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Retinal degeneration, apoptosis and the c-fos gene

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Abstract In the past few years, the interest in the research field of apoptosis in the retina has been growing rapidly. We will give a short overview of apoptosis in the context of retinal degeneration and summarize recent data obtained in our laboratory. Based on our findings, we will also discuss possible future strategies to influence apoptotic cell death in the retina and to modulate the time course of retinal dystrophies. Apoptosis is the final common pathway of photoreceptor cell death in several retinal dystrophies as well as in light-induced photoreceptor degeneration. We investigated potential signal transducers for apoptosis in our laboratory and found an essential role of the immediate-early gene product c-Fos in light-induced photoreceptor degeneration. This is of particular interest in light of the finding that c-fos is continuously upregulated concomitant with apoptotic photoreceptor death in animal models of the retinal dystrophy retinitis pigmentosa. Interference with c-fos expression or function might therefore represent a novel means to influence the time course of retinal dystrophies, which are at present incurable diseases.

Key words Apoptosis; light damage; retinal degeneration; gene expression; transcription factors

Apoptosis: a particular mode of cell death Apoptosis is a regulated mode of single cell death and means that the decision to die is made by the cell itself. This decision is triggered by endogenous and/or exogenous stimuli. Apoptosis occurs under physiological and pathological conditions in a large variety of systems. The execution of the apoptotic death program may be performed by continuously present effector systems for apoptosis or may require de-novo gene expression. Apoptosis plays a physiological role during development, is an important natural defense mechanism against cancer, and is also the mode by which cells die in a variety of degenerative disorders such as Parkinson’s disease, Alzheimer’s disease, retinitis pigmentosa (RP), and light-
induced photoreceptor degeneration.\textsuperscript{3-5} Therefore, this mode of cell death is of outstanding interest to scientists in different research fields.

Apoptosis can clearly be distinguished from necrosis, the latter involving lysis of cells and organelles and collateral tissue responses. Characteristic morphological features of apoptosis are the condensation of chromatin and cytoplasm of individual cells, followed by fragmentation of the cell and phagocytosis of the apoptotic bodies by macrophages or neighboring cells.\textsuperscript{5,7} In addition to light and electron microscopy, apoptosis can be demonstrated by other methods. One of these is the \textit{in-situ} nick end labeling of DNA fragments (TUNEL method; Terminal Transferase - dUTP nick-end labeling),\textsuperscript{8} where cells carrying fragmented DNA are visualized in a histological section. Another important method is the detection of internucleosomal DNA fragmentation. During apoptosis, the DNA forms fragments of oligonucleosomal length (180 base pairs (bp) or a multiple). Visualized by gel electrophoresis, these fragments form the so-called 'ladder'. For confirmation of apoptosis, at least two of these methods should demonstrate the described changes.

A broad spectrum of endogenous and exogenous stimuli and mediators of apoptosis has been identified.\textsuperscript{9,10} Intracellular signaling pathways may include increased calcium levels, protein kinase C-activation, oxidative stress and lipid hydroperoxydes, activation of proteases and endonucleases and, in general, altered gene expression. Several genes appear to be involved in the regulation of cell death by either preventing or promoting the death program, for example, c-myc, glucocorticoid receptor, p53, bcl-2, and \textit{c-fos}.\textsuperscript{2,3,6,11} The expression of other genes, for example stromelysin, ubiquitin, clusterin, and others, coincides with apoptosis, but their contribution to cell death remains to be determined.

A possible approach to influence cell death by apoptosis and thereby to alter the time course of, e.g., degenerative diseases, could be to modulate the expression of regulatory genes and/or to interfere with the function of the respective gene products.

\textbf{Apoptosis in the retina} In the past few years, apoptosis was found to be the mode of photoreceptor cell death under a variety of different conditions such as histogenesis during development,\textsuperscript{12} light-induced photoreceptor degeneration,\textsuperscript{5,13,14} and in animal models of retinitis pigmentosa (RP).\textsuperscript{15,16} Similar to the findings in rodents, apoptosis was also observed in donor eyes from human patients who had suffered from RP.\textsuperscript{17}

Since apoptotic cell death in the retina is under control of as yet largely unknown genes, research has focused on the identification of control and regulatory mechanisms for apoptosis. The idea is to retard or even prevent photoreceptor degeneration by blocking ‘cellular suicide’. In retinal dystrophies like RP, however, only small populations of photoreceptors die at a given time thereby making the study of mechanisms underlying retinal degeneration very difficult. We used our \textit{in-vivo} model system\textsuperscript{18} where apoptosis of photoreceptors and subsequent retinal degeneration is induced by diffuse white fluorescent light in a very synchronized manner.\textsuperscript{13} Using this system in transgenic mice enabled us to specifically investigate the effect of different gene products.
on retinal photoreceptor apoptosis. One potential regulatory gene was the immediate-early gene (IEG) c-fos.

**The immediate-early gene c-fos** The c-fos gene encodes a nuclear phosphoprotein that forms a heterodimeric complex with members of the Jun family of proteins to constitute the transcription factor activator protein 1 (AP-1). Although there is evidence for c-fos being a mediator of apoptosis in some systems, its precise role is unclear. In the retina, the c-fos gene is physiologically expressed in a diurnal manner and is inducible by light pulses.

In a first step, we have investigated whether c-fos is upregulated in our model system of light-induced apoptosis. Following light exposure, we found elevated levels of c-fos expression in retinal photoreceptors prior to apoptotic cell death. To investigate the possible involvement of c-fos in light-induced photoreceptor degeneration, we exposed genetically normal control mice (wild-type) (Fig. 1c,e,g) and mice lacking c-fos (c-fos knockouts) (Fig. 1d,f,h) to bright light and compared them to unexposed littermates of both genotypes (Fig. 1a,b). At 12 and 24 h after light exposure, wild-type mice displayed severe retinal degeneration whereas the retinas of c-fos knockouts showed virtually no damage.

Apart from morphological analysis, we performed TUNEL staining (Fig. 2) to detect DNA strand breaks in photoreceptor nuclei and an
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Both methods confirmed the occurrence of apoptosis in the retinas of wild-type controls but not in c-fos−/− mice. Thus, the c-fos gene is essential for light-induced apoptosis in the mouse retina. In other words, although bright light as the damaging stimulus was present, the photoreceptor cells did not execute the apoptotic program.

**Inherited retinal dystrophy and the c-fos gene** Apoptosis is the mode of cell death common to a variety of different diseases. In the retina, apoptosis occurs in light-induced photoreceptor degeneration4,13 and in animal models of RP, an inherited retinal dystrophy.24,25
The best-known mouse model for RP is the retinal degeneration (rd) mouse.26,27 This animal carries a mutation in the gene coding for the beta-subunit of the cGMP phosphodiesterase, thus rendering this phototransduction-specific enzyme nonfunctional.28 Remarkably, the same defect is also found in human patients suffering from autosomal recessive RP.29

Rendering the c-fos gene in the rd mouse nonfunctional: a new approach to inhibit retinal degeneration

Several lines of evidence led us to study the c-fos gene in the rd mouse. First, as in our model of light-induced degeneration, photoreceptors die by apoptosis.30 Second, the c-fos gene is upregulated prior to apoptotic cell death in the photoreceptors of the rd mouse.31 Therefore, ongoing studies in our lab intend to generate c-fos−/−rd/rd double mutant mice. The aim is to influence the rate of apoptotic cell death and thereby retarding the time course of photoreceptor degeneration in this hereditary retinal dystrophy. Furthermore, the exact role of c-fos in apoptosis of retinal photoreceptors remains to be elucidated: is it linked to an apoptosis-specific pathway or is it involved in the phototransduction system in an as yet unknown manner?

References


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