Molecular Vision 2017; 23:1015-1028 <http://www.molvis.org/molvis/v23/1015>
Received 22 May 2017 | Accepted 25 December 2017 | Published 25 December 2017

β-amyloid expression in age-related cataract lens epithelia and the effect of β-amyloid on oxidative damage in human lens epithelial cells

Jie Xu,[1,2] Dan Li,[1,2] Tianyu Zheng,[1,2] Yi Lu[1,2]

(The first two authors contributed equally to this work.)

[1]Department of Ophthalmology, EYE and ENT Hospital of Fudan University, Shanghai, China; [2]Shanghai Key Laboratory of Visual Impairment and Restoration, Shanghai, China

Purpose: To evaluate the changes in β-amyloid (Aβ) expression in age-related cataract (ARC) lens epithelia and the effect of Aβ on oxidative damage in human lens epithelial cells (HLECs).

Methods: Specimens of lens epithelia and aqueous humor were obtained from 255 cataract surgery patients and 48 healthy donor eyes. The ARC samples were divided into four groups according to the Lens Opacities Classification System III, with increasing severity from Group I to Group IV. The HLECs were cultured under healthy or oxidative conditions with or without Aβ pretreatment. Western blot, immunofluorescence, real-time PCR, and enzyme-linked immunosorbent assay were performed to detect Aβ and β-amyloid precursor protein (APP) expression. β-secretase activity was analyzed in lens epithelia and HLECs. The effect of Aβ on the viability of HLECs under oxidative conditions was investigated using a cell viability assay.

Results: Compared with the healthy group, the Aβ 1–42 expression levels in lens epithelia and Aβ 1–40 expression levels in aqueous humor decreased in Groups I, II, and III ($p<0.05$) but were unchanged in Group IV. In contrast, APP expression levels increased in Groups I, II, and III ($p<0.05$) compared with those in the healthy group but were unchanged in Group IV. H_2O_2-treated HLECs exhibited decreased amounts of Aβ 1–42 and increased amounts of APP. β-secretase activity decreased in the lens epithelia of all four subgroups of ARCs compared with that in the lens epithelia of healthy subjects and decreased in H_2O_2-treated HLECs. Furthermore, treatment with nanomolar concentrations (0.2 nM to 10 nM) of Aβ could protect cell viability from oxidative damage.

Conclusions: Aβ and APP expression levels exhibited differential changes during the development of ARC, indicating active feedback of this protein processing. Decreased expression of physiologically generated Aβ in the early and mid-stages of ARC development might be one of the potential mechanisms accelerating oxidative stress in HLECs during cataractogenesis.

β-amyloid (Aβ) is generated from amyloid-β precursor protein (APP) through sequential cleavages by β- and γ-secretase [1]. There are two major C-terminal variants of Aβ, "long-tailed" Aβ 1–42 and "short-tailed" Aβ 1–40, with Ala-42 and Val-40 as C-terminal residues, respectively [2]. Abnormal Aβ aggregation in the brain is an important characteristic of Alzheimer disease (AD) [3]. Previous studies have suggested that Aβ might also be associated with cataract formation in patients with AD [4]. When Goldstein et al. stained the lenses of patients with AD with Congo Red (a common method for detecting amyloid deposition), they observed enhanced amyloid immunoreactivity in the same supranuclear regions in which cataracts were identified with slit-lamp examinations, suggesting that Aβ played a role in supranuclear cataract formation in patients with AD [4]. In addition, cataracts manifest in the Aβ-transgenic AD mouse model [5]. Furthermore, Moncaster et al. confirmed Aβ as a key pathogenic determinant linking lens and brain pathology in AD and Down syndrome (DS) [6]. Characteristic lens pathology is regarded as a distinctive early onset ocular phenotype in patients with DS that may be clinically detectable early in life [7-9]. Researchers identified a DS-related pathogenic pathway associating progressive age-dependent Aβ accumulation in the lens and supranuclear cataracts with corresponding cerebral Aβ accumulation and neuropathology in the brain [6]. In addition, positive APP and Aβ staining has also been reported in the cataract lens epithelia of subjects without AD or DS [10]. The fact that the single layer of lens epithelia is essential for maintaining the metabolic homeostasis and transparency of the lens has been well acknowledged [11,12]. Oxidative damage to lens epithelia results in cell apoptosis, DNA damage, and protein degradation and ultimately induces ARC [11-15]. Of note, Aβ has also been

Correspondence to: Yi Lu, Department of Ophthalmology, Eye and ENT Hospital of Fudan University, 83 Fenyang Road, Shanghai, China, 200031; Phone: 86-21-64377134; FAX: 86-21-64377151; email: luyieent@126.com

strongly associated with oxidative stress [16-18], which is considered a significant contributor to the pathogenesis of AD [19-21]. Therefore, Aβ may play a role in cataract formation through the oxidative stress response pathway.

However, studies on the relationship between Aβ and oxidative stress in AD pathogenesis have obtained controversial results [17,22,23]. According to the classic amyloid cascade hypothesis, Aβ might be the main pathogenic factor accelerating oxidative damage in the development of AD [22,24-27]. The consequences of increased Aβ in the brain include enhanced oxidative stress, neuronal death, neurofibrillary tangles of hyperphosphorylated tau, amyloid plaque formation, and the onset and advancement of AD [22]. However, researchers have demonstrated that a proper amount of Aβ may be beneficial to various cellular functions; for example, Aβ could function as an antioxidant against metal-induced oxidative damage [28] and exhibits increased expression in response to various cellular injuries [29-31]. The absence of endogenous Aβ causes neuronal cell death, which can be prevented by the introduction of picomolar to nanomolar concentrations of Aβ [32]. Other evidence for the beneficial role of Aβ includes the production of Aβ in healthy brain tissue during normal neuronal activity, with physiologically important functions in learning and memory [33-35]. Additionally, several clinical trials seeking to treat AD by reducing Aβ have failed [29,36,37]. Therefore, the roles played by Aβ under different physiologic and pathological conditions need to be studied.

To investigate the possible role played by Aβ in ARC development, the current study evaluated the expression of Aβ and APP in the lens epithelia and aqueous humor of healthy subjects and patients with ARC. We also explored the expression pattern of Aβ and APP in cultured human lens epithelial cells (HLECs) under oxidative conditions. Finally, we investigated the effect of the introduction of Aβ at nanomolar concentrations on the viability of HLECs under oxidative stress.

METHODS

This study was approved by the Ethics Committee of the EYE and ENT Hospital of Fudan University in accordance with the Declaration of Helsinki and the ARVO statement on human subjects. Written informed consent was obtained from all participants.

Subjects: A total of 255 cataract patients (128 males, 127 females, 61.8 ± 4.2 years) operated in the EYE and ENT Hospital of Fudan University and 48 normal donor eyes (23 males, 25 females, 60.6 ± 4.5 years) obtained from the eye bank of the EYE and ENT Hospital of Fudan University were recruited in the present study. A diagnosis of ARC was defined as a cataract in a patient aged 50 to 70, with no other accompanying systemic (including AD), corneal, or retinal disease and with no history of corneal or intraocular surgery. The axial lengths of the patients with ARC who were included in this study were within the normal range (22–25 mm). Cataract diagnosis was performed via slit-lamp examination of the eyes. To avoid introducing other pathogenic factors, certain classes of cataracts were divided into separate groups, including posterior subcapsular cataract (PSC), cataract with myopia (defined by an axial length longer than 26 mm), and cataract with diabetes. The cortical (C), nuclear (N), or posterior subcapsular (P) opacity of the cataract was classified with the Lens Opacities Classification System III (LOCS III). A healthy lens, without any ocular or systemic diseases, had less than C2, N2, and P2 opacity. The specimens that met the inclusion criteria were divided into the following eight groups: Group I, ARC of C2–3N1–2; Group II, ARC of C3–4N2–3; Group III, ARC of C4–5N3–4; Group IV, ARC of C4–5N4–5; Group V, PSC (P2–P4, with C<2 and n<2); Group VI, cataract with myopia; Group VII, cataract with diabetes; and a healthy group as a control.

Tissue preparation: Anterior lens epithelia samples (approximately 5 mm in diameter) were obtained from 234 consenting patients (aged 50 to 70) with cataracts by continuous curvilinear capsulorrhexis during cataract surgery by the same surgeon (YL). Aqueous humor samples were aspirated from 42 patients with cataracts before surgery by corneal paracentesis, which was performed by inserting a 26-gauge needle into the anterior chamber. Forty-five healthy lens epithelia samples were obtained from the Eye Bank of the EYE and ENT Hospital of Fudan University (donor eyes were from individuals aged 50 to 70 who had no diagnosed ocular or systemic diseases, with less than C2, N2, and P2 opacity). Aqueous humor samples of healthy controls from the eye bank were aspirated by inserting a 26-gauge needle into the anterior chamber from the limbus. All specimens were stored at −80 °C until analysis. Tissues from three lens epithelia were combined as one sample for the western blot and β-secretase activity analyses. Four lens epithelia were combined as one sample for real-time PCR (RT–PCR) experiments.

Cell culture: The human lens epithelial cell line (SRA01/04) was obtained from the Cancer Institute of the Chinese Academy of Medical Science (Beijing, China). Authentication testing of the SRA01/04 cell line was performed by Shanghai Biowing Applied Biotechnology Co. Ltd via short tandem repeat (STR) profiling (Appendix 1). Cells were maintained in RPMI-1640 (11,875; Gibco, Thermo Fisher Scientific Inc., Waltham, MA) supplemented with 15% fetal bovine serum

(10100; Gibco, Thermo Fisher Scientific Inc.) and seeded at a density of 1 × 10^5 cells/ml 1 day before the experiments. To induce oxidative stress, the cells were exposed to 200 µM H_2O_2 in cell culture medium and then incubated for 24 h before harvesting. To investigate the effect of Aβ, cells were pretreated with various concentrations of Aβ 1–42 (A9810; Sigma-Aldrich, St. Louis, MO) or Aβ 1–40 (A1075; Sigma-Aldrich) 24 h before oxidative shock induced by H_2O_2. Aβ 1–42 and Aβ 1–40 were solubilized in dimethyl sulfoxide (DMSO) and PBS (1X; 154 mM NaCl, 5.6 mM Na_2HPO_4, 1 mM KH_2PO_4; PH 7.2), respectively, and then added to cultured HLECs to a final concentration of 0.2, 0.5, 1.0, and 10 nM without incubation. Unlike incubated Aβ, which contains many oligomers, this freshly dissolved Aβ contains mostly monomers, especially when the concentrations of Aβ we applied were low [23,28,38].

Western blot analysis: Protein sample preparation and immunoblotting were performed according to our previous work [39]. The primary antibodies used for western blot were APP (1:1,000; SIG-39320, Biolegend, San Diego, CA) and anti-Aβ 1–42 (1:1,000; ab10148, Abcam, Cambridge, UK). Three independent experiments were performed.

Immunofluorescence: Lens epithelia specimens were fixed in 4% paraformaldehyde and permeabilized in 0.1% Triton X for 10 min before blocking in 1% serum and 0.1% bovine serum albumin (BSA) for 30 min at 21 °C. The antibodies included APP (1:80; MAB348, EMD Millipore, Billerica, MA) and Aβ 1–42 (1:100; ab10148, Abcam). Alexa Fluor® 488 goat polyclonal to mouse immunoglobulin G (IgG) and Alexa Fluor® 488 goat polyclonal to rabbit Ig were used as the secondary antibody, respectively. Fluorescent images were obtained and analyzed using a confocal laser microscope (TCSSP8, Leica Microsystems GmbH, Wetzlar, Germany).

RT–PCR experiments: Total cellular RNA was isolated from the lens epithelia using a RNA Extraction Kit (CW0584, CWBIO, Beijing, China). After treatment with RNase-free DNase for 15 min, total RNA was reverse transcribed using oligo d(T) primers. APP expression relative to β-actin was determined using a Power SYBR Green Reagents Kit (Applied Biosystems, Foster City, CA). PCR was performed in a final volume of 20 µl containing 2 µl of cDNA and 10 µM primers with the ABI ViiATM7 system (Applied Biosystems). The following conditions were used: initial denaturation of 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The relative gene expression was calculated with the ΔΔCt method. The following primers were used: APP (forward (F), 5′-CCG ACC GAG GAC TGA CCA CT-3′, reverse (R) 5′-TGA CGA TCA CTG TCG CTA TG-3′); β-actin (forward (F)5′-AAG GTG ACA GCA GTC GGT T-3′, reverse (R) 5′-TGT GTG GAC TTG GGA GAG G-3′).

Enzyme-linked immunosorbent assay: The Aβ 1–42 and Aβ 1–40 expression levels in aqueous humor were determined using human Aβ 1–42 enzyme-linked immunosorbent assay (ELISA) kits (KHB3441; Invitrogen, Life Technologies) and human Aβ 1–40 ELISA kits (KHB3482; Invitrogen, Life Technologies), respectively, according to the manufacturer's instructions. Optical density was read at 450 nm within 30 min on a microplate spectrophotometer. Concentrations were calculated according to the standard curve. The experiment was repeated three times.

β-secretase activity assays: A β-secretase assay kit (k360–100; BioVision, San Francisco, CA) was used to measure β-secretase activity in the soluble fraction of the pooled lens epithelia and cultured HLEC homogenates according to the manufacturer's instructions. In brief, soluble protein fractions at the final concentration of 1 µg/µl were incubated at 37 °C for 1 h with β-secretase-specific substrate peptides conjugated to the fluorescent reporter molecules. After incubation, light emitted at 510 nm was detected in a fluorescent plate reader after excitation at 350 nm. All experiments were performed three times.

Cell viability assays: The HLECs were plated in 96-well plates at a density of 1 × 10^4 cells/100 µl/well and treated as described in detail above. Next, 10 µl of Cell Counting Kits-8 (CCK-8; DOJINDO, Kyushu, Japan) was added to each well, and the cells were incubated for 2 h. Absorbance was measured at a wavelength of 450 nm. Three independent experiments were performed.

Statistical analysis: Statistical analysis was performed using SPSS 22.0 (IBM Corp., Armonk, NY). All data are presented as the means ± standard deviations. The chi-square test was used to examine differences in sex distribution. One-way ANOVA and least significant difference (LSD) tests were used to detect differences in age distribution, to compare the Aβ and APP expression and β-secretase activity in the human samples between healthy subjects and subgroups of ARCs with different severities, and to compare cell viability among different interventions. The independent samples *t* test was used to compare the Aβ and APP expression and the β-secretase activity between untreated HLECs and H_2O_2-treated HLECs. Differences were considered statistically significant when $p<0.05$.

RESULTS

Demographic data: The demographic characteristics of the study subjects are presented in Table 1. There were no statistically significant differences among the groups in terms of age or sex (all p>0.05, ANOVA and least significant difference (LSD) tests for age; chi-square tests for sex).

Aβ and APP expression in ARC and healthy eye tissues: Lens epithelia and aqueous humor samples were collected from cataract surgery patients and the Eye Bank, and categorized into seven cataract groups and a healthy group. Samples from Groups I to IV were collected from cataract surgery patients with increasing severity of cataract, the Group V samples were from PSCs, the Group VI samples were from cataracts with myopia, and the Group VII samples were from diabetic cataracts. The western blot and immunofluorescence results indicated that the Aβ 1–42 protein expression levels decreased in Groups I, II, and III in the ARC lens epithelia compared with those in the healthy tissue specimens (p<0.05 compared with healthy subjects; Figure 1A), but there was no statistically significant difference in Group IV (p>0.05 compared with healthy subjects; Figure 1A). Additionally, Aβ 1–42 staining was mainly localized to the cell nucleus in healthy tissues but transferred more to the cytoplasm in the ARC tissues (Figure 1B). The Aβ 1–42 expression levels were lower in the PSC (Group V), cataract with myopia (Group VI), and diabetic cataract (Group VII) groups than those in the healthy tissues (Figure 1A). However, the concentrations of Aβ 1–42 were low in aqueous humor when examined with ELISA, showing no statistically significant differences among all eight groups (Appendix 2). We further detected the Aβ 1–40 expression levels in aqueous humor using specific ELISA kits. The results showed that the Aβ 1–40 concentration was much higher than that of Aβ 1–42, suggesting Aβ 1–40 is the major form in aqueous humor. Similar to the changing tendency of Aβ 1–42 expression levels in the lens epithelia, the Aβ 1–40 expression levels decreased in Groups I, II, and III in ARC aqueous humor compared with those in the healthy tissue specimens (p<0.05 compared with healthy subjects; Figure 1C) but increased to healthy levels in Group IV (p>0.05 compared with healthy subjects; Figure 1C). Lower Aβ 1–40 expression levels were detected in the PSC (Group V), cataract with myopia (Group VI), and diabetic cataract (Group VII) groups than those in healthy tissues (Figure 1C).

In contrast, the RT–PCR, western blot, and immunofluorescence analyses showed the lowest mRNA and protein expression levels in healthy lens epithelia, which were increased in Groups I, II, and III (p<0.05 compared with healthy subjects; Figure 2A,B) but showed no statistically significant difference in Group IV (p>0.05 compared with the healthy subjects Figure 2B). The APP expression levels in the PSC, diabetic cataract, and cataract with myopia groups were all higher than those in healthy tissues (Figure 2B).

β-secretase activity in ARC and healthy eye tissues: To explore the underlying mechanism of the reversal of Aβ and APP expression levels, β-secretase activity was further detected in ARC and healthy eye tissues. The results demonstrated that the activity of β-secretase decreased by 27.3%, 34.0%, 27.8%, and 26.1% of the healthy group in Groups I, II, III, and IV, respectively (all p<0.05 compared with healthy subjects, Figure 3). No statistically significant differences were seen among the four ARC subgroups.

Aβ and APP expression and β-secretase activity in H_2O_2-treated HLECs: To investigate the changes in the Aβ system in cultured HLECs under oxidative stress (a significant contributor to the pathogenesis of ARC), Aβ 1–42 and APP expression levels and β-secretase activity were measured in H_2O_2-treated HLECs. After a 200 μM H_2O_2 treatment for 24 h, western blot analysis showed that Aβ 1–42 expression decreased (p<0.05, Figure 4A) and APP expression increased (p<0.05, Figure 4B) in H_2O_2-treated HLECs. H_2O_2 treatment also reduced β-secretase activity by 25.3% in cultured HLECs (p<0.01, Figure 4C).

The protective effect of Aβ in cultured HLECs under oxidative conditions: First, to test whether nanomolar concentrations (0.2 nM to 10 nM) of Aβ were toxic, we monitored the viability of the cultured HLECs using the CCK-8 assay, observing no substantial change when cells were exposed to 0.2 nM to 10 nM synthetic Aβ 1–42 or Aβ 1–40 peptides for as long as 24 h (p>0.05, Figure 5). Then the effect of Aβ 1–42 or 1–40 on oxidative damage in cultured HLECs was investigated. The cell viability of HLECs was measured after incubation with 200 μM H_2O_2, with or without various concentrations of Aβ pretreatment. The investigation of cell morphology using an inverted microscope revealed that cells in the healthy control group were regular hexagonal or round cells with relatively high cell density (Figure 6A), while cell granularization, shrinkage, and an apparent cell density reduction were induced in the H_2O_2- and H_2O_2+ vehicle-treated groups, with cell viability decreased about 65.9% to 72.1% of the healthy control group (Figure 6A–C). Aβ 1–40 pretreatment protected the HLECs against the morphology changes induced by H_2O_2 treatment in a concentration-dependent manner (Figure 6A). Accordingly, cell viability was increased to 75.6%, 82.3%, 87.2%, and 77.1% by Aβ 1–40 pretreatment with concentrations of 0.2, 0.5, 1.0, and 10 nM, respectively (all p<0.05 compared with the vehicle control, Figure 6C), corroborating the morphological study.

Table 1. Demographic data for all participants.

Group	n	Age (mean ± SD, y)	Gender (males/females)
I (ARC of C2–3N1–2)	48	61.4±4.4	24/24
II (ARC of C3–4N2–3)	48	62.0±4.1	25/23
III (ARC of C4–5N3–4)	48	62.2±4.2	25/23
IV (ARC of C4–5N4–5)	48	62.3±4.0	22/26
V (Posterior Subcapsular Cataract, P2-P4, with C<2 and n<2)	21	62.1±4.8	11/10
VI (Cataract with Myopia)	21	60.7±4.2	9/12
VII (Cataract with Diabetes)	21	61.0±4.4	12/9
Normal	48	60.6±4.5	23/25

ARC=age-related cataract, C=cortical, n=nuclear, p=posterior subcapsular, SD=standard deviation. Group I to IV were divided according to the Lens Opacities Classification System III with increasing cataract severity.

In addition, cell viability was increased to 76.0%, 87.7%, 89.1%, and 76.6% of the healthy control group by Aβ 1–42 pretreatment with concentrations of 0.2, 0.5, 1.0, and 10 nM, respectively (all $p<0.05$ compared with the vehicle control, Figure 6B). The protective effect peaked with an Aβ concentration of 1 nM (all $p<0.05$ compared with other concentrations of Aβ, except 0.5 nM Aβ 1–42). Despite the higher mean cell viability by Aβ 1–42 pretreatment with concentrations of 1 nM, no statistically significant difference was seen between the 1 nM and 0.5 nM Aβ 1–42 pretreatments.

DISCUSSION

Accumulated evidence has indicated that Aβ plays a role in the pathogenesis of AD and cataracts [3-5,10]. Although the function of Aβ in AD remains controversial, Aβ is still the most well-known causative factor for the disease and a drug target in AD research. However, the relationship between ARC and Aβ has not been clarified.

To understand the functional role of Aβ in ARC, we designed experiments to evaluate three aspects of this issue: 1) the expression pattern of Aβ and APP in ARC and healthy samples (including lens epithelia and aqueous humor); 2) the

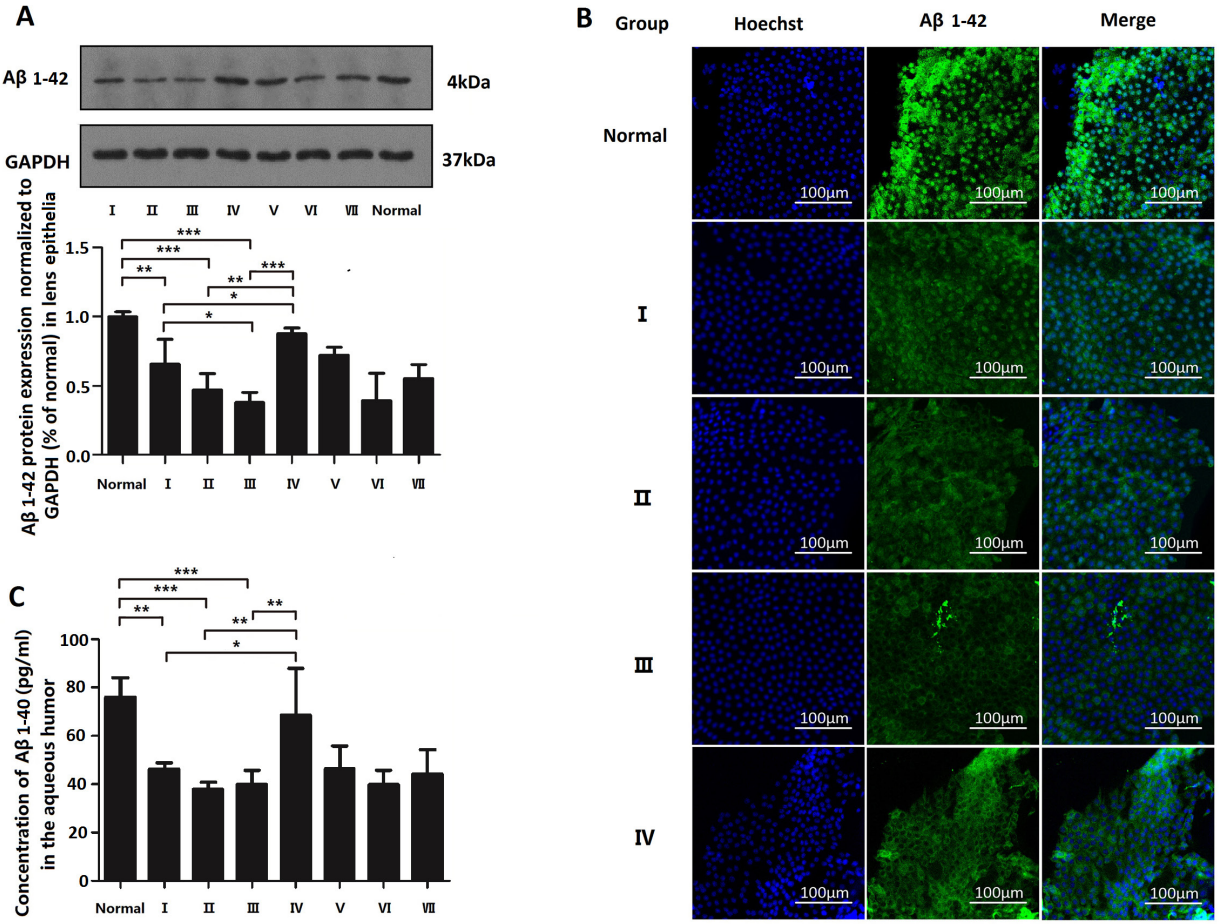

Figure 1. Aβ expression levels in lens epithelia and aqueous humor of healthy and cataract eyes. **A**: Western blot analysis of β-amyloid (Aβ) 1–42 in lens epithelia. **B**: Immunofluorescence of Aβ 1–42 and Hoechst nuclear staining in lens epithelia. **C**: Enzyme-linked immunosorbent assay (ELISA) of Aβ 1–40 in the aqueous humor. The cortical (C), nuclear (N), or posterior subcapsular (P) opacity of the cataract was classified with the Lens Opacities Classification System III (LOCS III), with an increasing cataract severity from Group I to Group IV. Group I: C2–3N1–2; Group II, C3–4N2–3; Group III, C4–5N3–4; Group IV, C4–5N4–5; Group V, posterior subcapsular senile cataract (P2–P4, with C<2 and n<2); Group VI, cataract with myopia; Group VII, cataract with diabetes; and the healthy group, healthy tissue samples. In each group, three independent experiments were performed. The data are the means ± standard deviation (SD). *p<0.05, **p<0.01, ***p<0.001, with one-way ANOVA and least significant difference (LSD) tests among Groups I to IV and the healthy group. Healthy samples were used as normal controls. Aβ 1–42 protein expression (% of healthy) in the lens epithelia was normalized to GAPDH.

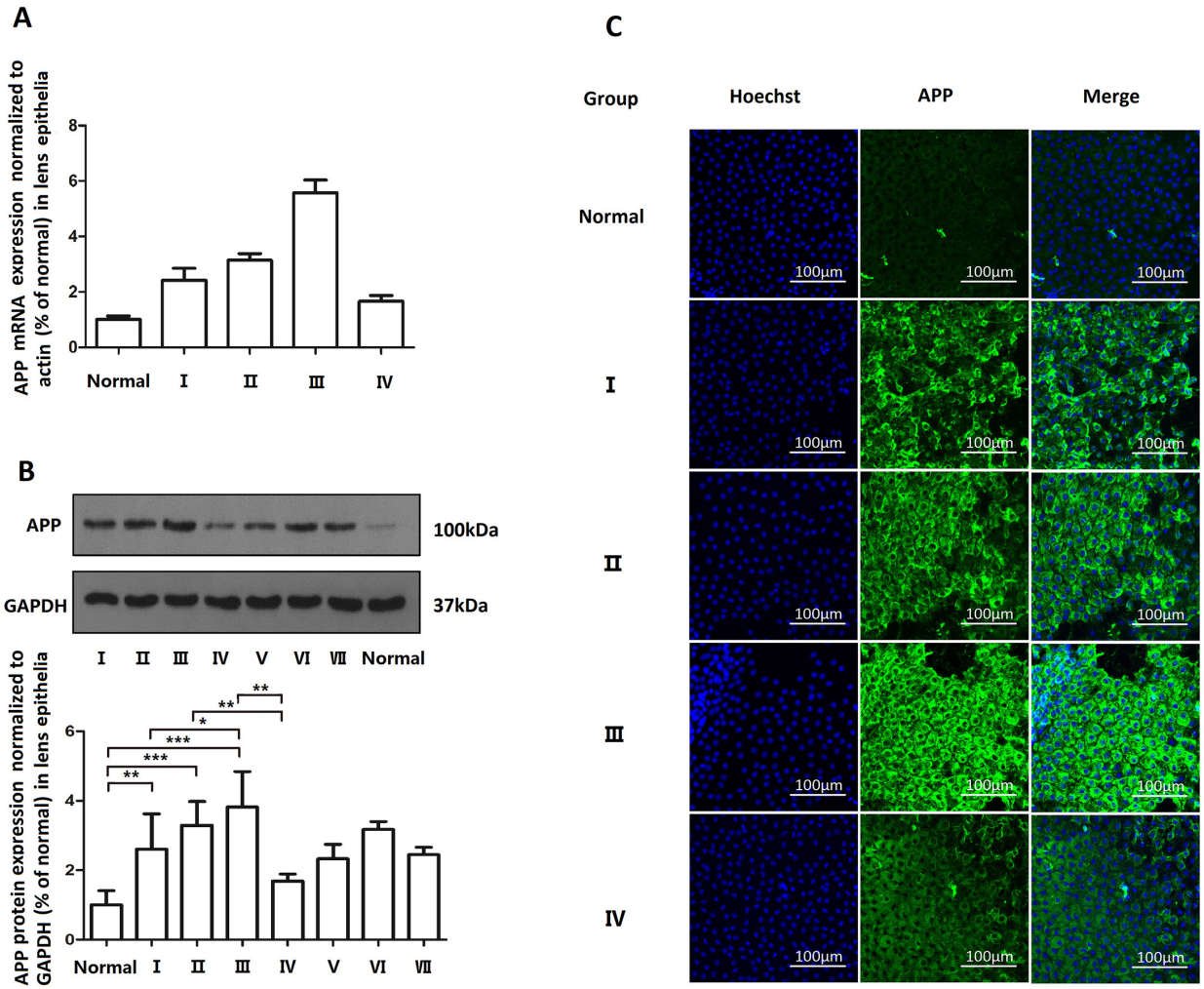

Figure 2. APP expression levels in lens epithelia of healthy and cataract eyes. **A**: Real-time PCR analysis of amyloid-β precursor protein (APP) in lens epithelia. **B**: Western blot analysis of APP in lens epithelia. **C**: Immunofluorescence of APP and Hoechst nuclear staining in lens epithelia. The cortical (C), nuclear (N), or posterior subcapsular (P) opacity of the cataract was classified with the Lens Opacities Classification System III (LOCS III), with increasing cataract severity from Group I to IV. Group I: C2–3N1–2; Group II, C3–4N2–3; Group III, C4–5N3–4; Group IV, C4–5N4–5; Group V, posterior subcapsular senile cataract (P2-P4, with C<2 and n<2); Group VI, cataract with myopia; Group VII, cataract with diabetes; and the healthy group, healthy tissue samples. In each group, three independent experiments were performed. The data are the means ± standard deviation (SD). *p<0.05, **p<0.01, ***p<0.001, by one-way ANOVA and least significant difference (LSD) tests among Groups I to IV and the healthy group. Significant differences between the ΔΔCt values of each group in Figure 2A were found with one-way ANOVA (p<0.001) and LSD tests (p<0.05). Healthy samples were used as normal controls. The APP mRNA and protein expression levels (% of healthy) were normalized to actin and GAPDH, respectively.

expression pattern of Aβ and APP in HLECs under oxidative stress, and 3) the effect of various concentrations of Aβ on HLECs under oxidative stress.

Previous studies identified Aβ 1–42 and Aβ 1–40 in the lens [4]. As the Aβ 1–42 peptide was more widely investigated in Alzheimer disease (AD) studies, we mainly assessed Aβ 1–42 expression in lens epithelia and aqueous humor. However, we found that the concentrations of Aβ 1–42 were low in aqueous humor. Then we further detected the Aβ 1–40 expression levels in aqueous humor, showing that the Aβ 1–40 concentration was much higher than the Aβ 1–42 concentration. Consistently, Goldstein et al. and Prakasam et al. identified Aβ 1–40 as the predominant soluble species in

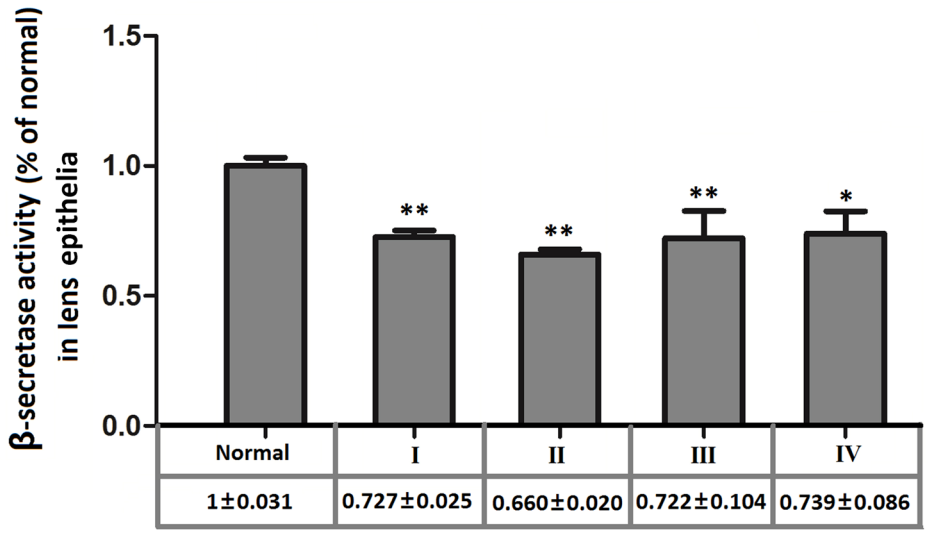

Figure 3. β-secretase activity assay in lens epithelia of healthy and cataract eyes. The cortical (C) and nuclear (N) opacity of the cataract was classified with the Lens Opacities Classification System III (LOCS III), with increasing cataract severity from Groups I to IV. Group I: C2–3N1–2; Group II, C3–4N2–3; Group III, C4–5N3–4; and Group IV, C4–5N4–5. The data are the means ± standard deviation (SD). The experiment was repeated three times in each group. *p<0.01, **p<0.001, with one-way ANOVA and least significant difference (LSD) tests. No statistically significant differences among the four subgroups of age-related cataracts (ARCs; from Group I to Group IV) were found (p>0.05).

aqueous humor [4,40]. Previous studies even demonstrated that Aβ 1–40 was the major species secreted from cultured cells and found in biologic fluids [2,41].

Consistent with previous reports demonstrating reduced Aβ activity in different tissues of patients with AD and AD rats [42-44], the present results showed lower Aβ 1–42 expression levels in lens epithelia and lower Aβ 1–40 expression levels in aqueous humor in the early and mid-stages (Groups I to III) of ARC compared with those in healthy samples. We also found a statistically significant decrease in the activity of β-secretase in all four ARC lens epithelia subgroups and HLECs cultured under oxidative stress, which is a well-accepted contributor to ARC [11-13]. Therefore, we speculated that the reduced Aβ expression observed in the early and mid-stages of ARC formation was due to a reduction in β-secretase activity under oxidative stress,

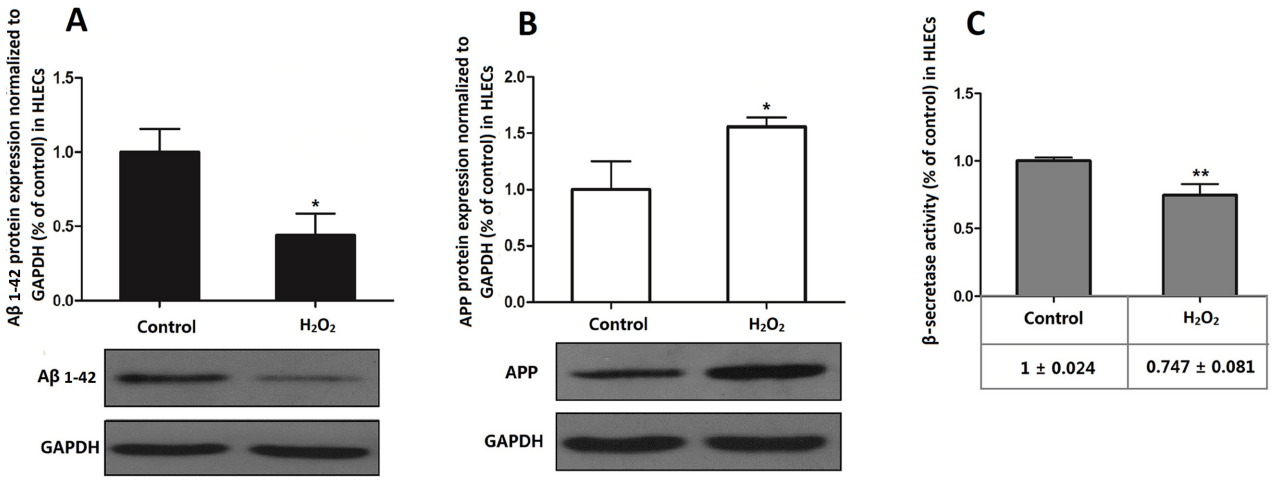

Figure 4. Aβ 1–42 and APP expression levels and β-secretase enzyme activity in H_2O_2-treated HLECs. **A**: Western blot analysis of β-amyloid (Aβ) 1–42 in cultured human lens epithelial cells (HLECs). **B**: Western blot analysis of amyloid-β precursor protein (APP) in cultured HLECs. **C**: β-secretase activity analysis in cultured HLECs. The concentration of H_2O_2 was 200 μM. The data are the means ± standard deviation (SD). Three independent tests were performed in each group for each experiment. *p<0.05, **p<0.01 with the independent samples *t* test.

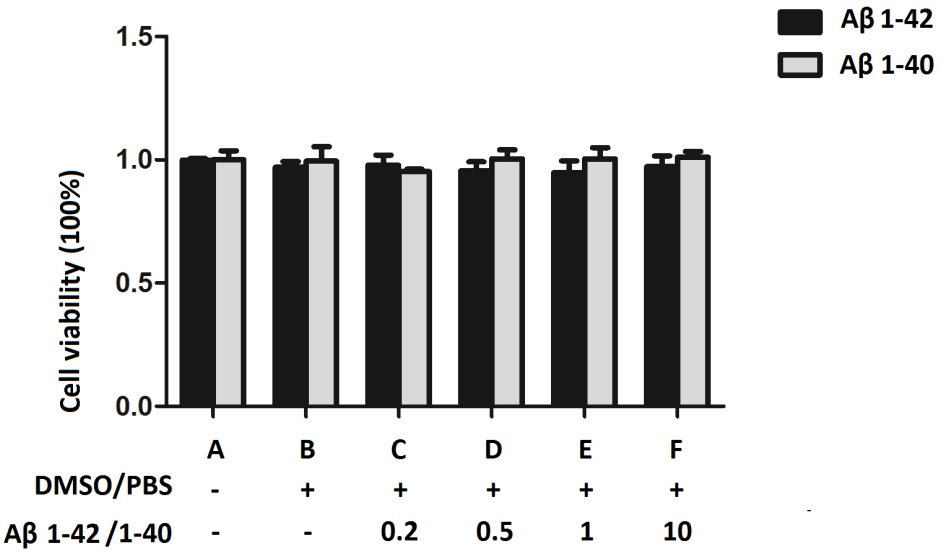

Figure 5. HLEC viability assay with intervention of β-amyloid (Aβ) peptide alone. A treatment with nanomolar concentrations (0.2 nM to 10 nM) of Aβ 1–42 or Aβ 1–40 peptides alone for 24 h incubation left cell viability unimpaired. The data are the means ± standard deviation (SD). In each group, three independent tests were performed. No statistically significant differences were found comparing the Aβ treatment groups with the untreated control group, with one-way ANOVA and least significant difference (LSD) tests ($p>0.05$).

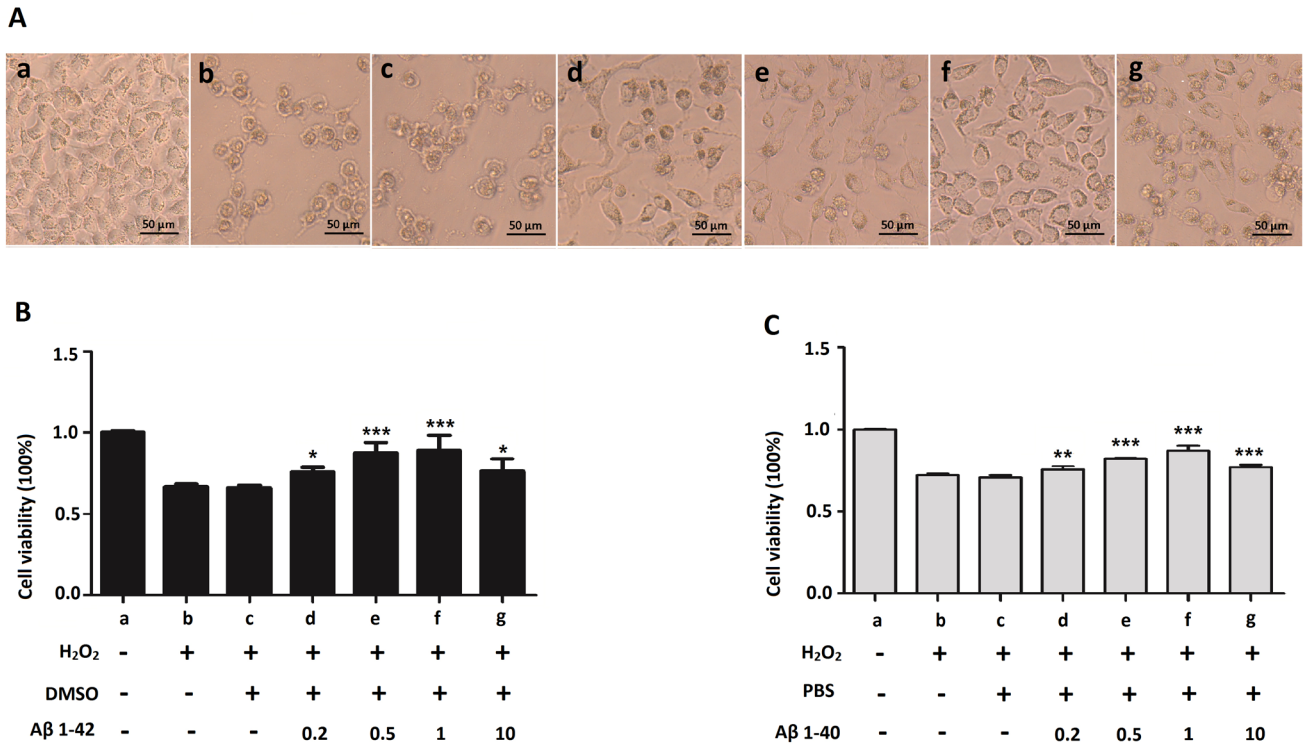

Figure 6. HLEC viability assay with or without Aβ intervention under oxidative stress. **A**: Inverted microscope investigation of cell morphology with or without β-amyloid (Aβ) 1–40 pretreatment under oxidative stress. **B**: Human lens epithelial cell (HLEC) viability assay with or without Aβ 1–42 pretreatment under oxidative stress. **C**: HLEC viability assay with or without Aβ 1–40 pretreatment under oxidative stress. The concentration of H_2O_2 was 200 µM. Aβ concentrations were 0.2 nM to 10 nM as listed in (**B**) and (**C**). The data are the means ± standard deviation (SD). In each group, three independent tests were performed. *p<0.05, **p<0.01, ***p<0.001 comparing the Aβ intervention groups with the vehicle control group, with one-way ANOVA and least significant difference (LSD) tests.

which is in agreement with a previous study demonstrating the association between decreased β-secretase activity and another age-related disease, frontotemporal dementia [45]. In contrast, in a lens study, Nagai et al. observed increased Aβ expression in the lens epithelia of UPL rats (a hereditary model for cataracts) [46]. We attribute these discrepancies to the different specimens examined in these studies. Nagai et al. used a hereditary cataract rat model that exhibits changes in the biologic and pathological characteristics of the lenses that do not entirely correspond to those of human ARC.

Of note, in the severe stage (Group IV) of ARC, Aβ 1–42 and Aβ 1–40 expression levels increased compared with those in the early and mid-stages (Groups I to III) of ARC, representing the restoration of the healthy levels. One potential explanation is the feedback effect; the decrease in Aβ in the early and mid-stages of ARC could induce a compensatory increase in the expression of its precursor protein, APP. In turn, this upregulation of APP may lead to an increase in Aβ during the severe stage (Group IV) of ARC, which conversely weakened the feedback loop and resulted in reduced APP. This hypothesis is consistent with AD studies that proposed that the overproduction of Aβ in AD is the result of a compensatory effect [31,42]. However, the current study did not include subjects at the terminal stage of cataract, the hypermature cataract, which is rarely seen in the clinic currently; thus, whether the production of Aβ would continue to increase from Group IV to hypermature cataract, becoming higher than that in the healthy group and leading to abnormal Aβ aggregation, which is observed in AD [47-49], is unknown.

In the present study, we found nanomolar concentrations (0.2 nM to 10 nM) of Aβ exhibit no toxicity on cultured HLECs, consistent with previous studies in SH-SY5Y cells [50,51]. Furthermore, we found that Aβ 1–42 and Aβ 1–40 at low nanomolar concentrations (0.2 nM to 10 nM) reduced oxidative damage in HLECs. Aβ pretreatment could protect HLECs against the morphology changes and cell viability reduction induced by H_2O_2 treatment in a concentration-dependent manner. Consistently, previous studies have demonstrated that low concentrations of Aβ (10 pM to 1 nM) could play a protective role against the toxicity caused by the inhibition of Aβ production in neuronal cells [32]. Kontush et al. also suggested that Aβ may well function as a physiologic antioxidant for cerebrospinal fluid (CSF) lipoproteins at the concentrations of 0.1–1 nM [23]. Decreased CSF concentration of Aβ frequently detected in AD along with a positive correlation between the Aβ level and CSF resistance to oxidation are in line with these findings [23,44]. One possible mechanism of this protective function could involve the gene regulatory role of Aβ. As presented in Figure 1B, Aβ was highly expressed in HLEC nuclei in healthy subjects and accumulated more in the cytoplasm in severe ARC samples, indicating that Aβ might be more active in gene regulation in healthy lenses. In agreement with this hypothesis, a previous study demonstrated the nuclear localization of the internalized Aβ peptide [50]. Aβ likely contains a helix–loop–helix structure, which is common in certain transcription factors [50,52]. In addition, chromatin immunoprecipitation (ChIP) experiments have confirmed that Aβ is a regulator of gene expression [50]. Another recent microarray analysis identified changes in the expression levels of 225 genes in response to Aβ treatment, including upregulation of *insulin-like growth factor binding protein-3/5* (*IGFBP3/5*) [51], which has a known antioxidative effect [53,54]. Therefore, we speculated that the decreased expression of physiologically generated Aβ in the early and mid-stages of ARC development might be one of the mechanisms accelerating the oxidative damage in HLECs in cataractogenesis. The mechanism by which nanomolar concentrations of Aβ protect HLECs against oxidative damage merits additional investigations.

In contrast, other studies have shown that Aβ at micromolar concentrations (5, 10, and 25 μM) can increase the apoptosis rate in neuronal cells, together with decreased cell viability, increased reactive oxygen species levels, and mitochondrial defects [55-57]. In addition, Aβ at concentrations of 20 μg/ml (equal to 5 μM) and 0.2 μM was observed to induce the apoptosis and degeneration of LECs and lens opacity [10,18]. Another example of the dosage-dependent bilateral role of Aβ is that high concentrations of Aβ are detrimental to cognition, whereas low concentrations of Aβ play a positive, modulatory role in neurotransmission and memory [35]. The opposing roles of Aβ could be attributed to the conformational changes in Aβ at different concentrations and conditions. High levels of incubated Aβ were used in these studies to identify toxic actions of Aβ. After incubation, increased Aβ accumulation leads to the formation of oligomers, followed by fibrils, and ultimately, plaques [28,58,59]. Consistently, Zou et al. proposed a novel concept that the biologic action of Aβ is dualistic. Aβ monomer functions as an antioxidant molecule, preventing the generation of oxygen radicals, whereas oligomerized or aggregated Aβ not only loses its antioxidant activity but also promotes the generation of oxygen radicals, disrupts lipid homeostasis, and ultimately, exhibits neurotoxicity [28]. Zou et al. assumed that oxygen radicals generated in an age-dependent manner contributed to generation of Aβ in patients with AD, which may protect neurons against oxygen radical toxicity. However, with the increasing amount of Aβ serving as an antioxidant, Aβ aggregates in extracellular local fluid with longer incubation

periods and in turn, exhibits neurotoxicity [28]. Giuffrida et al. even raised a "loss-of-function" hypothesis, suggesting that the pathological aggregation of Aβ may induce neurodegeneration by depriving neurons of the protective activity of Aβ monomers [60]. However, few studies on the protection of Aβ monomers against oxidative stress on HLECs have been conducted. Further studies are needed to determine whether the monomer and polymer forms of Aβ exert different influences on cell functions of HLECs.

The limitation of the present study was that we did not include a group with an increasing severity than Group IV (the last stage of cataract), including the cataracts of N6 and hypermature cataracts, which were rarely seen in our clinic, due to the earlier timing of the current cataract surgeries. The lack of investigation of the last stage of cataract limits the full understanding of the changing tendency of Aβ and APP during the progress of cataract. Further studies comparing Aβ and APP expression at all stages of ARC should be conducted, based on a continuous and long-term collection of specimens.

In summary, Aβ and APP expression levels exhibited differential changes during the development of ARC, indicating active feedback of this protein processing. The decreased expression of physiologically generated Aβ in the early and mid-stages of ARC development might be one of the mechanisms accelerating the oxidative damage in HLECs during cataractogenesis.

APPENDIX 1. STR ANALAYSIS.

To access the data, click or select the words "Appendix 1"

APPENDIX 2. B-AMYLOID (AB) 1-42 EXPRESSION LEVELS IN AQUEOUS HUMOR OF HEALTHY AND CATARACT EYES.

The cortical (C), nuclear (N) or posterior subcapsular (P) opacity of the cataract was classified with the Lens Opacities Classification System III (LOCS III), with an increasing cataract severity from Group I to Group IV. Group I: C2-3N1-2; Group II, C3-4N2-3; Group III, C4-5N3-4; Group IV, C4-5N4-5; Group V, posterior subcapsular senile cataract (P2-P4, with C<2 and N<2); Group VI, cataract with myopia; Group VII, cataract with diabetes; and the healthy Group, healthy tissue samples. In each group, three independent experiments were performed. The data are the means ± standard deviation (SD). No significant differences among all eight groups were seen, by one-way ANOVA and least significant difference (LSD) tests ($p>0.05$). Healthy samples were used as normal controls. To access the data, click or select the words "Appendix 2"

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (NSFC; grant numbers 81,270,989 and 81,300,747), the Specialized Research Fund for the Doctoral Program of Higher Education (SRFDP; grant number20130071120096) and the Excellent Young Doctor Training Program of Shanghai (2015). We would like to thank all the participants and the staff for their valuable contribution to this research. The authors declare that there are no conflicts of interest to disclose regarding this work, including no personal financial interests and no commercial financial support from a for-profit company. Tianyu Zheng (susu0102@163.com) and Yi Lu (luyieent@126.com) are both the co-corresponding authors of this work.

REFERENCES

1. De Strooper B, Annaert W. Proteolytic processing and cell biological functions of the amyloid precursor protein. J Cell Sci 2000; 113:1857-[PMID: 10806097].
2. Ida N, Hartmann T, Pantel J, Schroder J, Zerfass R, Forstl H, Sandbrink R, Masters CL, Beyreuther K. Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. J Biol Chem 1996; 271:22908-14. [PMID: 8798471].
3. Carrell RW, Lomas DA. Conformational disease. Lancet 1997; 350:134-8. [PMID: 9228977].
4. Goldstein LE, Muffat JA, Cherny RA, Moir RD, Ericsson MH, Huang X, Mavros C, Coccia JA, Faget KY, Fitch KA, Masters CL, Tanzi RE, Chylack LT Jr, Bush AI. Cytosolic beta-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer's disease. Lancet 2003; 361:1258-65. [PMID: 12699953].
5. Melov S, Wolf N, Strozyk D, Doctrow SR, Bush AI. Mice transgenic for Alzheimer disease beta-amyloid develop lens cataracts that are rescued by antioxidant treatment. Free Radic Biol Med 2005; 38:258-61. [PMID: 15607908].
6. Moncaster JA, Pineda R, Moir RD, Lu S, Burton MA, Ghosh JG, Ericsson M, Soscia SJ, Mocofanescu A, Folkerth RD, Robb RM, Kuszak JR, Clark JI, Tanzi RE, Hunter DG, Goldstein LE. Alzheimer's disease amyloid-beta links lens and brain pathology in Down syndrome. PLoS One 2010; 5:e10659-[PMID: 20502642].
7. Haargaard B, Fledelius HC. Down's syndrome and early cataract. Br J Ophthalmol 2006; 90:1024-7. [PMID: 16672328].
8. Robb RM, Marchevsky A. Pathology of the Lens in Down's syndrome. Arch Ophthalmol 1978; 96:1039-42. [PMID: 148880].
9. Malt EA, Dahl RC, Haugsand TM, Ulvestad IH, Emilsen NM, Hansen B, Cardenas YE, Skøld RO, Thorsen AT, Davidsen EM. Health and disease in adults with Down syndrome. Tidsskr Nor Laegeforen 2013; 133:290-4. [PMID: 23381164].

10. Lee KW, Seomun Y, Kim DH, Park SY, Joo CK. Possible role of amyloid beta-(1–40)-BSA conjugates in transdifferentiation of lens epithelial cells. Yonsei Med J 2004; 45:219-28. [PMID: 15118992].

11. Wang X, Simpkins JW, Dykens JA, Cammarata PR. Oxidative damage to human lens epithelial cells in culture: estrogen protection of mitochondrial potential, ATP, and cell viability. Invest Ophthalmol Vis Sci 2003; 44:2067-75. [PMID: 12714645].

12. Li WC, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, Spector A, Leib M, Cotliar AM, Weiss M, Espy J, Howard G, Farris RL, Auran J, Donn A, Hofeldt A, Mackay C, Merriam J, Mittl R, Smith TR. Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. J Cell Biol 1995; 130:169-81. [PMID: 7790371].

13. Zhang J, Wu J, Yang L, Zhu R, Yang M, Qin B, Shi H, Guan H. DNA Damage in Lens Epithelial Cells and Peripheral Lymphocytes from Age-Related Cataract Patients. Ophthalmic Res 2014; 51:124-8. [PMID: 24457594].

14. Zhang Y, Zhang L, Zhang L, Bai J, Ge H, Liu P. Expression changes in DNA repair enzymes and mitochondrial DNA damage in aging rat lens. Mol Vis 2010; 16:1754-63. [PMID: 20808729].

15. Pendergrass W, Penn P, Possin D, Wolf N. Accumulation of DNA, nuclear and mitochondrial debris, and ROS at sites of age-related cortical cataract in mice. Invest Ophthalmol Vis Sci 2005; 46:4661-70. [PMID: 16303963].

16. Takagane K, Nojima J, Mitsuhashi H, Suo S, Yanagihara D, Takaiwa F, Urano Y, Noguchi N, Ishiura S. Abeta induces oxidative stress in senescence-accelerated (SAMP8) mice. Biosci Biotechnol Biochem 2015; 79:912-8. [PMID: 25612552].

17. Swomley AM, Forster S, Keeney JT, Triplett J, Zhang Z, Sultana R, Butterfield DA. Abeta, oxidative stress in Alzheimer disease: evidence based on proteomics studies. Biochim Biophys Acta 2014; 1842:1248-57. [PMID: 24120836].

18. Frederikse PH, Garland D, Zigler JJ, Piatigorsky J. Oxidative stress increases production of beta-amyloid precursor protein and beta-amyloid (Abeta) in mammalian lenses, and Abeta has toxic effects on lens epithelial cells. J Biol Chem 1996; 271:10169-74. [PMID: 8626578].

19. Cervellati C, Wood PL, Romani A, Valacchi G, Squerzanti M, Sanz JM, Ortolani B, Zuliani G. Oxidative challenge in Alzheimer's disease: state of knowledge and future needs. J Investig Med 2016; 64:21-32. [PMID: 26755810].

20. Niedzielska E, Smaga I, Gawlik M, Moniczewski A, Stankowicz P, Pera J, Filip M. Oxidative Stress in Neurodegenerative Diseases. Mol Neurobiol 2016; 53:4094-125. [PMID: 26198567].

21. Cencioni C, Spallotta F, Martelli F, Valente S, Mai A, Zeiher AM, Gaetano C. Oxidative stress and epigenetic regulation in ageing and age-related diseases. Int J Mol Sci 2013; 14:17643-63. [PMID: 23989608].

22. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci 1991; 12:383-8. [PMID: 1763432].

23. Kontush A, Berndt C, Weber W, Akopyan V, Arlt S, Schippling S, Beisiegel U. Amyloid-beta is an antioxidant for lipoproteins in cerebrospinal fluid and plasma. Free Radic Biol Med 2001; 30:119-28. [PMID: 11134902].

24. Murphy MP, Beckett TL, Ding Q, Patel E, Markesbery WR, St CD, LeVine H 3rd, Keller JN. Abeta solubility and deposition during AD progression and in APPxPS-1 knock-in mice. Neurobiol Dis 2007; 27:301-11. [PMID: 17651976].

25. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 2003; 300:486-9. [PMID: 12702875].

26. Selkoe DJ. Alzheimer's disease is a synaptic failure. Science 2002; 298:789-91. [PMID: 12399581].

27. Chen G, Chen KS, Knox J, Inglis J, Bernard A, Martin SJ, Justice A, McConlogue L, Games D, Freedman SB, Morris RG. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. Nature 2000; 408:975-9. [PMID: 11140684].

28. Zou K, Gong JS, Yanagisawa K, Michikawa M. A novel function of monomeric amyloid beta-protein serving as an antioxidant molecule against metal-induced oxidative damage. J Neurosci 2002; 22:4833-41. [PMID: 12077180].

29. Reitz C. Alzheimer's Disease and the Amyloid Cascade Hypothesis: A Critical Review. Int J Alzheimers Dis 2012; 2012:1-11. [PMID: 22506132].

30. Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI. Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. J Neurol Neurosurg Psychiatry 1994; 57:419-25. [PMID: 8163989].

31. Regland B, Gottfries CG. The role of amyloid beta-protein in Alzheimer's disease. Lancet 1992; 340:467-9. [PMID: 1354793].

32. Plant LD, Boyle JP, Smith IF, Peers C, Pearson HA. The production of amyloid beta peptide is a critical requirement for the viability of central neurons. J Neurosci 2003; 23:5531-5. [PMID: 12843253].

33. Blair JA, Siedlak SL, Wolfram JA, Nunomura A, Castellani RJ, Ferreira ST, Klein WL, Wang Y, Casadesus G, Smith MA, Perry G, Zhu X, Lee HG. Accumulation of intraneuronal amyloid-beta is common in normal brain. Curr Alzheimer Res 2014; 11:317-24. [PMID: 24597504].

34. Morley JE, Farr SA, Banks WA, Johnson SN, Yamada KA, Xu L. A physiological role for amyloid-beta protein:enhancement of learning and memory. J Alzheimers Dis 2010; 19:441-9. [PMID: 19749407].

35. Puzzo D, Privitera L, Leznik E, Fa M, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. J Neurosci 2008; 28:14537-45. [PMID: 19118188].

36. Herrup K. The case for rejecting the amyloid cascade hypothesis. Nat Neurosci 2015; 18:794-9. [PMID: 26007212].

37. Castello MA, Jeppson JD, Soriano S. Moving beyond anti-amyloid therapy for the prevention and treatment of Alzheimer's disease. BMC Neurol 2014; 14:169-[PMID: 25179671].

38. Bemporad F, Chiti F. Protein misfolded oligomers: experimental approaches, mechanism of formation, and structure-toxicity relationships. Chem Biol 2012; 19:315-27. [PMID: 22444587].

39. Li D, Qiu X, Yang J, Liu T, Luo Y, Lu Y. Generation of Human Lens Epithelial-Like Cells From Patient-Specific Induced Pluripotent Stem Cells. J Cell Physiol 2016; 231:2555-62. [PMID: 26991066].

40. Prakasam A, Muthuswamy A, Ablonczy Z, Greig NH, Fauq A, Rao KJ, Pappolla MA, Sambamurti K. Differential accumulation of secreted AbetaPP metabolites in ocular fluids. J Alzheimers Dis 2010; 20:1243-53. [PMID: 20413851].

41. Asami-Odaka A, Ishibashi Y, Kikuchi T, Kitada C, Suzuki N. Long amyloid beta-protein secreted from wild-type human neuroblastoma IMR-32 cells. Biochemistry 1995; 34:10272-8. [PMID: 7640283].

42. Puzzo D, Gulisano W, Arancio O, Palmeri A. The keystone of Alzheimer pathogenesis might be sought in Aβ physiology. Neuroscience 2015; 307:26-36. [PMID: 26314631].

43. Yu Y, Sun X, Tang D, Li C, Zhang L, Nie D, Yin X, Shi G. Gelsolin bound β-amyloid peptides(1–40/1–42): Electrochemical evaluation of levels of soluble peptide associated with Alzheimer's disease. Biosens Bioelectron 2015; 68:115-21. [PMID: 25562737].

44. Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. NeuroRx 2004; 1:213-25. [PMID: 15717022].

45. Alcolea D, Carmona-Iragui M, Suárez-Calvet M, Sánchez-Saudinós MB, Sala I, Antón-Aguirre S. Blesa R1\, Clarimón J, Fortea J, Lleó A. Relationship between beta-Secretase, inflammation and core cerebrospinal fluid biomarkers for Alzheimer's disease. J Alzheimers Dis 2014; 42:157-67. [PMID: 24820015].

46. Nagai N, Ito Y. Excessive hydrogen peroxide enhances the attachment of amyloid beta1–42 in the lens epithelium of UPL rats, a hereditary model for cataracts. Toxicology 2014; 315:55-64. [PMID: 23941810].

47. Savioz A, Giannakopoulos P, Herrmann FR, Klein WL, Kovari E, Bouras C, Giacobini E. A Study of Abeta Oligomers in the Temporal Cortex and Cerebellum of Patients with Neuropathologically Confirmed Alzheimer's Disease Compared to Aged Controls. Neurodegener-Dis 2016; 16:398-406. [PMID: 27400224].

48. Ingelsson M, Fukumoto H, Newell KL, Growdon JH, Hedley-Whyte ET, Frosch MP, Albert MS, Hyman BT, Irizarry MC. Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. Neurology 2004; 62:925-31. [PMID: 15037694].

49. D'Andrea MR, Nagele RG, Wang HY, Peterson PA, Lee DH. Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer's disease. Histopathology 2001; 38:120-34. [PMID: 11207825].

50. Barucker C, Harmeier A, Weiske J, Fauler B, Albring KF, Prokop S, Hildebrand P, Lurz R, Heppner FL, Huber O, Multhaup G. Nuclear Translocation Uncovers the Amyloid Peptide A 42 as a Regulator of Gene Transcription. J Biol Chem 2014; 289:20182-91. [PMID: 24878959].

51. Barucker C, Sommer A, Beckmann G, Eravci M, Harmeier A, Schipke CG, Brockschnieder D, Dyrks T, Althoff V, Fraser PE, Hazrati LN, George-Hyslop PS, Breitner JC, Peters O, Multhaup G. Alzheimer amyloid peptide abeta42 regulates gene expression of transcription and growth factors. J Alzheimers Dis 2015; 44:613-24. [PMID: 25318543].

52. Sticht H, Bayer P, Willbold D, Dames S, Hilbich C, Beyreuther K, Beyreuther K, Frank RW, Rösch P. Structure of amyloid A4-(1–40)-peptide of Alzheimer's disease. Eur J Biochem 1995; 233:293-8. [PMID: 7588758].

53. Natsuizaka M, Kinugasa H, Kagawa S, Whelan KA, Naganuma S, Subramanian H, Chang S, Nakagawa KJ, Rustgi NL, Kita Y, Natsugoe S, Basu D, Gimotty PA, Klein-Szanto AJ, Diehl JA, Nakagawa H. IGFBP3 promotes esophageal cancer growth by suppressing oxidative stress in hypoxic tumor microenvironment. Am J Cancer Res 2014; 4:29-41. [PMID: 24482736].

54. Sokolovic A, Montenegro-Miranda PS, de Waart DR, Cappai RM, Duijst S, Sokolovic M, Bosma PJ. Overexpression of insulin like growth factor binding protein 5 reduces liver fibrosis in chronic cholangiopathy. Biochim Biophys Acta 2012; 1822:996-1003. [PMID: 22434064].

55. Kim DI, Lee KH, Gabr AA, Choi GE, Kim JS, Ko SH, Han HJ. Abeta-Induced Drp1 phosphorylation through Akt activation promotes excessive mitochondrial fission leading to neuronal apoptosis. Biochim Biophys Acta 2016; 1863:2820-34. [PMID: 27599716].

56. Cui W, Zhang Y, Yang C, Sun Y, Zhang M, Wang S. Hydrogen sulfide prevents Abeta-induced neuronal apoptosis by attenuating mitochondrial translocation of PTEN. Neuroscience 2016; 325:165-74. [PMID: 27026591].

57. Kim J, Nam Y, Jeon S, Han H, Suk K. Amyloid neurotoxicity is attenuated by metallothionein: dual mechanisms at work. J Neurochem 2012; 121:751-62. [PMID: 22404335].

58. Zhou Y, Liu L, Hao Y, Xu M. Detection of Aβ Monomers and Oligomers: Early Diagnosis of Alzheimer's Disease. Chem Asian J 2016; 11:805-17. [PMID: 26994700].

59. Rauk A. The chemistry of Alzheimer's disease. Chem Soc Rev 2009; 38:2698-[PMID: 19690748].

60. Giuffrida ML, Caraci F, Pignataro B, Cataldo S, De Bona P, Bruno V, Molinaro G, Pappalardo G, Messina A, Palmigiano A, Garozzo D, Nicoletti F, Rizzarelli E, Copani A. β -Amyloid Monomers Are Neuroprotective. J Neurosci 2009; 29:10582-7. [PMID: 19710311].

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 25 December 2017. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.