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CONSTITUTIVE PRO-APOPTOTIC MOLECULES ARE DIFFERENTIALLY EXPRESSED IN THE FETAL AND ADULT MOUSE EYE

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Abstract

Apoptosis is a major mechanism by which cells are dying, during development, other physiological processes, or due to certain types of damage. In this study we compared the gene expression of two groups of pro-apoptotic molecules in the fetal and adult mouse eye. Molecules of one group, that include Bad, Bak and Bax, belong to the Bcl-2 family and are involved in the intrinsic pathway of apoptosis, while molecules of the other group, Fas, FasL and TRAIL, that belong to the TNF family, participate in the extrinsic pathway. Molecules of the latter group contribute to the acquisition of immune privilege status of the eye. Molecule levels were measured by real-time PCR on RNA extracted from fetal and adult eyes. We found that Bad, Bak, and Bax, were more highly expressed in the fetal mouse eye, whereas Fas, FasL, and TRAIL had greater expression in the adult eye. The difference between the expression patterns of these two groups of pro-apoptosis molecules thus suggests that different mechanisms of apoptosis are prominent at the fetal and mature stages of the mouse eye.

Keywords

Apoptosis; intrinsic and extrinsic pathways; mouse eye; development; age

Programmed cell death (apoptosis) serves to eliminate excess, damaged, or infected cells throughout the lifespan of an organism. This is a critical process for development of all metazoans, and its dysregulation can elicit morphogenetic abnormalities and disease (Huang and Strasser, 2000; Ashkenazi and Herbst, 2008). A family of pro-apoptotic proteins has been implicated in the development of the nervous system, including the development of the eye. Removal of damaged cells by apoptotic mechanisms prevents inflammation, a process detrimental to vision and, therefore, apoptosis is essential for the normal eye (Cellerino et al., 2000; Hahn et al., 2004). Our study focused on two groups of pro-apoptotic molecules, belonging to the Bcl-2 and tumor necrosis factor (TNF) families. The Bcl-2 family molecules are involved in the intrinsic pathway of apoptosis, whereas the TNF family proteins participate in the extrinsic pathway.

The Bcl-2 family of proteins includes molecules that share homology across a single or several distinct structural domains (Lindsten et al., 2005). Pro- and anti-apoptotic molecules of this

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family play major roles in the development and maintenance of the nervous system including the eye (Cellerino et al., 2000; Lindsten et al., 2000; Lindsten et al., 2005). The pro-apoptotic molecules under study here include Bak, Bax, and Bad. Bak and Bax are required for mitochondrial release of apoptotic molecules (e.g., cytochrome c), the subsequent activation of the caspase cascade, and cell killing (Green and Reed, 1998; Kaufmann et al., 2007). Bad is a member of the BH3-only proteins and shares sequence with only the BH3 domain of Bcl-2 (Lindsten et al., 2005; Ness et al., 2006). Bad promotes apoptosis by inactivating anti-apoptosis molecules, such as Bcl-2, and amplifying other cell death inducers (Huang and Strasser, 2000).

Molecules of the TNF family tested here included Fas, Fas ligand (FasL), and TRAIL (tumor necrosis factor related apoptosis inducing ligand). When stimulated by unfavorable environmental conditions, or interaction between Fas and FasL on different cells, these groups of molecules activate the caspase cascade that brings about cell death (Griffith et al., 1995; Ferguson et al., 2002; Ashkenazi and Herbst, 2008). These molecules are constitutively expressed on various eye tissues and provide a major mechanism for the establishment and maintenance of the immune privilege of the eye (Griffith et al., 1995; Ferguson and Griffith, 2007).

In the present study we compared these two groups of pro-apoptotic molecules for their expression in fetal and adult mouse eyes.

To obtain eyes of mice at different stages of development, we mated BALB/c mice (Jackson Laboratory, Bar Harbor, ME) and collected eyes at embryonic days E14, E16, E18, post-natal day P1 and at 6–8 weeks of age. All procedures were under protocols approved by the NEI Institutional Animal Care and Use Committee. RNA was extracted from whole eyes as described elsewhere (Takase et al., 2006) and pooled within each group to obtain a sufficient amount, as well as to decrease biological variability. Real-time PCR assays were performed on the RNA samples, using the reagents provided by Applied Biosystems (Foster City, CA) and the procedures detailed elsewhere (Takase et al., 2006).

Real-Time PCR experiments were all carried out a minimum of three times. Samples used in each PCR experiment were tested in triplicate and normalized to β -actin expression. Normalized data were entered into a graphing and data analysis program (Graph Pad Prism Software, San Diego, CA) that includes determination of statistical significance, by a two-tailed, unpaired *t*-test.

Data of a representative experiment are shown in Fig. 1, while data of all repeated experiments are summarized in Fig. 2. The normalized expression level of each transcript in fetal and adult eyes is recorded in Fig. 1 as the “Relative Expression” value. We then calculated the ratios between the values obtained with fetal and adult eyes in all repeated experiments and recorded the mean $\pm$ S.E.M. in Fig. 2.

As seen in the two figures, members of the two families of pro-apoptotic molecules demonstrated different preferential expression in either the fetal eyes or adult eyes. Bcl-2 family molecules, Bad, Bak and Bax, were expressed in fetal eyes more than in adult eyes and the opposite preferential expression was found with the TNF family molecules, Fas, FasL and TRAIL, with higher expression in the adult eyes.

The remarkable difference between the two families of pro-apoptotic molecules is in accord with the known function and mode of action of these molecules. Bcl-2 family molecules are involved in the intrinsic pathway of apoptosis, a pathway that takes place in tissues with a high cell turnover, a critical process in developing fetal tissues. Indeed, data of Hahn et al (2003) showed that Bax and Bak are essential for photoreceptor apoptosis during development. On

the other hand, the TNF family molecules participate in the extrinsic apoptotic process that is activated following exposure to extra-cellular stimuli. This pathway plays a major role in processes that provide the immune privilege status to the eye and studies by Ferguson and coworkers have established the activities of Fas, FasL and TRAIL in these processes (Ferguson et al., 2002; Ferguson and Griffith, 2007). The function of the TNF family proteins in the eye is thus in line with our finding that these molecules are expressed in the adult eye more than in the fetal eye.

It is of note, however, that despite the differences in preferential expression of members of the two families of apoptosis related molecules, all tested transcripts were expressed at relatively high levels in both fetal and adult eyes. This observation is in accord with our previous finding that the expression of pro-apoptotic molecules in normal mouse eyes is higher than that of a large number of other constitutively expressed molecules (Takase et al., 2006).

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ABBREVIATIONS

TNF

tumor necrosis factor

TRAIL

tumor necrosis factor related apoptosis inducing ligand

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Figure 1.

Gene Expression of proapoptotic molecules at the fetal and adult stages of the mouse eye. Levels of RNA extracted from whole eyes were measured by real-time PCR, as described in the text. Data of a representative experiment, recording the transcript expression of the six molecules as mean $\pm$ SEM of three repeated measurements at the fetal and adult ages. Bad, Bak, and Bax are expressed higher at the fetal age, whereas Fas, FasL, and TRAIL show the opposite preferential expression.

Figure 2. The intrinsic and extrinsic proapoptotic groups of molecules are preferentially expressed at different ages. A summary of 3–5 independent experiments, recording the data as the ratio between the mean expression $\pm$ SEM of fetal/adult.