AAV-Mediated Gene Transfer of Human X-Linked Inhibitor of Apoptosis Protects against Oxidative Cell Death in Human RPE Cells

Haidong Shan,1 Dan Ji,1 Alun R. Barnard,1 Daniel M. Lipinski,1 Qisheng You,1 Edward J. Lee,2 Tengku Ain Kamalden,1 Xinghuai Sun,5 and Robert E. MacLaren1,2

PURPOSE. To determine whether human X-linked inhibitor of apoptosis (XIAP) enhances the survival of cultured human retinal pigment epithelial cells exposed to H2O2.

METHODS. ARPE-19 cells were exposed to H2O2 to induce oxidative cell death. Intracellular reactive oxygen species (ROS) were measured using 2',7'-dichlorofluorescein diacetate. MTT assay was performed to quantify mitochondrial stress. Cell apoptosis was determined by TUNEL assay. Human XIAP was delivered with bicistronic expression of green fluorescent protein (GFP), using recombinant adenovirus vector (AAV-XIAP-GFP). The null vector, containing identical sequences but without XIAP, was used as a control (AAV-NULL-GFP). Transduced cells underwent fluorescence-activated cell sorting. XIAP overexpression was examined by immunostaining and Western blot analysis.

RESULTS. ARPE-19 cells exposed to 0.25 mM H2O2 for 1 hour showed increased TUNEL staining compared with non-stressed cells (17 ± 1.4 vs. 1.8 ± 0.4 cells per 20 × field; P = 0.000006), accompanied by a significant increase in intracellular ROS (207 ± 46% vs. 100 ± 9.5%; P = 0.0002). The AAV-XIAP-GFP transduced cells had 11-fold higher XIAP expression than the AAV-NULL-GFP controls (1300 ± 126% vs. 120 ± 10%; P = 0.0006). XIAP overexpression significantly reduced the number of apoptotic cells after stress compared with the AAV-NULL-GFP controls (3.2 ± 0.6 vs. 18 ± 1.6 cells per 20 × field; P = 0.00003). Mitochondrial stress was reduced by AAV-XIAP-GFP, but did not reach a statistical significance (68 ± 3.5% vs. 74 ± 3.8%; P = 0.24).

CONCLUSIONS. Overexpression of human XIAP protects ARPE-19 cells against H2O2-induced oxidative cell death by acting downstream on the apoptotic pathway. XIAP gene therapy using AAV may provide a means of reducing the effect of oxidative stress to RPE cells in age-related macular degeneration. (Invest Ophthalmol Vis Sci. 2011;52:9591-9597) DOI:10.1167/iovs.10-6850

A ge-related macular degeneration (AMD) is a major cause of sight loss in older adults in developed and developing countries. It can be classified into two types, either as exudative pathology characterized by choroidal neovascularization, or geographic macular atrophy; both result in reduced central vision.1 Oxidative stress is thought to play a critical role in the pathogenesis of AMD. Retinal pigment epithelial (RPE) cells may be particularly susceptible due to accumulation of lipid molecules secondary to the phagocytosis of photoreceptor outer segments, high oxygen tension, light illumination, and other types of stress such as complement activation, all of which generate reactive oxygen species (ROS).2 On the other hand, antioxidative enzymes in RPE cells decrease with age, potentially allowing ROS to cause mitochondrial DNA damage and ultimately leading to the apoptosis of RPE cells.2 Therefore, an approach to delay oxidative RPE cell death could be helpful for preventing the progression of AMD. Indeed, large-scale randomized controlled clinical trials have already been set up to investigate the role of antioxidants in reducing the risk of AMD progression and the associated vision loss.3

Apoptosis is the process of programmed cell death that may occur under oxidative stress.4 X-linked inhibitor of apoptosis (XIAP) belongs to the family of inhibitor of apoptosis proteins. XIAP can block cell death by interfering with the activity of caspase-3, 7, and 9 which play essential roles in caspase-dependent apoptosis.5 Studies in vivo have shown that XIAP can preserve photoreceptors, retinal ganglion cells, and optic nerve axons in animal models of retinal detachment,6 glaucoma,7 optic nerve trauma,8 retinitis pigmentosa,9 and N-methyl-N-nitrosourea-induced retinal degeneration.10 Despite these promising results, however, there are few data on the role of XIAP in neuroprotection of the RPE undergoing oxidative stress, which arguably would be highly relevant to a potential treatment strategy for AMD.

In the present study, we therefore developed an assay of oxidative stress-driven apoptosis in a human RPE cell line and used it to examine the neuroprotective effects of XIAP delivered by adenovirus-associated viral vector (AAV). We provide novel data showing that XIAP overexpression can protect human RPE cells against H2O2-induced oxidative cell death in vitro.

From the 1Nuffield Laboratory of Ophthalmology, University of Oxford and Oxford University Hospitals NIHR Biomedical Research Centre, Oxford, United Kingdom; 2Moorfields Eye Hospital-UCL Institute of Ophthalmology NIHR Biomedical Research Centre, London, United Kingdom; and 3Eye and ENT Hospital of Fudan University, Shanghai, China.

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Corresponding author: Robert E. MacLaren, Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford OX3 9DU, UK; enquiries@eye.ox.ac.uk
**METHODS**

**Cell Culture**

The human RPE-derived cell line ARPE-19 was maintained in Dulbecco's modified eagles minimum nutrient mixture F12 Ham (DMEM:F12; Sigma-Aldrich, Dorset, UK) containing 1% fetal bovine serum, 1% L-glutamate, 100 U/ml penicillin G potassium, and 0.1 mg/ml streptomycin sulfate in 175 culture flasks in humidified atmosphere of 5% CO₂ at 37°C. Differentiated cells were obtained within 4 to 6 weeks after confluence, exhibiting typically morphologic and biochemical markers of RPE cells in vivo such as hexagonal packing of pigmented, polarized epithelia, expression of CRALBP and RPE65.

**Vectors and Transduction**

A plasmid encoding the human XIAP coding sequence was commercially purchased (Origene, Rockville, MD) and packaged into a serotype 2 AAV vector. This plasmid contained the chicken beta actin promoter and the coding sequence for green fluorescent protein (GFP) fused downstream to XIAP, expressed bicistronically through an internal ribosome entry site sequence as previously described. Hence in this construct (AAV-XIAP-GFP), the GFP reporter gene is coexpressed with XIAP in cells, but the proteins are not fused. A null vector, containing identical regulatory sequences including GFP but without XIAP, was used as a control (AAV-NULL-GFP). Both vectors were purified against immobilized heparan sulfate proteoglycan and the final concentrations were diluted to an identical titer of 1.0 × 10¹² genomic particles/mL.

**Transduction and FACS**

The ARPE-19 cells were seeded in 6-well plates at a density of 2.5 × 10⁴/ml before the AAV transduction. The vectors were diluted with prewarmed growth medium and added to the plates to reach a multiplicity of infection of 4 × 10⁴ genomic particles per cell.

The transduced cells were incubated for 3 days to reach maximal GFP intensity, then washed twice in PBS and resuspended in PBS containing 1% FBS before flow sorting with a cell sorter (Beckman Coulter Ltd, High Wycombe, UK) based on the GFP fluorescence. Sorted cells were plated in culture medium and left in 96-well plates overnight before being subjected to H₂O₂ stress and further examination of XIAP expression and neuroprotection.

**H₂O₂-Induced Cell Death**

To induce oxidative stress, fresh H₂O₂-conditioned medium was made by dissolving 30% H₂O₂ (Sigma-Aldrich) in culture medium. This was applied to the transduced and flow sorted ARPE-19 cells prepared in 96-well plates as described above. Before H₂O₂ stress, the culture medium was removed and freshly prepared H₂O₂-conditioned medium was added to each well. The cells were then incubated at 37°C for 1 hour before being carefully washed twice with PBS and replaced with growth medium. The cells were cultured for an additional 24 hours before further assays and experiments.

**ROS Measurement**

Intracellular ROS was measured using 2',7'-dichlorofluorescein diacetate (DCFH-DA, Sigma-Aldrich). DCFH-DA enters cells and is deacetylated to 2',7'-dichlorofluorescein, which reacts with ROS to form the fluorescent dichlorofluorescein (DCF). Cells were loaded with 5μM DCFH-DA for 30 minutes at 37°C before H₂O₂ stress. The DCF fluorescence intensity was determined immediately afterwards using spectrofluorometer with excitation and emission settings of 485 and 530 nm (Thermo Scientific, Basingstoke, UK).

**MTT Assay**

Cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) assay to determine mitochondrial stress as described previously. Briefly, MTT was added to the ARPE-19 cells at a final concentration of 0.5 mg/mL and incubated for 2 hours at 37°C. The medium was then removed and 100 μL of dimethyl sulfoxide was added to solubilize the blue formazan produced. After mild agitation of the plates for 5 minutes, the density of blue formazan was measured using a microplate photometer with a 570 nm test wavelength (Thermo Scientific).

**TUNEL Assay**

To detect cell apoptosis, the terminal deoxynucleotidyltransferase dUTP nick-end labeling (TUNEL) assay was performed using a commercial kit (Roche, Burgess Hill, UK) according to the manufacturer's protocol. TUNEL has the advantage of detecting the late stages of apoptosis before membrane lysis, complementing the MTT assay which detects mitochondrial stress (an earlier stage of apoptosis). Positive and negative controls were set up each time. Apoptotic cells were identified by positive nuclear staining under inverted fluorescence microscope with an excitation wavelength of 560 nm (Leica, Milton Keynes, UK). The number of apoptotic cells was counted as TUNEL positive cells in 20 × field and was repeated in 5 separate fields.

**Western Blot**

Sorted GFP cells (AAV-NULL-GFP and AAV-XIAP-GFP, respectively) were centrifuged at 1200 rpm for 5 minutes at 4°C before being harvested by RIPA lysis buffer (Millipore, Watford, UK) with freshly mixed protease inhibitors, containing 0.5 M Tris-HCl (pH 7.4), 1.5 M NaCl, 2.5% deoxycholic acid, 10% NP-40, 10 mM EDTA, 1 mM PMSF, 1 g/mL aprotinin, 1 g/mL leupeptin, 1 g/mL pepstatin, 1 mM Na3VO4 and 1 mM NaF. The lysate was transferred into 1.5 mL Eppendorf tubes and ultrasonically homogenized for 10 seconds. It was then clarified by centrifugation at 14,000g for 15 minutes at 4°C. The supernatant was immediately collected in a fresh tube and stored at −20°C. Protein concentrations were determined using a UV-spectrophotometer at 280 nm (Thermo Scientific).

Samples were boiled at 98°C for 10 minutes before loading into a 10% SDS polyacrylamide gel. The proteins were fractionated by electrophoresis and then transferred to nitrocellulose membrane. The nitrocellulose blots were blocked with freshly prepared 5% nonfat milk for 1 hour at room temperature, incubated with mouse monoclonal XIAP antibody (1:1000, Abcam, Cambridge, UK) and rabbit anti-β-actin (1:2000, Sigma-Aldrich) overnight at 4°C. Appropriate secondary antibodies conjugated to horseradish peroxidase were incubated for 1 hour at room temperature. Blot membranes were developed using the enhanced chemiluminescence technique. Quantification was determined by analyzing protein bands from 4 separate lanes using software (ImageJ; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html).

**Immunocytochemistry**

The transduced cells were fixed with 4% paraformaldehyde, incubated for 10 minutes with PBS containing 0.25% Triton X-100 and then with 1% BSA in PBST for 30 minutes to block nonspecific binding of the antibodies. A mouse monoclonal XIAP antibody (1:200, Abcam) was used as the primary and Alexa Fluor 555 as the secondary. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich). Immunoreactivity was examined under an inverted fluorescence microscope (Leica).

**Statistical Analysis**

The data in MTT, ROS assay, and Western blot analysis were shown as relative values of the nonstressed, nontransduced cells. Independent samples T test was performed between two groups. One-way ANOVA with Bonferroni post-hoc multiple tests was performed among multiple groups. The level of significance was set at 0.05. All data were analyzed by a comprehensive system (PASW Statistics 18; IBM Ltd., Portsmouth, UK) and presented as mean ± 2SEM.
RESULTS

H$_2$O$_2$-Induced Oxidative RPE Cell Death

We examined the H$_2$O$_2$-induced oxidative cell death by measuring mitochondrial stress, TUNEL staining and ROS in the differentiated ARPE-19 cells. A wide range of different concentrations of H$_2$O$_2$ were tested to determine an appropriate H$_2$O$_2$ stress which could trigger significant cell toxicity and therefore might be a good oxidative stress threshold against which to evaluate XIAP protection.

We first examined mitochondrial stress by MTT assay after increasing the concentration of H$_2$O$_2$ from 0.015 mM to 8 mM (0.015 mM, 0.03 mM, 0.06 mM, 0.125 mM, 0.25 mM, 0.5 mM, 1 mM, 2 mM, 4 mM, and 8 mM, respectively). The results confirmed that H$_2$O$_2$ toxicity in the ARPE-19 cells was dose-dependent (Fig. 1). Incubated for 1 hour, cells treated with 0.125 mM or lower concentration of H$_2$O$_2$ had similar cell viability than the nonstressed cells (88 % vs. 100 % for 0.125 mM H$_2$O$_2$; P = 0.17). However, there was a significant reduction of cell viability when cells were treated with 0.25 mM or higher dose of H$_2$O$_2$ compared with the nonstressed cells (69 % vs. 100 % for 0.25 mM H$_2$O$_2$; P = 0.0001).

To further determine whether H$_2$O$_2$ induced cell toxicity via apoptosis, the TUNEL assay was performed in the nonstressed, 0.125 mM, 0.25 mM, 0.5 mM, and 1 mM H$_2$O$_2$-treated cells. Cells treated with 0.125 mM did not show a significance in apoptotic cells compared with the nonstressed cells (2.6 ± 1.0 vs. 1.8 ± 0.4; P = 0.5). However, cells treated with 0.25 mM or higher dose of H$_2$O$_2$ showed significantly more apoptotic cells than the nonstressed cells (17 ± 1.4 vs. 1.8 ± 0.4 for 0.25 mM H$_2$O$_2$; P = 0.000006) (Fig. 2).

The DCFH-DA assay was also performed to measure the intracellular ROS in the nonstressed, 0.125 mM, 0.25 mM, 0.5 mM, and 1 mM H$_2$O$_2$-treated cells (Fig. 3). The results showed that cells treated by 0.125 mM, 0.25 mM, 0.5 mM, and 1 mM H$_2$O$_2$ all had significantly increasing ROS compared with the nonstressed cells (165 ± 20%, 207 ± 46%, 220 ± 11%, and 353 ± 32% vs. 100 ± 9.5%; P < 0.001, respectively).

Therefore, we identified ARPE-19 cells incubated by 0.25 mM H$_2$O$_2$ for 1 hour as the optimal oxidative stress challenge for triggering a significant and reproducible threshold of cell apoptosis in our experiments.

XIAP Overexpression Mediated by Gene Transduction

The serotype 2 adeno-associated virus (AAV2)-inverted terminal repeat ITR plasmids used to generate the AAV-XIAP-GFP and AAV-NULL-GFP vectors are shown in Figure 4. To determine whether XIAP can be successfully overexpressed, we transduced two groups of ARPE-19 cells independently with these two AAV vectors, but under similar conditions. The cells were then sorted based on the GFP expression using fluorescence-activated cell sorting. The results showed that the transduction rate was 55% in the AAV-XIAP-GFP and 58% in the
AAV-NULL-GFP groups (Fig. 5). This confirmed the XIAP and null vectors had similar properties with regard to transduction and transgene expression.

Western blot was performed to analyze the XIAP protein level in the GFP sorted cells collected separately from the AAV-XIAP-GFP and AAV-NULL-GFP transduced cells. The AAV-XIAP-GFP transfected cells had an 11-fold higher XIAP expression than the AAV-NULL-GFP controls (1300/11006126% vs. 120/1100610%; P/0.0006) (Fig. 6). This confirmed that XIAP expression from the vector was specifically due to the XIAP transgene and could be identified by GFP fluorescence in the AAV-XIAP-GFP transduced cells, as predicted by the bicistronic alignment of these two coding sequences in the construct.

Immunocytochemistry was also performed to examine XIAP expression in the transduced ARPE-19 cells. We detected a markedly increased staining of intracellular XIAP in the GFP coexpressing cells transduced by AAV-XIAP-GFP, while there was a much lower level of immunostaining of endogenous XIAP expressed in the AAV-NULL-GFP transduced controls and nontransduced cells nearby (Fig. 7 and Supplementary Figs. S1, S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-6850/-/DCSupplemental).

These results confirmed that we successfully achieved XIAP overexpression delivered by the AAV-XIAP-GFP vector. In addition, we would predict this slightly more than one log unit increase in XIAP expression in individual ARPE-19 cells might act broadly within the physiological range. Certainly at this level, the cytoplasmic distribution of the overexpressed XIAP protein has a very similar pattern to that seen in the AAV-NULL-GFP controls.

**XIAP Protection against H2O2-Induced ARPE-19 Cell Death**

In the flow sorted AAV-XIAP-GFP transduced cells subjected to 0.25 mM H2O2 for 1 hour, the MTT results showed that mitochondrial stress was numerically attenuated compared with the sorted AAV-NULL-GFP cells, but this was not statistically significant (68 ± 3.5% vs. 74 ± 3.8%; P = 0.24). Both sorted GFP populations had significantly more mitochondrial stress.

![FIGURE 3.](image3.png) **FIGURE 3.** ROS measurement by DCFH-DA assay. The DCF fluorescence intensity confirmed that intracellular ROS significantly increased in cells treated by 0.125 mM, 0.25 mM, 0.5 mM, and 1 mM H2O2. Error bar, ± 2 SE, n = 8. ***P < 0.001.

![FIGURE 4.](image4.png) **FIGURE 4.** Schematic drawing of the AAV vectors used in this study. BGH, bovine growth hormone; CAG, a combination of the cytomegalovirus (CMV) early enhancer element and chicken β-actin promoter; IRES, internal ribosome entry; WPRE, Woodchuck posttranscriptional regulatory element.

![FIGURE 5.](image5.png) **FIGURE 5.** Fluorescence-activated cell sorting data of the AAV transduced ARPE-19 cells, showing the AAV-XIAP-GFP (A) transduction rate was 58% and the AAV-NULL-GFP (B) was 58% based on the GFP expression.
compared with the nonstressed cells (68 ± 3.5% and 74 ± 3.8% vs. 100 ± 2.9%; P = 0.00003 and 0.0003, respectively) (Fig. 8).

In contrast, the TUNEL assay showed a significant improvement of cell survival in the sorted AAV-XIAP-GFP transduced cells after H2O2 stress. The AAV-XIAP-GFP transduced cells had significantly fewer apoptotic cells under H2O2 stress compared with the AAV-NULL-GFP controls (3.2 ± 0.6 vs. 18 ± 1.6 cells per 20 × field; P = 0.00003), while the latter had a similar number of apoptotic cells compared with the nontransduced cells (18 ± 1.6 vs. 17 ± 1.4; P = 0.66). Additionally, the number of TUNEL positive cells was similar in the sorted AAV-XIAP-GFP transduced and nonstressed cells (3.2 ± 0.6 vs. 1.8 ± 0.4; P = 0.8), in keeping with a strong protective effect at this threshold of oxidative stress (Fig. 9).

DISCUSSION

In the present study, we first define a reproducible model of oxidative stress that triggers apoptotic cell death in a human RPE cell line and we subsequently show a significant neuroprotective effect when human XIAP is overexpressed in these cells mediated by AAV gene transfer. The lack of improvement after transduction with a specific control vector confirmed that the protective effects were specific for the XIAP protein and were not secondary to GFP expression or nonspecific effects of AAV transduction on cell homeostasis. Furthermore apoptosis with the control vector was similar to nontransduced cells, which also demonstrates that the mechanism of cellular transduction with AAV does not itself appear to accelerate apoptotic cell death in this model of human RPE oxidative cell death.

A recent study examined the molecular changes after H2O2-induced oxidative stress in the ARPE-19 cell line.14 That study showed that stress induced the translocation of serine protease HtrA2/Omi from the mitochondria to the cytoplasm, which in turn cleaved endogenous XIAP to activate apoptosis via a caspase-3 dependent mechanism. The investigators also showed a modest reduction in apoptosis when cells were also exposed to the HtrA2/Omi inhibitor, UCF-101. In our study however, we have gone a step further, because AAV mediated gene delivery does not just prevent the degradation of endogenous XIAP, it results in overexpression of XIAP (approximately one log unit increase in XIAP expression). In a study in transgenic mice, overexpression of XIAP was achieved in retinal bipolar and cerebellar Purkinje cells through use of the L7 promoter and resulted paradoxically in neuronal degeneration.15 It may be difficult to extrapolate this directly, because caspase-3 has a key role in neuronal development, particularly with regard to long-term depression and NMDA synapse modeling.16 Hence, overexpression of XIAP during development might lead to secondary neuronal death through mechanisms of developmental failure that would not be relevant to the classic apoptotic pathway. Nevertheless we were keen to quantify XIAP levels as accurately as possible and the 11-fold

FIGURE 6. Western blot analysis of XIAP in the ARPE-19 cells with or without AAV transduction. The histograms showed that XIAP protein level in the AAV-XIAP-GFP transduced cells was significantly higher than the nontransduced and AAV-NULL-GFP controls. Error bar, ± 2 SEM, n = 4. ***P < 0.001.

FIGURE 7. XIAP immunocytochemistry in ARPE-19 cells after AAV transduction shows qualitatively higher XIAP expression above background levels in GFP coexpressing cells in the AAV-XIAP-GFP transduced cells (A, B, arrows). As expected, in AAV-NULL-GFP transduced cells, no similar increase in XIAP was seen in cells expressing GFP (C, D, asterisk). See also Supplementary Figures S1, S2 (http://www.iovs. org/lookup/suppl/doi:10.1167/iovs.10-6850/-/DCSupplemental). Scale bar, 25 μm.

FIGURE 8. MTT results of AAV transduced ARPE-19 cells after H2O2 stress, showing that mitochondrial stress was numerically attenuated compared with flow sorted AAV-NULL-GFP cells after H2O2 stress, but this was not statistically significant. Both sorted AAV transduced populations had significantly less mitochondria activity than the nonstressed cells. Error bar, ± 2 SE, n = 8. ***P < 0.001.
higher expression we observed compared with the AAV-NULL-GFP controls was clearly neuroprotective. In this model however, an upper limit of XIAP protein expression above which it might become proapoptotic was not investigated.

Another interesting observation from our study was that while TUNEL assay outcome measures were significantly altered with XIAP, the MTT assays did not show a statistically significant protective effect. A plausible explanation for this is evident if one considers the mechanism of the assays and the action of XIAP. In our RPE oxidative stress system, one mechanism of action of XIAP is to inhibit activity of caspases-3, 7, and 9, which is one of the final steps in the apoptotic pathway and leads to DNA fragmentation. The mitochondria however acts upstream and releases proapoptotic proteases such as HTRA2/Omi and Smac/Diablo, which inhibit XIAP to induce apoptosis indirectly by release of active caspases. Other apoptotic regulators such as Bax and Bcl-2 also act upstream within the mitochondria. The TUNEL assay is a measure of DNA fragmentation and hence represents the final step in apoptosis after caspase activation. The MTT assay however measures cell viability indirectly through mitochondrial stress, by quantifying the reduction of a bromide salt (MTT) by mitochondrial succinate dehydrogenase. Hence, because XIAP acts largely downstream of the mitochondria, it might be predicted to have much less effect on mitochondrial stress (MTT) than on DNA fragmentation (TUNEL), which is directly influenced by caspase activity.

Theoretically inhibiting apoptosis at the final effector steps of caspase activity may be more effective than targeting upstream mitochondrial mechanisms. An advantage of targeting caspases is that they represent a final common effector of several apoptotic pathways. Here we have observed that XIAP has a marked effect in preventing DNA fragmentation despite mitochondrial stress, at least in the short term (24 hours after the H$_2$O$_2$ stress). Others have shown a long-term effect of XIAP neuroprotection in rodent monogenic degeneration models. A potential question arises however as to how a cell might behave if undergoing mitochondrial stress but unable to trigger apoptosis due to XIAP-mediated caspase inhibition. Surviving but completely nonfunctional cone photoreceptor cells have been observed in patients with end-stage retinitis pigmentosa and there is evidence that these cells may improve morphologically in animal models when stimulated with ciliary neurotrophic factor (CNTF) which acts externally on the cell receptors via the JAK/STAT pathway upstream of mitochondria. A disadvantage of XIAP therefore might be that it could act too far downstream to influence many of the upstream cellular stress responses, such as inhibition of translation which may be reversed by trophic factors. This might result in the preservation of cells that remain highly stressed and have no useful function.

Alternative strategies targeting mitochondria with antioxidants have not been particularly effective in clinical trials for preventing RPE cell loss in AMD, as evidenced by continued progression of geographic atrophy in AREDS (Age-Related Eye Disease Study). For clinical trial use, XIAP would need to be delivered by gene transfer as it has an intracellular action. While AAV may be safe as a vector, ectopic expression of XIAP in dividing cells could pose a significant risk of malignancy. Hence it might be preferable to use an inducible promoter or RPE specific serotype to limit ectopic transgene expression, such as AAV4. Nevertheless gene therapy to the outer retina requires relatively small doses of AAV compared with other organs and has very limited systemic spread. Clinical trial neuroprotection endpoints are also clear as there is a fellow eye for comparison. Hence AMD might be a good disease in which to assess the therapeutic potential of XIAP delivered by AAV-mediated gene transfer. Here we have shown neuroprotective effects of XIAP in RPE cells undergoing oxidative stress in vitro, with no detrimental effects of the AAV vector.

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References


