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Neurobiology of Glaucomatous Optic Neuropathy

Diverse Cellular Events in Neurodegeneration and Neuroprotection

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Abstract

Although glaucomatous optic nerve degeneration is a leading cause of worldwide blindness, neither the precise cellular mechanisms underlying neurodegeneration in glaucoma, nor effective strategies for neuroprotection are yet clear. This review focuses on diverse cellular events associated with glaucomatous neurodegeneration whose balance is critical for determination of ultimate cell fate. An improved understanding of the site of primary injury to optic nerve, the mediator pathways of apoptotic cell death and intrinsic protection mechanisms in retinal ganglion cells, the role of glial activation on the survival and death of retinal ganglion cell bodies and their axons, and the protective and destructive consequences of immune system involvement can facilitate development of effective neuroprotective strategies in glaucoma.

Index Entries: Apoptosis; glaucoma; neurodegeneration; neuroprotection; retinal ganglion cells.

Introduction

Primary open angle glaucoma, a chronic neurodegenerative disease characterized by progressive optic nerve atrophy and loss of visual field sensitivity, is a leading cause of irreversible blindness worldwide. Intraocular pressure elevation, which is commonly detected in glaucomatous eyes, is thought to be an important stress factor for the initiation or progression of the optic nerve degeneration in these eyes *(1)*. Glaucoma affects approximately 0.5% of the American population, and is prevalent in 1.3% of white and 4.7% of black Americans over the age of 40 (1.6 million persons) *(1–4)*. Because conventional treatment to reduce intraocular pressure does

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not always prevent progression of glaucomatous neurodegeneration *(5)*, recent research in glaucoma has been focused on the alternative treatment strategies for neuroprotection, which can be adjunctive to the intraocular pressure lowering treatment. However, neither the precise pathogenic mechanisms of glaucomatous neurodegeneration nor effective strategies for neuroprotection are yet clear.

Optic nerve degeneration in glaucoma is characterized by a progressive loss of retinal ganglion cells and their axons. Retinal ganglion cell death during glaucomatous neurodegeneration is thought to occur by an apoptotic mechanism, as shown in different animal models (6–8) and in human donor eyes with glaucoma *(9,10)*. In addition to elevated intraocular pressure, primary and/or secondary ischemia in the optic nerve head and/or retina has been suggested to be another stress factor in glaucomatous eyes (11–14). Multiple causative events, triggered by elevated intraocular pressure or ischemia, are thought to be associated with glaucomatous degeneration of the optic nerve. In this review, we focus on diverse cellular events associated with the glaucomatous neurodegeneration whose balance is critical for determination of ultimate cell fate. Elucidation of the intracellular cascades that regulate retinal ganglion cell death and survival is a crucial prerequisite for the development of effective neuroprotective strategies in glaucoma.

Site of Primary Injury: Optic Nerve Axons vs Cell Bodies

Retinal ganglion cell death in glaucoma is commonly thought to be associated with the injury of optic nerve axons at the optic nerve head, and subsequent retrograde degeneration of the cell bodies. For example, secondary to the elevated intraocular pressure, the blockade of axoplasmic transport at the optic nerve head and the resultant blockade of neurotrophin transport to the retinal ganglion cell bodies has been suggested to be a mechanism of retinal ganglion cell death in glaucoma *(15–18)*. Production of reactive oxygen species (ROS) may contribute to retinal ganglion cell death following neurotrophin deprivation *(19)*. Nitric oxide-induced damage has also been implicated in the glaucomatous injury of retinal ganglion cell axons *(20)*. Although axonal damage at the level of optic nerve head may explain selective loss of ganglion cell bodies by retrograde degeneration, there are regional *(21–23)* and cellular *(24,25)* differences in the susceptibility of individual retinal ganglion cells to glaucomatous damage, which are not well-understood. Intraretinal events, including chronic ischemia *(11,13,14)*, glutamate excitotoxicity *(26)*, and an autoimmune mechanism *(27,28)*, may also facilitate primary and/or secondary degeneration of retinal ganglion cells in glaucoma. Simulation of these noxious conditions, in vitro or ex vivo, indeed results in the apoptotic death of retinal neuronal cells in a caspase-dependent manner *(28–30)*.

Recent evidence suggests that neurons have distinct programs for selective axonal degeneration or apoptotic death of their cell bodies *(31)*. Although the loss of optic nerve axons during the glaucomatous neurodegeneration is accompanied by the apoptotic death of retinal ganglion cell bodies *(6–8)*, the primary site of injury still remains controversial. Potential differences in the effective period of neuroprotective interventions in the case of axonal or neuronal injury *(32)* suggest the importance of the precise identification of injury site in glaucoma. However, there is also considerable evidence that the primary damage of optic nerve in glaucoma is followed by delayed secondary degeneration of neurons that were initially spared *(33,34)*. Development of such secondary neuronal degeneration makes the differentiation of the primary injury site yet more uncertain. From the perspective there is a continuum of neurodegeneration with widespread damage to neurons beyond the initial injury site, whether the identification of the initial injury site is essential for therapeutic gain in glaucoma is not entirely clear.

Glial Activation: Neuroprotective vs Neurodestructive Effects

Histopathologic studies demonstrate that glial cells located either in the retina *(35–37)* or in the optic nerve head *(38)* exhibit an activated phenotype in glaucomatous eyes, as characterized by their hypertrophic morphology and increased protein expression, including glial fibrillary acidic protein. Glial cells are known to support neuronal tissue by supplying metabolites and growth factors, by scavenging toxic agents *(39),* and by providing guidance to the axons *(40).* Therefore, glial activation may be an initial cellular attempt to limit the extent of injury and to promote tissue repair in glaucoma. For example, it has been proposed that retinal glial cells, the primary cell type responsible for the removal of glutamate from the extracellular space *(41),* are initially activated to prevent excessive glutamate accumulation that may underlie glaucomatous neurodegeneration secondary to excitoxicity *(42).*

Activated glial cells may subsequently have various noxious effects on neuronal tissue. For example, proteolytic activity of optic nerve head astrocytes is upregulated in glaucomatous eyes, as characterized by their increased expression of matrix metalloproteinases *(43,44).* Another observation in the optic nerve head of glaucomatous eyes is that the astrocytes located in the specialized connective tissue columns within the optic nerve head, migrate into the openings in which optic nerve axons travel when they exit the eye *(45).* Recent in vitro studies demonstrated that the migratory ability of optic nerve head astrocytes is activated in response to glaucomatous stressors, such as elevated pressure, and is accompanied by an activated proteolytic degradation of the extracelluar matrix *(46).* These cellular events have been implicated in the tissue remodeling of the glaucomatous optic nerve head, which results in the characteristic clinical appearance of the optic nerve head in glaucomatous eyes called optic disc cupping.

Alterations in the structural configuration and biomechanical properties of the optic nerve head tissue during the remodeling events that occur in the glaucomatous optic nerve head may contribute to the injury of neuronal tissue in glaucomatous eyes by stretching or compressing optic nerve axons or changing their microenvironment by degradation of the extracellular matrix around the axons. In addition, there is considerable evidence suggesting that the activated glial cells may also have direct neurotoxic influences in glaucoma. For example, activated glial cells in glaucomatous eyes have been shown to produce neurotoxic substances, such as nitric oxide synthase (NOS) *(47)* and tumor necrosis factor-α (TNF-α) *(43,48).* In vitro observations have shown that in response to stressors, such as elevated pressure or ischemia, activated glial cells may facilitate retinal ganglion cell apoptosis due to increased production of TNF-α and nitric oxide *(30).* Thus, glial cells play a significant role in retinal ganglion cell survival during glaucomatous neurodegeneration due to their ability to either protect retinal ganglion cells against stress stimuli or cause further injury. Better understanding of the precise molecular mechanisms of these opposing effects can facilitate development of improved strategies for neuroprotection.

Upregulation of TNF-α: Mediation of Neuronal Cell Death vs Induction of an Adaptive Response

Apoptotic cell death represents ordered biochemical events, and the proteolysis is mediated by a conserved group of cysteine aspartic acid proteases (caspases) that are related to mammalian interleukin 1β (IL-1β) converting enzyme and to nematode CED-3 *(49,50).* Different stimuli initiate the caspase cascade by ligand binding, such as TNF death receptor binding. For example, following binding of TNF-α to TNF receptor-1 (p55), a death receptor, its cytoplasmic tail binds to downstream

adaptor proteins and a receptor-interacting protein containing a protease domain (caspase-8), and thus initiating the caspase cascade directly *(51).* In addition to the receptor-mediated pathway of apoptotic cell death, also called the extrinsic pathway, translocation of mitochondrial cytochrome *c* from mitochondria into the cytoplasm starts another chain of events during the early phases of apoptosis, which also play initiates the proteolytic cascade via cascade activation *(52–54).* The bcl-2 family of proteins tightly regulates mitochondrial events in this intrinsic pathway of apoptotic cell death, which can promote (e.g., bax and bad) or inhibit (e.g., bcl-2 and bcl-xL) apoptosis induced by certain triggering events *(55–58).* Caspase-3 is a final common caspase in both the death receptor-mediated and the mitochondria-mediated pathways of apoptotic cell death *(59).* As a consequence, caspases contribute to cell death through degradation of DNA repair enzymes and structural elements, such as nuclear lamina and cytoskeleton, and indirect activation of chromosomal endonucleases *(60).*

Apoptosis of retinal cells induced by different glaucomatous stressors shares a common caspase cascade *(28–30),* including the activation of caspase-3, which can be blocked using specific inhibitors of caspases *(29).* Control of apoptotic cell death in the retina is regulated by various members of the bcl-2 family of proteins, predominantly by bcl-xL *(61–63).* Transcription factor p53, which activates bax *(64,65),* and is required for cytochrome *c* release from the mitochondria, has also been proposed to be involved in neuronal apoptosis in glaucoma *(66).* Furthermore, there is evidence suggesting that the mitochondrial cell death pathway may contribute to apoptosis in a rat model of high-pressure glaucoma *(67,68).*

Although both caspase-dependent and -independent components of mitochondrial cell death pathway are involved in TNF-α-mediated cell death, activation of caspase-8 is commonly accepted to be a hallmark of TNF receptor family cell death pathway *(51).* Several in vitro studies have demonstrated activation of retinal caspase-8 in response to glaucomatous stressors *(28,29).* In addition, retinal caspase activation has been studied in vivo, in rats with elevated intraocular pressure. These studies found that caspase-8 activation accompanies retinal cell death cascade in rat eyes with elevated intraocular pressure following cautery of episcleral vessels (G. Tezel, unpublished observation). Recent studies confirm the activation of retinal caspase-8 in rats with elevated intraocular pressure following obstruction of aqueous humor outflow with laser coagulation or limbal hypertonic saline injection *(69).* Observation of retinal caspase-8 activation, in vitro and in vivo, supports the hypothesis that TNF-α may be an important mediator of cell death in glaucomatous neurodegeneration. Subsequently, histopathologic studies in postmortem eyes revealed an upregulation of both protein and mRNA of TNF-α and TNF receptor-1 in glaucomatous optic nerve head and retina compared to age-matched control eyes *(43,48,70).* The presence of TNF receptor-1 in the retinal ganglion cells and their axons indicates that these cells are sensitive to the cytotoxic effects of TNF-α Increased production of TNF-α by glial cells in glaucoma may therefore participate in the death of retinal ganglion cells via direct activation of the apoptotic cell death cascade.

Using a co-culture system, in which retinal ganglion cells and glial cells are grown on different layers, but share the same culture medium, influences of glial cells on survival of retinal ganglion cells have been studied under stress conditions identified in glaucomatous eyes. These studies found that following exposure to elevated hydrostatic pressure or simulated ischemia—two prominent stress factors identified in the eyes of patients with glaucoma—glial cells secrete TNF-α, as well as other noxious agents such as nitric oxide, into the co-culture media and facilitate apoptotic death of retinal ganglion cells. Furthermore, retinal ganglion cell death in these cultures were attenuated approx 70% by inhibition of the bioactivity of TNF-α *(30).* Thus, these findings identify TNF-α as a potentially important

mediator of retinal ganglion cell death in glaucoma, and provide a novel target for neuroprotection studies.

Further evidence supporting the hypothesis that TNF-α signaling may play a prominent role in glaucoma comes from a recent genetic study *(71).* This study has identified a mutation of *optineurin* gene that was present in approx 15% of open angle glaucoma patients. Since optinuerin protein is apparently associated with TNF-α signaling, these findings warrant further in vivo studies to better identify the pathogenic significance of TNF-α mediated cell death, and its inhibition as a neuroprotective strategy in glaucoma.

What remains unclear about the role of TNF-α signaling in glaucoma is that in different cell types, or even within the same cell type, responses to TNF-α are quite different, and may result in either cell death or defensive-protective adaptations and survival. In most cells, TNF receptor-1 occupancy by TNF-α induces apoptosis by activating the apoptotic caspase cascade. However, under certain conditions it may provide protection by induction of survival genes, including NF-κB and heat-hock proteins *(72–77).* It is apparent that there is a critical balance between positive and negative regulators modulated by selective signaling pathways initiated by TNF-α binding to its specific receptor. This homeostasis or its disruption may determine the survival or demise of retinal ganglion cells.

Immune System Involvement: Autoimmune Disease vs Protective Immunity

Compelling evidence has provided insight as to the potential role for the immune system in glaucomatous neurodegeneration. For example, an increased prevalence of monoclonal gammopathy *(27);* retinal immunoglobulin deposition *(10);* elevated serum autoantibody titers to optic nerve head glycosaminoglycans *(78)* or to retinal antigens, including rhodopsin *(79),* heat-shock proteins *(80,81),* gamma-enolase *(82)* and gluthione S-transferase *(83),* have been reported in some patients with glaucoma. In these patients, an autoimmune mechanism may be responsible for eliciting glaucomatous neurodegeneration, since autoimmune damage to neuronal tissue may occur directly by autoantibodies *(28),* or indirectly by a "mimicked" autoimmune response to a sensitizing antigen, which in turn results in neuronal injury *(80,84).* The clearest evidence that autoantibodies may induce retinal cell death is the observation that antibodies to small heat-shock proteins, in concentrations similar to those found in the sera of many glaucoma patients, elicit apoptosis when applied exogenously to human retina, ex vivo *(28),* or in cultured retinal cells, in vitro *(81).*

A prominent and progressive atrophy of the retinal pigment epithelium adjacent to the optic nerve head in glaucoma patients, termed peripapillary chorioretinal atrophy, has been proposed to allow communication and access of circulating antibodies to the retina in these eyes *(10).* Additional findings supporting the involvement of the immune system in glaucoma include the presence of HLA-DR positive microglia in the parapapillary region of the glaucomatous optic nerve head *(85),* the induction of HLA-DR expression and antigen-presenting capacity of optic nerve head astrocytes in glaucoma *(86),* and alterations in the cellular immune system, such as increased percentage of CDS + T lymphocytes and altered serum levels of soluble IL-2 cytokine receptors, in some patients with glaucoma *(87).*

There is also evidence demonstrating that an immune activity in response to the central nervous system (CNS) injury is not always harmful. It has been proposed that a protective immunity is evoked in injured optic nerve to reduce the secondary degeneration of neurons, which can be induced by active or passive immunization with self-antigens *(88,89).* Better understanding of the regulation of balance between this protective autoimmune response and induction of an autoimmune disease appears to be critical to take advantage of the immune system for neuroprotective strategies in glaucoma.

Induction of Retinal Heat Shock Proteins: Intrinsic Protection Mechanisms vs Antigenic Stimuli

As in other systems, intrinsic protection mechanisms are known to be critical to overcome cell death stimuli in the retina. In addition to the members of the bcl-2 family of proteins that can regulate the processing and activation of apoptotic cell death, heat-shock proteins are implicated as a component of the endogenous protection mechanisms in all mammalian cells *(90).* Both the bcl-2 family of proteins *(66)* and heat-shock proteins have been associated with glaucomatous neurodegeneration *(91,92).*

Heat-shock proteins, which are classified into families based on their molecular weight, including hsp90, hsp70, hsp60, and small (25–30 kDa) a heat-shock protein families, are known to be associated with an early response against stress, which facilitates restoration of damaged areas after injury *(93–95).* It has been reported that heat-shock proteins, including hsp27 and hsp60, are upregulated in the retinal ganglion cells in glaucoma *(91).* This suggests that these proteins play a role as a native defense mechanism in response to stressed or injured neurons in glaucoma. One of the protective mechanisms attributed to heat-shock proteins, particularly to hsp27, is that they counteract disruption of actin architecture and enhance cellular resistance to oxidative stress and apoptotic cell death *(96–100).* In vitro studies have revealed that retinal cells are more resistant to apoptosis in higher-density cultures, in which cytoskeletal structures are better developed, and the expression of hsp27 is greater in higher-density cultures compared to lower-density cultures *(63).* In addition, recent studies have provide evidence that exogenously applied hsp27 antibody can induce apoptosis in retinal ganglion cells, in vitro and ex vivo *(28,81),* by inactivating or attenuating the ability of native hsp27 to stabilize actin cytoskeleton *(28),* which confirms the protective role of hsp27 in these cells. Over expression of another heat-shock protein, hsp72, has also been suggested to have a neuroprotective role on retinal neurons *(92,101).*

Although heat-shock proteins function as endogenous protectants of retinal neurons in response to a variety of stressors, including those associated with glaucoma, they may have the ability to elicit an activated immune response as well. For example, heat-shock proteins are known to be highly antigenic, and immune responses to heat-shock proteins are implicated in the development of a number of human autoimmune diseases *(102).* Glial cells of the retina and optic nerve head likely function as antige-presenting cells, and along with their activation in glaucoma, their antigen-presenting capacity is induced *(86).* Therefore, enhanced expression of heat-shock proteins in the glaucomatous eyes may be an immunostimulatory signal. Therefore, although increased expression of heat-shock proteins in glaucomatous eyes may initially serve to protect retinal cells from further destruction and facilitate their repair, they may subsequently recruit immune responses that may contribute to the progression of disease.

Conclusion

As in other forms of neurodegeneration, development of effective neuroprotective treatment strategies in glaucoma appears complicated due to complex interactions of diverse cellular events that determine ultimate cell fate. It is predictable that even if one of the pathways identified to be associated with glaucomatous neurodegeneration is blocked, cell death can still be mediated through alternative pathways. Therefore, neuroprotective strategies may require impeding a wide range of cell death pathways. Alternatively, neuroprotective strategies may be directed at enhancing intrinsic protective mechanisms.

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