Structural and functional analysis of SNP rs76740365 G>A in exon-3 of alpha A-crystallin gene in lens epithelial cells

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Research Article

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Abstract

Age-related cataract (ARC) is the leading cause of visual impairment and blindness worldwide among older adults. As a major structural protein in lens, alpha A-crystallin (CRYAA) plays a crucial role in maintaining lens transparency. Previously, we have identified a single nucleotide polymorphism in the exon-3 of CRYAA gene associated with the susceptibility of ARC, especially posterior subcapsular cataract. To clarify the effect of E156K mutation on the properties of CRYAA, we investigated the structure and function of human recombinant wild-type and E156K CRYAA. The mass spectrometric detection revealed that E156K mutation had no significant effect on the apparent molecular mass of CRYAA oligomeric complex. Evaluation of the structures of CRYAA by far- and near-UV circular dichroism spectra and intrinsic tryptophan fluorescence analysis indicated that E156K mutation did not significantly affect the secondary structures, while caused the perturbations of the tertiary structure. The mutant CRYAA displayed an increase in chaperone-like activity by heat-induced aggregation of β L- and γ -crystallins and DTT induced insulin aggregation, which might be related to the increase of the surface hydrophobicity. Transfection studies of lens epithelial cells revealed that the E156K mutant impeded cell apoptosis, which was associated with the activation of p-AKT signal pathway and down-regulation of Casepase3. Taken together, our results for the first time showed that E156K mutation in CRYAA associated with ARC resulted in enhanced chaperone-like function by inducing its surface hydrophobicity, which was directly related to the activation of its anti-apoptotic function.

Introduction

Age-related cataract (ARC) remains the leading cause of visual impairment worldwide and the largest contributor to blindness in adults 50 years of age and older [1, 2]. At present, surgical removal of the opaque lens is the primary therapy for restoring sight in cataract patients. However, the mechanism involved in the cataractogenesis and the process of ARC has not been fully elucidated, and there is no proven method of prevention for cataract.

Alpha A-crystallin (CRYAA) is the main structural protein in the lens which plays a crucial role in maintaining lens transparency, constituting approximately 35% of all lens crystallins. As a molecular chaperone and a member of the small heat shock protein family, CRYAA helps maintain the solubility of the other lens proteins, such as β - and γ -crystallins, and protects them from aggregation. CRYAA knockout mice display a small lens, lens epithelial cell death, reduced proliferation, cataract, and inhibition of pathological neovascularization [3]. Previous studies demonstrated that the levels of CRYAA decreased in the nuclear capsule of ARC patients [4–6] compared to those of controls, while the mechanisms underlying the downregulation of CRYAA in the lens remained unclear.

CRYAA is involved in multiple cellular functions, including apoptosis, cell migration, and so on. However, mutations in CRYAA can detrimentally affect the function. To date, numerous genetic variants have been identified in the *CRYAA* gene, the majority of which are single nucleotide polymorphisms (SNPs). Bhagyalaxmi et al. reported an association of G > A transition found in exon-1 of the *CRYAA* gene with

ARC and differential risk of genotypes for individual types of cataract [7]. In the exon-2 of the *CRYAA* gene, the F71L mutation resulted in defective chaperone-like function, intimately linked to ARC [8]. SNP rs727846 in the *CRYAA* gene has been reported to be related to ARC through decreasing transcriptional activity of the *CRYAA* promoter [9]. Our previous study revealed that SNPs identified in the 5' untranslated region (5' UTR) of *CRYAA* were associated with susceptibility to ARC in a Han Chinese population, especially the nuclear subtype [10].

Recently, our team has reported that the SNP (rs76740365 G > A) in the exon-3 of *CRYAA* gene is closely involved in cataract formation, especially posterior subcapsular cataract (PSC). The mutation in rs76740365 is a missense mutation that causes the amino acid substitution from glutamic acid (E) to lysine (K), which localizes in the 156th amino acid residues of *CRYAA*. However, its functional role in the pathogenesis of ARC remains unclear. In the present study, we aimed to further elucidate the underlying mechanism of E156K mutation in causing cataract at the molecular level and cellular level.

Methods

Expression of wild type and mutant α A-crystallin and purification

cDNA encoding human *CRYAA* was cloned into pSmart-I vector (Invitrogen) and used as a template to generate the E156K mutation in the *CRYAA* gene using a quick-change site directed mutagenesis kit (Stratagene, La Jolla, CA). The E156K mutation was confirmed by automated DNA sequencing. The pSmart-I plasmids were introduced into *Escherichia coli* BL21 (DE3) for protein expression using IPTG. The recombinant proteins of wild type and E156K *CRYAA* were purified using a Superdex G200 gel filtration column (GE Healthcare). The homogeneity of purified protein was identified by SDS-PAGE and immunodetection as described previously [11].

MALDI-TOF mass spectrometry

The mass spectrometric analysis was carried out using Bruker BIFLEX matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometer. A saturated solution of 10 mg/mL of sinapinic acid in 50% acetonitrile/water containing 0.1% trifluoroacetic acid was used as the matrix. About 1 μ L volume of sample solution and the matrix solution at a ratio of 1:1 was applied to MALDI target plate and air dried. Mass spectra were obtained in linear mode and generated by using Flex-control Software (Bruker, USA). Finally, all acquired spectra were processed with Flex Analysis v.3.3 (Bruker Daltonics).

Circular dichroism (CD) measurements

The secondary and tertiary structure of α A-crystallin was examined by far-UV and near-UV CD spectra at 25°C in a Jasco-810 spectropolarimeter (Jasco, Inc., Japan). Spectra were recorded using a bandwidth of 1 nm at a scanning speed of 200 nm/min. Far-UV CD spectra were collected from 195 to 250 nm in a 1 mm path length quartz cuvette, and near-UV spectra were collected from 250 to 300 nm in a 10 mm path

length quartz cuvette. Protein concentrations of 0.2 mg/ml and 1 mg/ml in 10 mM phosphate buffer at pH 7.2 were used for recording the far- and near-UV CD spectra respectively. The reported spectra were an average of five accumulations and the data were analyzed using CDNN (Circular Dichroism analysis using Neural Networks) software.

Fluorescence measurements

The fluorescence measurements were recorded on a fluorescence spectrophotometer (RF-6000, Shimadzu, Japan). The selective tryptophan excitation wavelength was set at 295 nm and the emission was collected in the range 200–400 nm using 5nm/5nm slits. The hydrophobic probe, 4,4'-Bis-1-anilino naphthalene 8-sulfonate (bis-ANS) (Molecular Probes, USA), was used to check the surface hydrophobicity of proteins. Bis-ANS emission spectra were detected using an excitation wavelength of 395 nm, and spectra were recorded from 200–560 nm using 5nm/5nm slits. Three scans were averaged and buffer spectra were subtracted from protein spectra to calculate each spectrum.

Structural modeling

Protein structural modeling was performed using Maestro molecular modeling software (Schrödinger) [12]. The structural graphics were prepared using PyMOL Molecular Graphics System software (Version 1.3 Schrödinger, LLC).

The chaperone-like function of wild type and mutant human α A-crystallin

The chaperone-like ability of wild-type and E156K mutant CRYAA was investigated against the heat-induced aggregation of β L-crystallin and the DTT-induced aggregation of insulin. Aggregation was monitored by measuring light scattering at 360 nm using the Biotek microplate reader according to previously described methods [8, 13].

Cell culture and transfection

The human lens epithelium (HLE) SRA01/04 cells (ATCC; Rockville, MD, USA) were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 20% fetal bovine serum (FBS) at 37°C in a humidified 5% CO₂ incubator. For transfection, cells were seeded into six-well plates 24h before and grown to 70–80% confluence. The transfection mixture consisting of 2 μ g of plasmid DNA and 4 μ l Lipofectamine™ 2000 (Invitrogen) reagent in 2 ml of serum-free medium was then added in a six-well plate. Transfected cells were incubated at 37°C and CO₂ for 48 h before use.

Establishment of stable CRYAA knock-down cell lines

To knock down the endogenous *CRYAA*, shRNAs targeting four unique sequences were constructed (sh1–sh4) using the lentiviral vector PHY-310. The target sequences were as follows: human *CRYAA* sh1, GGGACAAGTTCGTCATCTTCC; human *CRYAA* sh2, GCAGGACGACTTTGTGGAGAT; human *CRYAA* sh3, CCGGCATCTCTGAGGTTTCGAT; human *CRYAA* sh4, CCTCGTCCTAAGCAGGCATTG. Selection of infected

cells was achieved with puromycin (2 µg/ml). Knockdown efficiency of the shRNA candidates was measured by quantitative PCR (qPCR).

Plasmid construction

The human *CRYAA* was amplified using primers, as described above and inserted into the PHY-LV-OE1.6-FLAG vector (Invitrogen) using the restriction enzymes Hind III and KpnI (Takara). Rs76740365 SNP mutant of *CRYAA* (E156K) was generated by site-directed mutagenesis.

Western blot

For western blots, cells were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer (R0278, Sigma) supplemented with protease inhibitor cocktail (#78425, Thermo Scientific). The lysate was centrifuged at 12,000 ×g for 15 min at 4°C, the supernatant was collected and stored for further analyses. To ensure equal protein load, protein concentration was measured using a bicinchoninic acid (BCA) assay. The protein samples were then denatured by boiling at 100°C for 5 min in 5X loading buffer containing 10% SDS and 100 mM dithiothreitol. Equal amounts of protein were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) before being electrotransferred to 0.45 µm pore-size polyvinylidene difluoride (PVDF) membranes (IPVH00010, Merck). After blocking with 5% nonfat milk prepared in Tris-Buffered Saline with 0.1% Tween (TBS-T) for 1 h at room temperature, the PVDF membranes were probed overnight with the following primary antibodies at 4°C: *CRYAA* (ab181866, Abcam), β -actin (A3854; Sigma), AKT (#9272; CST, Ipswich, MA), p-AKT (#4060; CST, Ipswich, MA), Caspase 3 (#9662; CST, Ipswich, MA), and Caspase 9 (#9502; CST, Ipswich, MA). On the next day, the membranes were washed with TBS-T three times for 10 min and incubated with peroxidase-conjugated goat anti-mouse IgG (H + L) or anti-rabbit IgG (H + L; Yeasen, China) for 1 hour. Signals were visualized by Super Signal West Pico (NCI5079, Thermo Scientific). Images from western blotting experiments were processed using a Kodak Imaging System and quantitated using ImageJ program.

Quantitative real-time PCR (qRT-PCR)

RNA isolation and qRT-PCR analysis were performed as previously described [14]. Total RNA was isolated TRIzol (15,596, Thermo Fisher) and quantified on NanoDrop 2000 (Thermo Fisher). The extracted RNA was transcribed into cDNA using a reverse transcription kit (KR106-02, Tiangen, China) and the expression of *CRYAA* was determined using SYBR Premix (FP205-02, Tiangen) on an ABI 7500 PCR machine following the manufacturers' manual. The sequences of primer pairs were described below: *CRYAA*, 5'- CTGAGGACGATGTGTCTAACCTC - 3' (forward) and 5'- AGGCCTGGA CTCAGCTGA - 3' (reverse); β -actin, 5'- TTG TTA CAG GAA GTC CCT TGC C -3' (forward) and 5'- ATG CTA TCA CCT CCC CTG TGT G -3' (reverse).

Apoptosis

Cell apoptosis was determined using an Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) apoptosis detection kit (Beyotime, Shanghai, China). After the indicated treatments, the cells were collected through trypsinization followed by washing with PBS twice. For analysis of apoptosis, the

HLECs were stained with Annexin V- FITC for 15 min at room temperature and then incubated with PI on ice. After incubation, all samples were analyzed by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA). Cells stained positive with Annexin V and negative with PI were considered as early apoptosis.

Statistical analysis

All experiments were repeated three times independently with similar results and the data were shown as mean $\pm$ Standard Deviation (SD). Significant difference was assessed using two-tailed Student's t-test, ANOVA two-way test with Bonferroni post-test, or Fisher's exact test, depending on the types of the variables. The statistical analysis and the calculation of odds ratios (ORs) were conducted using SPSS for Windows, version 17.0 (SPSS, IBM Inc., Chicago, IL, USA). SHEsis software platform was used to estimate Hardy–Weinberg equilibrium (HWE), linkage disequilibrium (LD), and haplotype frequencies [15]. Differences were considered statistically significant at P value < 0.05 .

Results

Effect of Mutation E156K on the Molecular Mass of CRYAA

To characterize the structural significance of the E156K mutation, we cloned both wild-type (WT) and mutant genes expressed in *E.coli*. The mutant proteins were purified as described for WT and SDS-PAGE profile of WT and E156K mutant proteins after purification and homogeneity of recombinant CRYAA were shown in Fig. 1. The molecular mass of E156K CRYAA was then determined by using MALDI-TOF mass spectrometer (Fig. 2). Mass spectrometry analysis showed similar molecular weight both for the mutant and wild-type proteins, indicating insignificant differences in the molecular masses (Table 1).

Table 1 Molecular weights of wild-type α A-crystallin and E156K mutant α A-crystallin.			
Proteins	m/z	Intens	Mass
α A	20054.43	2852.25	20053.42
α A E156K	20053.59	3012.43	20052.583

Secondary structural changes induced by E156K mutant in CRYAA

To determine the secondary structure of WT and E156K mutant CRYAA, two proteins were analyzed using Far-UV Circular Dichroism (CD) spectroscopy. As shown in Fig. 3, both the WT and mutant proteins showed a characteristic minimum around 217 nm, which was indicative of a β -sheet structure. The CDNN analysis of the CD data revealed that E156K CRYAA possessed increased β -sheet content with a concomitant decrease in α -helix compared with WT CRYAA, while no significant difference was found between two groups (Table 2). These results suggested that the E156K mutation has no significant effect on the secondary structure of CRYAA.

Table 2
Percent secondary structure content in wild type and E156K α A-crystallin.

Protein	α -helix	β -sheet	β -turn	Radom coil
α A	24.40%	38.70%	18.30%	35.90%
α A E156K	22.20%	41.90%	18.90%	35.40%

Tertiary structural changes induced by E156K mutant in CRYAA

Near-UV CD spectra of proteins reflect differences in conformation and can be used to probe the tertiary structure of proteins [16, 17]. In our study, the near-UV spectrum of WT protein exhibited significantly different in peaks beyond 260 nm and 277 nm from E156K mutant protein, suggesting a minimal alteration in the signal for tryptophan in the 270–290 nm region and phenylalanine in the 250–270 nm region in the E156K mutant compared to the WT protein (Fig. 4A). To further corroborate the findings from near-UV spectrum, the tertiary structure alterations were analyzed by tryptophan fluorescence measurements (Fig. 4B). The WT and mutant CRYAA did not show any variation in emission maxima of tryptophan fluorescence. However, the fluorescence intensity decreased in mutant proteins, indicating a potential alteration of the microenvironment of aromatic amino acid residue in mutant proteins when compared to WT proteins. Taken together, the results revealed that E156K mutation was likely to affect the tertiary structure.

Surface hydrophobicity

To evaluate the surface hydrophobicity of WT and mutation proteins, Bis-ANS fluorescence was employed and showed that the fluorescence emission peak position for both proteins were similar (Fig. 5). However, the fluorescence intensity of bis-ANS was found slightly higher for the mutant protein compared to the WT protein, a difference indicating a gain of surface hydrophobicity in the E156K mutant CRYAA. In order to understand the structural features that contribute to the increased surface hydrophobicity of E156K CRYAA, we performed molecular modeling on the WT and the mutant proteins. The model of dimer structure illustrated that E156K mutation induced a clear change in the protein structure (Fig. 6). As a result, their negatively charged surface changed to positively charged, which was the possible reason for the disturbance to the surface hydrophobicity of E156K CRYAA.

The chaperone activity of E156K mutant CRYAA

We further detected the chaperone-like activity (CLA) of CRYAA of two proteins using DTT-induced aggregation of insulin as well as heat-induced aggregation of β L-crystallin as model systems (Fig. 7). In DTT-induced aggregation of insulin, the CLA of E156K mutant protein was increased by approximately 15.62% as compared to WT. Similarly, the E156K mutant showed about 9.35% higher CLA in heat-induced aggregation of β L-crystallin when compared to WT CRYAA. These findings suggested that the E156K mutation leads to an increase in the chaperone activity of CRYAA.

Bioinformatics functional analysis proteins interacting with CRYAA

In our previous study, a total of 343 proteins interacting with CRYAA were identified by using protein microarrays [18]. To further investigate the functions of these proteins, we performed protein chip enrichment analysis and found 143 proteins that were significantly different. Next, Gene Ontology (GO) enrichment analysis was performed using DAVID Bioinformatics Resources [19, 20] to obtain a biological view of these differentially expressed proteins and the protein-protein interaction network was constructed according to the functional relationships annotated in the KEGG database. The results revealed that the proteins interacting with CRYAA were related to chaperone, hydroxylase, proliferation, transcription regulation (Fig. 8).

E156K mutant CRYAA induced apoptosis in HLECs

The bioinformatics functional analysis of proteins interacting with CRYAA showed enrichment for proteins that were associated with apoptosis such as AKT. Furthermore, to investigate the cellular apoptosis induced by mutant E156K CRYAA in human lens epithelium cells (HLECs), we knocked down CRYAA expression through lentivirus transduction. Figure 9A showed that all four CRYAA shRNAs suppressed expression of CRYAA protein in HLECs. CRYAA knockdown lens epithelial cells (CRYAA-sh1) were selected for further experiments because it had a better inhibitory effect on CRYAA expression. Subsequently, we transfected the CRYAA-sh1 HLECs with WT CRYAA plasmid or CRYAA mutant E156K plasmid. As shown in Fig. 9B, the expression of CRYAA protein in the WT group and the mutant E156K group was higher than that in the vector groups, whereas the level of CRYAA protein in the vector group was similar to that in the control group.

Cell apoptosis was assessed with Annexin V-FITC/propidium iodide staining and the percentage of apoptotic cells in the HLECs transfected with mutant E156K was less than that in the WT group, indicating that the antiapoptotic effect induced by E156K mutant was stronger than WT CRYAA (Fig. 10A). AKT/ p-AKT and apoptosis-related proteins were then analyzed with a western blot assay (Fig. 10B). The expression of p-AKT was elevated in mutant E156K group and the opposite results was observed in expression of casepase-3. Together, mutant E156K CRYAA exerted an anti-apoptotic effect in HLECs, being associated with the activation of AKT signal pathway and down-regulation of casepase-3.

Discussion

A recent study by our group demonstrated that the SNP (rs76740365 G > A) in the CRYAA exon region was associated with the susceptibility of ARC, especially posterior subcapsular cataract (PSC), and probably increased the risk of PSC development. Furthermore, in an attempt to gain an insight into the structural variations caused by the missense mutation E156K in CRYAA, we have constructed, expressed, and purified human recombinant E156K CRYAA.

It is observed that E156K mutation did not affect the structure of the proteins in terms of the apparent molecular mass and the secondary structure, while it can alter the tertiary structure resulting in the disturbance to surface hydrophobicity of CRYAA. Ito et al. reported that phosphorylation of alpha B-crystallin caused dissociation of large oligomers to smaller sizes molecules [21]. Other studies have demonstrated that R21C, R54C and R116H mutations in *CRYAA* are associated with congenital cataracts and have an important effect on the molecular weight [22, 23]. However, no major difference in molecular weights were found in our study, suggesting that the mutation of E156K had no detectable effect on size of the oligomers. It is possibly because the E156K mutation is located in the short C-terminal regions in *CRYAA*, which are suggested to play an important role in the structure and chaperone activity but have a negligible impact on oligomeric size [24, 25]. The results from far UV measurements indicated that E156K mutant did not perturb the secondary structure of proteins and all the spectra reflected characteristics of dominant β -sheet structure, which was consistent with previous studies on the structure of wild-type *CRYAA* [26]. In the study by Validandi et al. and Bhagyalaxmi et al., F71L mutation did not significantly affect the apparent molecular mass, secondary and tertiary structures, and hydrophobicity of *CRYAA*, but had a substantial influence on functional properties [8, 13]. However, in this study, the Glu156 residue in *CRYAA*, mutated to Lys (K), induced significant alterations in the protein tertiary structure, resulting in an increase in surface hydrophobicity. As exhibited in other lens proteins, hydrophobicity is associated with crystallin activities and increased hydrophobic interaction could lead to the reduction of the solubility or abnormal folding, which are suggested the differences between the mutant and wild-type proteins.

CRYAA acts as a molecular chaperon protecting other crystallins from aggregation or inactivation and traps aggregation-prone denatured proteins. Sharma et al. identified a 19-amino acid peptide sequence from α A-crystallin which possessed the hydrophobic site and anti-aggregation property, suggesting a critical role for the α A-crystallin domain of heat shock proteins in the chaperone-like activity [27]. Later, they reported that the residues 70–88 in α A-crystallin prevented the oxidation and UV-induced aggregation of γ -crystallins, further confirming that the polypeptide sequence was indeed a functional element of α A-crystallin, responsible for its chaperone-like property [28]. Previous studies demonstrated that the decrease in chaperone activity of alpha-crystallin possibly promoted the aggregation of lens proteins, which could lead eventually to cataract formation [29]. However, Biswas et al. reported an enhancement in the chaperone function of α -crystallin due to increased surface hydrophobicity by methylglyoxal (MGO) modification [25]. Others proved that α -dicarbonyl compounds like MGO were the major sources of advanced glycation end-products (AGEs), which accumulated in lenses during the aging process and even more rapidly in cataractous lenses [30, 31]. Together, these findings suggested that the development of cataract disease was not necessarily correlate with the decrease in the activity of alpha-crystallin. In our study, the E156K mutation markedly enhanced the chaperone function of *CRYAA*. Considering our previous findings that the SNP (rs76740365 G > A) in the exon-3 of *CRYAA* gene was related to the PSC development, we speculated further that the increased chaperone function of E156K *CRYAA* was probably associated with the pathogenesis of PSC. However, experiments with E156K mutant *CRYAA* are required to fully elucidate the mechanisms by which it is involved in the PSC formation in the future.

To further investigate the proteins interacting with CRYAA and the potential roles that these protein interactions play in the function of CRYAA, we performed the bioinformatics functional analysis and the results revealed that these proteins were associated with chaperone, hydroxylase and involved in the processes of transcription regulation and proliferation. Xi et al. reported slower proliferation and higher apoptosis in CRYAA-knockout lens epithelial cells than wild-type cells, suggesting a role for CRYAA in regulating cell proliferation [32]. However, we found no evidence for aberrant proliferation between wild type and E156K CRYAA, suggesting that E156K mutation in CRYAA did not significantly affect cell proliferation levels (Supplementary Fig. S1-S2). The protein-protein interaction analysis also indicated that CRYAA interacted with various proteins that may contribute to its potent antiapoptotic protection against oxidative damage. Our previous study demonstrated that CRYAA provided protection against oxidative stress [33]. It is also well known that CRYAA are distinct antiapoptotic regulators and prevent stress-induced apoptosis by regulating multiple signaling pathways [34]. In the present study, CRYAA induced anti-apoptotic ability in HLECs, which could be enhanced by E156K mutation. In addition, a significant elevation of p-AKT signaling pathway and down-regulation of caspase-3 were observed in the E156K mutant HLECs. One previous study by Hu et al. suggested that CRYAA could regulate caspase-3 or Bax and activate the AKT signaling pathway to suppress apoptosis induced by oxidative stress [35]. However, Pasupuleti et al. demonstrated that the antiapoptotic function is directly related to the chaperone function [36]. In their study, the anti-apoptotic function of human CRYAA was enhanced in R21A mutant protein that showed increased chaperone activity through promotion of Akt phosphorylation [36]. Consistent with their conclusions, we also found that E156K mutation enhanced chaperone function of mutant proteins. Together, we concluded that that E156K mutant might increase anti-apoptotic function of HLECs by enhancing the chaperone activity through the activation of AKT signaling pathway and the inhibition of Caspase-3.

In summary, E156K mutation in CRYAA did not result in a significant loss of molecular mass and secondary structure of the CRYAA protein. However, E156K mutant altered the tertiary structure and enhanced the chaperone function of CRYAA by inducing its surface hydrophobicity. Furthermore, we found that among the proteins interacting with CRYAA identified by bioinformatics functional analysis, AKT/P-AKT was activated in mutant E156K group, which seemed to be the possible mechanism underlying the activation of the anti-apoptotic function induced by E156K mutation.

Declarations

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Conflict of interest The authors have no conflicts of interest to declare.

Author Contributions Zhennan Zhao, Yang Sun, Qi Fan contributed equally to this work. Yi Lu was responsible for conception and design. Zhennan Zhao designed and performed experiments. Qi Fan collected the literatures, extracted and analyzed the data. The first draft of the manuscript was written by

Yang Sun and all authors commented on previous versions of the manuscript. Yongxiang Jiang was involved in reviewing the manuscript.

Data availability The datasets generated or analyzed during the current study are included in this article. Further any enquiries can be directed to the corresponding author.

Ethical approval This research was approved by the Ethics Committee of Eye & ENT Hospital of Fudan University and followed the tenets of the Declaration of Helsinki.

Consent to publication The manuscript is approved by all authors for publication.

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Figures

Figure 1

SDS-PAGE of purified wild-type CRYAA (A) and E156K CRYAA (B). M represents molecular weight marker. Lane α A represents wild-type α A-crystallin and Lane α A E156K represents recombinant mutant α A-crystallin

Figure 2

Mass spectrometry of wild-type (A) and E156K CRYAA (B)

Figure 3

Near-UV CD spectra of wild-type (solid curve) and E156K mutant (dashed curve) CRYAA

Figure 4

Tertiary structural studies on the wild-type and E156K CRYAA. Near-UV CD spectra (A) and tryptophan fluorescence spectra (B) of wild type (solid curve) and E156K mutant (dashed curve)

Figure 5

ANS fluorescence spectra of wild-type (solid curve) and E156K mutant (dashed curve)

Figure 6

The predicted structure of wild-type CRYAA and E156K CRYAA. **A, B** Loop-helix structures. **C, D** Surface structures. Positively charged residues are colored in blue and negatively charged residues are colored in red. The red box highlights structural differences in the amino acid residues differences between wild-type CRYAA and E156K CRYAA

A

B

Figure 7

Chaperone-like activity of E156K mutant in CRYAA compared to wild-type CRYAA in (A) DTT-induced aggregation of insulin and (B) heat-induced aggregation of β L-crystallin

Figure 8

Functional protein association network determined using DAVID Bioinformatics Resources with respect to the target proteins

Figure 9

A Construction of the CRYAA knock down HLECs (CRYAA-sh1). CRYAA mRNA expression was significantly down-regulated in HLECs transfected with CRYAA-sh1, CRYAA-sh2, CRYAA-sh3 and CRYAA-sh4. **B** Expression of CRYAA protein was determined with western blot assays and the CRYAA protein expression was significantly up-regulated in CRYAA-sh1 transduced with wild type CRYAA plasmid and CRYAA mutant E156K plasmid

Figure 10

E156K mutant CRYAA decreased cell apoptosis. **A** Annexin V-FITC/ propidium iodide staining was performed to analyze cell apoptosis, and Annexin V-negative/ propidium iodide-positive cells underwent apoptosis. **B** Western blot assays were used to detect the expression of AKT, p-AKT, casepase-3 and casepase-9 protein

Supplementary Files

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