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Retinal Endothelial Cell Apoptosis

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Abstract

Retinal endothelial cell apoptosis occurs in response to a number of stressors, including high glucose, oxidative stress, hypoxia. Because these stressors are common factors in a number of ocular diseases, it is critical to understand the cellular mechanisms by which apoptosis occurs in retinal endothelial cells. This review discusses the various models of retinal endothelial cells used in ophthalmological research. The mechanisms responsible for retinal endothelial cell apoptosis are discussed, as well as potential therapeutics currently under development to prevent retinal endothelial cell apoptosis. The primary goal of this review is provide the reader with a background knowledge of the current state of research ongoing in retinal endothelial cell apoptosis and potential avenues for future testing.

The retinal endothelial cell (REC) is one of the key cell types to be significantly affected in many ocular diseases. REC comprise 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.

REC CULTURE MODELS

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 a large number of cells from an individual eye. Because BREC form confluent layers in culture they are used commonly for examining blood-retinal barrier function, as they have specific properties that are key for barrier studies [1]. 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 [2], cytokine signaling [3], and insulin-like growth factor pathways [4]. 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 macrovascular 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 glucose results in loss of REC by apoptosis. In tests of the direct effects

of hyperglycemia on REC, most studies using primary cultures of HRECs[2, 5] or BREC[6, 7] report an increase in apoptosis when cells are exposed to high glucose. Similar results were seen in the TR-iBRB2 rat REC cellline [8, 9]. These studies suggest that hyperglycemia affects REC directly by increasing their vulnerability to apoptosis. 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 (20–25mM) did not increase apoptosis [3]. In another study to compare the effects of high glucose on HREC versus human aortic endothelial cells, Duffy et al (2006) found that hyperglycemia actually reduced apoptosis, reporting an increase of 14.9% in cell viability and a decrease in 33% of apoptotic cells [10]. 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. In support of this view, a number of hyperglycemia-induced changes have been shown to induce REC apoptosis.

A by-product of excessive glucose is increased glycation of proteins and the formation of advanced glycation end-products (AGEs). In late stages, these glucose adducts are irreversibly formed and have been suggested to result in the retinal changes noted in diabetic retinopathy [11]. Exposure of BREC to 5uM AGE-bovine serum albumin leads to a significant increase in apoptotic rate [12]. Subsequent studies have identified a specific tripeptide (RGD sequence) within AGE products that is involved in the retinal cell apoptosis [13].

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 [14]. The direct pro-apoptotic effects of ROS have been noted in BREC[15] and the Tr-iBRB rat retinal endothelial cell line[16]. 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.

In addition to high glucose and ROS, several other factors are known to result in retinal endothelial cell apoptosis. In the work mentioned above, Busik et al (2008) found that hyperglycemia-induced inflammatory cytokines increased REC apoptosis, more so than high glucose alone [3]. Some components of the basement membrane (type IV collagen noncollagenase 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 [17]. Similar to the actions of ROS, ischemia has also been reported to induce apoptosis of retinal endothelial cells [18, 19].

Ideally, future studies should include both *in vitro* and *in vivo* preparations so that REC apoptosis can be defined under specific conditions *in vitro* and then translated into the context of the intact tissue. The ultimate goal will be to understand the mechanisms underlying REC apoptosis, especially as applied to our understanding of the full complement of factors that contribute to major REC-related conditions such as diabetic retinopathy. This understanding would lead the way to development of therapeutic options for retinal disease.

APOPTOTIC PATHWAYS

A significant increase in the cleavage of caspase 3 along with DNA fragmentation is consistently observed in response to the majority of factors known to induce apoptosis in REC[8, 14, 15, 18]. Based on these observations, the REC cell death can be definitively documented as apoptosis, distinct from other mechanisms of cell death such as necrosis or

autophagy. A number of different REC pathways may lead to activation of caspase 3-mediated apoptosis, including mitochondrial pathways, Fas- and Fas ligand-dependent pathways, and inflammatory-mediated (TNF, IL-1) pathways. There is evidence for all three of these pathways in REC apoptosis in diabetic retinopathy. A number of studies indicate that mitochondrial changes and mitochondrial damage are key components of diabetic retinopathy, suggesting that modulation of the mitochondrial apoptotic pathways is highly involved. Based on a thorough analysis of the changes in mitochondrial morphology that occurs in response to high glucose, researchers found that mitochondrial apoptotic pathways, including cytochrome c, were significantly increased in REC from rat [20]. In BREC, exposure to high glucose or oxidative stress led to mitochondrial damage, activated mitochondrial apoptotic pathways, and promoted BREC apoptosis [7, 21, 22]. The traditional death pathways involving Fas and Fas ligand have also been reported to be activated by high glucose and to induce REC apoptosis [2]. TNF and IL-1 are also able to activate cell death signaling in HREC [3, 23, 24].

PREVENTION OF APOPTOSIS

The primary purpose of the study of REC apoptosis is to determine the mechanisms involved and suggest treatment strategies to block cell death and protect these important cellular elements of the retinal vasculature. 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 our expanded understanding of REC apoptotic pathway, a number of new pathway-specific treatments have also been investigated. Mohammed and Kowluru (2012) have recently reported that regulation of matrix metalloproteinase 9 can prevent retinal endothelial cell apoptosis, focusing on changes specific to the mitochondria [6]. Other studies have shown that use of β -adrenergic receptor agonists can protect REC against apoptosis, likely through a reduction in TNF [24, 25]. Similar findings have been reported for IL-1 [3]. Additionally, novel drugs that prevent formation of ROS have been investigated for their ability to prevent retinal endothelial cell death [14]. Some established drugs are also effective in reducing REC apoptosis through reduction of reactive oxygen intermediates [26]. Further drug development for treatment of diabetic retinopathy, based on greater understanding of REC-specific pathways, holds promise for tackling retinal disease.

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