gene expression specifically in Müller cells (143). For targeting OLs, promoters for the genes encoding proteolipid protein (144), myelin basic protein (145), and the myelin-associated glycoprotein (146) are used. Presently, foundational research efforts striving to facilitate the regeneration of RGC axons and synapses are categorized into two strategies: the promotion of regenerative factors and the prevention of inhibitory factors hindering regeneration. One example of the former strategy is BDNF. As previously mentioned, glial cells serve as the primary source of neurotrophic factors, including BDNF, and they maintain close associations with RGC axons and dendrites. The AAV-mediated expression of BDNF in astrocytes and Müller cells could potentially result in efficient delivery to axons and synapses, respectively. On the other hand, an example of the latter strategy involves insulin-like growth factor (IGF). Insulin and IGF share receptors and downstream signaling pathways (147) both of which are linked to RGC protection and the regeneration of dendrites, synapses, and axons (148–151). Notably, IGF-binding protein (IGFBP), which binds to IGF and hampers its signaling, becomes upregulated in astrocytes during neurodegenerative conditions and neurodevelopmental diseases (152–154). Given that the inhibition of astrocytic IGFBP partially restores neuronal function in the brain (152), the suppression of IGFBP signaling in astrocytes and/or Müller cells may prove beneficial for safeguarding and rejuvenating RGCs by enhancing IGF signaling.

The use of targeted gene therapy in microglia presents itself as an appealing candidate for the treatment of glaucoma. Previously, inducing gene expression in microglia using AAV posed a challenge, but cutting-edge techniques now enable us to achieve such gene induction (155–157). Lin et al. initiated the evolution of the AAV capsid protein (AAV-cMG) in conjunction with the Cre-LoxP system, resulting in selective gene induction in microglia *in vivo* (28). Okada et al. utilized a 1.7-kb putative promoter region of the *Iba1* gene for inducing gene expression in microglia/macrophage cells (156). Young et al. achieved enhanced selectivity for microglia/tissue-resident macrophages by inserting a random 21-mer into the AAV9 capsid (157). It is well-established that microglia exhibit high motility and accumulate at injury sites following ONC (34). These inherent characteristics of microglia allow us to utilize them as vectors for delivering molecules to the site

TABLE 1 Techniques for Glial Cell regulation: advantages and Disadvantages.

Techniques	Advantages	Disadvantages
1. AAV	a. Promoter and capsid-mediated cell specificity b. Prolonged therapeutic effect c. Already employed in clinical treatments	a'. Potential off-target effect b'. Impact on the innate immune system
2. PLX	a. Mainly affects microglia in the nervous system b. Well-regulated temporally c. Renewal and resetting of endogenous microglia	a'. Potential impact on border-associated macrophages and a subset of peripheral macrophages b'. Lacks tissue selectivity c'. Efficacy might be altered if microglial CSF1R expression were modified
3. Glial transplantation	a. iPSC-derived cells are applicable b. Grafted cells exhibit relatively long-term survival c. No need for immune suppression (via transnasal transplantation)	a'. May be influenced by the microenvironment of the host tissue b'. Lack tissue selectivity (via transnasal transplantation) c'. Invasive (injection-based transplantation)
4. TES	a. Already applied in clinical treatment b. Non-invasive c. Stimulation is selectively applied to the cornea	a'. Parameters should be optimized for glaucoma b'. Inappropriate settings may be detrimental to patients

of injury. Furthermore, we can employ proinflammatory gene promoters to activate the expression of the target gene (Figure 4A). For instance, promoters associated with interleukins and tumor necrosis factor α (TNFα) can be employed within this system, given that these molecules are produced by microglia at the lesion core (15). In cases of glaucoma and post-ONC, the ONH sustains damage, leading to the accumulation of microglia at the injury core. This, in turn, triggers the proinflammatory program in AAV-treated microglia, subsequently inducing the production of target genes (Figure 4B). Promoting remyelination emerges as an appealing strategy for vision recovery in glaucoma. The induction of connexin (Cx) genes, such as Cx32 and Cx47, has been associated with a protective effect against leukodystrophy (158, 159), indicating a potential impact on optic nerve remyelination. Moreover, the deletion of the *Chrm1*gene, which encodes muscarinic receptor 1, a negative regulator of OPC differentiation, leads to increased myelination and axon density (116). The limitation of this technique for the clinical application might lie in the efficiency of AAV delivery to the target tissue and cells. For example, achieving efficient AAV delivery to the optic nerve remains a challenging issue.

10.2 Pharmacological tools for controlling microglia: PLX compounds

PLX compounds, originally developed by Plexxikon Inc., serve as potent antagonists for the colony-stimulating factor 1 receptor (CSF1R). Oral administration of PLX3397 for either 7 or 21 days results in a reduction in brain microglia numbers by 80-90% and over 95%, respectively (160). Several analogs of PLX compounds, including PLX3397, PLX5562, and PLX647, have been developed. Oral PLX treatment also leads to a significant decrease in the number of retinal microglia (161, 162). This effect is reversible, with microglia repopulating after the discontinuation of PLX compounds (Figure 5). In the case of retinal microglia, the rate of recovery varies among retinal layers, namely the NFL/GCL, IPL, and OPL (163). Upon removal of PLX, microglia spontaneously repopulate through proliferation in both the brain and retina. The removal and repopulation of microglia induce an anti-inflammatory response and promote brain recovery following injury (164–167). Numerous studies have demonstrated that microglia alter their phenotypes to become neurotoxic, and the removal and repopulation of microglia elicit a neuroprotective effect in models of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease (168–171). Additionally, aside from brain diseases, microglia also play a role in neurodegenerative ocular injuries and diseases (172–174). Given that PLX treatment exhibits a protective effect on RGCs against *N*-methyl-D-aspartate (NMDA)-mediated toxicity (162), this compound may also have potential applications in the treatment of glaucoma. Although studies have shown that axonal regeneration after ONC is unaffected by the absence of microglia (175), this condition conceals both the neurodegenerative and supportive capabilities of microglia. PLX-mediated repopulation generates 'new' microglia with their phenotypes and functions reset, even in pathological conditions. For example, repopulation of aged microglia converts their cellular characteristics to a more youthful state, rescuing age-associated deficits in synapses and brain functions (176). Immature microglia possess the potential for anti-inflammatory responses and tissue regeneration (48). Such a 'microglial reset' could also prove valuable in restoring synapses and

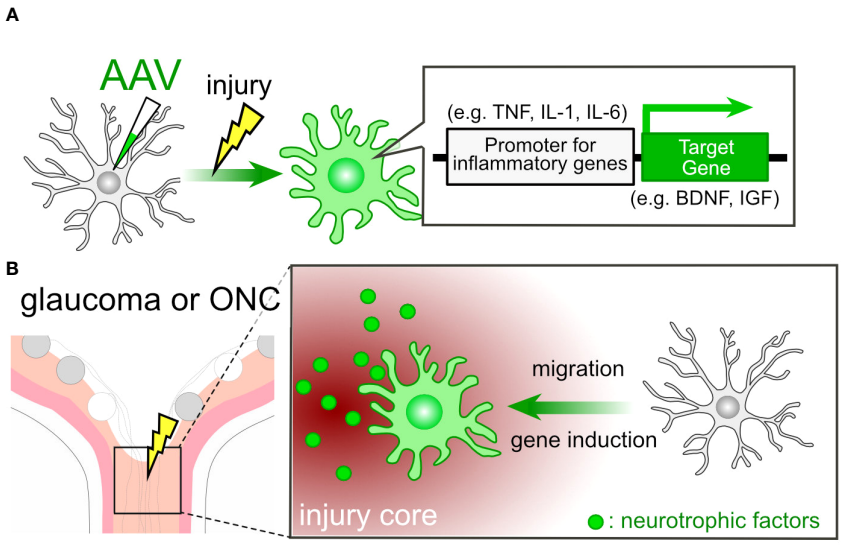

FIGURE 4
AAV-Mediated Cell Engineering and Target Molecule Delivery by Microglia. **(A)** AAV-mediated induction of neurotrophic factor genes in microglia. AAV-mediated engineering can induce the expression of neurotrophic factor genes in microglia under pathological conditions. By employing promoters associated with proinflammatory genes, microglia can produce neurotrophic factors like BDNF and IGF in response to pathological conditions. **(B)** Microglial Response in Glaucoma or ONC: In cases of glaucoma or ONC, the injury core triggers the microglial proinflammatory program, resulting in the induction of neurotrophic factor genes in microglia treated with AAV.

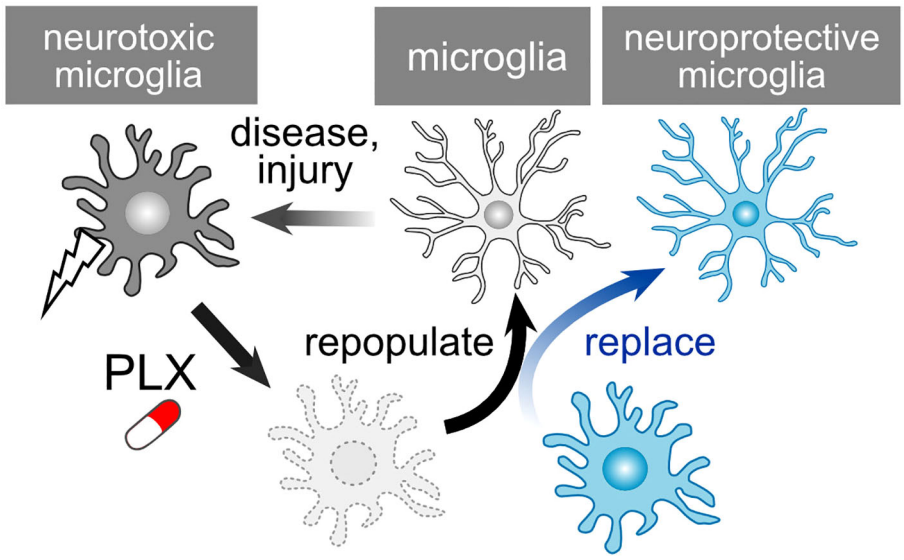

FIGURE 5
PLX-Mediated Repopulation and Replacement of Microglia. Microglia undergo a shift towards neurotoxic phenotypes following injury or in pathological conditions. PLX treatment depletes these microglia, and they subsequently repopulate once treatment is ceased. The newly generated microglia reset their neurotoxic characteristics. However, considering that repopulated microglia could revert to a neurotoxic phenotype, the replacement of microglia presents an additional strategy to maintain their health.

visual function in the context of glaucoma. Of note, PLX treatment can also be detrimental in certain situations. Microglia depletion from glaucoma model mice using PLX compounds exacerbates RGC damage (177, 178). Since microglia dynamically change their phenotypes, and there might be a neuroprotective glaucoma-associated microglial subcluster, techniques for more precise control of microglia are required, which could also pose a clinical limitation.

10.3 Cell transplantation

A recent study has demonstrated the feasibility of transplanting exogenous microglia into the CNS following microglial depletion induced by PLX compounds (179, 180). By combining the depletion of neurotoxic microglia with the transplantation of healthy, normal microglia, a replacement strategy can be employed (Figure 5). The depletion process appears to be crucial, as microglia extend their processes and establish their own territorial domains with an approximate diameter of 50 μm in both mouse and human brains (181). The absence of endogenous microglia permits the exogenously transplanted microglia to infiltrate and integrate into the nervous tissue. When combined with AAV-mediated functionalization of microglia (Figure 4B), this approach enables the precise delivery of specific molecules to designated sites, enhancing the efficiency of recovery while minimizing potential side effects. Transplantation can also be accomplished by introducing human iPS cell-derived microglia (iPSMG) into the mouse retina (182). Beyond microglia, intravitreal astrocyte transplantation may prove beneficial in safeguarding RGCs against damage induced by kainic acid (183). Additionally, transplantation of OPCs contributes to the neuroprotection and regeneration of the optic nerve. OPC transplantation has been shown to protect RGC in glaucoma model animal (184). Furthermore, the transplantation of OPC-rich neurospheres induces the myelination of the optic nerve (185–187). One limitation of clinical application may involve the duration for which the grafted cells survive in the host tissue. In the case of the iPSMG, transnasal transplantation to the brain maintained the grafted microglia for at least 60 days in mice (179, 180). Injected iPSMGs in the mouse retina survived over 200 days (182). However, it is not clear how long they would survive in the human tissue. Another concern is whether the grafted cells maintain their healthy phenotypes, as their phenotypes can be influenced by the microenvironment of the host tissue.

10.4 Transcorneal electrical stimulation

TES represents a non-invasive technique that administers electrical stimulation to the retina via the cornea. This approach has demonstrated therapeutic efficacy in both human patients and animal models afflicted with various injuries and diseases, including ischemic and traumatic optic neuropathies (188), axotomy (189, 190), retinal artery occlusion (191), ischemic damage (192), and photoreceptor degeneration (193, 194). TES has also exhibited a protective effect on RGCs in mouse models of glaucoma (195) and holds the potential to enhance visual function in human patients with glaucoma (196). While the precise mechanisms underlying TES are not fully elucidated, one of its neuroprotective mechanisms involves actions mediated by glial cells. For instance, TES suppresses pro-inflammatory responses by microglia (190, 197, 198) (Figure 6A). Simultaneously, TES induces the expression of various neurotrophic factors, including fibroblast growth factor 2

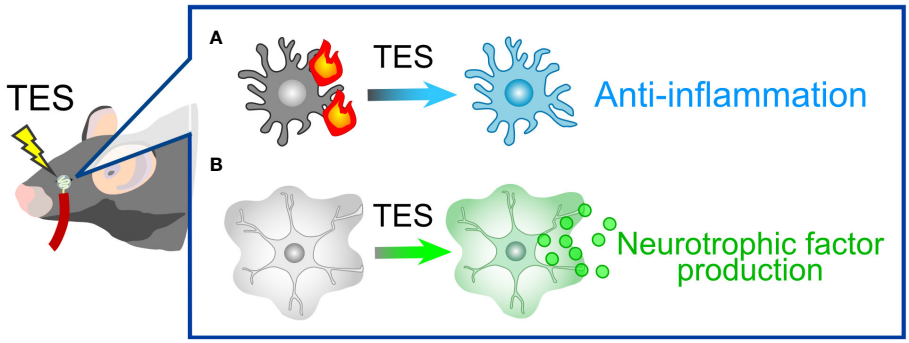

FIGURE 6
TES-Mediated Alterations in Glial Cells. **(A)** Microglial response. In pathological conditions, microglia tend to adopt pro-inflammatory phenotypes. TES effectively suppresses microglial inflammatory responses. **(B)** Müller Cell Expression: TES leads to an upregulation in the expression of neurotrophic factors, including FGF2, BDNF, and IGF, in Müller cells.

(FGF2), BDNF, and IGF, in Müller cells (54–56) (Figure 6B). The combination of anti-inflammatory effects and neurotrophic support is likely the primary mechanism behind RGC protection in glaucoma. As previously mentioned, these neurotrophic factors contribute not only to the protection of RGCs but also to the regeneration of dendrites, synapses, axons, and visual functions. Ongoing research aims to refine the parameters of TES for optimal neuroprotection and functional recovery (189, 199). TES is already employed in human patients with retinitis pigmentosa and has demonstrated safety and efficacy in improving visual function (200, 201). A current limitation of TES in the context of glaucoma treatment might be the absence of defined parameters. Optimized TES parameters will contribute to the development of a safe and effective treatment for glaucoma.

10.5 Extracellular vesicles

The EVs encompass membrane-derived vesicles with heterogenous groups, including exosomes and microvesicles (202). Initially described as a means to eliminate intracellular unneeded components to the extracellular space, subsequent studies have revealed their capacity for intercellular communication via transporting various molecules, including nucleic acids, lipids, and proteins. EVs are released from various cells and tissues including ocular cells and tissues. Due to their high stability and permeability to the blood-brain barrier (203), they represent attractive tools for drug delivery and hold significant potential as biomarkers for various diseases. In the ocular tissues, it has been reported that both retinal microglia and Müller cells release EVs (204–206). Considering the neuroprotective effects demonstrated by EVs from glial progenitors after traumatic brain injury (207), it is plausible that EVs from neuroprotective glial cells would similarly confer neuroprotection to RGCs. Moreover, embryonic stem cell-derived EVs, capable to delivering BDNF to other cells (208), could be employed as a therapeutic tool in glaucoma, for instance, by utilizing EVs derived from neurotrophin-overexpressing glia.

11 Concluding remarks

Glial cells are widely distributed throughout the nervous system, including ocular tissues. Pathological changes in glial cells play a pivotal role in driving RGC damage and resulting in visual deficits. Given that neuroprotective glia produce essential neurotrophic factors that impact both neuroprotection and neurodegeneration, glial cells represent an appealing therapeutic target for addressing glaucoma. With the aid of state-of-the-art techniques, we can precisely regulate glial functions, effectively suppressing neurotoxicity while enhancing neuroprotection and regeneration. Several glia-related molecules have already advanced to clinical trials, and we anticipate further advancements in drug discovery research aimed at targeting glial cells.

Author contributions

YS: Funding acquisition, Validation, Visualization, Writing – original draft, Writing – review & editing. KN: Conceptualization, Validation, Writing – review & editing. XG: Funding acquisition, Validation, Writing – review & editing. TH: Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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