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Comparative Optic Nerve Head Physiology: Glaucoma Induced Retinal Ganglion Cell Apoptosis by Disruption of the Translaminar Pressure Gradient and Reduced Neurotrophic Signaling

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One Sentence Summary: Glaucoma is associated with decreased neurotrophic signaling at the optic nerve head; an understanding of which may lead to novel therapeutic approaches to reverse this blockade.

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Abstract:

Glaucoma is the leading cause of irreversible blindness, and the second leading vision loss neurodegenerative disease affecting millions worldwide. Glaucoma is characterized as a group of eye disorders which are initially asymptomatic but may progress to significant optic nerve head damage and vision loss with time. Early stages of glaucoma often go undetected, leading to irreversible damage to the optic nerve head prior to patients seeking medical care. Glaucoma leads to vision loss via death of retinal ganglion cells. Retinal ganglion cell apoptosis is thought to begin due to interference with the normal transmission of neurotrophic signals that arrive by retrograde axonal transport from target cells. The physiologic mechanism of retinal ganglion cell death still remains unknown, and currently no treatment to reverse the damage and restore vision exists. Emphasis in research has been placed on the contribution of the lamina cribrosa and the trans-laminar pressure differences in the pathophysiology of glaucoma. A growing body of evidence suggests that glaucoma occurs due to and elevated trans-laminar pressure difference (TLPD) secondary to an intraocular pressure and cerebrospinal fluid pressure imbalance. This review extensively focuses on the underlying mechanisms of axonal blockade, which interfere with neurotropic signaling at the optic nerve head. The review also explores additional mechanisms of cell death at the optic nerve head that produce optic nerve damage

which mimics glaucoma. Further insight and research into these areas may lead to therapeutic reversal of this blockade and decrease the burden of glaucoma worldwide.

Introduction

The second leading cause of blindness worldwide, glaucoma is a group of neurodegenerative diseases which lead to progressive optic neuropathy and irreversible loss of retinal ganglion cell (RGC) axons.[1] Glaucoma costs the U.S economy an estimated $2 billion every year in direct costs and productivity, hence the emphasis on regular eye exams for early diagnosis and intervention.[2] The glaucomatous optic nerve head (ONH) presents with a characteristic excavated appearance of the optic disk, collapse and remodeling of the lamina cribrosa (LC),[1] and activation of astrocytes[3,4]. The pathological mechanisms responsible for glaucoma development remain unknown. However, elevated intraocular pressure (IOP) has been identified as a potential causative and the only modifiable risk factor in glaucoma[5]. Chronic elevation of IOP results in optic nerve head (ONH) alterations,[5] including the compression of retinal ganglion cell axons at the lamina cribrosa (LC), restriction of axoplasmic flow, and retrograde neurotrophic transport blockade to RGCs.[1] Of note, patients may have elevated IOP and never develop glaucoma. Likewise, patients may develop glaucoma in spite of having normal or even low IOP. In addition to exposure to IOP, the RGC axons are exposed at the ONH to intracranial pressure (ICP), which is transmitted to the optic nerve at multiple points. The interaction between these two pressures has been linked to the development of glaucomatous damage at the optic nerve head.

The lamina cribrosa (LC) is an important mesh-like structure which resides within the posterior scleral opening through which RGCs exit the globe and continue as the retrobulbar optic nerve (ON) (See figure A-1 for diagram). The laminar plates contain precisely organized

extracellular matrix proteins including collagen and elastin fibers, which comprise the supportive framework and elasticity of the ONH. IOP- induced conformational distortion within the scleral lamina cribrosa results in rotation, compression, misalignment and collapse of the laminar pores and channels such that axoplasmic flow is reduced and ultimately blocked causing death of RGCs.[6] Apoptotic cell death in glaucoma is initiated by interference with the transmission of neurotrophic signals that normally arrive by retrograde axonal transport from target cells.[7]

Various methods have been employed to elicit a complex relationship between astrocytic, neural and fibrous elements of the lamina cribrosa. The cribriform plates are lined by basement membranes consisting of fibrillar collagen and elastic fiber filled cores. Anterior to the LC, the ONH is subjected to IOP. Posterior to the LC, the neural tissue is exposed to the cerebrospinal fluid pressure (CSFp).[2,4] The pressure across the LC is referred to as the translaminar pressure gradient (TLPD=IOP-CSFp). The difference between IOP and CSFp creates a pressure gradient across the LC that induces stress in the anteroposterior direction.[8] The RGCs pass through the anterior and posterior regions of the LC and are exposed to the TLPD at the ONH. Exposure to the TLPD allows them to play a potential role in the development of glaucoma when neurotrophic signaling is reduced.[9]

Methods

A literature search conducted for the years 1981-2017 included pertinent optic nerve axonal transport information under normal physiological and pathophysiological conditions from the review of citations retrieved from Web of Science Database, EBSCO Database, National Library of Medicine, SciFinder Database, and PubMed Database. Complete copies of each of the relevant articles were obtained, and after thorough analysis each was based on the strength of the

study design and relation to neurotrophic signaling in glaucoma. Keywords searched included: glaucoma, optic nerve head, retinal degeneration, signal transduction, translaminar gradient.

Normal Optic Nerve Characteristics

The prelaminar optic nerve is known as the optic disc, optic nerve head or optic papilla. The concentration of RGC axons into a single optic nerve makes the optic nerve an extremely vulnerable segment of the visual pathway.[10] The optic nerve is comprised of four major regions, shown in figure 1.[10] The intraocular optic nerve includes the retinal ganglion cell layer, the nerve fiber layer, the optic nerve head or optic disc and the intralaminar region within the sclera. The intraorbital optic nerve, intracanalicular optic nerve and the small intracranial optic nerve, which merges into the optic chiasm are all located posterior to the intralaminar region.[10]

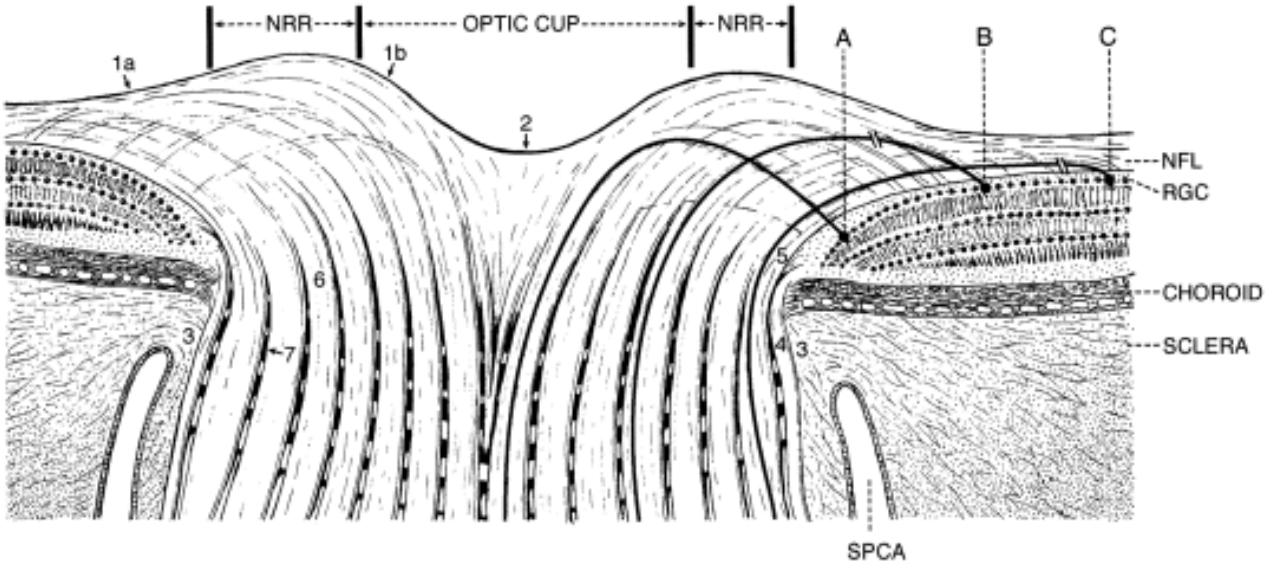

Figure 1. The optic cup, neuroretinal rim (NRR), nerve fiber layer (NFL), retinal ganglion cell (RGC) layer, choroid, sclera, and short posterior ciliary arteries (SPCA) are all illustrated. Retinal ganglion cells with cell bodies located near the optic disc **(A)**, midperipheral retina **(B)** and peripheral retina **(C)** send their axons to the central, middle and peripheral regions of the optic disc and nerve.[10]

Physiology of the Normal Optic Nerve

The optic nerve axons are made up of an outer sheath of myelin, a hollow, thin, collapsible, bilayer lipid cell wall, and a viscoelastic axoplasm containing transmitter molecules, proteins, microtubules and organelles.[10] The axoplasm moves along an intra-axonal pressure gradient. Axoplasmic flow is orthograde from the RGC cell body toward the synapse but is retrograde from the synapse toward the RGC cell body. Mitochondria located within each axon segment provide the necessary metabolic energy for axoplasmic flow and are subject to ischemic episodes. Anterograde axoplasmic flow is for the forward movement of cargo from the cell body to the axon tip. In contrast, retrograde axoplasmic flow is for movement of neurotrophins and waste products of the retinal ganglion cells backwards to the cell body.[10] Axoplasmic flow may be slow for purposes of cell membrane maintenance, or fast for synaptic transmission.[10]

The internal ophthalmic artery is the primary source of blood flow to the eye. It branches into the central retinal artery and several ciliary arteries. The central retinal artery and retinal arterioles supply blood to the prelaminar optic nerve. The primate lamina cribrosa receives blood primarily from the short posterior ciliary arteries.[10] Blood flow in the retina and prelaminar ONH circulations is autoregulated and capable of responding to IOP-induced ocular perfusion pressure (OPP) changes.[10]

Translaminar Pressure Gradient and Role in Glaucoma

There is significant interest in both the translaminar pressure difference (TLPD) and lamina cribrosa (LC) and their pathologic contribution to glaucoma. A growing body of evidence suggests that glaucoma occurs primarily due to an elevated TLPD, and secondarily due to an imbalance between IOP and CSFp. The main support for this theory originates from the two large retrospective studies by Berdahl et al.[7,11] These studies reported that subjects with open-

angle or normal-tension glaucoma had lower CSFp (and higher TLPD) compared to controls. Furthermore, subjects with ocular hypertension had elevated CSFp compared to controls and may be protected from developing glaucoma. Additional evidence of this theory was provided by a prospective study from Ren at al. evaluating subjects undergoing lumbar puncture: patients with NTG (9.5 ± 2.2 mmHg) had the lowest CSFp, followed by POAG (11.7 ± 2.7 mmHg) and then non-glaucomatous controls (12.9 ± 1.9 mmHg).[12] Once a significant translaminar pressure gradient results in enough optic nerve damage, irreversible optic neuropathy and vision loss may occur.

Retinal Ganglion Cell Characteristics

The optic nerve is composed principally of the axons of retinal ganglion cells. Retinal ganglion cell axons project from the retinal nerve fiber layer through the optic chiasm and optic tracts to either the lateral geniculate nucleus, RGCs and their optic nerve axons provide the sole connection between photoreceptors of the retina and the central components of the visual system.[10] The immature astrocytes and blood vessels enter the retina at the optic nerve head and colonize the entire RNF.[10] RGC axons guide the formation of an astrocytic network that subsequently directs vessel development.[13] Migrating astrocytes play an important role in the formation, development, and health of the retinal nerve fiber layer have been found in close association with the axons of RGCs. As shown in figure 2, RGCs are neighbors to astrocytes in the RNFL.[13] RGCs are necessary to provide directional information to migrating astrocytes. Astrocytes exhibited polarization defects, failed to colonize the peripheral retina and display fine-scale spatial patterning.[13] The fine-scale spatial pattern established by astrocytes is confirmed to be abnormal when they lack RGC guidance signals, confirming a model in which

RGCs are required for astrocytic colonization of the retina, and astrocytes are in turn essential for normal vascular development.[13]

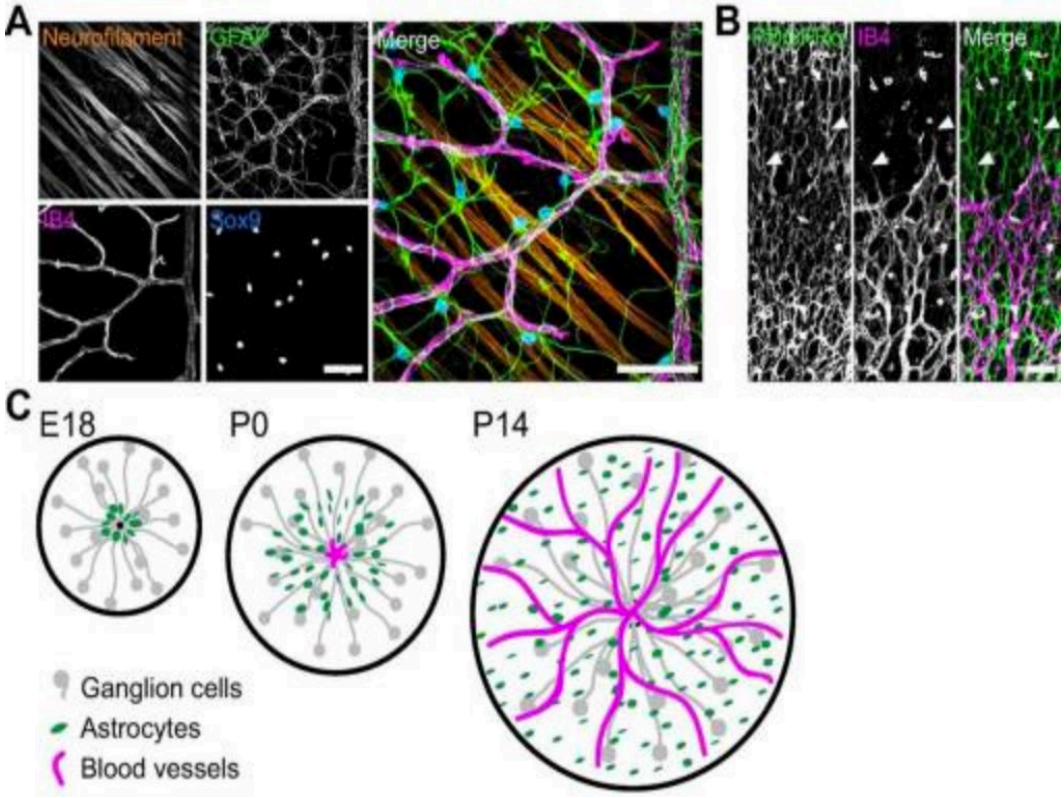

Figure 2. Cellular composition and development of the nerve fiber layer. Schematic image indicates RGCs (gray), astrocytes (green) and blood vessels (magenta).

Retinal Ganglion Cells in Glaucoma

A number of authors have recognized that glaucoma is characterized by death of retinal ganglion cells and by a typical excavated appearance of the optic nerve head. However, it is significant to note that axon apoptosis can occur in other ocular disease besides glaucoma.[1,14,15] Glaucoma is associated with reduced optic nerve axoplasmic flow and compromised circulation such that RGC death due to neurotrophin deprivation result.[10] In a landmark study, elevated IOP and subsequent chronic glaucoma was induced in one eye of 12 monkeys, either by the injections of red blood cells (RBC) fixed in glutaraldehyde to the anterior chamber, or Aargon laser treatment to the trabecular meshwork.[15] All 12 monkeys with induced chronic glaucoma

appeared to have RGCs with similar histological features including condensation of nuclear chromatin at the nuclear edge compatible with induced apoptosis.[15]

Additionally, the retinas of 5 monkeys that underwent unilateral optic nerve transection were evaluated in intervals after axotomy. The findings of the study are summarized in table 1. In the study, at 2 and 4 weeks all 5 demonstrated abnormal ganglion cells with apoptotic bodies and features identical to those with induced chronic glaucoma. Remarkably, at the 10-12 week interval few ganglion cells remained in the retina and therefore no abnormal cells were detected post axotomy.[15] The similarity in findings between induced glaucoma and total axotomy provide interesting insight into the process of retinal ganglion cell death. However, a number of questions regarding the mechanisms underlying development of glaucoma remain.

Table 1. Histologic Study Following Transfection of Monkey Eyes.

Time After-Transection (weeks)	*% Positive Cells*	*Degree of Axon Loss*
2	7	Mild
3	3	Mild
3	13	Mild
3	1	Moderate
4	8	Moderate
10	0	Severe
10	0	Severe
12	0	Severe
12	0	Severe

Vascular Regulation of Optic Nerve Head Perfusion

The vascular endothelium of the optic nerve is an active participant in the maintenance of vascular tone and regulation of blood flow through myogenesis (formation of muscle tissue).[10] Endothelial cells are responsible for detecting changes in the microenvironment and responding

accordingly with the synthesis of various vasoactive factors to maintain the delicate balance between vasoconstriction and vasodilation.[10] Endothelial-derived relaxing factor, prostacyclin (PGI_2) causes increased intracellular cAMP levels in vascular smooth muscle which reduces intracellular calcium, induces myorelaxation, and ultimately increases optic nerve head blood flow.[10] Endothelin-1 (ET-1) is responsible for vasoconstriction and decreasing the optic nerve head blood flow by increasing the release of intracellular calcium.

Pathologic Intracellular Signaling in Retinal Ganglion Cell Apoptosis

The survival of neurons depends on trophic signals from neighboring cells to control the continuous inhibition of an intrinsic apoptotic cell suicide program. The understanding of the stimuli that promote the survival of RGCs in vivo is therefore of critical relevance to developing methods to save adult RGCs from optic nerve diseases such as glaucoma which induce RGC death.[16] Promotion of central nervous system (CNS) neuronal survival in culture appears to require simultaneous stimulation by converging trophic peptides. In contrast, a single peptide trophic factor is sufficient to inhibit apoptosis of peripheral nervous system neurons.[16] The neurotrophic hypothesis suggests that, *in vivo*, the developing neurons compete for limited amounts of target-derived trophic factors which ultimately controls their survival.[17] However, Shen et al. attested that the hypothesis is too simple to apply to the developing CNS because it is necessary for the RGCs to be depolarized in order to respond to trophic factors. The simplest explanation for why RGCs lose trophic responsiveness following axotomy is that their intracellular levels of cAMP quickly decrease.[16]

The second messenger cyclic adenosine 3',5'-monophosphate (cAMP) is a regulator of biological processes in the optic nerve and retina.[16] cAMP levels control the trophic receptors of RGCs. Responsiveness of RGCs, measured by mitogen- activated protein (MAP) kinase

activation, in intact retinas was found to be nearly abolished by blockade of electrical activity, by adenylyl cyclase inhibition, and by axotomy.[16] Since physiological levels of cAMP play a critical role in controlling RGC response to peptide trophic factors, a decrease in cAMP levels could simply occur if a reduction in the activity of axotomized RGCs occurs.

The loss of responsiveness folloing axotomy could simply be decrease in RGC activity. The effects of peptides and cAMP elevation on RGC survival after axotomy were further explored in an adult rat model.[16] A calcium channel agonist, BayK8644, successfully mimicked the ability of CPT-cAMP to promote the survival of axotomized RGCs when combined with trophic factors. However, BayK8644 treatment alone did not save axotomized RGCs.[16] The ability of RGCs to activate and translocate MAP kinase from their cytoplasm into their nucleus can be used to assess the RGCs trophic responsiveness in vitro. Only when RGCs cAMP levels were elevated did peptide trophic factor provoke the translocation of MAP kinase into the nuclei of RGCs.[5] The treatment of the retinas with SQ22356 nearly abolished the translocation of MAP kinase into the nucleus according to Shen et al.

An additional trophic factor mediator, P38 MAP kinase contributes to the progression of retinal ganglion cell degeneration. Although increasing age is a significant risk factor for glaucoma, it was hypothesized by Dapper and Calkins that the effects of age were different from the effects of ocular pressure on p38 MAPK activation.[18] To study their hypothesis, Dapper and Calkins compared age naive mice to an inbred mouse model that had increases intraocular pressure. [18] This study found that ocular pressure plays a more significant role than advanced age on p38MAPK activation in the retina of experimental glaucoma models.[18] It is possible that again may decrease the threshold for signal pathway activation and lead to subsequent neuronal injury rather than by directly activating the p38 MAPK pathway.[18]

Actions of Transforming Growth Factor on Neurotrophic Signaling

Transforming growth factor-β2 is a protein that helps with controlling cell proliferation, differentiation, motility and apoptosis as part of the TGF- β signaling pathway.[5] Several studies suggest that ONH cells respond to elevated IOP by increasing TGF- β2 synthesis in the lamina cribrosa, which causes altered ECM protein expression.[5,19,20] Following the treatment of dissected human ONH and LC cells with recombinant TGF- β2, TGF-β2 was found to be significantly increased in the LC region of the ONH in eyes with induced glaucoma compared to age-matched normal eyes by an immunohistochemical evaluation. This is shown in figure 3.[5] Elevated TGF-β2 levels result in the pathological deposition of ECM proteins[5]. The TGF-β-dependent apoptosis signaling pathway has a critical role in glaucomatous neuropathy by increased synthesis and secretion of extracellular matrix(ECM) proteins and remodeling of the ONH.[5] ONH astrocytes and LC cells secrete TGF-β2, indicating that these cells may be an *in vivo* source of TGF-β2 in the human ONH.[5,19–21] Chronic elevation of IOP induces ONH changes including retinal ganglion cell axon compression at the LC and axoplasmic flow and neurotrophic transport blockage to RGCs.[5] Patients with glaucoma have increased levels of TGF-β2 in their aqueous humor and raised TGF-β2 levels result in the pathological deposition of ECM proteins in human trabecular meshwork cells.[5]

TGF-β2 expression has been shown to be increased in the human glaucomatous ONH by examining 4 age-matched normal and glaucomatous ONH tissues (figure 3).[5] Compared to normal tissues, the glaucomatous ONH demonstrated increased TGF-β2 immunostaining (red) intensity merged with glial fibrillary acidic protein (GFAP). Additionally, glaucomatous nerves had increased co-localization of the two in ONH (Figure 3B) and a the level of the LC (figure 3C).[5]

The GFAP gene encodes for the construction of glial fibrillary acidic protein, responsible for binding together to form intermediate filaments found in astroglial cells.[22] Zode et al. claimed to have presented the first results independently verifying increased immunohistochemical expression of TGF-β2 in the glaucomatous ONH.[5] *In vitro* and *in vivo* findings from this research provided concluding evidence that TGF-β2 is involved in ECM remodeling by cells of the ONH in humans. Many studies assume that ONH astrocytes and LC cells are able to respond to elevated IOP by increasing TGF-β2 synthesis and secretion, which in turn alters ECM protein expression and induces structural changes in the ONH.[5,20,23]

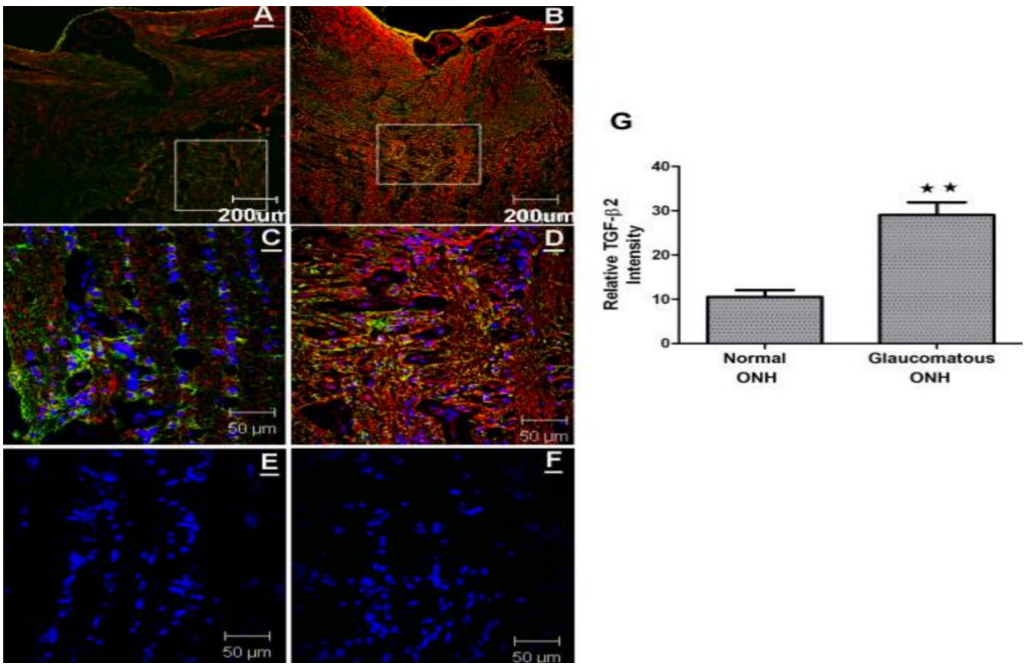

Figure 3. Immunohistochemical evaluation of transforming growth factor (TGF-β2) expression in age-matched normal and glaucomatous optic nerve head (ONH) tissues. **(A)** ONH tissue section from a normal human donor. **(B)** ONH tissue sections from glaucomatous human eyes. (**C, D)** Showing part of the lamina cribrosa (LC) at a higher magnification. **(E)** Control (no primary antibody for TGF-β2 and GFAP) merged with DAPI in normal optic nerve head, and **(F)** in glaucoma-induced optic nerve head. **(G)** Relative intensity measurements of TGF-β2 in the LC region in four age-matched normal and glaucomatous ONH tissues.

Mediation of Gadd45b in Retinal Ganglion Cell Apoptosis

Gadd45b is a growth arrest and DNA damage-inducible protein that is intrinsically expressed in retinal ganglion cells protecting RGCs from various neuronal pathologies, including glaucoma. Gadd45b is a mediator of the activation of the p38 mitogen-activated protein kinase pathway and its products are involved in regulation of growth and apoptosis.[19] Yoo et al. identified Gadd45b as an effector of TGF-β-dependent apoptosis. Northern blot analysis of RNA isolated from AML12 murine hepatocytes underwent apoptosis as early as 8 hours following exposure to TGF- β detected by ELISA (figure 4A) and TUNEL assays (figure 4B). As shown in figure 4C, Gadd45b mRNA was present in these cells and was rapidly induced following treatment with TGF- β.[19] Up-regulation of Gadd45b expression was found to be transcriptionally dependent, because the addition of actinomycin D, a transcription inhibitor, inhibited the TGF- β Gadd45b expression (Figure 4D). Therefore Gadd45b is an immediate early gene product of TGF- β cells in response to TGF- β induced apoptosis.[19]

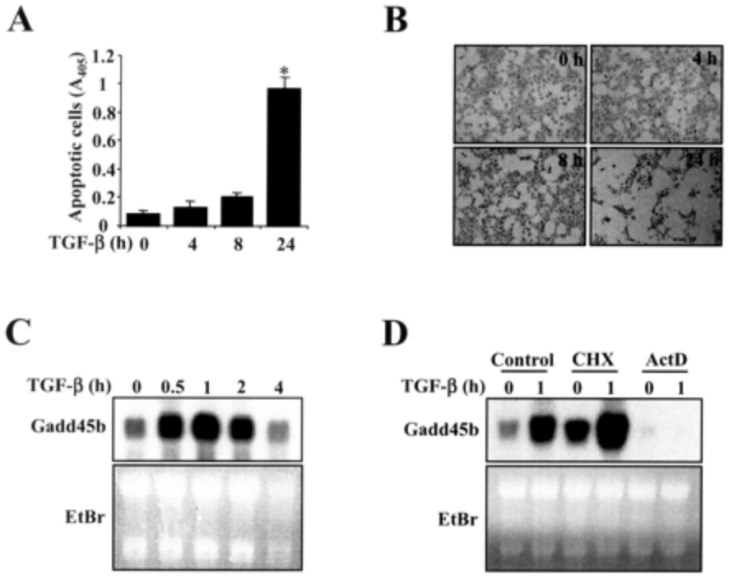

Figure 4. TGF- β induced apoptosis up-regulates Gadd45b expression. 4A and 4B: Induction of apoptosis in AML12 cells by *TGF- β. DNA fragmentation was detected by ELISA **(A)** and TUNEL assay **(B)**.* **(C)** *Northern blot analysis of Gadd45b RNA expression in AML12 cells stimulated with TGF- β for the indicated times.* (***D***) *Effect of cycloheximide or actinomycin on TGF- β induced Gadd45b expression in AML12 cells.*

Smad Signaling in Retinal Ganglion Cells

Smads are a group of intracellular proteins that are responsible for delivering signals from TGF- β ligands to the cell nucleus.[5] Neurotrophins and ECM proteins are synthesized by ONH astrocytes and LC cells in order to support RGC axons[5]. TGF-β2 dimers bind the type II receptor, resulting in transphosphorylation of the type I receptor. The activated type I receptor is responsible for phosphorylating Smads (Smad 2 and 3) and triggers heterodimerization with Co-Smad4 and translocation of the complex to the nucleus which leads to activation of specific target genes.[5,19] ONH astrocytes and LC cells have been concluded to possess autocrine TGF-β2 mediated Smad signaling. Zode et al. demonstrated the presence of TGF-β2, endogenous pSmad2 and 3 as well as their association with Co-Smad4 via co-localization.[5] ONH astrocytes and LC cells incubated with TGF- β2 at specific time intervals and phosphorylation of Smad2 and Smad3 was examined by western blot immunoassay. Recombinant TGF- β2 increased the phosphorylation of Smad2 and Smad3 in ONH astrocytes and LC cells compared to the controls.[5] Figure 5A shows total Smad2, Smad3 and actin levels did not change upon treatment with TGF- β2. This suggests that TGF- β2 can activated Smad2/3 in ONH and LC cells.[5] ONH astrocytes and LC cells were found to have autocrine TGF- β2 mediated Smad signaling.[5]

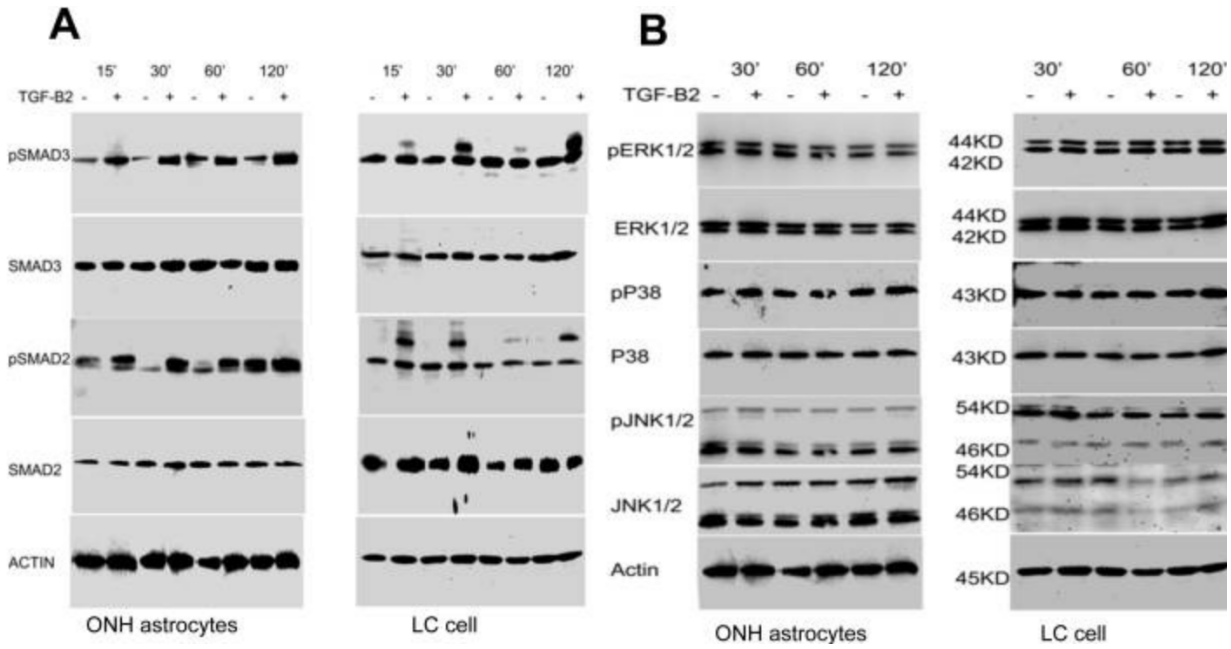

Figure 5. Effect of TGF- β2 on activation of Smad2/3 in optic nerve head (ONH) astrocytes and lamina cribrosa (LC) cells following TGF- β2 ncubation for 0, 15, 30, 60 and 120 minutes. **(A)** Cell lysates were analyzed for phosphorylated Smad3, total Smad3, phosphorylated Smad2, total Smad2 and actin. **(B)** ONH astrocytes and LC cells treated with or without TGF- β2 for 30, 60 and 120 minutes.

In contrast to Zode et al., recent studies suggest that p38 MAPK signaling is involved in apoptotic signaling in several cell types including RGCs.[18,19,24,25] P38 mitogen-activated protein kinases (MAPKs) are a class of mitogen-activated protein kinases that are responsive to stress stimuli and involved in apoptosis. The p38 MAPK family consists of four main isoforms, designated; α, β, γ, and δ. The homologous p38α and β isoforms have been associated with CNS degeneration following ischemia, RGC injury, and in neonatal brain astrocyte apoptosis and oxidative stress.[24,26,27] Little is known about p38 MAPK's potential pro-apoptotic downstream targets.[19] It has been reported that both Smad-dependent and Smad-independent pathways are capable of activating p38 MAPK.[19] P38 MAPK activation is significant to induce TGF-β mediated apoptosis. The involvement of the p38 signaling pathway in TGF-β induced apoptosis in AML12 cells was studied by using a specific inhibitor of p38, SB203580. Increased p38

MAPK phosphorylation was strongly detected following the exposure to reactive oxidative stress (ROS) indicating its pro-apoptotic expression is up-regulated under stress.[19,27]

Additional Mechanisms for Retinal Ganglion Cell Apoptosis

Glutamate Cytotoxicity

Glutamate is an amino acid that normally functions as a CNS neurotransmitter. However, it can become cytotoxic when its physiological concentrations are exceeded.[28,29] It has been suggested that glutamate toxicity in retinal ganglion cells plays a role in the neuronal loss in glaucoma. Glaucoma selectively damages larger retinal ganglion cells first. Dreyer et al. sought to explore whether glutamate-mediated cell death was also more definite in larger RGCs. They found that glutamate is significantly more toxic to larger retinal ganglion cells in glaucoma.[29] Glutamate exerts its toxic effect on larger retinal ganglion cells by its N-methyl-D-aspartate subtype of glutamate receptor (NMDA) in tissue culture. More importantly, the same findings were more importantly reciprocated in RGCs of the intact normal non-glaucomatous rat eye. It was found that glutamate-mediated loss first effected larger RGCs, similar to the pattern loss identified in glaucoma.[29]

Ocular Ischemic Syndrome

In primates, blood moves from the periphery of the retina and optic nerve head to the single central retinal vein located in the center of the optic disc. This centripetal vascular supply pattern to the optic nerve predisposes the retina and optic nerve to ischemic disorders if obstruction of venous drainage occurs.[10]

Patients with carotid atherosclerosis can present with ophthalmic symptoms. In atherosclerosis, thrombus formation from a ruptured atherosclerotic plaque can break off and travel distally into the ophthalmic artery. The thrombus can cause narrowing of the lumen and

chronic distal ischemia in the newly diseased vessel.[30] The central retinal artery (CRA) is an end artery dividing into four major branches supplying non-overlapping sectors of the retina they supply.[30] When an embolus enters the central retinal artery CRA causing occlusion, it tends to lodge in the narrowest portion of the artery at the lamina cribrosa which interferes with cellular signaling. More recently, retinal ischemia was induced by ligature in ophthalmic vessels of adult rats to investigate retrograde axonal transport in RGCs.[31] This induced retinal ischemia was concluded to result in glaucoma-like RGC loss and alterations of the retrograde axonal transport via surviving RGCs.[31]

Mechanisms for Axonal Blockade Reversal

Targeting TGF- β2 Downstream Signaling

TGF- β2 and downstream signaling provided in vitro and in vivo evidence supporting the conclusion that TGF- β2 is in fact involved in ECM remodeling by cells of the ONH.[5] The TGF-β2 driven ECM stimulation was concluded to require activation of recognized Smad signaling pathway. ECM proteins were stimulated in the ONH astrocytes and LC cells following the inhibition of type I TGF- β or knockdown of either Smad2 or Smad3 proteins. Therefore, inhibition of either of these downstream signals may prove to be a vital therapeutic target to prevent ECM remodeling present in the ONH under glaucomatous conditions.[5]

Antibody-Mediated TrkB Agonism

Brain-derived neurotrophic factor (BDNF) is a neurotransmitter modulator that plays a significant role in supporting differentiation, maturation and neuron survival.[14] BDNF signaling pathways activated transcription factors are important for the regulated gene expression for genes encoding proteins involved in neural plasticity, stress resistance and cell survival. TrkB receptor dimerization results from the ligand, BDNF binding and the auto-phosphorylation of the tyrosine

residues. This provides a docking site for src-homology 2-domain containing adaptor protein (Shc) and phospholipase C (PLC).[14] Bathina et al. concluded guanine nucleotide-releasing factor SOS binds SHC to the adapter protein, Grb2. Ras is linked to Grb2 by SOS and activates numerous cell signaling pathways: Ras/MAPK-ERK pathway, PI3-K pathway and PLC pathway.[14]

BDNF mRNA expression is strikingly reduced in the visual cortex by monocular deprivation.[14] Anti-BDNF antiserum and TrkB-IgG construct have been found to successfully antagonize the action of BDNF in neurotrophic pain and inflammatory hypersensitivity. These findings validated the potential for antibody-mediated TrkB agonism as a potential therapeutic approach to enhance RGCs survival after optic nerve damage.[14] In a trauma induced central nervous system model, and in vivo injection of BDNF in an adult rat retinal ganglion cell enhanced neuronal survival.[14] These studies suggest that BDNF is correlated with prompting RGC survival and if signaling is blocked, stress resistance and cell survival is reduced.

Conclusion

In conclusion, the pathophysiology of glaucoma is complex, and multiple downstream signaling cascades are involved in the development of retinal ganglion cell apoptosis at the level of the optic nerve head. The transforming growth factor-β-dependent apoptosis signaling pathway, Gadd45b and p38 MAPK all play critical roles in glaucomatous neuropathy. Additional mechanisms of disease, including glutamate cytotoxicity and ocular ischemic syndrome, produce glaucomatous-like neuropathy on the RGCs of the optic nerve head. Significantly, inhibition of type I TGF- β or knockdown of either Smad2 or Smad3 proteins may prove to be therapeutic targets to prevent ECM remodeling present in the ONH under glaucomatous conditions.

Additionally, the potential for antibody-mediated TrkB agonism to induce BDNF expression may be a therapeutic approach to enhance RGC survival following optic nerve damage.

Implications

The implications of the review expand on how glaucoma should not be confined as a disease solely of the ocular system, but as a disease of pressure differences throughout the body altering downstream signaling. A translaminar pressure difference is capable of inducing optic nerve head changes that affect retrograde axonal transport and ultimately interfere with retinal ganglion cell signaling. The review illustrates a potential mechanistic downstream signaling cascade that plays a critical role in retinal ganglion cell apoptosis in glaucoma. Furthermore, research involving cellular mechanisms responsible for glaucoma and retinal ganglion cell apoptosis are promptly evolving in the science community. Further research in the efforts of discovering mechanistic details responsible for glaucoma in order to identifying targets for reversing retinal ganglion cell death or translaminar pressure differences is of priority to evolve disease prevention.

Limitations

An ongoing issue that continues to hinder understanding the mechanism of RGC death in glaucoma and development of RGC therapeutics is the lack of a reliable RGC model. A reliable model would be used to facilitate *in vitro* screening of potential neurotrophic factors or therapeutic delivery systems in an efficient and cost-effective manner. Primary RGCs would ideally be isolated from post-mortem human retinas. Alternatively, mice or monkeys could serve as models. However, studying ONH tissue samples are limited in the low abundance of RGCs present in the retina, the difficult of efficient isolation, and more importantly, the short duration

of isolated RGC cultures *in vitro* limits the range of testing and applications that can be performed using these primary cells. A reliable immortalized RGC line is urgently needed.

Future Research

Considering all experimental evidences, translaminar gradient should be regarded as a significant area of future research. Studies in animal models and human optic nerve head samples will potentially allow for the relationship between ICP, IOP and glaucoma to be defined. Standardizing non-invasive and surrogate techniques to identify ICP values will be of significance since current methods are invasive. Future research focused on obtaining the ability to visualize the translaminar gradient change in optic nerve heads a the lamina cribrosa under glaucomatous conditions should correlate with identifying changes in neurotrophic signaling. This could lead to the development of understanding which specific cell type to target downstream the glaucoma apoptotic cell signaling mechanism to prevent continued RGC death and potentially reverse its damaging effects.

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Data and Materials Availability

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Supplementary Methods

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Appendix A

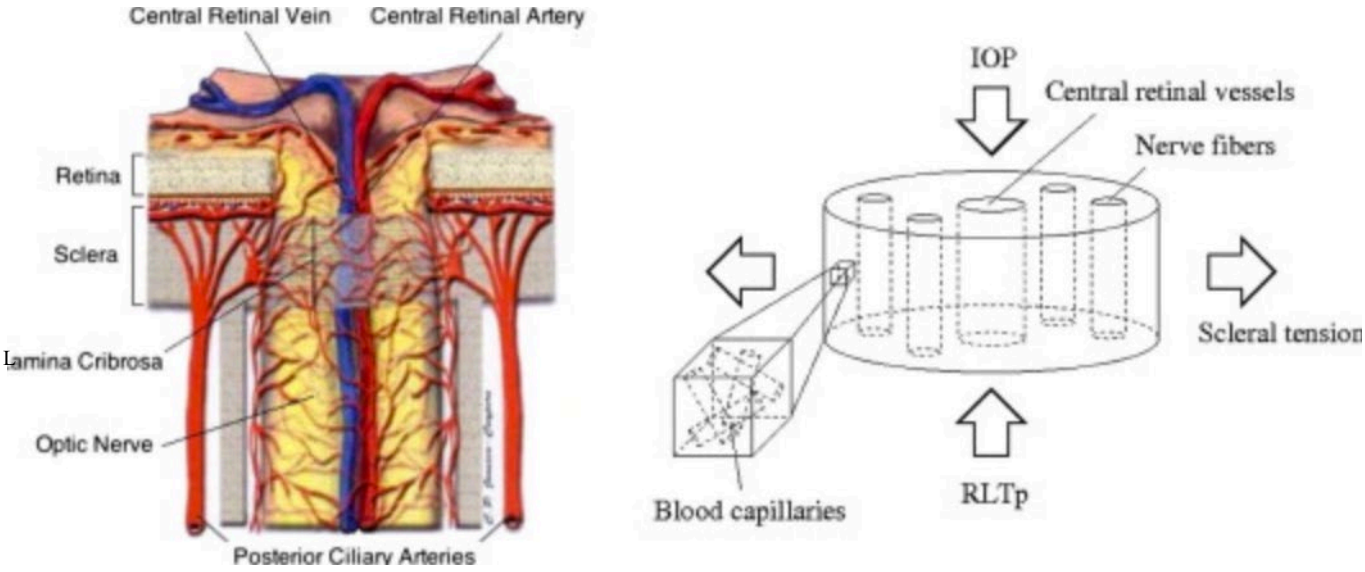

Figure A- 1: Left: Anatomy of the optic nerve head regions with the lamina cribrosa. Right: microscale view of the lamina cribrosa[32].

Appendix B

Acronyms and Abbreviations

RGC	Retinal ganglion cell
ONH	Optic nerve head
LC	Lamina cribrosa
IOP	Intraocular pressure
ICP	Intracranial pressure
ON	Optic nerve
CSFp	Cerebrospinal fluid pressure
OPP	Ocular perfusion pressure
TLPD	Translaminar pressure difference
NTG	Normal tension glaucoma
POAG	Possible open angle glaucoma
RNF	Retinal nerve fiber
RNFL	Retinal nerve fiber layer
PGI_2	Prostacyclin
cAMP	Cyclic adenosine 3’,5’-monophosphate
ET-1	Endothelin-1
CNS	Central nervous system
MAPK	mitogen-activated protein kinase
TGF- β2	Transforming growth factor-β2
ECM	Extracellular matrix
GFAP	Glial fibrillary acidic protein

Gadd	Growth arrest and DNA damage-inducible protein
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
AML	Alpha mouse live
NMDA	N-methyl-D-aspartate subtype of glutamate receptor
BDNF	Brain-derived neurotrophic factor