Review Article

Cell and Molecular Mechanisms of Retinal Ganglion Cell Degeneration in Glaucoma

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Abstract

Glaucoma is an eye disorder in which intraocular pressure is elevated and retinal ganglion cells therefore degenerate. It is a multifaceted ailment with multiple cell types and pathways involved, all working together and giving rise to optic nerve degeneration. Current drugs used in the treatment of glaucoma all work by lowering intraocular pressure and only slowing the progression of the optic nerve damage. No drugs have yet been shown to effectively target retinal ganglion cells and help regain the lost vision. It is of great importance to understand the cellular and molecular processes involved in glaucomatous neurodegeneration to be able to identify potential targets of treatment. The current review attempts to provide insight into these processes. First, an overview of the disease is provided and then, cell types other than retinal ganglion cells (RGCs) that contribute to the neurodegeneration process (including lamina cribrosa cells, astrocytes, oligodendrocytes, and microglia) and cellular and molecular events in the RGCs leading to their degeneration and death (such as mitochondrial dysfunction, axonal transport disruption, calcium dyshomeostasis, oxidative stress, apoptosis, and endothelial reticulum stress) are explained.

Keywords: Glaucoma; Retinal Ganglion Cell; Neurodegeneration; Apoptosis; Cellular Components; Signaling Pathways.

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Introduction

Glaucoma is one of the leading causes of permanent blindness worldwide and it affects about 76 million people around the globe. Increased intraocular pressure (IOP) is a major risk factor for the damage of retinal ganglion cells (RGCs) in the optic nerve, which leads to blindness [2, 3]. Other risk factors include Old age, family history, black or Asian race, diseases such as diabetes and hypertension, and use of systemic or topical corticosteroids [4, 5].

There are several types of glaucoma including open-angle, angle-closure, and normal-tension glaucoma. Open-angle glaucoma is the result of the ineffective outflow of the aqueous humor through trabecular and uveoscleral pathways in the anterior chamber of the eye and in contrast to angle-closure, the angle between the iris and the cornea is normal in this type of glaucoma. It is the most common type and patients suffering from it are usually asymptomatic until the damage to the optic nerve is severe and peripheral vision is lost. In angle-closure glaucoma, on the other hand, closure of this angle occurs due usually to age-related thickening of the lens and as a result, the drainage of the aqueous humor through the trabecular meshwork is blocked. IOP increases rapidly in angle-closure glaucoma and can cause vision loss within a day of the onset of aqueous outflow blockage. The decreased aqueous outflow in angle-closure and open-angle glaucoma then results in increased IOP which then damages the optic nerve. Unlike these two types, IOP is not elevated in normal-tension glaucoma and it is believed that the optic nerve damage could be the consequence of either pressure-sensitivity of the optic nerve or nerve ischemia due to vascular insufficiency. Accordingly, all types of glaucoma cause neurodegeneration in the retinal nerve fiber layer [4].

The optic nerve consists of RGC axons, glial cells, the connective tissue of the lamina cribrosa, and blood vessels. Although glaucomatous neurodegeneration occurs throughout the whole visual pathway from the retina to the brain, the optic nerve head (the location where RGC axons exit the eye to form the optic nerve) seems to be the initial site of RGC injury in glaucoma [4]. Figure 1 demonstrates the structure of the optic nerve head, its cellular components, and the effects of increased IOP on them.

The only drug treatments shown to be efficacious in glaucoma, reduce the intraocular pressure and include prostaglandin analogs, beta-blockers, alpha-2 agonists, carbonic anhydrase inhibitors, miotic agents, and more recently Rho-kinase inhibitors and nitric-oxide donating medications [6]. These medications only prevent the progression of the disease and do not reverse vision loss [7]. Moreover, the IOP-lowering effect of these medications wears off in some patients and despite controlled intraocular pressure, the IOP fluctuation in response to these drugs could lead to the progression of vision loss [8]. Many drugs including antioxidants, neuroprotective and immunomodulatory agents have been tested in clinical trials but none were shown to be efficacious [9] and the efforts made to develop effective medications targeting the optic nerve have been unsuccessful due to a lack of precise understanding of the underlying molecular events in glaucomatous neuropathy. Some pathways and events such as inflammation, ECM remodeling, RGC axonal degeneration, and apoptosis are proven to participate in glaucomatous RGC degeneration. However, the importance of some events such as RGC excitotoxicity and declined activity of insulin receptor signaling pathway is not quite clear and other pathways involved in the

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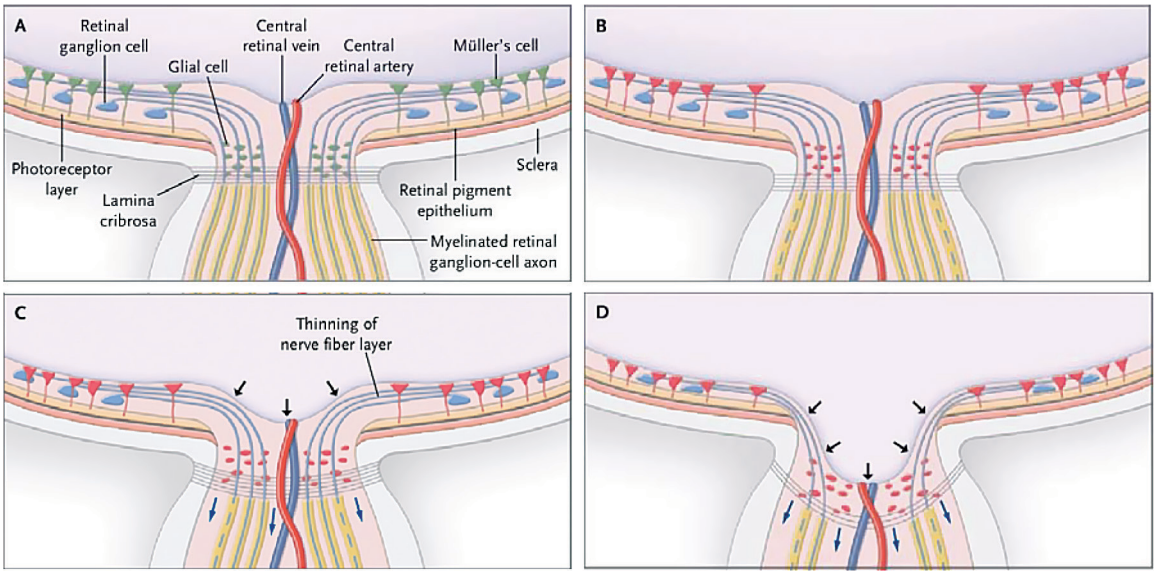

Figure 1: The Optic Nerve Head and Events Contributing to IOP-induced RGC Axonal Degeneration and Apoptosis. A) A normal optic nerve head with inactivae glial cells (green), B) IOP-induced stress (black arrows) on the retinal ganglion cells and activation of glial cells, C) Axonal demyelination and degeneration and apoptosis of some retinal ganglion cells due to glial activation, D) Optic nerve head cupping, backward displacement of lamina cribrosa, glial-induced neuroinflammation and death of most retinal ganglion cells. Adopted from: (1)

pathogenesis of glaucomatous neuropathy are yet to be discovered.

The purpose of the current review is to provide a clear view of the cellular processes known to participate in the optic nerve degeneration following increased intraocular pressure observed in most glaucoma patients. First, we will discuss the cell types and then the cellular and molecular events in RGCs and these cell types which have important roles in glaucomatous neurodegeneration. A summary of the upcoming sections is shown in table 1.

Cell types involved in glaucomatous neurodegeneration

Multiple cell types other than RGCs are involved in the neurodegeneration process in glaucoma. Normally, they are responsible for providing biomechanical, trophic, metabolic, and immunomodulatory protection to RGCs but due to changes in glaucoma, they tend to increase RGCs vulnerability to injury or even cause the injury themselves. These cell types, which are all present in the optic nerve head, include lamina cribrosa cells, glial cells (astrocytes, microglia, and oligodendrocytes), and vascular endothelial cells [10]. Figure 2 depicts the signaling pathways and cellular events of these cell types involved in glaucomatous neurodegeneration and each one is discussed in the following sections.

Lamina cribrosa

The optic nerve head is provided with mechanical support by lamina cribrosa which consists of parallel series of fibroelastic connective tissue plates (see figure 3). It also provides a scaffold for different cell types like lamina cribrosa cells, glial cells, and vascular endothelial cells. Elevated IOP causes a structural reconfiguration in the lamina cribrosa which is the reason for the cupping

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Table 1: Cellular and molecular events involved in glaucomatous neurodegeneration of retinal ganglion cells, cell types involved in each, and their inducers and consequences. Up and down arrows indicate increase and decrease of the item on their right, respectively

Cellular or molecular event	Cell(s) involved	Inducer(s)	Consequence(s)
Activation of stretch-activated K+ channels	Lamina cribrosa cells	Mechanical stress	Intracellular calcium ↑
Axonal transport dysfunction	RGCs	ATP ↓	Mitochondrial dysfunction, NMNAT ↓
Bax activation	RGCs	NF-κB activation, JNK pathway activation, insulin receptor activation, ER stress	Caspase cascade activation
Caspase cascade activation	RGCs	Bax activation, ER stress	Apoptosis
Cytoskeleton degeneration	RGCs	Oxidative stress, proteasome activation	Axonal degeneration
ECM production ↑	Lamina cribrosa cells, astrocytes	Mechanical stress, TGF-β, oxidative stress ↑	Tissue deformation
ER stress	RGCs	Abnormal protein aggregation	Activation of pro-apoptotic proteins, mitochondrial dysfunction
Extracellular ATP	RGCs	RGC damage	Purinergic receptor activation
Extracellular HSP	RGCs	RGC damage	TLR activation
IL-1β receptor activation	RGCs	IL-1β secretion ↑	NF-κB activation
IL-1β secretion ↑	Microglia	Vanilloid receptor activation, NLRP3 activation	IL-1β receptor activation
Insulin receptor activation ↓	RGCs, Microglia, Astrocytes	Unknown	Mitochondrial dysfunction, expression of pro-inflammatory mediators, apoptosis, impaired astroglial metabolic support
Intracellular calcium ↑	Lamina cribrosa cells RGCs	Activation of stretch-activated K+ channels ↓ ATP, NMDA receptor activation, SARM1 activation, NAD+ ↓	Expression of profibrotic proteins Proteasome activation
JNK pathway activation	RGCs	axonal cytoskeleton distortion, neurotrophin deprivation, energy failure, or neuroinflammation	Activation of pro-apoptotic proteins
Mechanical stress	RGCs, lamina cribrosa cells, astrocytes, microglia, oligodendrocytes, vascular endothelial cells	IOP ↑	Tissue deformation in the optic nerve head, ECM production, ↑ TGF-β ↑

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Cellular or molecular event	Cell(s) involved	Inducer(s)	Consequence(s)
Mitochondrial dysfunction	RGCs	ER stress, Axonal transport dysfunction, vascular dysfunction, activation of pro-apoptotic proteins	ATP, oxidative stress ↓
MMP ↑	Astrocytes	TGF-β ↑	Tissue deformation
Neurotrophin deprivation	RGCs	Axonal transport dysfunction	Apoptosis
NF-κB activation	RGCs	TNF-α receptor activation, IL-1β receptor activation	Bax activation
NMDA receptor activation	RGCs	Unknown	Intracellular calcium ↑
NMNAT ↓	RGCs	Axonal transport dysfunction, JNK signaling	SARM1 activation, NAD+ ↓
NLRP3 activation	Microglia	Purinergic receptor activation	IL-1β ↑
Oxidative stress	RGCs, lamina cribrosa cells, astrocytes, microglia, oligodendrocytes, vascular endothelial cells	Mitochondrial dysfunction	ECM production, damage to proteins & DNA, cytoskeleton degeneration ↑
Proteasome activation	RGCs	Intracellular calcium ↑	Cytoskeleton degeneration
Purinergic receptor activation	Microglia	Extracellular ATP	NLRP3 activation
SARM1 activation	RGCs	NMNAT ↓	Intracellular calcium ↑
Tenascin-C production	Astrocytes	IOP	TLR activation
TGF-β ↑	Lamina cribrosa cells	Mechanical stress	ECM production, ↑ MMP ↑
Tissue deformation	RGCs, lamina cribrosa cells, astrocytes, microglia, oligodendrocytes, vascular endothelial cells	MMP, ↑ IOP, ↑ ECM ↑ production	Activation of stretch-activated K+ channels, TLR activation, vascular dysfunction
TLR activation	Microglia	Tenascin-C production, tissue deformation	TNF-α ↑
TNF-α receptor activation	RGCs	TNF-α secretion ↑	NF-κB activation
TNF-α secretion ↑	Microglia	TLR activation, Vanilliod receptor activation	TNF-α receptor activation
Vanilloid receptor activation	Microglia	IOP ↑	IL-1β, ↑ TNF-α ↑
Vascular dysfunction	RGCs, lamina cribrosa cells, astrocytes, microglia, oligodendrocytes, vascular endothelial cells	↑ IOP, ↓ insulin signaling, ECM production, tissue deformation	Oxidative stress

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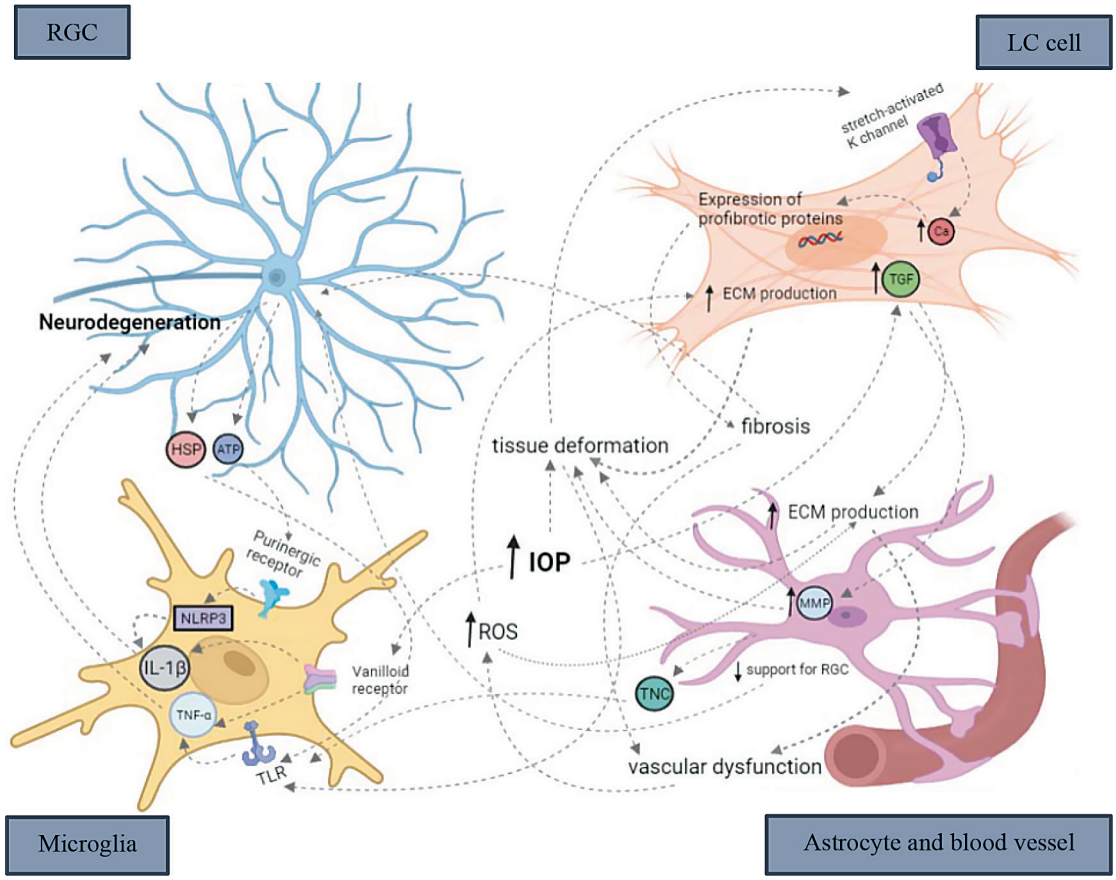

Figure 2: Signaling pathways and other cellular events of retinal ganglion cells, lamina cribrosa cells, microglia, and astrocytes. Proteins and other entities demonstrated in this figure mostly participate in pathways leading to inflammation (with proteins such as IL-1β, TNF-α, TLR, and NLRP3 complex) and extracellular matrix remodeling (with proteins such as MMP and TGF). Abbreviations: ECM: extracellular matrix, HSP: heat-shock protein, IL-1β: Interleukin-1β, IOP: intraocular pressure, LC: lamina cribrosa, RGC: retinal ganglion cell, ROS: reactive oxygen species, TGF: transforming growth factor, TLR: toll-like receptor, TNC: Tenascin-C, TNF-α: tumor necrosis factor. Created with BioRender.com

and enlargement of the optic disc observed in glaucomatous eyes [11]. Elevated IOP generates mechanical stress on the lamina and sclera and results in the backward displacement of lamina cribrosa which then deforms the resident cells [12] and eventually, damages the RGC axons and optic nerve head capillaries [13]. Due to individual differences in extracellular matrix components and therefore differences in the biomechanical properties of the sclera [14], the stress experienced by RGC axons, and glial and vascular cells' response to stress could vary [11]. This explains the racial differences in susceptibility of RGCs to IOP-induced injury [15].

Lamina cribrosa cells, which are located between the connective tissue plates of the lamina cribrosa [16], secrete components of the extracellular matrix (ECM) including fibronectin, collagen, and elastin and along with astrocytes, which are explained later, these cells are responsible for ECM remodeling and fibrosis in the optic nerve head. The production of ECM proteins by lamina

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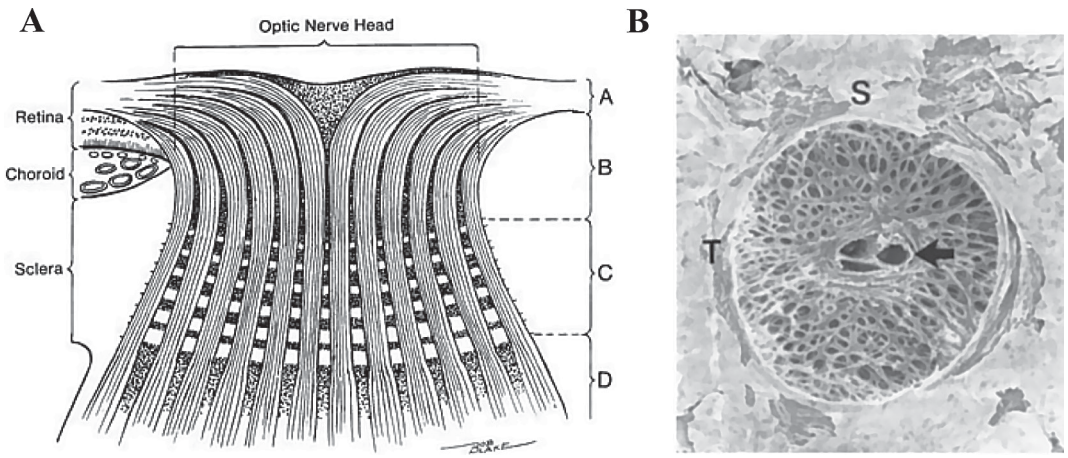

Figure 3: Longitudinal section (A) and cross-section (B) of lamina cribrosa in the optic nerve head. A) Surface nerve fiber layer (A), Prelaminar region (B), Lamina cribrosa region containing connective tissue and elastic fibers (C), and Retrolaminar region (D). B) Fenestrated structure of lamina cribrosa with blood vessels passing through the central openings (arrow) and passage of retinal ganglion cell axons through the sorriunding fenestrae. S: superior, T: temporal. Adopted from: Entokey.com

cribrosa cells and astrocytes is increased in response to IOP-induced mechanical stretch, TGF-β, and oxidative stress. Secretion of TGF-β is induced by increased IOP, while elevated levels of reactive oxygen species and oxidative stress are a result of reduction of the optic nerve head blood flow [12].

Lamina cribrosa cells are also able to sense the IOP-induced deformation and after their stretch-activated potassium channels open in response to the increased pressure, intracellular calcium levels elevate [17] and promote the pro-fibrotic processes [18].

In rodents and non-human primates, the mechanical support for the optic nerve is provided by a network of astroglial processes (called glia lamina), and not lamina cribrosa, as it isn't well developed in these species [19]. The topography of RGC damage in rodents matches the localized damage of the axon bundles at the glia lamina, and a similar pattern is seen in ocular hypertension-induced RGC damage, regardless of the structural differences of the optic nerve head. Thus, lamina cribrosa is not required for glaucomatous neuropathy, and glial pro-fibrotic and cellular processes (mostly related to astrocytes) are responsible for the observed optic nerve head damage [11].

Glial cells

Glial cells are important elements of the nervous system and provide support for all neurons including retinal ganglion cells. There are several types of glial cells such as astrocytes and microglia which possess crucial roles in the central nervous system. Astrocytes are responsible for maintaining the blood-brain barrier and providing support for neurons [20], while microglia play important roles in inflammation and brain infections [21]. Evidence shows that astrogliosis (activation of astrocytes) and microgliosis (activation of microglia) play a major role in the development of glaucomatous neurodegeneration [11]. Astrogliosis and microgliosis also lead to the loss of oligodendrocytes, another type of glial cell responsible for myelination of neurons in the CNS, through secretion of inflammatory

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cytokines by microglia [22]. Two phenomena are known to activate glial cells in glaucoma: increased IOP-induced stress and signals from RGCs. Glial mechanosensitive ion channels, such as vanilloid receptors, purinergic receptors, and pannexin channels, sense the increased IOP and lead to the initiation of inflammatory signaling [23]. They can also be activated due to the release of damage-associated molecules like ATP [24] and heat-shock protein (HSP) [25] from injured RGCs as explained later in more detail. We will now discuss the inducers and consequences of astrocytes and microglia activation, respectively.

Increased IOP leads to alterations in the activity of astrocytes. This response by astrocytes is called reactive astrogliosis [26]. In short term, astrocytes provide more trophic and metabolic support to the uninjured axons and assist in tissue repair in the injured ones by eliminating the injured dendritic structures with the help of microglia, but the prolonged effects of reactive astrogliosis are destructive rather than protective. Prolonged activation of glial cells results in diminished structural, trophic, and bioenergetic support from astrocytes, and neuroinflammation and formation of a toxic environment for RGCs by microglia [27]. However, Diminished support for RGCs is not the only aspect of astrogliosis. When sensing the IOP-induced tissue deformation, astrocytes produce more extracellular matrix and cause an imbalance between the production of matrix metalloproteinase (proteolytic enzymes that degrade ECM components) and their inhibitors. The resulting extracellular matrix remodeling increases the mechanical stress on the optic nerve axons [28]. This could also explain the existing distress of vascular properties of the optic nerve. Initially, it was thought that the direct mechanical stress of elevated IOP on optic nerve head blood vessels is the reason behind vascular dysfunction in glaucoma, but it is now evident that it could also be explained by astrocytes activity. The basal lamina produced by astrocytes surrounds RGCs and microcapillaries which besides the tissue stiffening, alters the intracellular signaling between astrocytes and both RGCs and endothelial cells of the microcapillaries [13]. Increased inflammatory signaling is an example thatlipid leads to the cytotoxic and phagocytic activity of glial cells and optic nerve neurodegeneration [11].

Microglia are responsible for neuroinflammation and cellular death detected in RGCs of glaucomatous eyes, but they aren't the only phagocytes involved in this process. Monocytes in the blood can enter the optic nerve head via the activation of leukocyte transendothelial migration pathway in the early stages of glaucoma and together with microglia [29], secret pro-inflammatory cytokines such as TNF-α and IL-1β which activate the NF-κB pathway in retinal ganglion cells and result in apoptosis and axonal degeneration [27]. Secretion of IL-1β by microglia is a result of NLRP3 inflammasome activation through stimulation of purinergic receptors by damage-associated molecules like ATP and ROS production by the mitochondria [30]. TNF-α secretion is in response to the stimulation of Toll-like receptors (TLRs) which are a class of pattern recognition receptors (PRRs) that initiate the innate immune response of microglia [31] by sensing stress-related ligands like heat-shock protein and Tenascin-C secreted by RGCs and astrocytes, respectively [29, 32]. There are two mechanisms by which TNF-α can cause damage to RGCs: the caspase-dependent apoptosis pathway which is briefly explained later, and the caspase-independent pathway in which mitochondrial dysfunction and oxidative

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stress occur [33]. An alternate mechanism by which microglia can contribute to the loss of RGCs dendrites and synapses is through the activation of the complement system [34].

Microglia, like many other cells, communicate with each other via exosomes which are extracellular vesicles that may contain miRNAs, proteins, or lipids [35]. Active microglia produce these exosomes and result in the activation of other microglia and therefore, exacerbate the inflammation and RGC death [36]. ATP release from RGCs stimulates the exosome production by microglia which could explain why elevated IOP doubles the exosome release [37].

Besides innate immunity, glial cells can induce adaptive immunity which results in the abnormal activity of T-cells and increased production of autoantibodies against ocular antigens. Thus, the cytotoxicity mediated by the autoantibodies could also lead to toxicity to RGCs [38].

Cellular processes in RGCs involved in glaucomatous neurodegeneration

Multiple cellular processes are involved in glaucomatous neurodegeneration including impaired axonal transport and depletion of neurotrophic factors, SARM1-induced axonal degeneration, cytoskeletal disruption, neuroinflammation, vascular dysregulation, mitochondrial dysfunction and energy failure, oxidative stress, endothelial reticulum (ER) dysfunction, calcium dyshomeostasis, excitotoxicity, and insulin resistance [11]. These processes and how they are linked together are discussed in the following section and a summary of them is shown in figure 3.

Axonal transport and mitochondrial dysfunction

Increased IOP disrupts the axoplasmic flow which is crucial for the retrograde transport of neurotrophic factors from the brain and this lack of neurotrophic factors leads to apoptosis. Dysfunctional axonal transport also leads to the accumulation of some proteins like amyloid precursor protein (APP) and γ-synuclein [39] in the axons, which are proteins known for their contribution to neurodegeneration. The compromised transport could be a result of mitochondrial dysfunction which itself could either be caused by deficient vascular nutrient supply due to compromised capillaries in the optic nerve head or increased mitochondrial permeability (which occurs due to activation of pro-apoptotic proteins such as Bax and Bak resulting in the activation of the caspase cascade, as later discussed) and therefore mitochondrial swelling and oxidative stress due to activated self-destruction program [40]. The axonal self-destruction is activated because of the lack of anterograde axonal survival factors supplied from the brain [41]. Also, the compromised transport can lead to the accumulation of dysfunctional mitochondria which are normally eliminated from the cytoplasm in a process called mitophagy [11]. Mitophagy is decreased in aging [42] which could be one of the reasons why aging is a risk factor for glaucoma. Dysfunctional axonal transport also leads to decreased transport of mitochondria to sites of injury in the RGCs and therefore, fails to compensate for the increased energy demand in these sites [43]. Other than mitochondrial and capillary impairment, decreased metabolic supply by astrocytes is also speculated to cause inadequate energy supply [44].

Besides energy generation, mitochondria are critical for the regulation of intracellular calcium homeostasis, apoptosis signaling, and oxidative stress, all of which contribute to RGC degeneration in glaucoma [11]. Studies also suggest that dysfunctional mitochondria could stimulate neuroinflammation [45] by

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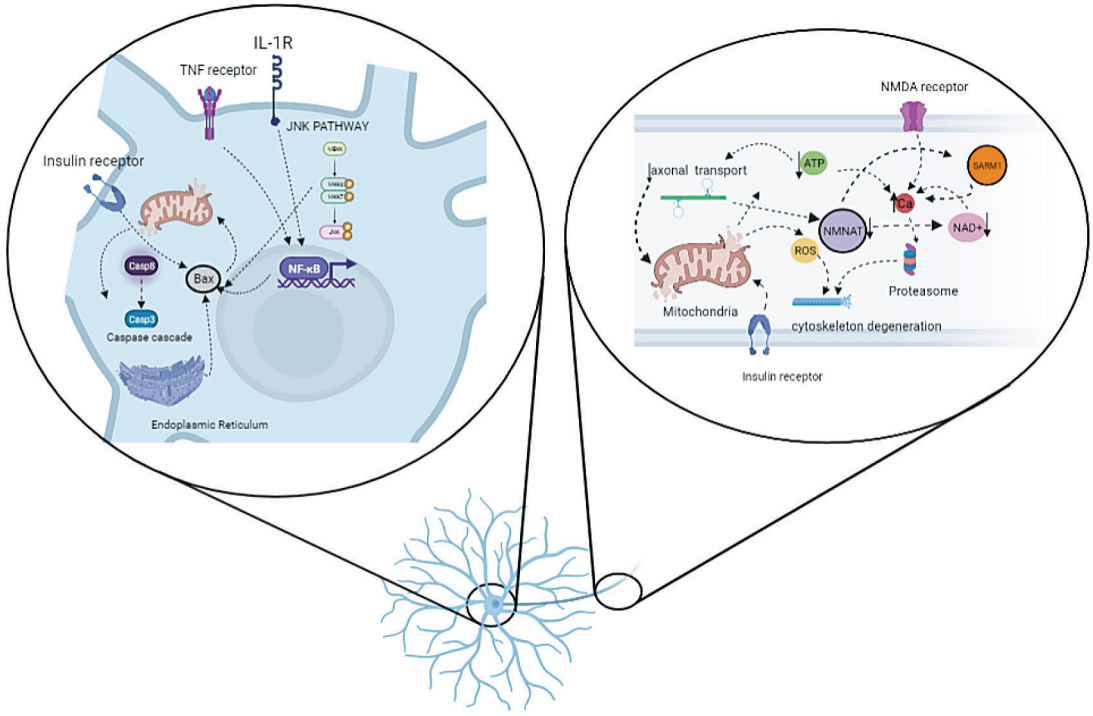

Figure 4: Cellular and signaling pathways involved in axons (right) and somas (left) of retinal ganglion cells in glaucoma and their relationships Created with BioRender.com

Abbreviations: ATP: adenosine triphosphate, Ca: calcium, Casp: caspase, IL-1R: interleukin-1 receptor, NAD+: nicotinamide adenine dinucleotide, NMNAT: nicotinamide mononucleotide adenylyltransferase, ROS: reactive oxygen species, SARM1: sterile alpha and TIR motif-containing protein 1, TNF: tumor necrosis factor

contributing to inflammasome activation [46]. Inflammasome activity may provoke mitochondrial injury and create a pathological cycle between mitochondrial dysfunction and neuroinflammation [47].

Calcium dyshomeostasis and cytoskeletal disruption

The enzyme nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) which is responsible for the production of nicotinamide adenine dinucleotide (NAD+), decreases due to injury-induced disrupted axonal transport. NAD+ is crucial in calcium regulation and its decreased amounts cause an elevation in the intracellular concentration of calcium. Dysregulation of calcium could also be a result of ATP depletion and therefore the reversed activity of the ATP-dependent Na+/Ca++ exchanger. The increased intra-axonal calcium then over-activates the ubiquitin-proteasome system and therefore leads to protease-mediated cytoskeletal breakdown and the destruction of axonal structure [41]. Calcium could also activate calpains (cysteine proteases) which also participate in proteolysis in the axonal cytoskeleton [48].

SARM1-dependent axonal degeneration

As explained before, axonal transport is disrupted in RGCs of glaucomatous eyes and consequently, turnover of the enzyme NMNAT2 is decreased. Normally, NMNAT2 inhibits the enzyme SARM1 (sterile alpha and TIR motif-containing protein 1) [49]

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which hydrolyzes NAD+ to form NAM (nicotinamide) and either ADPR (adenosine diphosphate ribose) or cADPR (cyclic ADPR) [50]. When the NMNAT2 amounts are reduced, SARM1 activates and produces more ADPR and cADPR. These two molecules then bind to calcium channels and increase calcium influx and therefore, as explained in the last section, lead to axonal degeneration [51]. Other mechanisms by which NMNAT2 activity is decreased are the activation of the JNK signaling pathway [52] and mitochondrial dysfunction in RGCs which could also decrease the activity of STMN2 (Stathmin 2), another SARM1 inhibitor [53]. TNF-α released from microglia can also activate SARM1 and lead to axonal degeneration [54].

The whole process of SARM1-induced axonal degeneration following lowered activity of NMNAT2 is known as Wallerian degeneration [53] which eventually leads to RGC axonal loss, RGC death, and oligodendrocyte loss. However, oligodendrocyte loss appears to be secondary to SARM1-induced axonal loss [54].

Oxidative stress

Increased generation of reactive oxygen species is another consequence of mitochondrial dysfunction. The resulting oxidative stress can impact cell survival through oxidative damage to proteins [33] and boost neurodegenerative inflammation [55]. Antioxidant treatment in experimental glaucoma models has been shown to act as an immunomodulator and protect RGCs somas and axons [56].

Apoptosis and other signaling pathways

Apoptosis in RGCs occurs following the activation of the caspase cascade. Caspase could either be triggered by Bax, a member of the pro-apoptotic Bcl-2 family of proteins which is activated by energy depletion due to mitochondrial dysfunction and neurotrophin deficiency, or by binding of microglia-derived TNF-α to its receptor on RGCs [57]. TNF-α could also act independently of caspase and cause mitochondrial dysfunction and oxidative stress [33]. When Bax and Bak are activated, they aggregate at the mitochondrial outer membrane (MOM), undergo conformational changes, and insert into the membrane. Then, they form homodimers and cause membrane destabilization and therefore, proteolipid pores are formed in the mitochondrial membrane [58]. As a result, pro-apoptotic signaling molecules such as cytochrome C exit the intermembrane space and enter the cytosol. This results in the activation of the caspase cascade which leads to apoptosis of the RGC [59]. Other important pathways include the JNK pathway and Death receptor-6 (DR6) signaling.

The JNK pathway is important in signal transduction after cellular stress. In glaucoma, it can be activated by distortion of axonal cytoskeleton, neurotrophin deprivation, energy failure, or neuroinflammation. It appears that this pathway is involved in both axonal degeneration and RGC soma apoptosis [60] by initiating the apoptotic transcriptional program and interacting with the Bcl-2 family of genes [61].

In the Death receptor-6 (DR6) signaling pathway, Activation of DR6 receptor by surface ligands like amyloid precursor protein (APP) due to axon injury results in the activation of caspase-6 (which is independent of the caspase cascade activated during apoptosis) and therefore activates axonal destruction program and leads to axonal degeneration [62]. APP is a protein involved in the pathophysiology of Alzheimer's disease. The involvement of APP, the accumulation of tau protein (a key mediator of neurotoxicity in Alzheimer's

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disease) in glaucoma [63], and other similarities between glaucoma and Alzheimer's disease demonstrate that these two are more related than previously thought.

ER stress

ER is the major intracellular organelle that senses cellular stresses and environmental changes, coordinates signaling pathways, and controls cell survival [64]. Studies indicate the presence of chronic ER stress in glaucoma. Abnormal protein aggregation which could be a result of aging leads to the activation of unfolded protein response (UPR) [65] by which the ER attempts to clear out the misfolded proteins. The UPR consists of the activation of chaperones, inhibition of mRNA translation, and transportation of the unfolded proteins to the cytosol for ubiquitination [66]. Some ER stress-related signaling proteins control cell fate either by activating pro-apoptotic molecules in response to cell burden [67].

ER and mitochondria are in contact at membrane contact sites (MCSs) [68]. These MCSs are important in some processes such as calcium transfer from the ER to the mitochondria (essential for many mitochondrial activities such as oxidative phosphorylation) and lipid transfer which are crucial for cell homeostasis [43, 69]. During the UPR, MCSs between the ER and the mitochondria are augmented and therefore, calcium transfer to the mitochondria is increased for the production of more ATP needed in the UPR. Calcium overload in the mitochondria then leads to increased release of cytochrome C to the cytosol and apoptosis [70]. Other than induction of apoptosis, loss of cytochrome C protein also leads to less ATP production and hence, production of ROS and cell damage [43]. The existence of more MCSs could also cause apoptosis due to more aggregation of Bax and Bak at these sites [70].

Excitotoxicity

The association of excessive NMDA glutamate receptor activation and therefore excitotoxicity with glaucoma is believed to be related to the OPTN gene which encodes Optineurin. Optineurin is a protein involved in controlling glutamate receptor signaling and is often mutated in patients with primary open-angle glaucoma (POAG) [71]. However, there isn't much evidence demonstrating excitotoxicity as a primary mechanism for RGC death in glaucoma.

Insulin receptor (IR) signaling

IR signaling has been shown to be important in the pathogenesis of glaucoma. In the RGC, declined activity of the insulin signaling pathway promotes dendritic retraction, mitochondrial dysfunction, tau hyperphosphorylation, and apoptosis. In microglial cells, insulin resistance induces the expression of pro-inflammatory mediators and therefore neuroinflammation in RGCs. It also contributes to vascular dysfunction by causing nitric oxide/endothelin-1 imbalance and endothelial cell apoptosis. In astrocytes, decreased insulin signaling causes depletion of glycogen stores which impairs astroglial metabolic support for RGCs. However, it is not quite clear whether insulin signaling directly contributes to the pathogenesis of glaucomatous neurodegeneration or is only a consequence of it [72].

Conclusion

Multiple cellular components are involved in glaucomatous neurodegeneration consisting of neuroinflammation, vascular dysfunction, mitochondrial and axonal transport dysfunction, cytoskeletal disruption, and many other

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processes. In glaucomatous neurodegeneration, different cellular pathways are activated in different cellular compartments, multiple cell types (including glial cells and lamina cribrosa cells) are involved and asynchrony of neurodegeneration is seen among different RGCs, meaning the degeneration does not affect all RGCs at once. Thus, recognizing the earliest molecular events in glaucomatous neurodegeneration is very challenging. Given the complexity of these processes, systems biology and integration of different '-omics' data could be tremendously helpful in understanding the precise sequence of cell type-specific events. The resulting spatio-temporal knowledge of pathological events in glaucomatous neurodegeneration could lead to the identification of new pharmacological targets in retinal ganglion cells that could slow the progress of degeneration more effectively or even reverse the optic nerve damage done before diagnosis.

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.
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