Retina and Apoptosis

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ABSTRACT

It is known that apoptosis plays a role in the pathogenesis of many retinal diseases. Diabetic retinopathy, age-related macular degeneration, retinitis pigmentosa, retinopathy of prematurity and retinoblastoma are examples of these diseases. Several ocular and extraocular neurodegenerative diseases share common feature early and pathological death of retinal cells. This provides a potential early diagnosis window in which to delay and possibly halt pathologic processes before they cause significant harm. Agents with mechanisms that reduce, or delay apoptosis are still being investigated as treatment modalities in retinal diseases.

Keywords: Apoptosis, Retinal endothelium, TUNEL technique.

Cell proliferation, apoptosis, and the balance between them are essential for proper tissue morphogenesis, homeostasis, and function.[1] Both cell proliferation and apoptosis are required for self-renewal, maintenance and adaptation of tissues to physiological stimuli by allowing an organism to control cell number and tissue volume.[1] Dysregulation of apoptosis has been reported in an increasing number of pathological conditions. In the retina, apoptotic cell death is seen in postnatal development, axotomy, ischemia, degenerative diseases, and experimental retinal detachment. Apoptosis has also been shown in human retina of retinoblastoma, pathological myopia, age-related macular degeneration, and traumatic retinal detachment.[2,3]

TdT-mediated dUTP nick and labeling (TUNEL) is a simple, sensitive, and reliable method that can selectively mark apoptotic cells in tissues.

p53 and Apoptosis

The protein p53 is perhaps best known for its role as a tumor suppressor. Furthermore, p53 may have important roles of in the retina during stressor disease although these potential roles remain unclear. Although p53 may be dispensable for light- or chemical stress-induced apoptosis and in certain animal models of retinitis pigmentosa (RP), p53 has been linked to retinal responses to irradiation, oxidative stress, and the development of retinoblastoma.[4] Developmental overexpression of p53 in the retina leads to the selective loss of rod photoreceptors but leaves the cone photoreceptor population apparently intact. The selective loss of rods also is consistent with the functional deficits shown by the diminished a and b waves of the rod-driven ERG in the super p53 mouse. The reduced numbers of cells in the INL of the super p53 mouse also might contribute to the reduced scotopic b wave amplitude, which reflects the activity of cells in the inner retina, particularly rod bipolar cells. The developmental overexpression of p53 also compromised cone function, as shown by the reduced amplitude of the photopic b wave, even though the cone population of the super p53 mouse retina appeared to be intact. The reduced photopic b wave in the super p53 mouse suggest that the loss of cells from the INL compromises cone-driven retina function, although secondary effects related to the reduced numbers of rods in the super p53 mouse retina also could indirectly contribute to the compromised cone-driven responses.[5]

Intense light can damage the retina and cause photoreceptor and retinal pigment epithelial cell apoptosis, an outcome characteristic of many retinal dystrophies. Although it is logical to expect the involvement of p53 in this process, the role of p53 in light-induced retinal apoptosis may be mixed.[6] These results suggested that although light-induced photoreceptor cell death is p53-independent, p53 may play

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Received: 01.12.2021
Accepted: 01.12.2021
Ret-Vit 2022; 31: 82-87
DOİ:10.37845/ret.vit.2022.31.15

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a role in the response of RPE cells to light. Of interested, age-related macular degeneration, a leading cause of legal blindness in the elderly, is often accompanied by lipofuscin accumulation in the RPE and by RPE cell death.[7] Given the role that p53 plays in lipofuscin-associated cell death in vitro and that inhibition of Mdm2, one of the main negative regulators of p53, has been shown to sensitize human RPE cells to apoptosis, it is reasonable to hypothesize that RPE cell death in AMD involves the p53 pathway.

The retina is particularly sensitive to oxidative stress because of its oxygen and lipid-rich environment. Damage owing to oxidative stress is thought to contribute to AMD, cataracts, primary open-angle-glaucoma, and other eye diseases.[8-10] p53 is also thought to play a role in oxidative-stress-mediated cell death in RGCs.

p53 may also regulate apoptosis in response to retinal ischemia. Hypoxic conditions may arise in the retina from ischemia, which involves a lack of blood flow to the eye.[11] Both p53 gene and protein expression have been shown to be upregulated in response to retinal ischemia, implying that the apoptosis that occurs in response to ischemia is p53 mediated.[12,13]

Retinal Ganglion Cells

Apoptosis of retinal cells is the common endpoint of different insults occurring in a variety of neurodegenerative disease.[14] The archetypal neurodegenerative disease of the retina is glaucoma, characterized by retinal ganglion cell (RGC) apoptosis, although other models of death have been proposed.[15-17] Pathological death of RGGs has also been detected in neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, optic neuritis, and multiple sclerosis. In contrast, other ophthalmic conditions may involve different cell populations; for example, in age-related macular degeneration (AMD), retinal pigment epithelium and photoreceptors progressively degenerate, leading to central vision loss.[18] RGC loss is a physiological process ubiquitously occurring due to ageing; however, the progression rate of RGC loss is significantly higher in subjects affected by glaucoma.[19] On average, a healthy subject has around 1.2 million RGC at birth[19], with approximately 20-40% thought to be lost before visual field defects are detected.[20] This leads to a diagnostic delay of up to 10 years.[21] Apoptosis-initiating events (such as raised IOP) are followed by cell shrinkage and blebbing, chromatin condensation and DNA fragmentation[22], but very early event in this process is the translocation of phosphatidyl-serine (PS) to the external leaflet of the cell membrane[23], this can be exploited by in vitro and in vivo diagnostic techniques.

Age-related macular Degeneration

A deregulated programmed cell death is thought to occur in other retinal diseases such as AMD[24], diabetic retinopathy (DR), and other retinal dystrophies. In all these conditions, monitoring apoptosis may represent a surrogate biomarker of disease activity and progression. AMD is the leading cause of irreversible blindness in the ageing population[25], and a major worldwide health problem. The primary insult in AMD occurs at the level of RPE, due to accumulation of yellowish auto-fluorescent lipofuscin deposits above Bruch's membrane and beneath RPE cells, known as drusen. Drusen are responsible for the distortion of central vision in "dry" AMD, the size of which may range from tiny dots up to 250 µm.[25] Larger drusen tend to fuse leading to pigment epithelial detachment. This last phenomenon represents a risk factor the development of the "wet" form of AMD, during which neovascularization from the choroidal circulation causes exudation and hemorrhage destructive to the anatomical order of the retinal layers. Secondary to RPE degeneration, rod and cone dysfunction also accounts for central vision loss, a characteristic feature of the pathology.[25] Notably, rods are more severely affected by AMD[26], with significant rates of photoreceptor and RPE apoptosis seen with TUNEL staining (terminal deoxynucleotidyl transferase dUTP nick-end labeling).[27]

Previous studies have demonstrated that injection of the reactive oxidant sodium iodate leads to death of retinal pigment epithelium (RPE) and a loss of vision. The injection of iodate into the circulation leads to rapid apoptosis of underlying photoreceptors, which depend upon the RPE, and loss of vision. Cones are lost preferentially compared the rods, implying that they are more sensitive to RPE loss than rods. This central pattern and time course of sequential RPE and photoreceptor loss following iodate injection was independent of exposure of light. These results then provide evidence that circulating oxidant is sufficient to specifically damage the central retina.[28]

Diabetic Retinopathy

Diabetic retinopathy (DR) is the most common cause of vision loss among working age groups and represents a huge socio-economic burden.[29] The most striking pathological changes of DR are the microvascular complications occurring in the retinal tissue; however, DR also involves an increased rate of apoptosis both in vascular and neuro-retinal cells, as shown by TUNEL assay-based studies.[30] The cell populations mostly involved in this phenomenon are RGCs and amacrine cells.[31]

The neuronal component of the retina is compromised, particularly glial cells are activated, and retinal ganglion cells die early in diabetic retinopathy. Several studies

reported increased levels of neurodegenerative metabolites and altered levels of neurotrophins in the diabetic retina.[32] Glutamate is one of the major excitotoxic metabolites found to be increased in the vitreous and retina of diabetic patients, and in animal models of diabetes which may implicate neuronal damage in the retina. This alteration in glutamate metabolism especially within Muller cells may cause an increase in synaptic glutamate levels in the retina leading to glutamate excitotoxicity and neuronal cell death. As a result of glutamate excitotoxicity, two major events seem to play an important role of death of neurons; the increase in the production of free radicals and the induction of the apoptotic cascade which may trigger pathological mechanisms leading to neuronal death.[32] Gabapentin may influence a decrease in the level of branch chain amino acids in the diabetic retinas, which in turn may lower glutamate synthesis and increase rates of glutamate oxidation. Thus, gabapentin might be a potential therapeutic drug in ameliorating oxidative stress and apoptosis in diabetic retina by inhibiting glutamate excitotoxicity. In this study, two-week oral treatment of gabapentin to diabetic rats was evaluated.[32] They found that, gabapentin also reduced the expression of proapoptotic caspase-3, a marker of apoptosis and increased anti-apoptotic marker Bcl-2 in diabetic retinas. Thus, these results suggest that gabapentin stimulates glutamate disposal and ameliorates apoptosis and oxidative stress in diabetic rat retina.[32]

Brain Disorders and Retina

The degeneration of some retinal cell populations has been associated with brain disorders such as AD and PD.[33] AD is by far the most common form of dementia, accounting for approximately 70% of all cases.[34] The main pathological and diagnostic feature is represented by the deposition of extracellular senile plaques and intracellular neurofibrillary tangles. Nowadays, diagnosis is primarily based on the patient's behavioral and clinical assessment[35], and secondarily, confirmed by either computed tomography or magnetic resonance imaging.[36] In AD, PD, Huntington's disease, and glaucoma there are common elements such as oxidative stress, mitochondrial dysfunction, excitotoxicity, and misfolded protein aggregation. Therefore, there is potential for apoptosis detection as a biomarker of disease diagnosis in all these diseases.[37]

Retinal Endothelial Cell

Retinal endothelial cell (REC) is one of the key cell types to be significantly affected in many ocular diseases. REC compromise the microvascular lining of retinal blood vessels prevalent in the outer plexiform layer and in the ganglion cell layer. Loss of REC presages retinal damage in the pre-proliferative form of diabetic retinopathy, oxygen-induced retinopathy, ischemia-reperfusion injury, as well as many other retinal disease models.[38]

Bovine retinal endothelial cells (BREC) have been widely used for studies of apoptosis in culture. These cells are easily obtained from local slaughterhouses, enabling researchers to isolate many cells from an individual eye. Because BREC form confluent layers in culture they are used commonly for examining blood-barrier function, as they have specific properties that are key for barrier studies.[39] To assist in translation to human disease, primary human retinal endothelial cells (HREC) isolated after medical enucleations or from cadaver eyes have become commercially available. HREC have been used for studies of apoptosis[40], cytokine signaling[41], and insulin-like growth factor pathways.[42] Primary cultures from mouse and rat have also been used successfully in a variety of studies. In addressing the role of REC in retinal disease, a key aspect regardless of the species, is that they are truly microvascular cells, with properties specific to the retina, in contrast to studies utilizing microvascular cells from human umbilical vein or human artery.

Hyperglycemic and Hypoxic Stimulation of REC Apoptosis

Although a variety of stressors have been shown to trigger REC apoptosis, one of the most widely studied is exposure to high glucose. In diabetic animals, exposure of the retinal vasculature to high dose glucose results in loss of REC by apoptosis. In test of the direct effects of hyperglycemia on REC, most studies using primary cultures of HRECs[39, 42], or BREC[43] report an increase in apoptosis when cells are exposed to high glucose. However, there are some studies to the contrary. For example, in primary cultures of HREC isolated from the National Disease Research Interchange, samples in high glucose (20-25 mM) did not increase apoptosis.[41] These conflicting results indicate that the response of REC to high glucose may be complex involving both direct and indirect effects, and therefore are likely to be influenced by differences in species, cell preparation, and culturing conditions.

Production of reactive oxygen species (ROS) either under control or diabetic conditions has been reported to increase REC apoptosis. In streptozotocin induced diabetic mice or in HREC, ROS significantly increased apoptosis.[44] The direct pro-apoptotic effects of ROS have been noted in BREC and the Tr-iBRB rat retinal endothelial cell line.[45] Thus any ROS generated under diabetic conditions might be expected to directly trigger REC apoptosis and/or enhance any direct pro-apoptotic pathways activated directly by hyperglycemia.

Busik et al[41], found that hyperglycemia-induced inflammatory cytokines increased REC apoptosis, more than high glucose alone. Some components of the basement membrane (type IV collagen non-collagenase 1 domain) have also been suggested to inhibit proliferation of mouse retinal endothelial cells and to stimulate apoptosis by decreasing the levels of key anti-apoptotic proteins, Bcl-xL and Bcl-2.[46] Similar to the actions of ROS, ischemia has also been reported to induce apoptosis of retinal endothelial cells.[47]

Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a genetically heterogeneous group of the eye conditions that exhibits similar clinical features. With a few exceptions, apoptosis of photoreceptors and RPE cells is a hallmark of RP and leads to thinning of the fundus. Because of the overall role of p53 in apoptosis, it is reasonable to hypothesize that p53 is involved in the retinal cell death associated with RP. However, results identifying the role of p53 in RP have been mixed.[10] TUNEL staining of retinal sections from *rd* mice with normal p53 expression showed rod photoreceptor and inner nuclear layer apoptosis kinetics similar to that in sections from *rd* mice that were p53 null. Furthermore, cone photoreceptor survival, measured by the total number of peanut agglutinin-positive cells in each eye, was no unaffected by the absence of p53.[1]

Retinoblastoma

Retinoblastoma (Rb) is a childhood cancer in which a malignant tumor develops in retinal progenitor cells. Patients with Rb have mutations in both copies of the RB1 tumor suppressor gene through somatic loss of heterozygosity, leading to the growth of death-resistant tumor cells.[48] p53 and other member of its pathway play critical role in tumor development.

ROP

Inflammation triggers angiogenesis by stimulation of endothelial cells. Various inflammatory mediators such as interleukin-6 (IL-6), c-reactive protein, IL-17, and IL-18 play an important role in the development of ROP and other ONDs. Apigenin has anti-inflammatory and antiangiogenic features. Mediators, such as HIF-1 and VEGF, are related to pathological ocular neovascularization. Apigenin inhibits the hypoxia-mediated upregulation of HIF-1a and VEGF on both transcription and translation levels.[49,50] Antiapoptotic and mitochondrial protective effect of apigenin can be analyzed effectively with Balb/c mice, with their immunocompromised state, which may a properly model to study immunomodulatory effects of apigenin affecting apoptosis and mitochondrial survival.

By using the reproducible model of oxygen-induced retinopathy as published, an investigation of different treatment modalities for retinal vascular diseases is possible.[51,52]

The antioxidative, antitumoral, antiproliferative, and anti-inflammatory effects of apigenin have been well described. Apigenin decreases the expression of hypoxia-inducible factor-1 (HIF-1) and VEGF genes, which are pivotal in the pathogenesis of retinal neovascular diseases.[51,52] Sezenoz et al., found that the Apigenin appears to be a promising candidate as a therapeutic agent in ONDs, given its demonstrated antiangiogenic, antioxidative, and cytoprotective effects on retinal endothelial cells.[52]

Prevention of Apoptosis

Standard treatments such as anti-inflammatory agents, antioxidants, and insulin have been developed and have shown varying degrees of success in preventing REC apoptosis. Based upon expanded understanding of REC apoptotic pathway, a few new pathway-specific treatments have also been investigated. Kowluru et al[43], reported that regulation of matrix metalloproteinase 9 can prevent retinal endothelial cell apoptosis, focusing on changes specific to the mitochondria. Other studies have shown that use of adrenergic receptor agonists can protect REC against apoptosis, likely through a reduction in TNF.[53] Similar findings have been reported for IL-1.[41] Additionally, novel drugs that prevent formation of ROS have been investigated for their ability to prevent retinal endothelial cell death.[44] Some established drugs are also effective in reducing REC apoptosis through reduction of reactive oxygen intermediates.[54]

There is an urgent need for therapies for retinal diseases; retinitis pigmentosa sufferers have no treatment options available and those targeted at other retinopathies have shown limited effectiveness. Th process of programmed cell death or apoptosis although complex, remains a possible target for the treatment of retinal diseases.[55]

Antioxidant scavenging of free radicals can protect the cellular environment simply by reducing oxidative damage to cellular constituents, but another possibility could be prevented loss signaling through PI3K/mTOR. Coupled with the fact that neuro-protective growth factors such as insulin activate signaling through mTOR in the rd1 model; a central role for PI3K/mTOR in mediating survival of retinal cells becomes a possibility. Growth factors as neuroprotectants have been well-studied in recent years, however little progress has been made. Treatments such as antioxidants, growth factors, *Epo* and insulin could converge on a common target, mTOR, modifying levels of autophagy and/or apoptosis.[55] This could help clarify

how different agents which are all proving useful in the prevention of retinal disease could be operating.

CONCLUSION

Several ocular and extraocular neurodegenerative diseases share common feature early and pathological death of retinal cells. This provides a potential early diagnosis window in which to delay and possibly halt pathologic processes before they cause significant harm. Apoptosis detection in retinal cells seems a plausible means to achieve this goal, with different strategies and technologies in the pipeline, with DARC already proven safe in humans. This transition from bench bedside may near future aid diagnosis, prognosis, follow-up, therapeutic tailoring, and drug development in the field of ophthalmology.

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