A novel silent mutation E83E of αA-crystallin in age-related cataract with impaired chaperone function

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Abstract

Background

Age-related cataract (ARC) is the leading cause of blindness of the aged, influenced by environmental factors and genetic factors. However, it is scanty in gene mutation studies. The study aims to expand knowledge of etiology of ARC.

Methods

Peripheral blood samples of ARC patients were collected to screen αA-crystallin (CRYAA) gene mutations by single strand conformation polymorphism assay. Cell morphology, protein expression level and chaperone function of CRYAA in HLE B-3 cell line influenced by mutant CRYAA were next analyzed. Chaperone function of mutant CRYAA was measured by aggregation assay in physiological status, heat treatment (42℃) and ultraviolet (UV) treatment. Cell morphology and protein expression level effected by UV in human lens epithelial B3 (HLE B-3) cells and human anterior lens capsules (ALCs) were further analyzed.

Results

We identified a novel silent mutation in CRYAA (c.249G > A, p.E83E) among 300 ARC patients. CRYAA-E83E may up-regulate the CRYAA expression levels of both mRNA and protein, and change the distribution of CRYAA in HLE B-3 cells. Compared to wild type CRYAA (CRYAA-WT), CRYAA-E83E showed lower chaperone function after 42℃ heat treatment and UV treatment. Meanwhile, after UV treatment, HLE B-3 cells and lens epithelial cells in human ALCs showed irregular and flatten morphology; decreased CRYAA expression levels; increased laminin γ2 expression levels, and changed epithelial-mesenchymal transition (EMT) related protein expression levels.

Conclusions

The E83E mutation of CRYAA is a potential pathogenic mutation, considering its influence on the expression level, intracellular distribution and chaperone function of CRYAA.

Background

Cataract is a leading cause of blindness worldwide. Itisanirreversible eye disease results from loss oftransparencyofcrystalline. About 80% cataracts are age-related,definedasage-related cataract (ARC). The formation ofARCismulti-factorial, and epidemiology researchshowedbothenvironmental factors (such as oxidative stress,thermalinjury,and UV irradiation) and genetic factors playing animportantrole[1,2].

The α-Crystallin is a major structural and refractiveproteininthe vertebrate lens [3, 4] and belongs to the small heatshockproteinfamily [5]. Thetwo subunits, αA and αB have57%sequence homology[6].αA-crystallin (CRYAA) comprises of 173aminoacid residues, has amolecular mass of 20 KDa, is encoded byCRYAAgene and is foundalmost exclusively in the lenstissues[7]. CRYAAgeneis located in chromosomes21 comprised of 3 exons. CRYAA hasthechaperone function likeother heat shock proteins and playsanimportant role in preventionof various substrateproteinsaggregation induced by UV andmaintaining transparency oflens[8,9]. CRYAA isdivided into 3 functiondomainsartificially: N-terminal domain(residues 1-143),α-crystallindomain (residues 64–144) andC-terminal domain (residues145–173) [10]. Ithasbeen reported that residues 70–88in CRYAA acted as aprimarysubstrate binding site and account forthe bulk of thetotalchaperone activity [11].

Several mutations of CRYAA gene have been reported toberelatedwith ARC such as D2D, R54K, R65QandF71L[12–15]. Up to now, D2D mutation is known tobetheonly silent mutation in the coding region of CYRAAassociatedwithcongenital and age-related cataract. The silentmutation ofD2Dresults in a compact CRYAA mRNA secondary structurecompared towildtype CRYAA mRNA [12]. Meanwhile, more researchprovedsilentmutations might influence the process oftranscription,proteinstructure or protein function[16,17].

It has been known for a long time that heat treatmentaffectedtheexpression, structure and function of heat shockproteinsincludingCRYAA [18–20]. CRYAA hadincreasedmembrane-boundfractions when cells were treated with heatshock at42℃ detectedby immunoblotting (IB) [21]. The results suggested thatthealtereddistributions in cellular of CRYAA might contribute totheonset ofARC. In the process of ARC, the accumulatedvariousstresses andsome mutations might affect the cellulardistributionsand cellularfunctions of CRYAA [21].

Lens tissues are formed by the proliferationanddifferentiationof the lens epithelial cells. Recently,humananterior lenscapsules (ALCs) models in vitro wasconstructedsuccessfully usingthe human lens epithelial B3 (HLE B-3)cell linebasement membrane(BM) [22]. Cellstransfected with CRYAA indicateditsability to prevent UVA inducedapoptosis in human lensepithelialcells[23–25].

Epithelial-mesenchymal transition (EMT) has been widelystudiedinthe posterior cataract, but there's no report showedtherelationshipbetween EMT and ARC. In vitro experiments showedthatUVB couldup-regulate expression levels of OrthodenticleHomeobox 2(Otx2) inSRA cell lines, and the increased Otx2 inducedEMT andup-regulatedSmooth muscle actin (SMA) [26]. Laminin γ2 is animportantstructuralcomponent of the epithelial BM in variousnormaltissues[27].

In the present study, we identified a novel silentmutationE83Eof CRYAA among 300 ARC patients. And furtherinvestigationwasperformed to study their potential contribution tothepathogenesisof ARC.

Methods

Peripheral blood samples collection and DNA extraction

A total of 300 nuclear ARC patients (aged21–92 yearsold)and 100 unrelated normal subjects (aged0–39 year old)wererecruited from the First Affiliated Hospitalof HarbinMedicalUniversity (Harbin, China). Peripheral bloodsamples werecollectedusing EDTA tubes and stored at -20℃. Thegenomic DNA wasextractedby TIANamp Genomic DNA Kit (TIANGEN,Beijing, China). Thisstudywas approved by the Internal Review Boardof HarbinMedicalUniversity. All the experiments were conducted inaccordancewithDeclaration of Helsinki Principles. Informed consentswereobtainedfrom all individuals involved.

Polymerase chain reaction (PCR)

PCR amplification was performed with 50 ng of genomicDNA,ina 25 μl reaction mixture containing12.5 μlof2 × PrimeSTAR Max Premix (Takara Bio Inc.,Dalian,China),0.2 μM of each primer. The PCR products were runon1.5%agarose gel. The primers used werelistedinTable 1.

Single strand conformation polymorphism(SSCP)andsequencing

Double-stranded DNA is denatured to single-stranded DNAinthetechnology of SSCP. Mutation screening was carried outbySSCPanalysis as described previously [9]. PCR products of affectedindividualswerepurified and sequenced by HaiGene (Harbin,China).

Cell culture

HLE B-3 stable cell line was obtained from theAmericanTypeCulture Collection (Manassas, VA, USA) and culturedaspreviouslydescribed [28,29].

Plasmid transfection

The plasmids of blank vector [pcDNA3.1(+)], wildtypeCRYAA(CRYAA-WT) and CRYAA-E83E were obtained fromBSgensCompany(Harbin, China). HLE B-3 cells were seeded in 6-wellplatesandwere transfected with 2 μg/well pcDNA3.1(+),CRYAA-WTandCRYAA-E83E plasmids using Invitrogen Lipofectamine®2000Reagent(Thermo Fisher Scientific Inc., Shanghai, China)asdescribedpreviously [28,29].

Reverse transcription-PCR (RT-PCR)

CRYAA (NM_001363766.1) mRNA levels were analyzed byRT-PCR.TotalRNA was isolated from HLE B-3 cells transfected withCRYAA-WTandCRYAA-E83E plasmid using TRIZOL reagent (HaiGene)according tothemanufacturer's protocol. RNA (1 μg) wasreversetranscribedusing PrimeScript™ II Reverse Transcriptase(Takara BioInc.)according to the manufacturer's protocol. PCR wasperformedwith5 μl of RT product, in a 25 μl reactionmixturecontaining0.625 U of TaKaRa Ex Taq (Takara Bio Inc.)and0.2 μM of eachprimer. The PCR products were run on1.5%agarose gel and visualizedby staining with Ethidium Bromide(SigmaAldrich; Merck KGaA). GAPDH(NM_001256799.3) was used asaninternal control. The primers usedwere listedinTable 1.

Antibodies

Rabbit polyclonal antibody (pAb) against CRYAA(catnos.abs116815, Absin), rabbit pAb against laminin γ2 (catnos.C30224,Assay Biotechnology Company), rabbit pAb againstfibronectin(catnos. 15613-1-AP, proteintech), rabbit pAb againstα-SMA (catnos.Ab5694, Abcam), mouse monoclonal antibody (mAb)againstβ-actin(cat nos. AF0003, Beyotime Institute ofBiotechnology),mouse mAbagainst GAPDH (cat nos. AF0006, BeyotimeInstituteofBiotechnology), mouse mAb against histone H3 (catnos.AF0009,Beyotime Institute of Biotechnology), mouse mAbagainstbcl-2 (catnos. sc-7382, Santa Cruz Biotechnology) and rabbitpAbagainst bax(cat nos. 50599-2-lg, proteintech) were used inthepresentstudy.

IB

Protein levels of CRYAA, histone H3, GAPDH, α-SMA,laminin γ2,fibronectin (FN), bax, bcl-2 in HLE B-3 celllysates,supernatants,BMs and human ALCs were analyzed by IB. Afterplasmidtransfectionfor 48 h, HLE B-3 cell lysates,supernatants andBMs wereharvested as described previously[25]. Human ALCs lysates werecollectedasdescribed below.

Chaperone function assay

Aggregation assay was chosen as the method toevaluatethechaperone function of CRYAA as describedpreviously[19]. HLE B-3 cellstransfectedwith CRYAA-WTand CRYAA-E83E plasmids were grown to 100%confluencyin a 6-wellplate. Cell lysates were collected,100 μl lysatescontainingequal amounts of proteins wereincubated at 55℃ in waterbath for3 h for protein aggregation.Soluble and insolublefractionswere separated by centrifuge with 18,000 × g/ 10 minat 4℃.The supernatant was as the solublesection. Sediment wasdissolvedby 25 μl 2 × sample buffer asthe insoluble section.Theexpression level of client proteins wasdetected by IB asdescribedabove.

Preparation of nuclear and cytoplasmic protein

HLE B-3 cells were seeded in 6-well plates andweretransfectedwith 2 μg/well CRYAA-WT and CRYAA-E83Eplasmids.The nuclearand cytoplasmic proteins were extracted byNuclear andCytoplasmicProtein Extraction Kit (Beyotime InstituteofBiotechnology,Shanghai, China) according to themanufacturer'sprotocols.

HLE B-3 cells treated with 42 ℃

The 42℃ treatment assay was performed asdescribedpreviously[25]. Briefly,HLE B-3cellstransfected with plasmids were cultured in 6-wellplates andputinto humidified atmosphere with 5% CO_2for24 h.Cells were cultured at 42℃ in humidifiedatmospherewithoutCO_2 for 4 h. With those culturedat 37℃inhumidified atmosphere without CO_2 for4 hasnegative control. Cells lysates were collectedasdescribedabove.

HLE B-3 cells treated with UV

HLE B-3 was cultured in 6-well plate grown to 95%confluency.Cellsupernatant was discarded and washed with PBStwice,500 μl PBSwas kept in each well. Cells were exposed toUV(253.7 nm, 100μW/cm^2) for 1 h.

Fornegativecontrol, cells werekept at room temperature in PBS withoutUVirradiation for 1 h.HLE B-3 cells transfected withplasmidswere cultured in low-glucoseDMEM containing 10% FBS at 37°Cinhumidified atmosphere with 5%CO_2 for 24 h.Cellswere then treated with UV for1 h as described above. HLEB-3cell lysates, supernatants andbasement membranes were harvestedasdescribed previously[25].

Human ALCs collection and treated with UV

Cataract severity of nuclear ARC were graded bytheEmery-LittleClassification System of nuclear opacity gradebeforesurgery.Human ALCs of nuclear grade Ⅲ were collected from20individualswith ARC at the First Affiliated Hospital ofHarbinMedicalUniversity (Harbin, China). Human ALCs were obtainedusingthecentral circular capsulorhexis method by asingleophthalmologistduring surgery. Human ALCs were storedinlow-glucose DMEM at 4°Cand immediately transferred tolaboratory.Human ALCs were cut into2 pieces at the same size andtiled in two35-mm dish containing750 μl PBS respectively. Onedish wastreated by UV(253.7 nm, 100 μW/cm^2)for30 min. Aftertreated, changed PBS with 1.5 mllow-glucoseDMED containing10% FBS, 100 units/ml penicillin and100 μg/mlstreptomycin.Human ALCs were cultured at 37°C with 5%CO_2for24 h. The UV group was again treated with UVfor30 min,turned-over each pieces of ALCs and treatedanother30 minwith UV. Another one dish without treated asthenegative control.Lysates of human ALCs was prepared asdescribedpreviously[22].

Coomassie brilliant blue (CBB) staining

HLE B-3 basement membranes were separated by 10%SDS-PAGE,thenstained by CBB staining R250 as describedpreviously[25].

Statistical analysis

Statistical analysis was performed using SPSS 18.0(SPSS,Inc.,Chicago, IL, USA). The quantitative data areshownasmean ± standard deviation. *P* values less than0.05wereconsidered as statistically significant.

Results

Screening and identification of CRYAA mutationsinARCpatients

Genomic DNA samples were isolated from peripheral bloodsamplesof300 ARC patients and 100 normal subjects. The SSCPassayshowedmobility shift pattern in a 63-year-old male ARCpatient(S220) inexon 2 of CRYAA gene*(*Fig. 1A*)*. Such changes werenotobservedamong 100 normal controls. Sequencing ofamplifiedproductsrevealed a novel heterozygous G > Anucleotidesubstitution inexon 2 of CRYAA (c.249G > A,NG_009823.1), whichdid not changethe amino acid sequence(Glutamicacid, Glu) at codon83(p.E83E)*(*Fig. 1B*)*. The Glu83 of CRYAA genearehighlyconserved among 8 species*(*Fig. 1C*)*.

Protein expressions, distribution andchaperonefunctioninfluenced by CRYAA-E83E

We construct CRYAA-WT and CRYAA-E83E plasmids toinvestigatethefunction of the novel silent mutation of CRYAA-E83E.In HLEB-3cells, compared to blank vector, transfection ofCRYAA-WTandCRYAA-E83E

showed significant increasedCRYAAgene(Fig. 2A)andprotein(Fig. 2B) expression levels by RT-PCRandIB,respectively; compared to CRYAA-WT, CRYAA-E83Eshowedhigherexpression levels of CRYAA gene and protein levelsdetectedbyRT-PCR (Fig. 2A)andIB(Fig. 2B), respectively. Toinvestigatetheprotein distribution of CRYAA, protein expressionlevelsinfluencedby CRYAA-E83E in nuclear and cytoplasmic proteinsof HLEB-3 cellswere analyzed. Histone H3 was used as internalreferenceof nuclearproteins. β-actin was used as internal referenceofcytoplasmicproteins. Compared to blank vector, theexpressionlevels of CRYAAcontributed by CRYAA-WT and CRYAA-E83Eweresignificantly higher innucleus and cytoplasm. Compared toCRYAA-WT,the protein expressionlevels of CRYAA by CRYAA-E83E waslower inboth nuclear andcytoplasmiclysates(Fig. 2C).

CRYAA is widely regarded as the small heat shockproteinwhichmainly suppress the aggregation of differentclientproteins.CRYAA-WT and CRYAA-E83E plasmids were transfectedinto HLEB-3cells. Cell lysates were centrifuged into solubleandinsolublefractions, the insoluble fraction indicates theexpressionlevelsof aggregated client proteins. Aggregation ofGAPDH, α-SMA,FN,laminin γ2 proteins in HLE B-3 cell lysates weredetected byIB.Compared to blank vector, HLE B-3 cells transfectedwithCRYAA-WTshowed higher expression levels of total, solubleandinsolubleCRYAA detected by IB; compared to CRYAA-WT, HLEB-3cellstransfected with CRYAA-E83E showed higher solubleCRYAAexpressionlevels and similar insoluble CRYAAexpressionlevels(Fig. 2D). Compared to blank vector,HLEB-3cells transfected with CRYAA-WT showed similar total,solubleandinsoluble GAPDH expression levels; compared to CRYAA-WT,HLEB-3cells transfected with CRYAA-E83E showed similartotalGAPDHexpression levels, lower soluble GAPDH expressionlevelsandsimilar insoluble GAPDHexpressionlevels(Fig. 2D). Compared to blank vector,HLEB-3cells transfected with CRYAA-WT showed similar total,solubleandinsoluble α-SMA expression levels; compared to CRYAA-WT,HLEB-3cells transfected with CRYAA-E83E showed similartotalα-SMAexpression levels, lower soluble and insolubleα-SMAexpressionlevels (Fig. 2D). Compared to blank vector,HLEB-3cells transfected with CRYAA-WT showed higher totalFNexpressionlevels, lower soluble FN expression levels andhigherinsoluble FNexpression levels; compared to CRYAA-WT, HLE B-3cellstransfectedwith CRYAA-E83E showed lower total FNexpressionlevels, highersoluble FN expression levels and lowerinsoluble FNexpressionlevels (Fig. 2D). Compared to blank vector,HLEB-3cells transfected with CRYAA-WT showed similar total,solubleandinsoluble laminin γ2 expression levels; comparedtoCRYAA-WT,CRYAA-E83E had similar total, soluble andinsolublelaminin γ2expression levels(Fig. 2D).

Morphology and chaperone function influencedbyCRYAA-E83Etreated with 42°C

To further investigate the chaperone functioninfluencedbyCRYAA-E83E, HLE B-3 cells transfected with CRYAA-WTandCRYAA-E83Eplasmid were cultured in medium at 37℃ for24 h,then changedinto 42℃ for 4 h, with those cultured at37℃ for4 h asnegative control. After treated, cells shrinked,becamesmall andturned round, and vacuoles were located aroundthecells(Fig. 3A). Aggregation of CRYAAandGAPDHwere detected by IB through chaperone function. Comparedtoblankvector, the expression levels of total, soluble andinsolubleCRYAAwere higher in HLE B-3 cells transfected withCRYAA-WTandCRYAA-E83E; compared to CRYAA-WT, HLE B-3 cellstransfectedwithCRYAA-E83E showed lower

soluble CRYAA expressionlevels andhigherinsoluble CRYAA expressionlevels*(*Fig. 3B*)*. Compared to blankvector, HLEB-3cells transfected with CRYAA-WT showed similartotal andsolubleGAPDH expression levels, slightly lower insolubleGAPDHexpressionlevels; compared to CRYAA-WT, HLE B-3 cellstransfectedwithCRYAA-E83E showed similar total GAPDH expressionlevels,lowersoluble GAPDH expression levels and higherinsolubleGAPDHexpression levels *(*Fig. 3B*)*.

Morphology and protein expression in HLE B-3 cellsandhumananterior lens capsules treated by UV

To investigate the influence of UV treatment onmorphologyandprotein expressions of HLE B-3 cells, cells weretreated withUVfor 30 min or 1 h, with cells withouttreatmentasnegative control. After UV treatment, HLE B-3cellsbecameirregular in shape and flatten, particularly thosetreatedwith UVfor 1 h *(*Fig. 4A*)*. In the earlystage(UV30 min), apoptosis with nuclear chromationcondensationandfragmentation as well as apoptotic bodieswereobserved*(*Fig. 4A). In the late stage (UV1 h),cellsbecame more flatten, and the apoptotic cells mightbephagocytizedby adjacent cells *(*Fig. 4A*)*. Compared tocellswithout UVtreatment, HLE B-3 cells treated with UV showedlowerexpressionlevels of CRYAA, FN and α-SMA, and higherexpressionlevels oflaminin γ2 and N-cadherin byIB*(*Fig. 4B*)*.

We futher collected human ALCs of nuclear grade Ⅲ from20ARCpatients. Human ALCs were treated with UV for 1 handculturedin medium at 37℃ for another 24 h, and thentreatedwith UVagain for 30 min. Human ALCs without treatedwere asnegativecontrol (without). A large number of lens epithelialcellsunderanterior capsules were observed. Cells becameirregular,flattenand vacuolization, the nucleus boundaries wereunclear,which issimilar to the changes of HLE B-3 cells treatedwith UV.Cellapoptosis was induced *(*Fig. 4C). IB of human ALCs showedlowerexpressionlevels of CRYAA and N-cadherin, and higherexpressionlevels oflaminin γ2 after treated withUV*(*Fig. 4D*)*.

Morphology and chaperone function influencedbyCRYAA-E83Etreated with UV

To further investigate the chaperone functioninfluencedbyCRYAA-E83E at different conditions, CRYAA-WTandCRYAA-E83Eplasmids were transfected into HLE B-3 cells.Thentreated HLE B-3cells with UV for 30 min, cellswithouttreatment as negativecontrol. HLE B-3 cells without anytreatmentand cells transfectedwith blank vector became irregularand flattenafter treated withUV. A large number of apoptotic bodieswereobserved in HLE B-3cells transfected with CRYAA-WT andCRYAA-E83Eafter UV treatment*(*Fig. 5A*)*. In HLE B-3 cells treatedwithUVfor 30 min, compared to blank vector, cellstransfectedwithCRYAA-WT and CRYAA-E83E showed significantlyhigherCRYAAexpression levels; compared to CRYAA-WT, the expressionlevelsofCRYAA contributed by CRYAA-E83Ewerehigher*(*Fig. 5B*)*. Compared toblankvector,Bcl-2/Bax ratio by CRYAA-WT was higher, suggestingCRYAA-WTmighthave function of anti-apoptosis; compared to CRYAA-WT,HLEB-3cells transfected with CRYAA-E83E showed higherBcl-2/Baxratio*(*Fig. 5B*)*, suggestingCRYAA-E83Emutationmight increase the ability of anti-apoptosis.Aggregationof CRYAAand GAPDH protein in HLE B-3 cell lysates weredetected byIBthrough chaperone function assay. Results showed that,comparedtoblank vector, the expression levels of total CRYAAwerehighercontributed by CRYAA-WT and CRYAA-E83; compared toCRYAA-WT,theexpression levels of total, soluble and insoluble CRYAAwashigherby CRYAA-E83E *(*Fig. 5C*)*. Compared to blank vector,HLEB-

3cells transfected with CRYAA-WT showed similar total,solubleandinsoluble GPADH expression levels; compared to CRYAA-WT,HLEB-3cells transfected with CRYAA-E83E showed lowersolubleGAPDHexpression levels and higher insoluble GAPDHexpressionlevels(Fig. 5C). Suggesting the impairedchaperonefunctionof CRYAA-E83E. To investigate the expressionlevels ofhistone H3,we collected BM of HLE B-3 cells treated withUV for30 min,with BM without treated as negative control. Inthe BMof untreatedHLE B-3 cells, compared to blank vector,theexpression levels ofhistone H3 contributed by CRYAA-WT werelower;compared to CRYAA-WT,the expression levels of histone H3byCRYAA-E83E were similar(Fig. 5D). CBB staining (bottom)ofBMproteins was used to beinternalreference(Fig. 5D). In the HLE B-3 cellBMtreatedwith UV, the expression levels of histone H3 levelbyCRYAA-E83Ewere significantlyup-regulated(Fig. 5D). To investigatetheexpressionlevels of secretory CRYAA, CRYAA-WT andCRYAA-E83Eplasmids weretransfected into HLE B-3 cells. Aftertreated with UVfor30 min, the supernatant of HLE B-3 cells wascollected,withsupernatant without treated as negative control. Inthesupernatantof HLE B-3 cells, compared to blank vector,theexpression levels ofCRYAA were higher contributed by CRYAA-WTandCRYAA-E83E; comparedto CRYAA-WT, the expression levels of CRYAAbyCRYAA-E83E were lowerbefore treated, but higher after treatedwithUV for 30 min(Fig. 5E).

Discussion

In the present study, we found a novelsilentheterozygousG > A nucleotide mutation in exon 2 ofCRYAA(c.249G > A,p.E83E) in an old male (S220) case among 300ARCpatients. Theage-at-onset of cataract was 63 in S220 consideredasearly onsetof ARC in view of the mean age of ARCcases(64.3 ± 12.9). To thebest of our knowledge, CRYAA-E83EandCRYAA-D2D were the only twosilent mutations in the coding regionofCRYAA related to ARC. Theexpression levels, distributionsandchaperone functions of CRYAAinfluenced by CRYAA-E83Ewereinvestigated in the presentstudy.

The expression levels of CRYAA gene and proteinwereup-regulatedin HLE B-3 cell lysates contributed byCRYAA-E83E,Prompting thatsilent mutation did not change the aminoacidsequence, but,influence processes of transcriptionandtranslation.

Karen L found the recombinant exogenous proteinswereco-localizedwith the native CRYAA in perinuclear regionsdetectedbyimmunohistochemistry [30]. The altered distributions incellularofCRYAA might contribute to the onset of ARC[21]. In thepresent study,thedistribution of CRYAA were more in cytoplasm thannucleus inHLEB-3 cell lysates transfected with CRYAA-WT. TheincreasedCRYAAlocated in cytoplasm may act as a functional protein.ComparedtoCRYAA-WT, the expression level of CRYAA was less both inHLEB-3cell nucleus and cytoplasm transfected with CRYAA-E83E,weproposedit may mainly distribute on the cytomembrane, butmoreexperimentsneed to be done. Histone H3 was widely used asinternalcontrol innuclear protein. Results showed histone H3 alsoexpressedin thecytoplasm of HLE B-3 cells in the present study.

It has been reported that the lens fiber cell markergeneCRYAAwas decreased in process of EMT [31]. However, whether CRYAAinfluencedtheexpression of EMT marker genes has never beenreported.

Inthepresent study, CRYAA-WT promoted the expression ofFN, whilemutantCRYAA had part of these functions. We proposed thereducedFNinduced by UV may regulated by CRYAA, but moreexperimentsstillneed to be done. Over-expression of CRYAA-WT didnot influencetheexpression of α-SMA and laminin γ2 in the presentstudy.

It has been reported that the chaperone activitydecreased50%with deletion of residues 70–76, whereas lossof100%chaperone-like activity with deletion of residues 70–88whenAdipicacid dihydrazide was used as thesubstrateprotein[17].Themutation at codon 83 (E83E) wasincluded in the residues70–88,which were reported as the mainchaperone-like activity areainCRYAA [17].Chaperone function measured atthetemperature of physiologicalshowed CRYAA were more insolublefraction of HLE B-3 cell lysatecontributed by CRYAA-E83E. Itdidnot show better chaperonefunction against aggregation ofGAPDH,α-SMA and laminin γ2 byCRYAA-WT at the physiologicaltemperature asdescribed previously[32]. Comparedto CRYAA-WT, chaperonefunctioninfluenced by CRYAA-E83E showed theability promoteGAPDHaggregation; suppress α-SMA and FNaggregation; noinfluencesagainst laminin γ2 aggregation.Chaperone functionmeasured byaggregation assay influenced by 42℃treatment and UVirradiationwere first constructed in this study.The heat treatmentresponseoccurred in HLE B-3 cells after treatedwith 42℃ for4 h. HLEB-3 cell lysates transfected withCRYAA-WT treated with42℃ showedlittle impact on chaperonefunction; HLE B-3 celllysatestransfected with CRYAA-E83E treatedwith 42℃ showeddecreasedchaperone function against GAPDHaggregation,

UV has been proved to up-regulate the expressionlevelofEMT-related molecules, such as FN, α-SMAandN-cadherin[26,33,34]. UV couldalso down-regulated theexpressionlevel of lens fiber cell markergene CRYAA in lensepithelial cellsof mouse [26].Laminin γ2 promotedmetastasis in lungadenocarcinoma via EMT[31]. Weidentified UV could induceapoptosis ofHLE B-3 cells. In the presentstudy, the expressionlevels oflaminin γ2 and N-cadherin were higherand the expressionlevels ofFN, α-SMA and CRYAA were lower aftertreated with UV inHLE B-3 celllysates. The reason ofdown-regulation of FN and α-SMAneeds to befurther explored. Theexpression levels of laminin γ2and N-cadherinin UV treated humanALCs have never been reported.After treatedwith UV, human lensepithelial cells showed similarchanges inmorphology (becameirregular and flatten) with HLE B-3cell line.The present studyshowed decreased CRYAA expressionlevel andincreased laminin γ2expression level in human ALCs,which weresimilar with HLE B-3 cellstreated with UV. But theincreasedN-cadherin expression level wasopposed to HLE B-3 cellstreatedwith UV.

HLE B-3 cells transfected with CRYAA-WT andCRYAA-E83Eplasmidswere treated with UV for 30 min. TheBcl-2/Bax ratiofrom highto low was CRYAA-E83E, CRYAA-WT, normal andblank vector,provedthe stronger anti-apoptosis function influencedby CRYAA-WTandCRYAA-E83E. HLE B-3 cell lysates transfected withCRYAA-WTtreatedwith UV showed little impact on chaperone function;HLE B-3celllysates transfected with CRYAA-E83E treated withUVshoweddecreased chaperone function against GAPDH aggregation,whichweresimilar to results treated with 42℃. It has been reportedtheHLEB-3 cell line BM constructed as human ALCs modelsinvitro[22]. InBMofHLE B-3 cells transfected with CRYAA-WT andCRYAA-E83Eplasmids,the expression levels of histone H3 were similarbefore UVtreatedin the present

study. The expression levels ofhistone H3wereupregulated by CRYAA-E83E after treated withUV,suggestingincreased expression levels of histone H3 inARCpatients' ALCs.Compared to CRYAA-WT, the expression levelsofsecretory CRYAA byCRYAA-E83E were lower before UV treatmentandupregulated after UVtreatment.

In summary, HLE B-3 cells transfected withCRYAA-E83Eshowedup-regulated expression levels of CRYAA;changeddistributions ofCRYAA in cells; impaired chaperone functionafter42°C heattreatment and UV irradiation. Genetic factorsofCRYAA-E83Emutation and environmental factors of heat treatmentandUVirradiation were possible underlying mechanisms in ARC.

Conclusions

CRYAA-E83E silent mutation up-regulated the expressionlevelofCRYAA; changed distributions of CRYAA and histone H3incells;impaired the chaperone function of CYRAA after 42°Cheattreatmentand UV irradiation. Meanwhile, UV induced cellapoptosisanddecreased the expression level of CRYAA in HLE B-3cells andhumanlens anterior capsules, might be an important aspectinthepathogenesis of ARC.

Abbreviations

ARC: age-related cataract; CRYAA: αA-crystallin;UV:ultraviolet;HLE B-3 cells: human lens epithelial B3cells;CRYAA-WT: wild typeCRYAA; EMT: epithelial-mesenchymaltransition;IB: immunoblotting;BM: basement membrane; Otx2:OrthodenticleHomeobox 2; SMA: Smoothmuscle actin; PCR: polymerasechainreaction: SSCP: single strandconformation polymorphism;RT-PCR:reverse transcription-PCR; pAb:polyclonal antibody;mAb:monoclonal antibody

Declarations

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Not applicable.

Author Contributions

P.L., X.L.T., H.Y.G. designed this study. F.Q.S.,D.R.L.,L.Y.S., C.W., X.W., H.R.L., X.L.T. and H.Y.G.harvestsamples andperformed the experiments. F.Q.S. wrotethemanuscript. P.L., H.Y.G. and X.L.T. revisedthemanuscript. All authorshave read and approved of thefinalversion.

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Availability of data and materials

The datasets used and/or analyzed during the currentstudyareavailable from the corresponding author onreasonablerequest.

Ethics approval and consent to participate

This study was approved from the Internal Review BoardofHarbinMedical University and followed the tenets ofDeclarationofHelsinki. Written informed consent was obtained fromalltheparticipants and from a parent or guardian forparticipantsunder18 years old after complete explanation.

Consent for publication

The potentially identifiable data was consented to publishbyallthe participants and the parent or guardian forparticipantsunder18 years old.

Competing interests

The authors have declared that no competing interests exist.

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Tables

Table 1. Primersusedinthe present study.

Primers	Nucleotidesequence(5′-3′)
CRYAA-exon1-F	GCTGGGGGCGGGCACTTG
CRYAA-exon-R	TGGGGACACAGGCTCTCG
CRYAA-exon2-F	GGTGACCGAAGCATCTCTGT
CRYAA-exon2-R	CGTGACCCCCTTGTCCTC
CRYAA-exon3-F	ACCCGGCCCCTGTGAGAG
CRYAA-exon3-R	AAAGGGAAGCAAAGGAAGACA
CRYAA-mRNA-F	TTTTGAGTATGACCTGCTGCC
CRYAA-mRNA-R	CAGAAGGTCAGCATGCCATC
GAPDH-mRNA-F	GCCAAGGTCATCCATGACAAC
GAPDH-mRNA-R	GTCCACCACCCTGTTGCTGTA

Figures

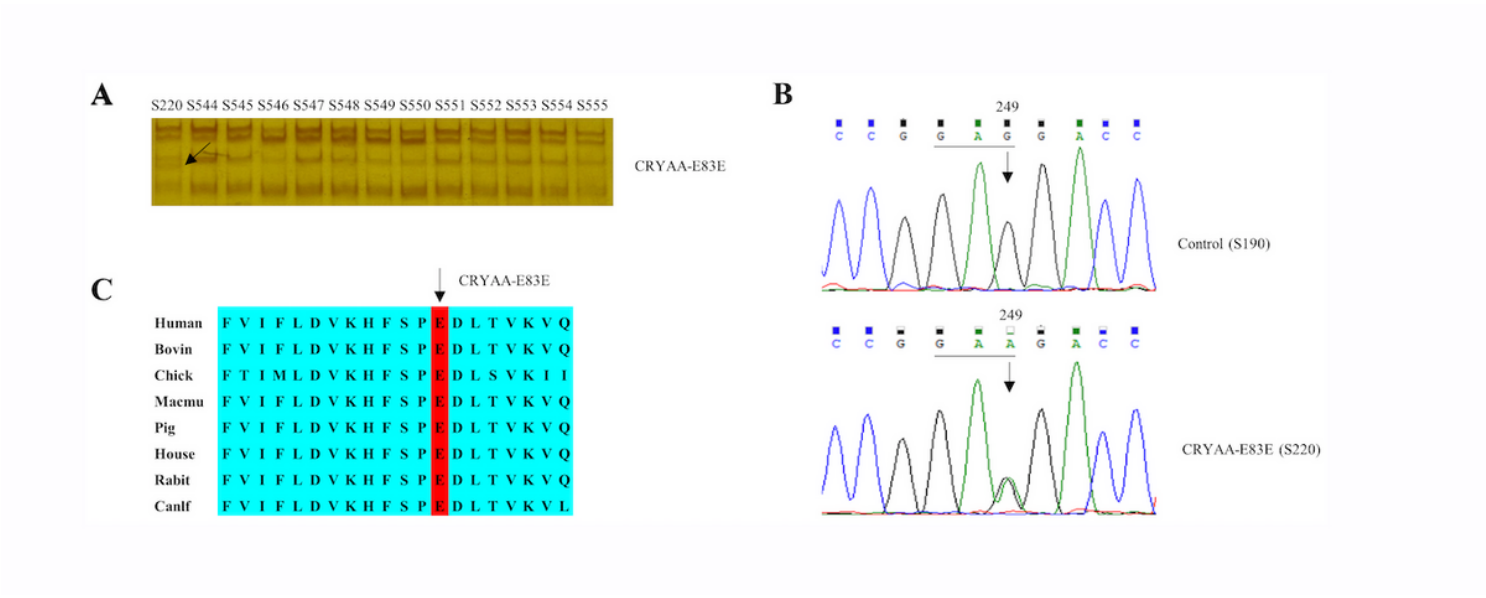

Figure 1

Screening and identification of CRYAA mutations in age-related cataract (ARC) patients. (A) Detection of CRYAA by PCR-SSCP assay. Dark arrow indicates the abnormal bands existed in affected individuals. (B) Sequence analysis of CRYAA in exon 2. The arrow showed a heterozygous G>A nucleotide change in exon 2 of CRYAA (c.249G>A), which leads to a silent mutation at codon 83 (p.E83E). (C) Multiple-sequence alignment in CRYAA at code 83 from different species.

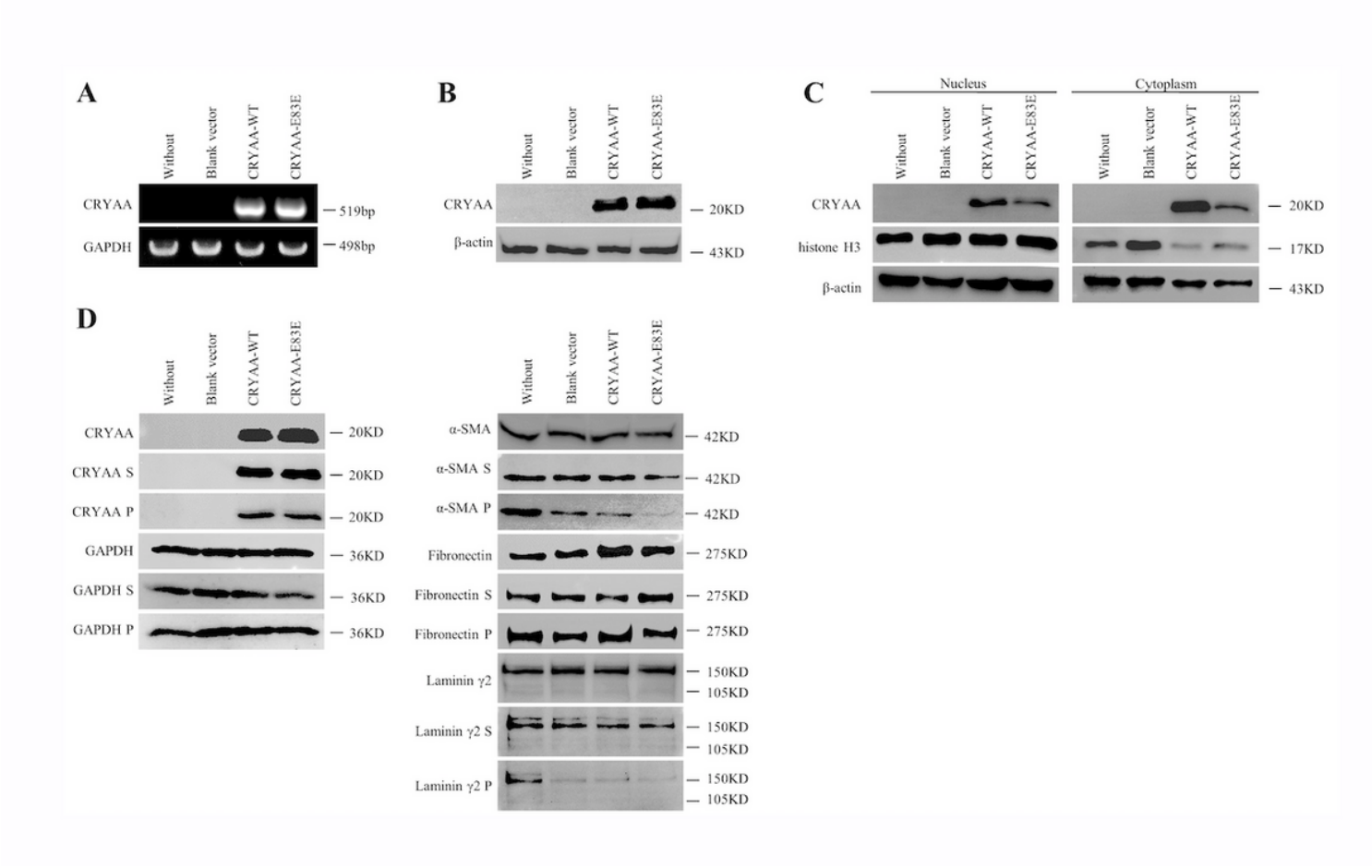

Figure 2

Protein expressions, distribution and chaperone function influenced by CRYAA-E83E. (A) Detection of CRYAA gene in HLE B-3 cells transfected with CRYAA-WT and CRYAA-E83E plasmid by RT-PCR. (B) Detection of CRYAA protein in HLE B-3 cell lysates transfected with CRYAA-WT and CRYAA-E83E plasmid by IB. (C) Detection of CRYAA and histone H3 in nuclear and cytoplasm of HLE B-3 cells transfected with CRYAA-WT and CRYAA-E83E plasmid by IB. (D) Detection of total, soluble (S) and insoluble (P) CRYAA, GAPDH, α-SMA, Fibronecin and laminin γ2 in HLE B-3 cells transfected with CRYAA-WT and CRYAA-E83E plasmid by chaperone function assay. Cell lysates incubated at 55℃ for 3 h were separated into soluble (S) and insoluble (P) fractions by centrifugation.

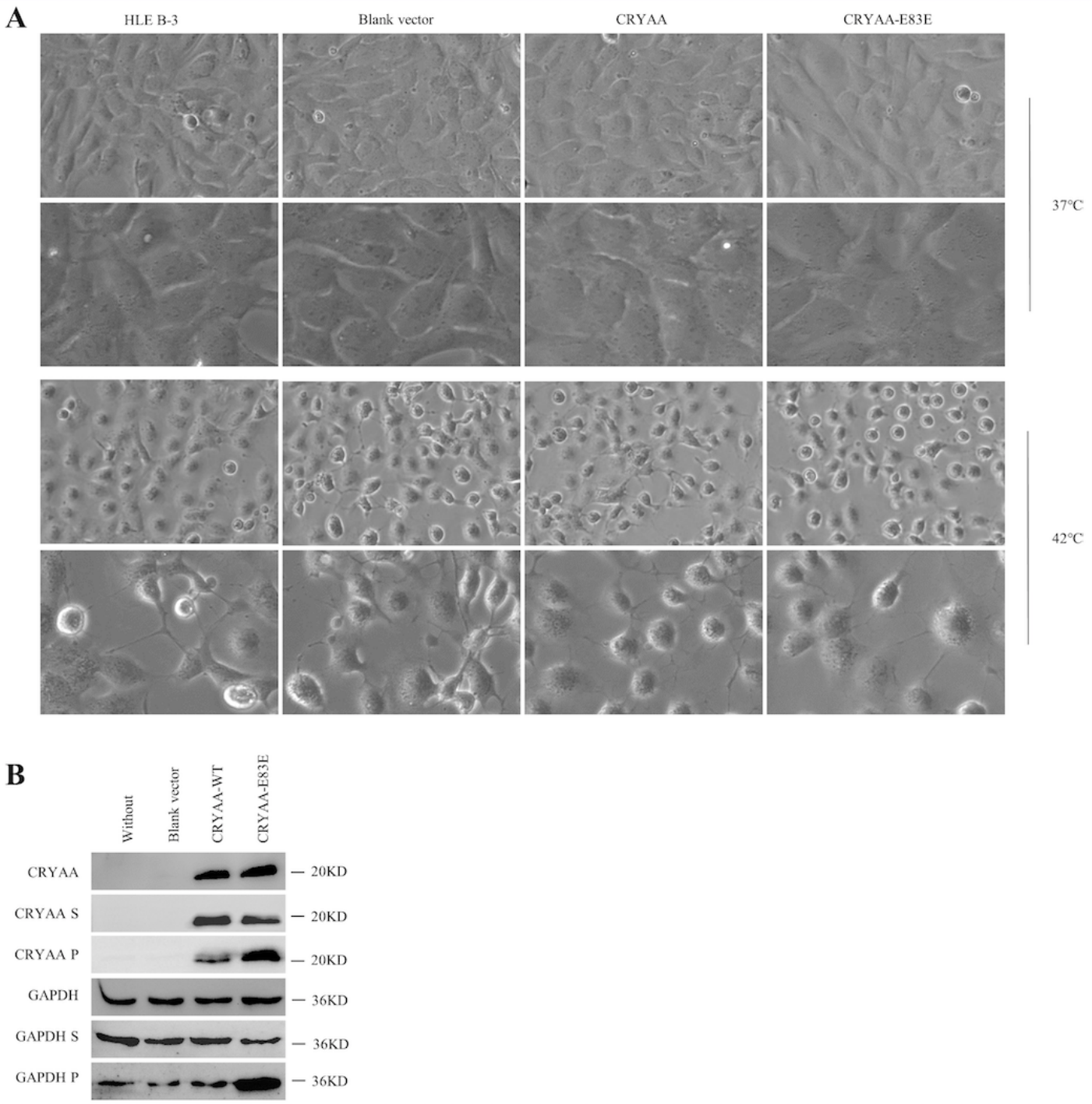

Figure 3

Morphology and chaperone function influenced by CRYAA-E83E treated with 42℃. HLE B-3 cells were cultured in medium at 37℃ for 24 h, then changed into 42℃ for 4 h, with those cultured at 37℃ for 4 h as negative control. (A) Cell morphology. Magnification: 100x for the first and third row and 200x for the second and forth row. (B) Detection of total, soluble (S) and insoluble (P) CRYAA and GAPDH in HLE B-3

cells transfected with CRYAA-WT and CRYAA-E83E plasmid treated with 42℃ by chaperone function assay. Cell lysates were treated as mentioned before.

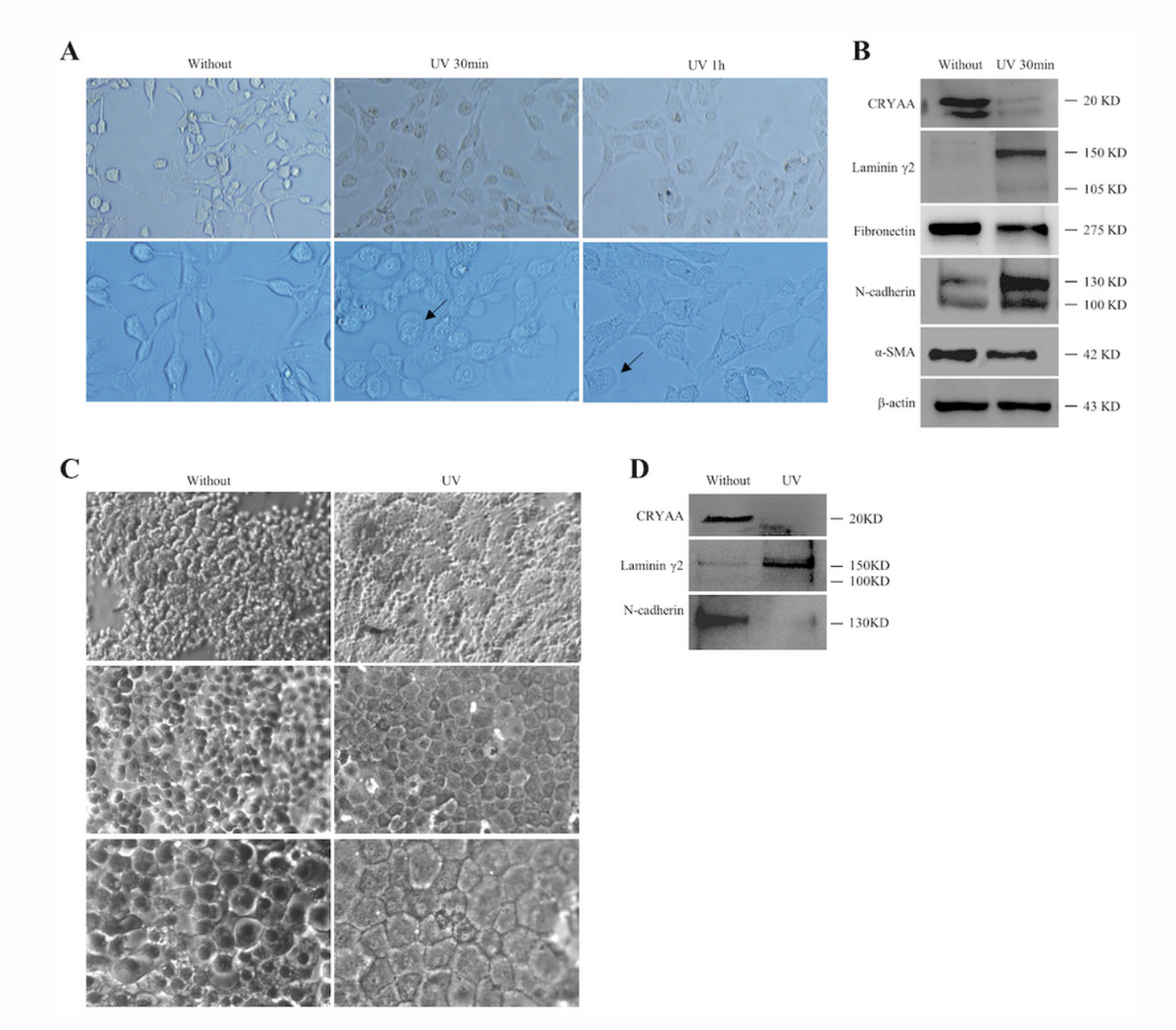

Figure 4

Morphology and protein expression of HLE B-3 cells and human anterior lens capsules treated by UV. HLE B-3 cells were treated with UV for 30 min or 1 h, with those cultured at room temperature for 1 h as negative control (without). (A) Morphology of HLE B-3 cells. Magnification: 100x for upper row and 200x for lower row. Arrows indicated the apoptotic cells. (B) Detection of CRYAA, laminin γ2, Fibronectin, N-cadherin and α-SMA in HLE B-3 cells treated with UV by IB. (C) Morphology of human lens epithelial cells. Human anterior lens capsules were first treated with UV for 1 h and then cultured in medium at 37℃ for 24 h, and then treated with UV for 30 min. Capsules without treatment with UV were as negative control

(without). Magnification: 40x for upper row,100x for middle row and 200x for lower row. (D) Detection of CRYAA, laminin γ2 and N-cadherin in human anterior lens capsules treated with UV by IB.

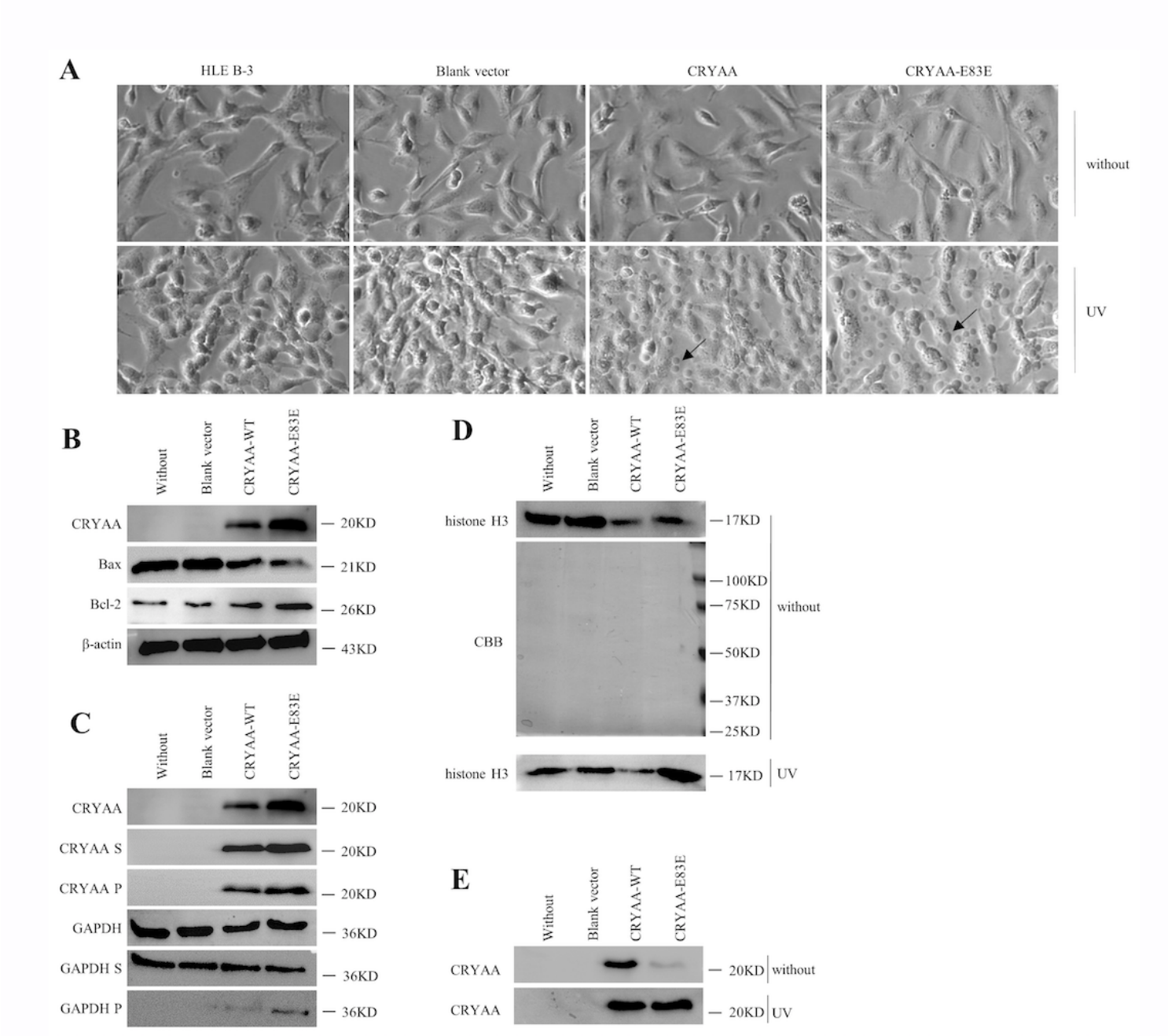

Figure 5

Morphology and chaperone function influenced by CRYAA-E83E treated with UV. HLE B-3 cells were cultured in medium for 24 h, then treated with UV for 30 min, with those cultured at room temperature for 30 min as negative control (without). (A) Cell morphology. Magnification: 200x. Arrows indicated the apoptotic bodies. (B) Detection of CRYAA, Bax and Bcl-2 in HLE B-3 cells transfected with CRYAA-WT and CRYAA-E83E plasmid by IB. (C) Detection of total, soluble (S) and insoluble (P) CRYAA and GAPDH in HLE B-3 cells transfected with CRYAA-WT and CRYAA-E83E plasmid by chaperone function assay. (D)

Detection of histone H3 by IB (top), and total proteins by CBB staining (bottom) in UV treated basement membranes of HLE B-3 cells transfected with CRYAA-WT and CRYAA-E83E plasmid. (E) Detection of CRYAA in HLE B-3 cell supernatant transfected with CRYAA-WT and CRYAA-E83E plasmid by IB.

Supplementary Files

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