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Possible Role of Amyloid β-(1-40)-BSA Conjugates in Transdifferentiation of Lens Epithelial Cells

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We investigated whether amyloid β (Aβ) aggregates have transforming growth factor β - like cytokine activity and cause transdifferention of lens epithelial cells, leading to certain types of cataract. In order to mimic Aβ aggregates, Aβ-(1-40) was crosslinked to bovine serum albumin (BSA) with disuccinimidyl suberate according to a previously described procedure. When human lens epithelial B-3 (HLE B-3) cells were treated with the Aβ-(1-40)-BSA conjugates, we observed the translocation of Smad-3, as well as the induced mRNA levels of fibronectin (FN), collagen type I (Col I), smooth muscle actin (SMA) and matrix metalloproteinase-2 (MMP-2). In addition, we investigated the morphology of rat whole lens cultured for 5 days in the presence of Aβ-(1-40)-BSA, and the immunohistochemical localizations of Aβ-(1-40)/amyloid precursor protein (APP) in human clinical tissues beneath the anterior capsules. In rat whole lens cultures, treatment with Aβ-(1-40)-BSA produced a transformed morphology that had multiple layers of lens epithelial cells. To compare the anterior capsules in anterior subcapsular cataracts with those in nuclear cataracts, immunohistochemical studies of Aβ/APP in human clinical tissues revealed that the predominant immunostaining of Aβ occurs in the anterior epithelial plaques, which likely produces the abnormal extracellular matrix. Thus, these findings suggest that Aβ aggregates *in vivo* are possibly involved in the regulatory process by which lens epithelial cells may transdifferentiate into fibroblast-like cells, as well as help understand the mechanisms which lead to certain types of cataractogenesis.

Key Words: Amyloid, cataractogenesis, epithelial cells, extracellular matrix, growth factors, lens differentiation

INTRODUCTION

Cataract is one of the most common causes of visual disturbance leading to partial or total blindness. Although cataract surgery is widely performed in order to eliminate the resulting blindness, the operation costs are high.[1] In addition, cataract is the leading cause of blindness in the world, affecting 16 - 20 million people in developing countries.[2] Cataract seen in the elderly is usually attributed to the normal aging process. However, cataract seen at a younger age is usually caused by many processes of eye-related diseases such as uveitis, other general diseases like diabetes, hereditary causes, injury, radiation and infections.

The lens surrounded by its capsule is composed of two types of epithelial cells: a monolayer of cells covering the anterior surface of the lens, and fiber cells which synthesize new crystallins and make up the body of the lens.[3] An interruption of the orderly process of mitosis and differentiation of lens epithelial cells could affect the transparency of the lens. Lens epithelial cells are known to transdifferentiate into mesenchyme-like cells, and lead to the formation of anterior subcapsular cataract (APC), and the posterior capsular opacification (PCO) that often arises from the lens epithelial cells that remain after cataract surgery.[4,5] Cytokines, such as transforming growth factor β

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(TGF-β), play an important role in the formation of these forms of cataract.[6] Abnormal extracellular matrix (ECM) components, such as fibronectin (FN) and collagen type I (Col I) are deposited and accumulate around the transdifferentiated cells,[7] and smooth muscle actin (SMA), a cytoskeletal component, is localized in spindle-shaped cells in these cataracts.[6] Thus, the biochemical and molecular changes in various kinds of lens epithelial cells and the clinical manifestations of cataracts are considered to play a central role in these types of cataractogenesis.

Amyloid β (Aβ) of a 39-43 amino acid proteolytic fragment is derived by proteolysis of an integral membrane protein identified as the amyloid precursor protein (APP),[8] and is the major constituent of senile plaques found in Alzheimer's disease (AD).[9,10] Huang et al.[11] have proposed that Aβ aggregates may act as TGF-β antagonists, and partial agonists based on a motif (FAED) of Aβ which is similar to the TGF-β active-site motif (WSXD). In their experiment, a multivalent synthetic analogue of Aβ inhibited TGF-β binding to TGF-β receptors 100-fold greater than did the Aβ-(1-40) monomer. On the other hand, Goldstein et al.[12] reported that Aβ-(1-40) occurs in lenses with and without Alzheimer's disease at concentrations comparable to those in the brain, and the levels of amyloid in aqueous humour are comparable to those in cerebrospinal fluid. Also in their study, Aβ-(1-40) accumulated in lenses from Alzheimer's patients as deposits located exclusively in the cytoplasm of supranuclear/deep cortical lens fiber cells, and the Aβ-promoted lens protein aggregation.

These findings led us to propose that Aβ aggregates with TGF-β-like activity in the lens mediate transdifferentiation of lens epithelial cells, potentially leading to the pathologic development of a distinct type of cataract involving lens epithelial cells. In the present study, using mRNA analysis, lens organ culture and immunohistochemical staining of Aβ/APP from clinically varied cataracts, we investigated the possibility of Aβ aggregates being involved in the regulatory process by which lens epithelial cells may transdifferentiate into fibroblast-like cells.

MATERIALS AND METHODS

Cell culture conditions

Human lens epithelial B-3 (HLE B-3) cell line was kindly provided by Dr. Usha Andley, and maintained as previously described.[13] Cultures were maintained in minimum essential medium (MEM; Invitrogen, Carlsbad, CA, U.S.A.) containing 20% fetal bovine serum (Invitrogen) and 50 μg/mL gentamycin (Sigma, St. Louis, MO, U.S.A.) at 37℃ in humidified 5% CO_2, 95% air.

Treatment of cells

HLE B-3 cells were seeded at 1×10^6 cells in 60 mm Petri dishes coated with type IV collagen (10 μg/mL; Sigma) (100 mm Petri dishes used in Smad-3 experiment), and incubated at 37℃ for 18 hours. Then, the culture medium was changed to MEM supplemented with 1% FBS, and the cells were returned to incubation. After incubation for 24 hours, the culture medium was changed to serum-free MEM, and the cells were treated with 400 pM human recombinant TGF-β1 (Sigma), or with varying concentrations of 1, 10, 100 or 200 nM Aβ-(1-40)-BSA conjugates for the indicated times.

Preparation of Aβ-(1-40)-BSA conjugates

Aβ-(1-40)-BSA conjugates were prepared according to Huang et al's method.[11] Briefly, 167 μl of H_2O containing 0.14 μmol of Aβ-(1-40) was mixed with 283 μl of 0.1 M $NaHCO_3$ (pH 8.0) in which 2.1 nmol of BSA were dissolved. After adjusting pH to 8.0, the solution was combined with 10 μl of 27 mM disuccinimidyl suberate in DMSO. Then, the reaction mixture was mixed at 4℃ for 16 hours followed by the addition of 50 μl of 1M ethanolamine with further mixing at room temperature for 2 hours. The mixture was dialyzed against three changes of 2L of 0.1 M $NaHCO_3$, pH 8.0. The BSA conjugates were kept at 4℃ prior to use, and 5-7 peptides per protein molecule were confirmed based on analyses of SDS-PAGE as Huang et al. have reported.[11]

Western blot analysis

To detect the Smad-3 translocation, the cytoplasmic and nuclear extracts were prepared separately. HLE B-3 cells were lysed in 500 μl of cytoplasmic extract buffer (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF)) containing 0.6% NP-40, and placed on ice for 15 mins. After centrifuging the suspension at 13,000 rpm for 10 mins, the supernatant was collected for cytoplasmic proteins. The pellet containing nuclear extract was resuspended in 50 μl of nuclear extract buffer (20 mM HEPES, pH 7.9, 400 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM PMSF), and incubated for 30 mins at 4℃. The supernatant was collected after centrifugation at 13,000 rpm for 20 mins at 4℃ as nuclear proteins, and stored at -70℃. The amount of protein was determined using BCA protein assay kit (Sigma). Protein (20 μg) from each sample was loaded on 10% SDS polyacrylamide gels for electrophoresis. After running the gel, the proteins were transferred to nitrocellulose membranes (Hybond-C, Amersham Pharmacia Biotech, Uppsala, Sweden) at 300 mA for two hours. Western blot analysis was performed after blocking with 5% skim milk. The membrane was incubated with rabbit anti-Smad 3 (1 : 1,000) (Zymed, San Francisco, CA, U.S.A.) for one hour, and washed three times in PBST (PBS containing 0.1% Tween 20) buffer. Then, the membrane was incubated with horseradish peroxidase (HRP) conjugated anti-rabbit IgG (1 : 20,000) (Zymed) for 50 mins. After washing for 10 mins with PBST buffer and repeating three times, the HRP activity was visualized by applying chemiluminescent substrate (ECL, Amersham, Arlington Heights, IL, U.S.A.) followed by exposing the membrane to X-ray film.

Amplification of reverse- transcribed RNA by PCR

Total cellular RNA was isolated from lens epithelial cells by using TRIzoL reagent (Invitrogen). One μg of RNA was reverse-transcribed in a 20 μL reaction mixture by using a kit (1st Strand cDNA Synthesis; Roche, Mannheim, Germany). Then, the prepared cDNA (0.2 to 1 μL) was amplified in a 20 μL reaction mixture. Conditions for PCR were as follows: 0.4 μM each 5′ and 3′ primers, 0.2 mM dNTP (Perkin-Elmer, Foster City, CA, U.S.A.), 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 1.0 U of Taq DNA polymerase (Perkin-Elmer, Norwalk CT, U.S.A.). Reaction mixtures were incubated in a thermal controller (Model PTC-100; MJ Research, Watertown, MA, U.S.A.) for 25 to 35 cycles (denaturation at 94℃ for 45 s, annealing at 60℃ for 30 s, extension at 72℃ for 45 s). The amounts of amplified products were analyzed using an image analyzer (ImageMaster VDS; Amersham Pharmacia Biotech). DNA size markers were run in parallel to validate the predicted sizes of the amplified bands (1 kb DNA marker; Invitrogen). The primer sequences specific for the genes examined and the predicted product sizes were as follows: 5′-atcatgtttgagaccttcaacacc-3′ (sense), 5′-catggtggtgccgccagacag-3′ (antisense), 350 bp for β-actin and 5′-ccatcaccaacaacatccag-3′ (sense), 5′-gagtttccagggtctgtcca-3′ (antisense), 660 bp for FN and 5′-tccaaaggagagagcggtaa-3′ (sense), 5′-gacca gggagaccaaactca-3′ (antisense), 693 bp for Col I and 5′-cccagccaagcactgtca-3′ (sense), 5′-tccagagtc cagcacgatg-3′ (antisense), 516 bp for SMA, and 5′-cttcttcagcatcaccaagg-3′ (sense), 5′-ctttgaccgaaac gaaaacc-3′ (antisense), 450 bp for APP. All primer sequences, except those for β-actin, were designed using Primer 3 primer-picking software obtained through a website operated by the Center for Genome Research at the Whitehead Institute for Biomedical Research (Cambridge, MA, U.S.A.).[14]

Northern blot analysis

Total RNA (10 μg/lane) was electrophoresed on formaldehyde/1% agarose gels, capillary transferred to a nitrocellulose membrane (Optitran BA-S; Schleicher & Schuell, Dassel, Germany) using TurboBlotter transfer systems (Schleicher & Schuell), and fixed by UV cross-linking (Stratalinker; Stratagene, La Jolla, CA, U.S.A.). cDNA probes (25 ng) for MMP-2 and β-actin were labeled with 50 μCi of [γ-^{32}P] dCTP (Amersham Pharmacia Biotech) by random priming (Random primed DNA labeling kit; Roche.). Blots were

prehybridized at 42℃ for 3 hours in 50% formamide, 5 × SSPE, 10 × Denhardt's solution, and 0.5% SDS, and then hybridized with [γ-^{32}P] dCTP-labeled probes for 16 hours. Blots were then washed with 0.2 × SSC and 0.1% SDS for 1 hour at 65℃ and exposed to autoradiographic film. Equal loading was assessed by hybridization with β-actin probe. Human MMP-2 cDNA clone was generously provided by Dr. Gregory I. Goldberg, Washington University School of Medicine, St. Louis, MO.

Lens organ culture and immunohistochemistry

All procedures in this study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Whole lenses were cultured as previously reported.[7,15] Briefly, lenses were dissected from rats (3-week-old Sprague-Dawley) and incubated in Medium 199 (Sigma) containing 0.1% bovine serum albumin and 1% penicillin-streptomycin. Aβ-(1-40)-BSA conjugates were added to the medium at 200 nM, and medium was changed every two days with the second addition of Aβ-(1-40)-BSA conjugates. Control lenses were cultured with the addition of 200 nM BSA. Damaged lenses that developed haziness after overnight incubation were discarded. After the indicated culture periods, the lenses were fixed in Carnoy's fixative (acetic acid/ethanol, 1 : 3, vol/vol) and embedded in paraffin. The paraffin-embedded lenses were sectioned on a microtome at a thickness of 5μm, deparaffinized in xylene for 10 mins, and rehydrated in alcohol for routine hematoxylin and eosin staining and immunolocalization of Aβ/APP. For the immunohistochemical analysis, the lens sections were incubated in 2% H_2O_2 for 5 mins, 20% normal horse serum for 10 mins, and 1 : 200 dilution of rabbit anti-pan β-amyloid antibody (Calbiochem, San Diego, CA, U.S.A.) for 2 hours. The sections were then incubated in anti- rabbit IgG-HRP (Amersham Pharmacia Biotech) for 10 mins, and then visualized according to the manufacturer's protocol using 3,3′-diaminobenzidine tetrahydrochloride substrate kit (Zymed).

RESULTS

Translocation of Smad-3 into nucleus by Aβ-(1-40)-BSA conjugates in HLE B-3 cells

The Smad family of proteins has been known to mediate the TGF-β signal from the cytoplasm to the nucleus.[16] Among Smads, Smad-2 or Smad-3 substrates are phosphorylated by the formation of TGF-β receptor/ligand complexes, followed by the addition of Smad-4, which allowed entry of the Smad complex into the nucleus. We investigated whether the translocation of Smad-3 from the cytoplasm into the nucleus would occur in HLE B-3 cells treated with 100 nM Aβ-(1-40)-BSA conjugates (Fig. 1). After 1 hour of exposure to either Aβ-(1-40)-BSA conjugates or TGF-β1, Smad-3 was translocated into the nucleus. However, when the control was treated only with BSA no translocation of Smad-3 was observed, suggesting that Aβ-(1-40)-BSA conjugates may have cellular effects through TGF-β receptors. Smad-3 levels, which appear as a doublet in the nuclear fraction, are possible due to cross reactivity of the antibody with another Smad.

Enhanced expression of FN, Col I and SMA mRNAs in HLE B-3 cells

Human lens epithelial cells of APC specifically

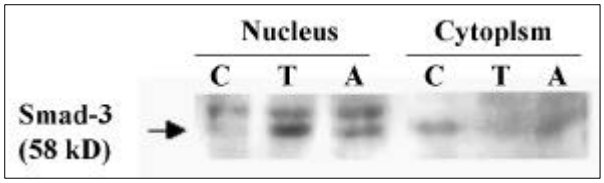

Fig. 1. Translocation of smad-3 by amyloid β-(1-40)-BSA conjugates (Aβ-(1-40)-BSA conjugates) in human lens epithelial cells (HLE-B3). HLE B-3 cells were seeded in 100 mm Petri dishes coated with type IV collagen at 1.0 × 10^6 cells/well, and incubated at 37℃ for 18 hours. And then the culture medium was changed to MEM supplemented with 1% FBS, and the cells were incubated. After incubation for 24 hours, the culture medium was changed to serum-free MEM, the cells were treated with or without 100 nM Aβ-(1-40)-BSA conjugates or with 400 pM TGF-β1. After exposure for 1 hour of treatment, nuclear and cytoplasmic proteins of the treated HLE-B3 cells were extracted for the analysis of Smad-3 translocation as described under "Methods". C, control cells; A, cells cultured with 100 nM Aβ-(1-40)-conjugates; T, cells cultured with 400 pM TGF-β1

express ECM-related markers such as FN, Col I and SMA mRNAs when compared with nuclear cataracts (NC).[6] We have investigated the production of mRNAs for the fibrotic markers in HLE B-3 cells in response to treatment with Aβ-(1-40)-BSA conjugates, as well as TGF-β1 (Fig. 2). Exposure to Aβ-(1-40)-BSA conjugates, as well as TGF-β resulted in significant induction of FN, Col I and SMA mRNAs.

MMP-2 mRNA induction by Aβ-(1-40)-BSA conjugates in HLE B-3 cell

TGF-β reportedly stimulates the proteinase cascades that degrade ECM during tissue remolding and cell migration, and induces MMP-2 specifically in primary cultures of chicken lens annular cells.[17] Also, our preliminary experiments showed that the treatment of HLE B-3 cells with TGF-β remarkably induced MMP-2 mRNA. Thus, we explored whether Aβ-(1-40)-BSA conjugates enhance the expression of the MMP-2 mRNA level. As shown in Fig. 3, the MMP-2 mRNA expression was most prominent in cultured HLE B-3 cells with 400 pM TGF-β1, and 100 or 200 nM Aβ-(1-40)-BSA conjugates also increased the mRNA level of MMP-2.

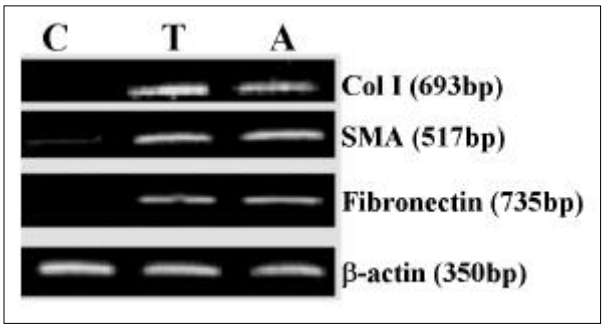

Fig. 2. Induction by amyloid β-(1-40)-BSA conjugates (Aβ-(1-40)-BSA conjugates) of fibronectin (FN), collagen type I (Col I) and α-smooth muscle actin (SMA) mRNAs in lens epithelial cells. HLE B-3 cells were seeded in 6-well plates coated with type IV collagen at 3.0×10^5 cells/well, and incubated at 37℃ for 18 hours. Then, the culture medium was changed to MEM supplemented with 1% FBS, and the cells were incubated. After incubation for 24 hours, the culture medium was changed to serum-free MEM, the cells were treated with or without 200 nM Aβ-(1-40)-BSA conjugates or with 400 pM TGF-β1. After 20 hours total RNA was isolated, and subjected to RT-PCR analysis to determine mRNA expression of the extracellular matrix proteins as described under "Methods". Data shown are from one of three independent experiments that produced similar results. M, molecular size standards (base pairs); C, control cells; A, cells cultured with 200 nM Aβ-(1-40)-conjugates; T, cells cultured with 400 pM TGF-β1.

Rat lens organ culture with Aβ-(1-40)-BSA conjugates

Rat lenses cultured without the addition of Aβ-(1-40)-BSA conjugates displayed a normal monolayer of lens epithelial cells (Fig. 4A). Lenses incubated for 5 days with Aβ-(1-40)-BSA conjugates developed the spindle-shaped lens epithelial cells in multilayers of 2 to 3 cells deep (Fig. 4B). This suggests that Aβ-(1-40)-BSA conjugates may participate in the pathogenesis of anterior subcapsular cataract.

Expression of APP mRNA and enhanced expression of Aβ/APP in human lens epithelial cells obtained from patients with APC

Next, we determined mRNA levels of APP in human lens epithelial cells of APC and NC. Fig. 5 indicates that the expression levels of APP mRNA were similar in lens epithelial cells from both types of cataracts. Subsequently, we examined whether APP is expressed in lens samples obtained from patients with APC and NC by

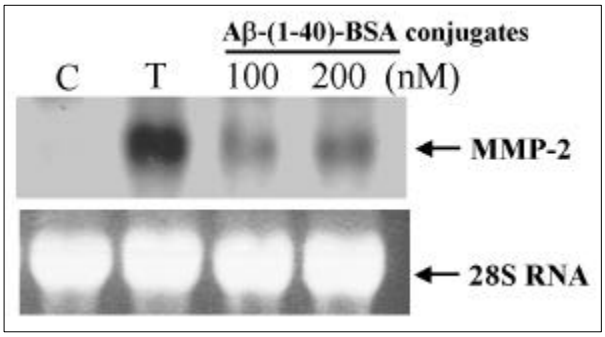

Fig. 3. Stimulation of matrix metalloproteinase 2 (MMP-2) mRNA levels by Aβ-(1-40)-BSA conjugates in cultured lens epithelial cells. HLE B-3 were seeded in 60 mm culture dishes coated with type IV collagen at 1.0×10^6 cells/well, and incubated at 37℃ for 18 hours. Then, the procedures for total RNA proceeded as described in Fig. 1. The total RNA was isolated and then subjected to Northern blot analysis to determine mRNA expression of MMP-2. Data shown are from one of two independent experiments that produced similar results. C, control cells; T, cells cultured with 400 pM TGF-β1.

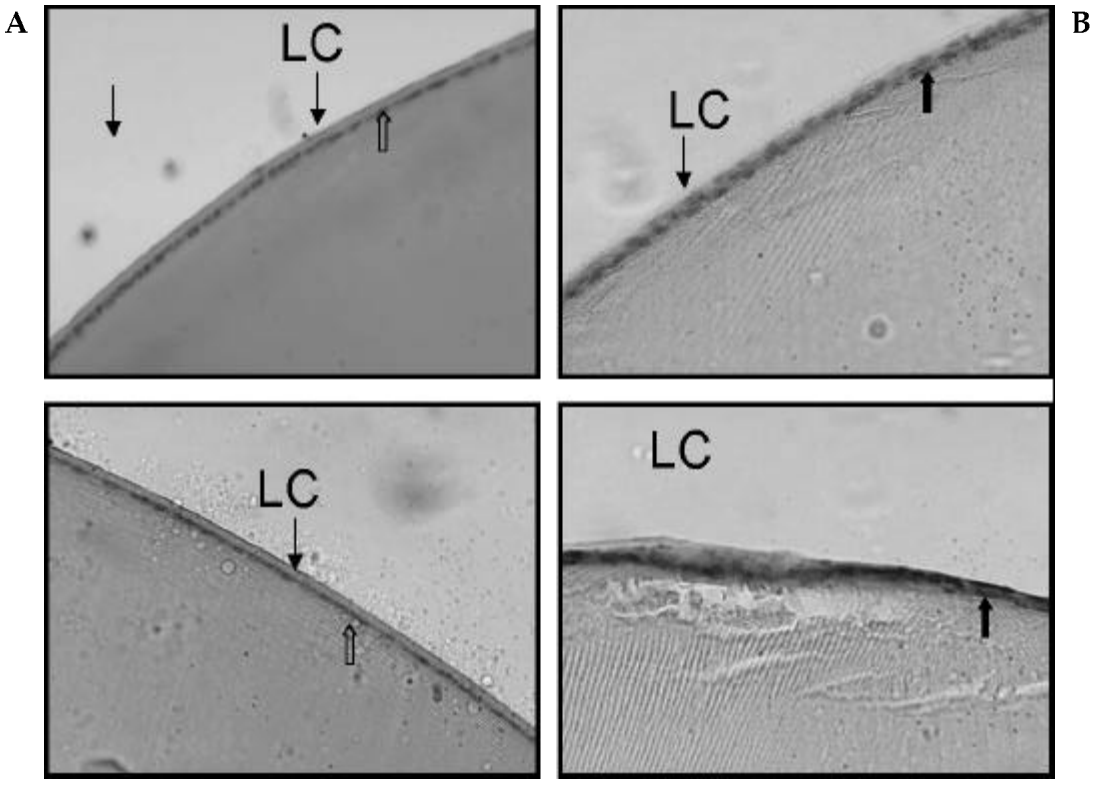

Fig. 4. Micrographs of intact rat lenses cultured without Aβ-(1-40)-BSA conjugates (A) or with Aβ-(1-40)-BSA conjugates (B). Whole lenses were dissected from rats (3-week-old Sprague-Dawley) and incubated in Medium 199 in the presence of 200 nM Aβ-(1-40)-BSA conjugates, and medium was changed every two days with the second addition of Aβ-(1-40)-BSA conjugates. Control lenses were cultured with the addition of 200 nM BSA. After 5 days of incubation, the lenses were fixed, embedded in paraffin, and sectioned for histologic analysis with hematoxylin & eosin staining. Lenses were photographed through the anterior subcapsular region (A, B). In the presence of Aβ-(1-40)-BSA conjugates, lenses showed transformation of lens epithelial cells such as myofibroblast-like cells (B, closed arrow). Without Aβ-(1-40)-BSA conjugates, lenses retained normal histology (A) with monolayer of lens epithelial cells (open arrow). The data shown are from two of three independent assays. LC, lens capsule.

using an immunohistochemical analysis with an anti-Aβ specific antibody (Fig. 6). This antibody, which is specific for the region of Aβ-(1-40) between amino acids 15 and 30 (manufacturer's information), is also able to detect the APP holoprotein, which is a transmembrane protein containing Aβ-(1-40) sequences. As shown in Fig. 6A and 6B, positive staining was observed on the lens epithelial cell membranes of both types of cataracts was observed, suggesting that the APP in human lens epithelial cells is similarly expressed among human cataract lenses. Sections incubated with normal rabbit serum in place of primary antiserum showed no reactivity (Fig. 6C and 6D).

DISCUSSION

Human lens epithelial cells have remarkably different morphological and immunohistochemical characteristics depending on the types of human cataract. Lens epithelial cells beneath the anterior capsule of NC show degenerative changes, such as a shrunken and vacuolated cellular matrix, and condensed nuclear chromatin while those of APC exhibit transdifferentiated changes, such as spindle-shaped fibroblast-like cells embedded within a fibrillar meshwork mass.[18] It has been reported that during embryonic development, the transdifferentiation of epithelium to mesenchyme (EMT) or vice versa occurs.[19] Lens epithelial cells transdifferentiate into fibroblast-

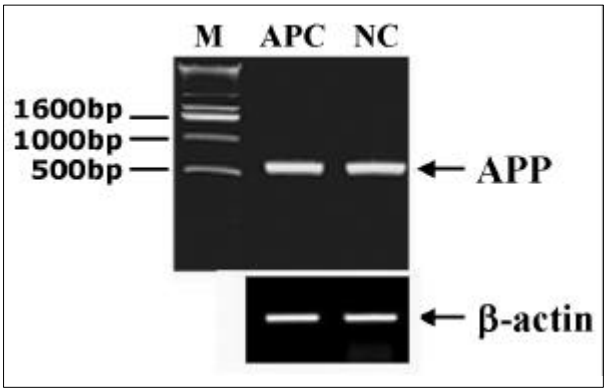

Fig. 5. Identification of amyloid precursor protein (APP) mRNA levels in clinical tissues of anterior polar cataract (APC) and nuclear cataract (NC) using RT-PCR. Total cellular RNA was isolated from lens epithelial cells attached to four combined anterior capsules of human lenses for each clinical type of cataracts. M, molecular size standards (base pairs).

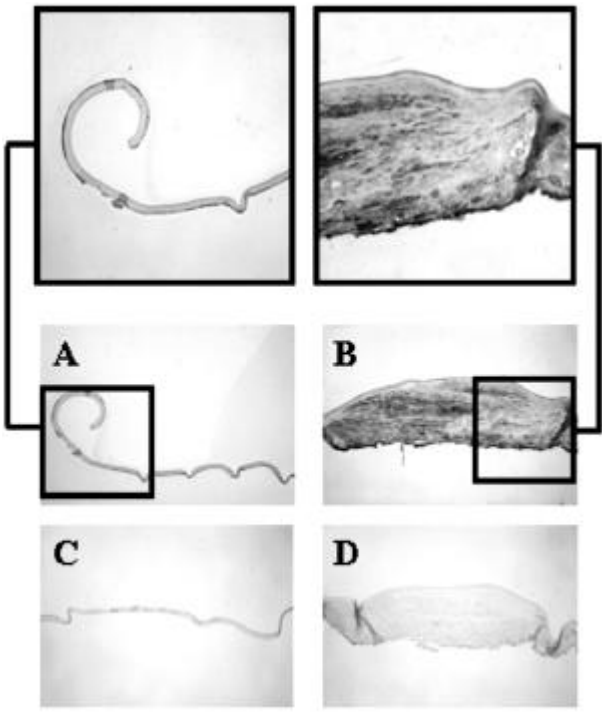

Fig. 6. Immunohistochemical localization of Aβ/APP in human clinical tissues beneath the anterior capsules in APC and NC. Anterior lens capsules with adhering cells from patients NC (A) or APC (B) were examined by immunohistochemical staining for Aβ (magnification, ×100; in magnified box, × 150). The data presented are from one of three independent assays that produced similar results.

like cells via the EMT process during APC formation, and opacification develops after cataract surgery.[4]

TGF-β is a multi-functional cytokine, and promotes EMT in many cells including mammary epithelial cells.[20] The overproduction of TGF-β and extracellular matrix by its sustained gene expression via the autocrine control of TGF-β are proposed to be an underlying mechanism of APC formation.[6] Huang et al.[11] reported that Aβ-(1-40)-BSA conjugate almost prevents ^{125}I-TGF-β1 from binding to TGF-β receptors at 100 nM in mink lung epithelial cells. Also, they showed that Aβ-(1-40)-BSA does not significantly affect DNA synthesis of R1B mink lung epithelial cells, which lack expression of the functional type I TGF-β receptors, compared with the wild-type cells. These studies led us to propose that Aβ-(1-40)-BSA conjugates have TGF-β-like activity in lens epithelial cells, and transform the characteristics of lens epithelial cells to those of fibroblast-like cells. Indeed, we confirmed the translocation of Smad-3 into the nucleus in HLE B-3 cells by Aβ-(1-40)-BSA conjugates, as well as TGF-β1 (Fig. 1). In addition, our experiments clearly showed that Aβ-(1-40)-BSA induces mRNAs of FN, Col I and SMA, which are established markers of fibrosis (Fig. 2). Thus, our results suggest that Aβ-(1-40)-BSA conjugates mediate at least some of the phenotypic changes of lens epithelial cells that lead to the development of APC. When whole lenses were cultured for 5 days in the presence of Aβ-(1-40)-BSA, these lenses developed opacities (data not shown), and the lens epithelial cells, with the characteristics of an epithelial monolayer, were generally present in multilayers of two to three cells deep (Fig. 4). This evidence may further support the role of Aβ conjugates in the formation of APC. On the other hand, since the abnormal expression of MMPs, which are involved in degrading ECM and tissue remolding, is known to play a role in pathological processes including rheumatoid arthritis,[21] tumor invasion and the metastasis of tumor cells,[22] these enzymes may contribute to the transdifferentiation of lens epithelial cells. TGF-β stimulates the proteinase cascades during tissue remolding and cell migration, and induces MMP-2 specifically in primary cultures of chicken lens annular cells.[17] In addi-

tion, Aβ-(1-40)-BSA also induced MMP-2 mRNA (Fig. 3). Thus, MMP-2 induction with Aβ-(1-40)-BSA conjugates may contribute to the EMT process via a similar cellular effect by TGF-β, and to the cell migration of the anterior lens capsule remnant epithelial cells onto the posterior capsule [23,24] during the formation of APC and PCO.

It is still unknown how Aβ occurs in the lens. The presence of both Aβ and APP in normal and cataractous human lenses has been reported, and they are detected predominantly in the epithelial or cortical regions of human and rat lenses, rather than the central nuclear regions of the lens.[12,25] Oxidative stress, such as H_2O_2 and UV254 irradiation, increased Aβ and APP in cultured intact lenses.[25] The level of H_2O_2 prevalent in the aqueous humour of patients with cataracts is relatively high; H_2O_2 being freely permeable, may affect the lens interior to form Aβ, and Aβ is actually present in primary aqueous humour at concentrations comparable to cerebrospinal fluid.[12,25] It has also been reported that reactive oxygen species are generated when the chromophores in the lens, having a sensitizing activity, are irradiated with UVA light.[26,27] Goldstein et al.[12] proposed that the cytosolic localization of Aβ in the lens could result from release of this peptide from other intracellular compartments during terminal differentiation of the lens epithelial cells as they mature into long-lived, post-mitotic lens fiber cells. During this process, β-APP and its metabolic products, including Aβ contained within organelles such as endoplasmic reticulum, Golgi apparatus, and trans-Golgi network, might be released into the cytosol, because these organelles disintegrate during terminal differentiation. Another alternative explanation might be endocytic Aβ reinternalisation, a clearance pathway that has been proposed as a possible initiation site for Aβ accumulation in the brain.

Proteolytic processing of APP mediated via α- or β-secretase leads to liberation of α-soluble APP (sAPP) or β-sAPP, which are part of the soluble NH_2-terminal ectodomain of APP, and believed to be non-amyloidogenic. The APP is cleaved at the N-terminous of Aβ-(1-40) sequence by β-secretase, releasing a large β-sAPP. Subsequent cleavage of the APP C-terminal fragment (C99) by γ-secretase generates Aβ-(1-40). In the case of α-sAPP, the APP is cleaved within the Aβ- (1-40) region by α-secretase, which cleaves predominantly between Lys 16 and Leu 17 (amino acid numbering from the N-terminus of Aβ-(1-40)) to generate this secreted derivative.[28] Alternative processing of APP through β-, γ-secretase leads initially to the formation of potentially amyloidogenic membrane-associated fragments, and ultimately to the formation of intact Aβ peptides, resulting in the secretion of the peptides. The APP holoprotein, which is a transmembrane protein containing Aβ-(1-40) sequences, can be recognized by the anti-Aβ antibody based on the information provided by the manufacturer. The limitations of the clinical sample specimens allowed us to perform APP mRNA and immunohistochemical studies with only anterior polar capsules of NC and APC, but we were also able to obtain plaques from the cortex regions, as well as capsules from APC. In clinical cataract samples, the positive staining on the capsule of NC appears to show APP holoprotein on the membrane (Fig 6A). It was expected because the expression levels of APP mRNA in NC, as well as in AP, was high (Fig. 5). Interestingly, the strong positive immunostaining against Aβ in the subcapsular plaque that was composed of ECM from APC (Fig. 6B) indicates the localization of Aβ-containing peptides within its subcapsular plaque. Our study used NC as a control, but we did not confirm whether normal capsules from normal lenses had Aβ. Goldstein et al.[12] identified Aβ 1-40 and Aβ 1-42 in lenses from human clinical samples with and without cataract extraction. Our data showed that Aβ (or APP) mRNA are present and abundant in the normal-appearing epithelial cells of NC specimens and in the plaques of APCs.

Further investigations are required to examine how Aβ affects the transdifferentiation of lens epithelial cells. Aβ may lead to the activation of the mitogen-activated protein kinase (MAPK) or Erk kinase superfamily and the p38 MAPK superfamily in microglia and THP1 monocytes, suggesting that these MAP kinase pathways lead to a respiratory burst and activation of transcription via such molecules as CREB.[29] The aggregated form of Aβ-(1-40) inhibits the activity of phospholipase C prepared from the cerebral cortex of adult rats,[30] suggesting that the aggre-

gated Aβ-(1-40) may mediate phosphoinositide signaling. It is necessary to clarify the mechanism by which Aβ-(1-40) conjugates interact with lens epithelial cells and cause the transdifferentiation of those cells.

We report here on the existence of APP in lens epithelial cells and the effects of Aβ-(1-40)-conjugates. Our data suggest that APP is possibly involved in the mechanism by which the induction of m-RNAs associated with transdifferentiation in lens epithelial cells takes place. Although further controlled experiments are required, this study showed the accumulation of Aβ in APC, the induction of cataract markers for abnormal ECM, and the transformation in lens epithelial cells by Aβ conjugates; findings that, by showing the possible causes of the plaques of APC, might offer an understanding of the mechanisms that lead to certain types of cataractogenesis.

REFERENCES

1. Bullimore MA, Bailey IL. Considerations in the subjective assessment of cataract. Optom Vis Sci 1993;70: 880-5.
2. Khadem M. Outcomes of cataract surgery: implications for the developing world. J Med Syst 1999;23:281-9.
3. Piatigorsky J. Lens differentiation in vertebrates: A review of cellular and molecular features. Differentiation 1981;19:134-53.
4. Novotny GE, Pau H. Myofibroblast-like cells in human anterior capsular cataract. Virchows Arch A Pathol Anat Histopathol 1984;404:393-401.
5. Cobo LM, Ohsawa E, Chandler D, Arguello R, George G. Pathogenesis of capsular opacification after extracapsular cataract extraction. An animal model. Ophthalmology 1984;91:857-63.
6. Lee EH, Joo CK. Role of transforming growth factor-β in transdifferentiation and fibrosis of lens epithelial cells. Invest Ophthalmol Vis Sci 1999;40:2025-32.
7. Hales AM, Chamberlain CG, McAvoy JW. Cataract induction in lenses cultured with transforming growth factor- beta. Invest Ophthalmol Vis Sci 1995;36:1709-13.
8. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 1985;82:4245-9.
9. Auld DS, Kar S, Quirion R. Beta-amyloid peptides as direct cholinergic neuromodulators: a missing link? Trends Neurosci 1998;21:43-9.
10. Selkoe DJ. Alzheimer's disease: genotypes, phenotypes, and treatments. Science 1997;275:630-1.
11. Huang SS, Huang FW, Xu J, Chen S, Hsu CY, Huang JS. Amyloid β-peptide possesses a transforming growth factor-β activity. J Biol Chem 1998;273:27640-4.
12. Goldstein LE, Muffat JA, Cherny RA, Moir RD, Ericsson MH, Huang X, et al. Cytosolic beta-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer's disease. Lancet 2003;361:1258-65
13. Andley UP, Rhim JS, Chylack Jr. LT, Fleming TP. Propagation and immortalization of human lens epithelial cells in culture. Invest Ophthalmol Vis Sci 1994;35:3094-102.
14. Wilson SE, Lloyd SA, He G, McCash CS. Extended life of human corneal endothelial cells transfected with the SV40 large T antigen. Invest Ophthalmol Vis Sci 1993; 34:2112-3.
15. Gordon-Thomson C, de longh RU, Hales AM, Chamberlain CG, McAvoy JW. Differential cataractogenic potency of TGF-beta1, -beta2, and -beta3 and their expression in the postnatal rat eye. Invest Ophthalmol Vis Sci 1998;39:1399-409.
16. Hu PP, Datto MB, Wang XF. Molecular mechanisms of transforming growth factor-beta signaling. Endocr Rev 1998;19:349-63.
17. Richiert DM, Ireland ME. Matrix metalloproteinase secretion is stimulated by TGF-beta in cultured lens epithelial cells. Curr Eye Res 1999;19:269-75.
18. Joo CK, Lee EH, Kim JC, Kim YH, Lee JH, Kim JT, et al. Degeneration and trans-differentiation of human lens epithelial cells in nuclear and anterior polar cataracts. J Cataract Refract Surg 1999;26:678-90.
19. Hay ED, Zuk A. Transformations between epithelium and mesenchyme: normal, pathological, and experimentally induced. Am J Kidney Dis 1995;26:678-90.
20. Miettinen PJ, Ebner R, Lopez AR, Derynck R. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. J Cell Biol 1994;127:2021-36.
21. Konttinen YT, Ainola M, Valleala H, Ma J, Ida H, Mandelin J, et al. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis 1999;58:691-7.
22. MacDougall JR, Matrisian LM. Contributions of tumor and stromal matrix metalloproteinases to tumor progression, invasion and metastasis. Cancer Metastasis Rev 1995;14:351-62.
23. McDonnell PJ, Stark WJ, Green WR. Posterior capsule opacification: a specular microscopic study. Ophthalmology 1984;91:853-6.
24. Kappelhof JP, Vrensen GF. The pathology of after-cataract. A minireview. Acta Ophthalmol Suppl 1992; 205:13-24.
25. Frederikse PH, Garland D, Zigler Jr JS, Piatigorsky J. Oxidative stress increases production of β-amyloid precursor protein and β-amyloid (Aβ) in mammalian lenses, and Aβ has toxic effects on lens epithelial cells. J Biol Chem 1996;271:10169-74.
26. Linetsky M, Ortwerth BJ. Quantitation of the singlet

oxygen produced by UVA irradiation of human lens proteins. Photochem Photobiol 1997;65:522-9.
27. Ortwerth BJ, Prabhakaram M, Nagaraj RH, Linetsky M. The relative UV sensitizer activity of purified advanced glycation endproducts. Photochem Photobiol 1997;65: 666-72.
28. Hooper NM, Karran EH, Turner AJ. Membrane protein secretases. Biochem J 1997;321:265-79.
29. McDonald DR, Bamberger ME, Combs CK, Landreth GE. Beta-Amyloid fibrils activate parallel mitogen-activated protein kinase pathways in microglia and THP1 monocytes. J Neurosci 1998;18:4451-60.
30. Zambrzycka A, Strosznajder RP, Strosznajder JB. Aggregated beta amyloid peptide 1-40 decreases Ca^{2+}- and cholinergic receptor-mediated phosphoinositide degradation by alteration of membrane and cytosolic phospholipase C in brain cortex. Neurochem Res 2000; 25:189-96.