REVIEWS

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Caspase-Dependent Apoptosis of Retinal Ganglion Cells During the Development of Diabetic Retinopathy

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Diabetic retinopathy constitutes the most frequent cause of vision loss in professionally active individuals. Progressive impairment of visual acuity results from massive fibrovascular proliferation involving the fundus of the eye, as well as from the apoptosis of the neuronal structures of the retina. The results of many clinical studies, both on experimental models and on human material, confirmed evident enhancement of this process in the course of diabetes. The programmed cell death of retinal ganglion cells predominantly occurs secondarily to caspase-dependent intracellular processes. This paper presents evidence for the considerable involvement of the caspase-dependent mechanism of apoptosis of retinal ganglion cells in the early stages of retinal changes associated with progressive impairment of visual acuity. The authors emphasize the necessity of comprehensive understanding of mechanisms that underlie the programmed death of neural cells in the eyes of patients with diabetes. This clinical problem becomes of vital importance in view of the constantly increasing incidence of diabetes and severe impairment associated with the disorders of carbohydrate metabolism. Identification of a key component involved in this process would enable attempts oriented at pharmacological blockade of apoptosis in the retinal ganglion cells of patients with diabetes (**Adv Clin Exp Med 2015, 24, 3, 531–535**).

Key words: apoptosis, retinal ganglion cells, diabetic retinopathy, caspase.

Civilization progress is associated with unfavorable changes in the lifestyle of the majority of human population. Improper diet, rich in fat and carbohydrates, and simultaneous limitation of physical activity can lead to the development of a severe metabolic disorder, diabetes. According to WHO estimates, the worldwide population of patients with diabetes will reach 20 million by 2030 [1]. Diabetic retinopathy is one of the most frequent complications of diabetes. Apart from widely analyzed microangiopathic disorders, the problem of changes in the neural structure of the eye, i.e. retina, is highlighted with increasing frequency. Retinal ganglion cells are the predominant components that undergo injury [1, 2]. Improper metabolic processes are also observed within amacrine cells, astrocytes, and photoreceptor cells [3, 4]. The disorders associated with diabetes lead to irreversible changes in the abovementioned neural structures of the retina, mostly through a programmed cell death [5]. This is reflected by markedly impaired perception of contrast and color which, together with accompanying microangiopathic complications, leads to progressive deterioration of visual acuity. These changes are irreversible as the neural cells are incapable of proliferation.

Apoptosis Under Conditions of General Homeostasis

Programmed cell death ensures the maintenance of balance between the processes of cellular proliferation and death. Uncontrolled proliferation (e.g. in neoplasms) or loss of cells (e.g. in degenerative changes) can result from mutations or influence of external factors. Biochemical pathways of apoptosis activation can be extra- or intracellular, and caspase-dependent or caspase-independent [6].

Caspases are enzymes belonging to the group of cysteine proteases. These proteins are actively involved in inflammatory processes and apoptosis. Caspase-2, -8, -9, and -10 initiate the programmed cell death. The "executive" caspases include caspase-3, -6, and -7 [7]. The extracellular activation of caspase system usually results from the activation of specific TNF-α receptors, which is reflected by the activation of caspase-8, followed by subsequent activation of caspase-3, which initiates the mechanisms of apoptosis. This results in the condensation of nuclear chromatin and fragmentation of DNA by endonucleases, followed by disintegration of cytoskeleton. Stimulation of the TNF-α receptor and activation of caspase-8 can lead to simultaneous activation of the mitochondrial pathway of cell apoptosis (intracellular programmed cell death). This process is associated with the involvement of pro-apoptotic protein *Bid* (*Bcl-2* protein family), leading to the release of cytochrome c from the mitochondrium [8]. Specific location of this protein within mitochondrial wall enables it to interact with other vital components involved in the activation of programmed cell death. These include *Bax* and *Bak* molecules which also belong to the *Bcl-2* family of pro-apoptotic proteins [9, 10]. Their activation is reflected by increased permeability of mitochondrial wall, loss of membrane potential and the resultant release of *cytochrome c* to cytosol. This process leads to formation of apoptosome, and involves *Apaf-1* (apoptotic protease activating factor) with secondary involvement of caspase-9 [8]. Activation of caspase-3 and the resultant programmed cell death constitute further stages of the process. The intracellular apoptosis can be also initiated without the involvement of *Bad* protein. In this latter case, the cascade of pro-apoptotic processes is initiated by an injury of a cell or severe oxidative stress [3].

Apart from the abovementioned caspase-dependent mechanism of programmed cell death, there is also a possibility of caspase-independent apoptosis. This process occurs secondarily to the increase in the intracellular concentration of calcium. Calpain, stimulating the release of *AIF* from the mitochondrium and caspase-12 from the endoplasmic reticulum, is a key component in this chain of molecular changes [11, 12] (Fig. 1).

Diabetic Retinopathy and Process of Apoptosis

The caspase-dependent intracellular model is a predominant mechanism of apoptosis involved in

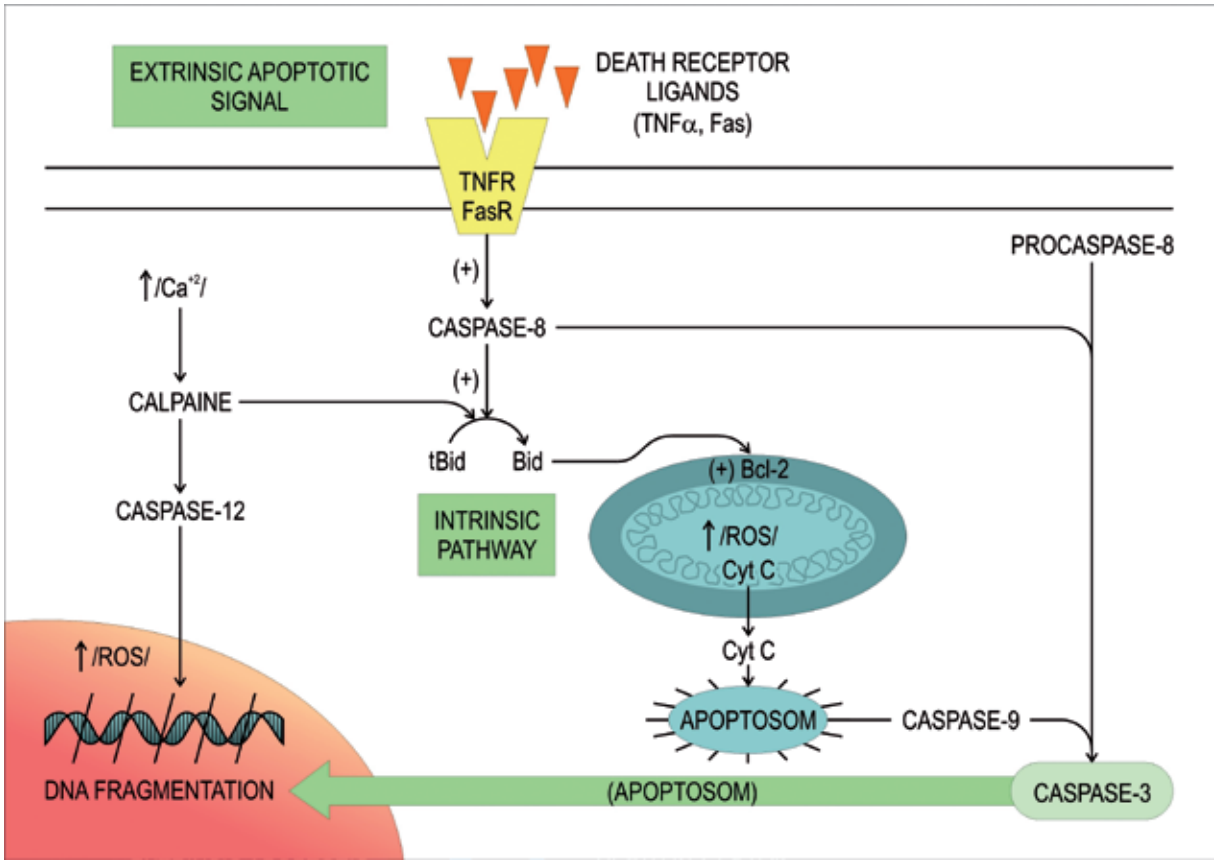

Fig. 1. Caspase-dependent and independent apoptotic pathways. (AIF – apoptosis-inducing factor, ROS – reactive oxygen species)

diabetic retinopathy. Programmed death of human vascular endothelial cells was studied extensively and widely described. In contrast, little is known on the apoptotic changes taking place within the cells forming the neural structures of the retina. As early as in 1960s, Bloodworth et al. [13] suggested that high glycemia exerts toxic effect on the retinal neurons. While functional changes of the retinal ganglion cells were detected as early as after 2 weeks of diabetes, the microaneurysms characteristic for simple retinopathy developed not earlier than after 6 months [1]. Abnormalities documented on ERG were associated with impaired perception of contrast and prolonged dark adaptation time [14, 15]. Immunohistochemical examination of the retina from patients with diabetes revealed the presence of caspase-3 [16], caspase-9 [16], *Bax* [3, 17, 18], *Bad* [17, 18], and *Fas* [3] in retinal ganglion cells (RGCs). Enhanced release of cytochrome c and *AIF* was documented both in RGCs and in photoreceptor cells [3, 19]. Müller cells and astrocytes also seem to undergo activation in the course of diabetes, as confirmed by the activation and expression of factors associated with caspase-dependent mechanism of programmed cell death [20, 21].

Most available data originates from studies of experimental models. A study of rats exposed to streptozotocin (STZ) revealed that the apoptosis-specific changes in retinal ganglion cells were observed as early as after one month of diabetes-specific metabolic disorders [3, 5]. Li et al. [1] confirmed increased concentration of caspase-3 in the retina of rats as early as 2 weeks after the induction of STZ. The highest concentration of this active protein was documented after one month of disorders, and the peak levels of caspase-3 were detected in the ganglion cells, nerve fiber layer, and outer photoreceptor layer [1]. These findings were confirmed during another stage of the study, i.e. intra-vitreal injection of specific inhibitor of caspase-3, DEVD-CHO. The intensity of apoptotic processes in the retinal ganglion cells was significantly reduced as early as 2 weeks after the administration of the active substance [1].

Barber et al. [22] tried to quantify the degree of retinal ganglion cell atrophy. They analyzed paraffin-embedded retinal specimens from STZ-exposed rats, obtained after 30 weeks of experimentally induced diabetes. A 10% reduction in the total number of retinal ganglion cells was documented, along with a 22% decrease in the thickness of the inner ganglionic layer of the retina, and a 14% decrease in the thickness of the inner nuclear layer. Interestingly, no changes in the thickness of the outer ganglion cell layer were documented, which suggests that the processes of apoptosis are more intense within the inner layers of the retina [22].

Also studies based on the TUNEL reaction revealed changes in the inner retinal layers. RGCs and photoreceptor cells were the most commonly injured cellular types, which was reflected by the results of electrophysiological examination (ERG). Noticeably, these changes were not associated with diabetic retinopathy-specific vascular injury [3, 21].

A study of mice with a mutation of insulin encoding gene (so-called *Ins2^{Akita} mouse*) revealed an increase in the concentration of caspase-3 after 4 weeks of diabetes-characteristic disorders [22]. Furthermore, a thinning of the inner retinal layers was observed after 22 weeks of the experiment [3]. Probably the changes documented in this experimental model also resulted from the caspase-dependent process of apoptosis. This is also suggested by OCT findings in patients with type 1 diabetes, in whom the reduced thickness of the inner retinal layers is associated with only minimal vascular lesions [24, 25].

Kowluru et al. [26] confirmed an increase in the cytosol concentration of cytochrome c, occurring secondarily to the activation of caspase system after 8 months of diabetes-specific metabolic disorders in STZ-exposed rats [3, 23]. The release of *cytochrome c* correlated with the transfer of *Bax* into the mitochondrium and initiation of apoptosis. This process can be inhibited *in vitro* through the reduction of superoxide concentration [3].

Apoptosis of Retinal Ganglion Cells in *Postmortem* Examination of Humans

The aforementioned findings from experimental models were confirmed in histological material (retinal specimens) obtained *postmortem* from patients with diabetes [16]. All analyzed specimens were free from PDR-characteristic lesions. Fluoro-Jade B (FJB) was used as a marker of injured retinal ganglion cells. Interestingly, the presence of the active form of caspase-9 and enhanced expression of *Bax* were revealed in the injured areas of ganglion cell layer (GCL), resulting in further increase in the concentration of caspase-3. These findings seem confirmatory to the involvement of *Bax* and caspase-dependent mechanism of apoptosis in the neurodegeneration of the retina in patients with diabetes [16].

Important data from *postmortem* studies was published by Abu El-Asrar et al. [27]. They proved enhanced expression of caspase-3, *Fas*, and *Bax* in the retinal ganglion cells of patients with diabetes. Hyperglycemia induces *de novo* expression

of pro-apoptotic factor *Bad* in GCL. Simultaneously, the expression of factors inhibiting the process of apoptosis, such as *Akt* (protein kinase B), Cox-2 (cyclooxygenase 2), and *Mcl-1*, was documented. *Akt* is a factor with confirmed protective effect on neural cells. This effect results from the influence of *Akt* on the synthesis of substances that regulate the life cycle of the cells [28]. The interaction between PI 3-kinase and *Akt* seems vital for this effect. Orike et al. [29] revealed that proper interaction between PI 3-kinase and *Akt* was a prerequisite of the survival of neural cells during an experimentally-induced deprivation of neurotrophic factors. A vital role of cooperation between these two factors was proved under hyperglycemic conditions: administration of insulin markedly reduced the number of retinal neural cells which underwent apoptosis, and this process was mediated by the activation of the PI 3-kinase/*Akt* system [30]. Furthermore, Abu El-Asrar et al. emphasized the association between the activation of the PI 3-kinase/*Akt* system and the increase in the cytoplasmic concentration of Cox-2 in retinal ganglion cells, and in the cells of retinal pigment epithelium and cilliary body epithelium [28]. Enhanced expression of Cox-2 is usually stimulated by such factors as bacterial lipopolysaccharides, pro-inflammatory cytokines, growth factors, hormones, and neoplastic cells. The effect of this factor is mediated by the synthesis of certain prostaglandins, including enhanced expression of apoptosis inhibitory factor Mcl-1 *via* the activation of PI 3-kinase/*Akt* system. Furthermore, the authors of this study documented the presence of *Bad*, a pro-apoptotic factor released in response to hyperglycemia and oxidative stress. The concentration of *Bad* was highest in retinal ganglion cells, thus confirming the theory on the enhanced expression of this factor in response to diabetes-induced neuronal injury. A similar response was observed in the course of neurotoxic injury of retinal ganglion cells, as well as during the transient retinal hypoxia associated with the occlusion of the central retinal artery [31].

Mitochondria are organelles which play a vital role in the process of apoptosis. Their regulatory function is associated with the ability to release pro-apoptotic factors, including cytochrome c and *AIF*. An increased concentration of cytochrome c and enhanced immunoreactivity of *AIF* were observed in *postmortem* retinal specimens.

Evidence presented in this paper, originating both from the experimental studies and from the examination of human *postmortem* material, unambiguously points to the involvement of apoptosis in the injury of retinal neuronal cells in patients with diabetes. However, the detailed pathogenic mechanism underlying the effects of hyperglycemia and associated oxidative stress remains unexplained. The presence of pro-apoptotic factors in retinal ganglion cells points to the predominant role of these compounds in the diabetic neurodegenerative disorders of the retina. This substantiates further efforts in search for a factor playing crucial role in the process of apoptosis of the retinal neural cells. Identification of such factors would raise a possibility of breaking the cascade of pathological reactions leading to atrophy of the retinal neural tissue.

Loss of vision in the course of diabetes is a growing worldwide problem of severe disability. The hereby presented metabolic pathway responsible for the injury of the organ of vision in the course of this condition undoubtedly constitutes a new target for clinical pharmacotherapy of diabetic retinopathy.

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