Diabetes Impairs the Neuroprotective Properties of Retinal Alpha-crystallins

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PURPOSE. α-Crystallins are small heat shock proteins that regulate cellular damage and cell survival. Expression of the proteins of the crystallin superfamily in the retina and their role in neuronal cell survival were investigated in two animal models of diabetes and retinal neurons in culture.

METHODS. Crystallin expression was assessed in streptozotocin-induced and Ins2(z/°ζa) diabetic mice using iTRAQ methodology and validated using immunoblotting. Protein-protein interactions, solubility properties, and subcellular localization of αA- and αB-crystallins were further analyzed in vivo and in a retinal neuronal cell model using immunoprecipitation and fractionation methods. Survival of retinal neurons to metabolic stress after overexpression and knock-down of α-crystallins was used to measure their neuroprotective properties.

RESULTS. All 10 of the crystallins identified in retinal lysates from both models of type 1 diabetes were strongly upregulated, coinciding with increased retinal cell death and expression of proapoptotic proteins Bax and Bcl-Xs. Diabetes strongly reduces the chaperone function of α-crystallins by reducing their solubility and disrupting the normal interaction of αA-crystallins with Bax. The same properties disrupted by diabetes were confirmed to be critical for the neuroprotective effect of the overexpression of α-crystallins in retinal neurons in culture.

CONCLUSIONS. Both chemically and genetically induced diabetic models are characterized by upregulation of α, β, and γ-crystallins in the retina. Despite being overexpressed, the molecular properties of α-crystallins are disrupted by diabetes and contribute to the loss of neuroprotective function. Identification and prevention of these alterations could lead to the emergence of new therapies for diabetic retinopathy.

Retinopathy is one of the major complications related to diabetes and develops to varying degrees in nearly all patients. Numerous studies have shown that many retinal cell types are affected by diabetes, including neurons and glia, but the pathophysiologic mechanisms that underlie this neurodegenerative process remain to be elucidated. Diabetic retinopathy includes microvascular, neuronal, glial, and microglial cell defects early in the course of the disease, before clinically visible vascular lesions. One of the major changes in the paradigm of the physiopathology of the disease has been in regard with the increased photoreceptor, inner nuclear layer, and ganglion cell death which starts within 2 to 4 weeks after the onset of diabetes in the streptozotocin (STZ)-induced diabetic rat and mouse models. We also recently showed that increased neuronal cell death correlates with a strong upregulation of several members of the crystallin family of proteins in the same model of diabetes.

Crystallins, the major water-soluble proteins of the lens, are also expressed in numerous nontelocellular types and tissues, including the brain and the retina. Three major classes of proteins make up the crystallins: α-, β-, and γ-crystallins. β-crystallins (βA1/βA3, βA2, βA4, βB1, βB2, and βB3) and γ-crystallins (γA-F and γS) are related to microbial proteins induced by physiologic stress, but their exact function, especially in the retina, remain unclear. The two α-crystallins, αA and αB, belong to the small heat shock protein family of molecular chaperones and participate in the regulation of apoptosis. The expression of αA- and αB-crystallins increases after retinal insult, such as light toxicity and retinal trauma, and coincide with increased levels of Bax, caspase-3, and other proteins involved in apoptosis and neurodegeneration.

α-crystallin can inhibit stress-induced apoptosis by interacting with proapoptotic members of the Bcl-2 family, Bax and Bcl-Xs, sequestering them in the cytoplasm to prevent them from altering mitochondrial integrity. Overexpression of αA-crystallin in retina during experimental uveitis has a neuroprotective effect on photoreceptor cells, mediated by the interaction of αA-crystallin with cytochrome c and procaspase-3, thereby preventing the activation of the latter. αB-crystallin can confer stress-induced apoptosis resistance to several retinal cell types, including retinal pigment epithelium, pericytes, endothelial cells, and astrocytes.

Crystallins are upregulated in rodent models of type 1 and type 2 diabetes, and this increased expression has been proposed to be an adaptive mechanism to protect retinal neurons from metabolic stresses. However, in the diabetic rodent retina, cells continue to die despite elevated crystallin expression. Therefore, this study was conducted to test the hypothesis that α-crystallin functions are impaired by diabetes, thereby preventing normal neuroprotective function. Here, we show that overexpression of α-crystallins is neuroprotective in retinal neurons in culture by binding Bax proteins and downregulating caspase activity. We also show in our two mouse models of type 1 diabetes that diabetes is characterized by increased levels of α, β, and γ-crystallin proteins in the retina. Despite their overexpression, α-crystallin function is reduced in diabetes as shown by the loss of solubility associated with a reduced interaction with the proapoptotic protein Bax. The impairment of the chaperone activity of αA- and αB-crystallins and the subsequent increased retinal neuron cell death points to a novel mechanism by which diabetes disrupts the physiologic adaptive response of retinal crystallins and leads to progressive neuronal cell death.
**METHODS**

**Induction of Diabetes**

Mice were housed under a 12-hour light/dark cycle with free access to standard mouse chow and water. All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Care and Use of Laboratory Animals. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ; 50 mg/kg; Sigma Chemical, St. Louis, MO) dissolved in sodium citrate buffer, pH 4.5 on five successive days while age-matched control male C57BL/6 mice (Charles River Laboratories, Wilmington, MA) received equivalent volumes of buffer alone.

C57BL/6J Ins2Akita heterozygote mice (Jackson Laboratory, Bar Harbor, ME) were housed under the same conditions described for the STZ-injected mice. Only males were used in the studies, because disease progression in females is slower and less uniform. In all experiments, the control groups were made up of siblings homozygous for the wild-type Ins2 gene.

In both models, mice were considered diabetic when exhibiting blood glucose levels >13.9 mM (250 mg/dL; One-Touch Meter; LifeScan, Milpitas, CA). The 12-week diabetes duration was chosen because of the previously reported increased neuronal cell death, microvascular leakage, astrocyte defects, microglial cell activation, and impaired insulin receptor signaling in rat models of type 1 diabetes5,18-21 and Ins2Akita mice.22

**Isobaric Tags for Relative and Absolute Quantitation Analysis**

8-Plex isobaric tags for relative and absolute quantitation (iTRAQ) was conducted on 100 μg of retinal lysate as previously described.6 MS Spectra were acquired and protein identification and quantification were performed using the Paragon algorithm as implemented in Protein Pilot 2.0 software (ABI/MDS-Sciex; Carlsbad, CA) with database searches carried out against the Mouse proteome subset of the National Center for Biotechnology Information database.

**Immunoblot Analysis**

Retinas were homogenized by sonication in immunoprecipitation (IP) buffer as previously described.23 Protein concentrations were measured with the BCA (bicinchoninic acid) protein assay (Thermo Fisher, Rockford, IL), and all samples were adjusted for equal protein concentration. Serum deprivation of R28 retinal neuronal cells was used as a model of retinal cell death.24-26 These cells were grown in DMEM containing 5 mM glucose supplemented with 10% fetal bovine serum (FBS; Flow Laboratories, Rockville, MD) and differentiated to neurons on laminin-coated plates with addition of cell-permeable cAMP (Sigma) as described previously.27 Cells were seeded at 1 × 10^4/well in 96-well plates 24 hours before transfection or treatments. For shRNA studies, cells were seeded at 1 × 10^5/well 24 hours before a first round of transfections. After being grown to confluency for another 24 hours posttransfection, the cells were reseeded into a new laminin-coated 96-well plate and transfected following the same protocol. Cells were then placed in serum-free DMEM for 3 hours before analysis. Cells were transfected 24 hours after seeding using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Briefly, 50 μL of the mix containing transfection reagent and plasmid DNA (0.2 μg) or smart pools of shRNA specific plasmids (0.1 μg; SABiosciences, Frederick, MD) was transferred into each well of the 96-well plate. Each well contained 100 μL of fresh medium. Media were replaced 8 hours after transfection. This method routinely

**Table 1. α, β, and γ-Crystallin Proteins Are Highly Upregulated in the Retina from Two Mouse Models of Diabetes**

<table>
<thead>
<tr>
<th>Ins2Akita Mice</th>
<th>STZ Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>Diabetic</strong></td>
</tr>
<tr>
<td>αA-crystallin</td>
<td>1.059 ± 0.041</td>
</tr>
<tr>
<td>βB-crystallin</td>
<td>1.008 ± 0.043</td>
</tr>
<tr>
<td>βA1-crystallin</td>
<td>1.153 ± 0.079</td>
</tr>
<tr>
<td>βA2-crystallin</td>
<td>0.858 ± 0.049</td>
</tr>
<tr>
<td>βB1-crystallin</td>
<td>1.297 ± 0.177</td>
</tr>
<tr>
<td>βB2-crystallin</td>
<td>1.048 ± 0.031</td>
</tr>
<tr>
<td>βB3-crystallin</td>
<td>0.977 ± 0.037</td>
</tr>
<tr>
<td>βA4-crystallin</td>
<td>0.677 ± 0.110</td>
</tr>
<tr>
<td>γB-crystallin</td>
<td>1.002 ± 0.122</td>
</tr>
<tr>
<td>γ-crytallin</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Crystallin proteins identified by MS using the iTRAQ method in Ins2Akita and STZ-induced diabetic mice 12 weeks after the onset of diabetes, and their relative changes compared to nondiabetic littermates. STZ, streptozotocin.
yields 70% to 80% transfection efficiency in R28 cells. Cells were grown for 72 hours incubated for another 48 hours for apoptosis studies.

Cell Death Assays

Apoptosis was measured by Cell DNA Fragmentation ELISA (Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s instructions. Briefly, after homogenizing the retinal tissue or cells in lysis buffer, samples were incubated and spun. Twenty μL of the supernatant, as well as of the positive and negative controls, were then transferred into the ELISA plate along with the immunoreagent complex. After incubation and washes, the colorimetric solution was added and incubated until the colorimetric reaction developed. After adding the stop solution, the colorimetric signal was measured with a fluorescence plate reader (SpectraMax Gemini EM; Molecular Devices, Sunnyvale, CA) with excitation at 405 and 490 nm.

Caspase-3/7 activity was measured using the ApoONE Assay (Promega, Madison, WI) as described previously.27

Statistical Analysis

For all immunoblot experiments, the data were normalized to the actin signal as control before analysis. ANOVA models with heterogeneous variances, adjusted for the replication of the experiment, were fit to the data to assess differences between diabetic and control mice. The mean ± SEM and statistically significant differences are reported. Analyses were performed using nonrepeated measures ANOVA followed by the Student-Newman-Keuls test for multiple comparisons or t-test for a single comparison.

RESULTS

Retinal Crystallin Upregulation is A Hallmark of the Retina in Diabetes

The iTRAQ method was used as an unbiased approach to study changes in the retinal proteome in two mouse models of type 1 diabetes. Results from the iTRAQ study were validated and confirmed by Western blot analysis using pan-specific antibodies raised against α- (A), β- (B) and γ-crystallin proteins (C), as well as specific antibodies raised against αA- (D) and αB-crystallin proteins (E) in STZ mice retina samples (black bars) and their nondiabetic littermates (open bars). Representative immunoblots and graphic representation of the corresponding quantification of the major bands are presented. Crystallin expression is presented normalized to actin levels and relative to the expression in the littermate controls (100%; n = 8/group). This analysis shows that α-, β-, and γ-crystallins are highly upregulated in the retina from diabetic STZ mice when compared to their littermate controls. C, Control; D, diabetic; 1–3, major bands labeled according to decreasing molecular weight. *Significantly different from control ([P < 0.05]).
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Diabetes specifically upregulates crystallins without general upregulation of heat shock proteins. Immunoblot analysis of the expression of Hsp90 and Hsp25 in STZ mice retina samples and their nondiabetic littermates is shown. Representative immunoblots and graphic representation of the corresponding quantification of Hsp90 (A) and Hsp25 (B) expression is presented normalized to actin levels and relative to the expression in the littermate controls (100%; n = 8/group; *significantly different from control (P < 0.05).

**Figure 2.** Diabetes specifically upregulates crystallins without general upregulation of heat shock proteins. Immunoblot analysis of the expression of Hsp90 and Hsp25 in STZ mice retina samples and their nondiabetic littermates is shown. Representative immunoblots and graphic representation of the corresponding quantification of Hsp90 (A) and Hsp25 (B) expression is presented normalized to actin levels and relative to the expression in the littermate controls (100%; n = 8/group; *significantly different from control (P < 0.05).

Diabetes Impairs Retinal Alpha-crystallin Interactions with Proapoptotic Proteins

Cell survival is controlled in part by the equilibrium between pro- and antiapoptotic factors, such as proteins of the Bcl-2 family. Different members of this family of proteins have been shown to potentially interact with α-crystallin in different stress conditions. Immunoblot analysis revealed that the expression of two proapoptotic members, Bax and Bcl-Xs, were significantly increased in diabetic retinas, but two antiapoptotic proteins, Bcl-2 and Bcl-XL, were not changed (Figs. 3B-F). When activated, Bax undergo conformational changes and is translocated to the mitochondria. Specific analysis of the activated form of Bax also revealed a large increase in retinal lysates from diabetic animals (Fig. 3D). Similar perturbations of the equilibrium between pro- and antiapoptotic members of the Bcl-2 family are known to result in increased cell death.

Bax and α-crystallin are binding partners, and to test whether diabetes affects their association we subjected Bax and α-crystallin immune complexes to immunoblot analysis using anti-αA- and αB-crystallin antibody. αA- and αB-crystallin strongly interact with Bax in the cytoplasm in control retinal tissue (Fig. 3F, C lanes). Despite the increased amount of all three proteins, this interaction was dramatically reduced in diabetic tissues, suggesting α-crystallins have lost effectiveness as chaperones to sequester Bax and protect against cell death (Fig. 3F, D lanes).

Diabetes Alters the Solubility of Retinal Crystallin Proteins

Solubility and subcellular localization, two factors known to be related to α-crystallin chaperone activity, were assessed using buffers differentiating between triton-X soluble and insoluble fractions. Whereas α-crystallins were detected almost exclusively
in the soluble fraction in control retinal tissues, >40% of their content was trapped in the nonsoluble fraction in diabetic retinal tissues (Fig. 4). Reduced crystallin solubility is associated with mutations and posttranslational modifications in the lens and leads to loss of chaperone function and cataract formation. Because crystallin interactions with Bax are lost during diabetes, and protein–protein interaction reflects and controls subcellular localization, the subcellular localization of the different proteins was analyzed. Bax and αA-crystallin, but not αB-crystallin, were partially translocated to the mitochondria in retinas from diabetic mice (Fig. 4). These data reveal profound alterations in crystallin protein intracellular localization and solubility along with the dramatic upregulation of expression. They strongly suggest that the functional capacity of crystallins to compensate for persistent metabolic stress and prevent cell death decreases in early diabetic retinopathy. Metabolic Stress-induced Retinal Neuron Cell Death is Downregulated by α-crystallin Overexpression through Interaction with Bax

The regulation and potential role of α-crystallins in retinal neurons during diabetes were investigated using R28 cells, E1A-transformed retinal neuronal cells that have been shown to differentiate into neurons when cultured on a laminin matrix with cyclic AMP. Basal levels of expression of the different crystallin proteins in retinal neurons were assessed by immunoblot analysis. The analysis shows that proteins of α-, β-, and γ-crystallin families were expressed at low basal levels in retinal neurons (Fig. 5A). The neuroprotective potential of αA-crystallin proteins in retinal neurons was tested by overexpressing αA- or αB-crystallin proteins. Retinal neurons were transfected with plasmids overexpressing native or GFP-tagged forms of αA- or αB-crystallin proteins. Transfection of R28 cells with either one of those plasmids led to a threefold or greater increase in α-crystallin expression (Fig. 5B).

Diabetic retinopathy is caused by a combination of cellular insults including metabolic stress, growth factor deprivation, and increased inflammatory cytokines. It was previously shown that metabolic stresses, such as serum deprivation but not hyperglycemia, can rapidly induce cell death of retinal neurons, and that increasing levels of inflammatory cytokines potentiate this effect. Because TNFα levels increase in human and rodent models of diabetic retinopathy, we also exposed R28 cells to TNF α (10 ng/mL) to partially mimic the metabolic stress conditions seen in diabetes.

**FIGURE 3.** Diabetes increases retinal cell death and expression of proapoptotic members of the Bcl-2 family while decreasing their association with α-crystallin proteins. DNA fragmentation assay results are shown comparing retinal cell death of controls and STZ mice (A). Immunoblot analysis of the expression of proteins of the Bcl-2 family in STZ mice retina samples and their nondiabetic littermates is shown. Representative immunoblots and graphic representation of the corresponding quantification of anti- and proapoptotic members of the Bcl-2 family. Bcl-2 (B), Bax (C), activated Bax (D), Bcl-XI (E), and Bcl-Xs (F) expression is presented normalized to actin levels and relative to the expression in the littermate controls (100%; n = 8/group; *significantly different from control (P < 0.05). Immunoblot analysis of Bax and α-crystallin association in retinal tissue from diabetic and littermate control mice (G). While αA- and αB-crystallin levels in retinal lysates were confirmed to be highly increased by diabetes, their interaction with Bax were strongly reduced in the retinal lysates from diabetic mice (G). C, Control; D, diabetic; IB, immunoblotting antibody; Ip, immunoprecipitation; Ip (-), no antibody control immunoprecipitation.)
forms of experiments were performed with GFP-tagged and nontagged ways. To control for a potential interference of the GFP-tag, suggesting that the protective mechanism used similar path-

5E). The sole knockdown of A/H9251 serum starvation, R28 cells with knockdown of A/H9251 were observed from overexpressing both A- and H9251-crystallin and demonstrated a similar neuroprotec-

FIGURE 4. Both A-crystallins are highly nonsoluble in retinal lysates from diabetic mice, while only A-crystallin translocate to the mitochondria. Triton X-100 (Tx) solubility assay (left panel) and mitochondrial translocation analysis (right panel) of control (C) and diabetic (D) mice retina samples were performed. All four fractions obtained were analyzed for the expression and subcellular localization of A-crystallin proteins, Bcl-2, and Bax. Representative immunoblots (A) and corresponding quantification (B) are presented normalized to actin levels and relative to the expression in the littermate controls (100%; C = 8/group). *Significantly different from control (P < 0.05). While Bcl-2 and Bax were nondetectable (ND) in the nonsoluble fractions, A- and B-crystallin were both found to be highly concentrated in the nonsoluble fraction from diabetic retinal lysates. Interestingly, diabetes also clearly induced the translo-
cation of a fraction of A-crystallin but not B-crystallin to the mitochondria. Loading and specificity were controlled using markers of the cytosol (γ-tubulin) or mitochondria (Cox IV) and actin, respectively (n = 8/group).

in vivo environment of human diabetic retinopathy. This model, while not perfectly mimicking the diabetic milieu, was chosen to study the neuroprotective potential of A-crystallins in retinal neurons. As previously shown, caspase-3/7 activity and DNA fragmentation levels increased more than twofold in cells exposed to 3 hours of serum starvation and more than fivefold when combined with high levels of TNFα. Overexpression of A- or B-crystallin significantly prevented the increase of both the DNA fragmentation and the caspase activity by 31% and 38% respectively, demonstrating the neuroprotective role of A- and B-crystallin proteins (Fig. 5C) and that this effect is caspase dependent (Fig. 5D). Interestingly, no additive effect was observed from overexpressing both A- and B-crystallin, suggesting that the protective mechanism used similar path-

ways. To control for a potential interference of the GFP-tag, experiments were performed with GFP-tagged and nontagged forms of A-crystallin and demonstrated a similar neuroprotec-
tive potential (data not shown).

Because retinal neurons express basal levels of A-crystallins, we also tested their potential neuroprotective function by knocking down their expression using smart pools of shRNA-
specific expressing plasmids. This method regularly reduced A- or B-crystallin expression by respectively 50 and 80% (Fig. 5E). The sole knockdown of A-crystallin resulted in a 30% increase in DNA fragmentation (Fig. 5F). These data demonstrate that A-crystallins play a role in basal mechanisms necessary for retinal cell survival. To test whether A-crystallin could protect retinal neurons by blocking pro-apoptotic proteins, co-immunoprecipitation experiments were performed on lysate from R28 cells overexpressing A-crystallins and subjected or not to metabolic stress. We first showed that similar levels of expression of GFP-tagged A- and B-crystallin proteins were achieved in both conditions (Fig. 6A). Further analysis of Bax immunocomplexes revealed that A-crystallins and Bax are associated in control condition and that this inter-
action was increased by metabolic stress (Fig. 6B). These data, in correlation with the increased cell survival of retinal neurons when exposed to serum starvation, suggest that A-crystallin overexpression can protect retinal neurons through interaction with the proapoptotic protein Bax.

DISCUSSION

In this study of the retinal proteome of two different murine models of type 1 diabetes, we showed the conserved upregu-
lation of multiple members of α-, β-, and γ-crystallin proteins in the retina during diabetes. We also showed that the increased levels of A- and B-crystallins correlate with increased content in the proapoptotic proteins Bax and Bcl-Xs, but reduced interactions of these proteins with crystallins. Furthermore, we showed that both A- and B-crystallins protect retinal neu-

rons from metabolic stress by sequestering proapoptotic pro-
teins, such as Bax. We also showed that the neuroprotective function of α-crystallins is impaired by diabetes through decreased protein solubility and destabilization of Bax/α-crystallin complexes, leading to caspase activation and subsequent apoptosis. Together, these findings provide new insights regarding the mechanism by which retinal neuron cell survival processes are dysregulated by diabetes.

We and others previously demonstrated that α-, β-, and γ-crystallins were dramatically upregulated in different rat models of type 1 and type 2 diabetes.3-8 Our study shows that the crystallin proteins upregulation is not only conserved across type 1 and type 2 diabetes but also across species and models of diabetes. Crystallins are indeed upregulated in the drug-induced (STZ) mouse, as well as in the genetic Ins2Akita model of type 1 diabetes.

Study of the STZ-induced diabetic rat model previously revealed the increased rate of neuronal cell death in the retina during diabetes.3-4 We showed in this study that STZ mice, like the Ins2Akita mice,22 demonstrate increased neuronal cell death after 3 months of diabetes. As shown by studies in neuronal cell cultures, increased expression of functional α-crystallins protects retinal neurons from metabolic stress-induced apoptosis but does not prevent it totally. These results mirror what is observed in diabetes, in which α-crystallin expression is increased as part of the adaptive response but does not totally prevent diabetes-induced neuronal cell death. Because of the transient nature of the stress studied in the cell culture model, it is possible that increased α-crystallin can rescue neurons that are exposed to short or limited stress, but not chronic stress. It is also possible that the α-crystallin neuroprotective function is partially impaired either in general or in specific cell types during diabetes. The pattern of migration identified previously in the rat model and confirmed in this study by Western blot analysis strongly suggests that α-crystallin proteins are posttranslationally modified, which has been shown to affect crystallin function.31,39 Crystallins have been long shown to be targeted by multiple types of posttranslational modifications, will lead to a better understanding of their function.31,39 Crystallins have been long shown to be targeted by multiple types of posttranslational modifications, which has been shown to affect crystallin function.31,39 Characterization of the change in patterns of glycation and phosphorylation, as well as other types of posttranslational modifications, will lead to a better understanding of their function in diabetes.

We showed that both αA- and αB-crystallin could protect retinal neurons from stress-induced cell death (Fig. 5) but, interestingly, overexpression of both did not have an additive effect. This result suggests that both proteins are neuroprotective using similar or, as suggested by Liu et al.,43 parallel pathways. Despite the fact that both proteins had an antiapoptotic effect against ultraviolet A light-induced cell death, this study has shown that the prosurvival pathways activated were different. While αA-crystallins activate the Akt pathway, the...
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A

GFP-αA-crystallin
GFP-αB-crystallin
αA-crystallin
αB-crystallin
Bax
actin

0% FBS
10% FBS
No FBS

IB: αB-crystallin

GFP- αB-crystallin antibody heavy chain
antibody light chain

IB: Bax

0% FBS
10% FBS
No FBS

Figure 6. The neuroprotective effect of αA- and αB-crystallin overexpression in retinal neurons in culture correlates with conserved interaction with the proapoptotic protein Bax. Representative immunoblot of Bax, endogenous, and GFP-tagged αA and αB-crystallin expression levels in R28 cells in culture under normal or serum depleted condition are presented (A). Representative immunoblot of αB-crystallin performed on samples resulting from coimmunoprecipitation of Bax and αB-crystallin proteins from R28 cells cultured in normal or serum depleted conditions (B). IB, Immunoblotting antibody; Ip, immunoprecipitation. Results are representative of triplicates in three independent experiments.

αB-crystallin protective function was related to the inhibition of the Raf/Mek/Erk pathway.

Both αA- and αB-crystallins interact with Bax in retinal tissue from nondiabetic animals. However, while all three proteins are overexpressed in diabetic conditions, the interaction between α-crystallins remains while their interaction with Bax is lost. We confirmed that α-crystallins and Bax can interact in retinal neurons and that the neuroprotective effect of α-crystallin overexpression in retinal neurons in culture was associated with an increased interaction with Bax. This result, along with the increased levels of Bax in the cytosolic fraction during diabetes, strongly suggests that α-crystallin upregulation can protect retinal neurons from cell death through the inhibition of Bax and that this function is partially impaired during diabetes, leading to increased cell death despite increased α-crystallin levels. These results corroborate those of Kim et al., who recently showed that α-crystallin could downregulate apoptosis in a model of type 2 diabetes by a similar mechanism.

Crystallins proteins in general—and αA- and αB-crystallin especially, by their role in controlling apoptosis and different survival pathways—are critical potential targets for the development of new therapeutic approaches to treat diabetic retinopathy. While this manuscript focuses on the role of crystallin in neurons, additional studies are necessary to further characterize the role of this family of proteins in the other retinal cells. Aside from their neuroprotective function demonstrated in this article, recent studies showed that α- and β-crystallins could be playing crucial roles in other aspects of retinal physiology and pathophysiology involving glial and vascular cells. The conservation of the upregulation of crystallins between species and models strongly suggests that understanding crystallin function in the retina is critical to the development of new treatments. Understanding the regulation of crystallin expression, as well as its functions, possibly through posttranslational modifications, could also lead to the identification of new targets for drug and therapy development, not only for diabetic retinopathy, but also for numerous retinal or cerebral neurodegenerative diseases.

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References


