Identification of proteins that interact with alpha A-crystallin using a human proteome microarray

Qi Fan,[1] Lv-Zhen Huang,[2] Xiang-Jia Zhu,[1] Ke-Ke Zhang,[1] Hong-Fei Ye,[1] Yi Luo,[1] Xing-Huai Sun,[1] Peng Zhou,[3] Yi Lu[1]

(The first three authors contributed equally to this work)

[1]Department of Ophthalmology, Eye and ENT Hospital of Fudan University, Shanghai, People's Republic of China; [2]Department of Ophthalmology, Peking University People's Hospital, Beijing, People's Republic of China; [3]Department of Ophthalmology, Parkway Health Hong Qiao Medical Center, Shanghai, People's Republic of China

Purpose: To identify proteins interacting with alpha A-crystallin (CRYAA) and to investigate the potential role that these protein interactions play in the function of CRYAA using a human proteome (HuProt) microarray.
Methods: The active full-length CRYAA protein corresponding to amino acids 1–173 of CRYAA was recombined. A HuProt microarray composed of 17,225 human full-length proteins with N-terminal glutathione S-transferase (GST) tags was used to identify protein–protein interactions. The probes were considered detectable when the signal to noise ratio (SNR) was over 1.2. The identified proteins were subjected to subsequent bioinformatics analysis using the DAVID database.
Results: The HuProt microarray results showed that the signals of 343 proteins were higher in the recombinant CRYAA group than in the control group. The SNR of 127 proteins was ≥ 1.2. The SNR of the following eight proteins was > 3.0: hematopoietic cell-specific Lyn substrate 1 (HCLS1), Kelch domain-containing 6 (KLHDC6), sarcoglycan delta (SGCD), KIAA1706 protein (KIAA1706), RNA guanylyltransferase and 5′-phosphatase (RNGTT), chromosome 10 open reading frame 57 (C10orf57), chromosome 9 open reading frame 52 (C9orf52), and plasminogen activator, urokinase receptor (PLAUR). The bioinformatics analysis revealed 127 proteins associated with phosphoproteins, alternative splicing, acetylation, DNA binding, the nuclear lumen, ribonucleotide binding, the cell cycle, WD40 repeats, protein transport, transcription factor activity, GTP binding, and cellular response to stress. Functional annotation clustering showed that they belong to cell cycle, organelle or nuclear lumen, protein transport, and DNA binding and repair clusters. CRYAA interacted with these proteins to maintain their solubility and decrease the accumulation of denatured target proteins. The protein–protein interactions may help CRYAA carry out multifaceted functions.
Conclusions: One-hundred and twenty-seven of 17,225 human full-length proteins were identified that interact with CRYAA. The advent of microarray analysis enables a better understanding of the functions of CRYAA as a molecular chaperone.

Alpha A-crystallin (CRYAA) is a member of the small heat shock protein (sHSP) family, also known as the sHSP 20 family [1]. In humans, the *CRYAA* gene encodes a 173 amino acid residue protein by single copy genes located on chromosome 21. CRYAA is one of the major lens proteins, accounting for 35% of all crystallins. Although it is a major component of the lens, it is also found in ganglion cells, inner nuclear layers, and photoreceptors of the eye, as well as in spleen, liver, kidney, adrenal, cerebellum, brainstem, and other organs [2,3]. We found previously that CRYAA is related to the formation of age-related cataracts [4]. We have also shown that CRYAA has high potency in protecting oxidative stress in a gene knockout animal model [5]. However, the mechanism of this protection needs further investigation.

Previous studies have shown that the antioxidant function of CRYAA is linked to the glutathione (GSH) level [6] and that its antiapoptotic function is directly interlinked with the chaperone function by reducing phosphatase tensin homolog (PTEN) activity and enhancing phosphoinositide 3 kinase (P13K) activity [7]. However, these studies have not elucidated the mechanism of the chaperone function of CRYAA. To understand the functions of CRYAA and how these are regulated, protein–protein interactions need to be investigated. CRYAA has been found to interact with caspase-3 [8], Bax [9], b-cell lymphoma-extra protein (Bcl-X) [9], methionine sulfoxide reductase [10], actin [11], amyloid-β peptides [12], and many other proteins. However, conventional methods of screening for the interaction of CRYAA with proteins are cumbersome and time consuming.

Correspondence to: Yi Lu, Department of Ophthalmology, Eye and ENT Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, People's Republic of China; Phone: +86-21-64377134; FAX: +86-21-64377151; email: luyi0705@yeah.net

Functional proteome microarrays were designed to screen interactions between proteins in a single experiment [13]. The major limitation of previously used microarrays is their lack of comprehensiveness. Recently, a new human proteome (HuProt) microarray was developed, with about 80% coverage of the human proteome [14,15].

In this study, we used the HuProt microarray to identify proteins that interact with CRYAA. Furthermore, we performed bioinformatics analysis to study the function of these interacting proteins.

METHODS

Recombinant protein and antibody of alpha A-crystallin: Active recombinant full-length CRYAA protein, corresponding to amino acids 1–173 of human CRYAA, and mouse monoclonal antibody to CRYAA were purchased from Abcam Inc. (Cambridge, MA, catalog numbers ab48778 and ab14821). Recombinant full-length CRYAA protein and alpha-A antibody specificity were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and western blotting (Appendix 1).

Human proteome microarrays: The HuProt microarray (CDI Laboratories, Inc., Mayaguez, Puerto Rico) was composed of 17,225 human full-length proteins with N-terminal glutathione S-transferase (GST) tags. With minor modification from previously described [14,15], the HuProt microarray was performed to the following procedure. In the CRYAA group, the microarray was incubated with blocking buffer (3% bovine serum albumin [BSA] in 1X PBS [KH_2PO_4 1.4 mM, Na_2HPO_4 8 mM, NaCl 140 mM, KCl 2.7 mM, 1000 ml distilled H_2O]with 0.1% Tween-20, [PBST], pH=7.2) at 4 °C for 1 h. After rinsing three times with 300 µl PBST (0.1% Tween-20 in 1X PBS), 500 µl of recombinant full-length CRYAA (2 µg of protein diluted in 500 µl 1X PBST, with 5 mM of dithiothreitol [DTT], pH=7.2) were added and incubated under a glass coverslip at 4 °C for 1.5 h. After washing three times with PBST, 500 µl of mouse anti-CRYAA monoclonal antibody (1:1,000 diluted in 500 µl 1X PBST) was added to the microarray slide and incubated at 37 °C for 1 h. After washing three times with PBST, the slide was incubated with 500 µl of goat anti-mouse immunoglobulin G (IgG)-Cy3-conjugated antibody (1:200, Jackson Laboratory, Bar Harbor, ME) in the dark at 37 °C for 1 h. After washing three times with 1X PBST and then three times with Milli-Q water, the microarray was centrifuged for 5 min in a 50 ml centrifuge tube.

In the control group, the procedure for the microarray assay was similar to that described above for the CRYAA group, but with the following modification. Instead of 500 µl recombinant full-length CRYAA, the microarray was incubated with the 500 µl PBST (with 5 mM of DTT, pH=7.2) after blocking.

The microarray was scanned with an Axon GenePix 4000B Microarray Scanner (Molecular Devices, LLC, Sunnyvale, CA), and the probe signals were acquired using GenePix Pro 6.0 software (Molecular Devices). The probes were considered detectable when the signal-to-noise ratios (SNRs) for both duplicates were over 1.2.

Bioinformatics analysis: The proteins with an SNR ≥1.2 were listed for subsequent bioinformatics analysis. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) Bioinformatics Resources 6.7 was used for the enrichment and pathway analysis as previously described [16,17].

Statistics: All statistical analysis was performed with SPSS 17.0 software. All p values were calculated by the chi-square test or Fisher's exact test when appropriate.

RESULTS

Identification of proteins interacting with alpha A-crystallin using human proteome microarrays: The HuProt microarray examined 17,225 probe sets and found 343 proteins where the signals at 532 nm were higher in the recombinant CRYAA group than in the control group (Figure 1). Of these, 127 proteins had an SNR ≥ 1.2. The 127 proteins are listed in Appendix 2. The SNRs of the following eight proteins were above 3.0: hematopoietic cell-specific Lyn substrate 1 (HCLS1), Kelch domain-containing 6 (KLHDC6), sarcoglycan delta (SGCD), KIAA1706 protein (KIAA1706), RNA guanylyltransferase and 5′-phosphatase (RNGTT), chromosome 10 open reading frame 57 (C10orf57), chromosome 9 open reading frame 52 (C9orf52), and plasminogen activator, urokinase receptor (PLAUR).

Bioinformatics functional analysis: Enrichment of biological processes was determined using the DAVID Bioinformatics Resources. This revealed 127 proteins associated with phosphoproteins, alternative splicing, acetylation, DNA binding, the nuclear lumen, ribonucleotide binding, the cell cycle, WD40 repeats, protein transport, transcription factor activity, GTP binding, and cellular responses to stress (Figure 2). Functional annotation clustering showed that they belong to cell cycle, organelle or nuclear lumen, protein transport, and DNA binding and repair clusters (Figure 3).
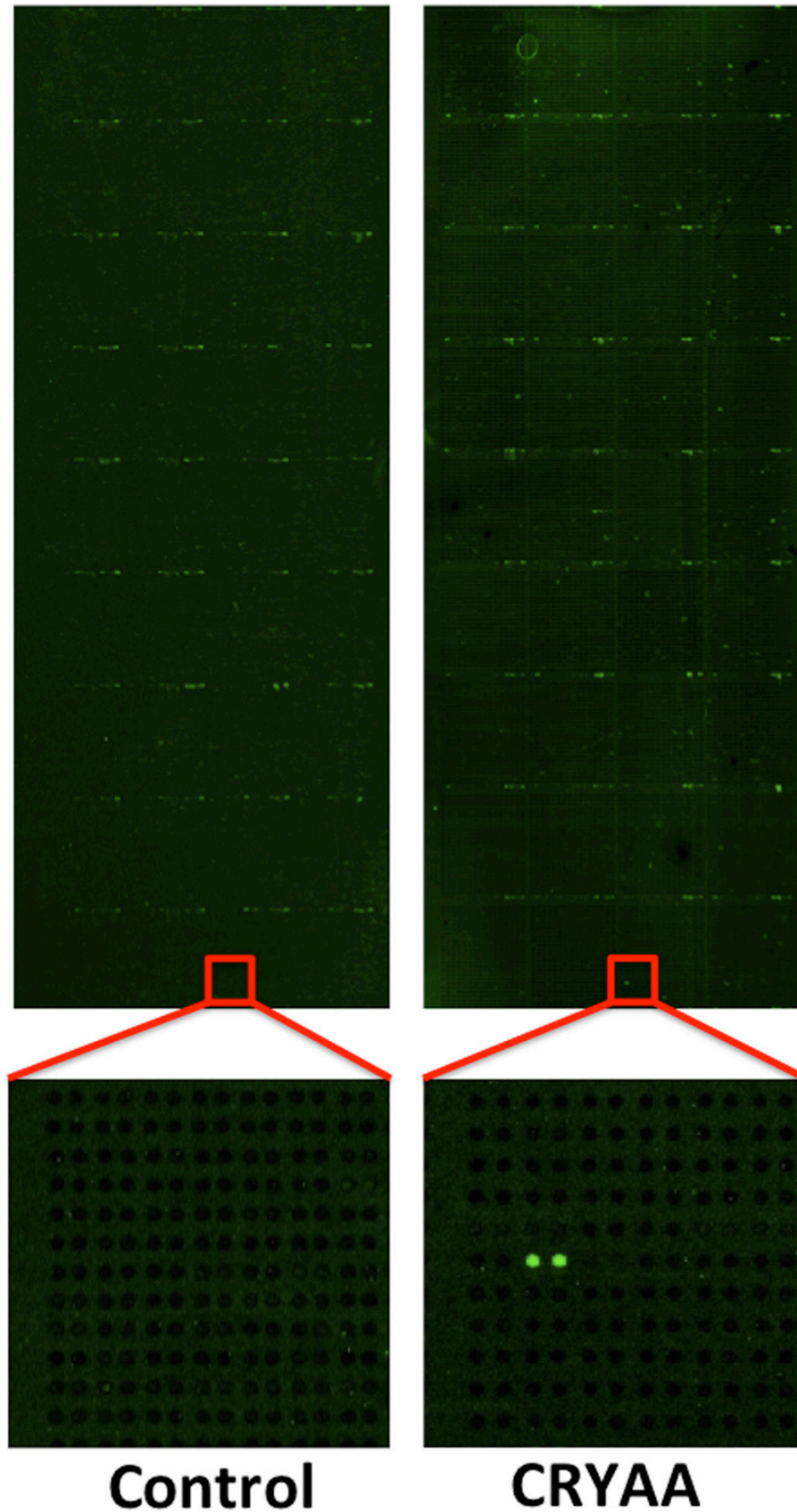
Figure 1. Identification of alpha A-crystallin (CRYAA)-interacting proteins in the protein array. The protein arrays were incubated with recombinant full-length CRYAA, followed by mouse anti-CRYAA monoclonal antibody and goat anti-mouse immunoglobulin G (IgG)-Cy3 conjugated antibody in the CRYAA group. One section of the protein array is presented, showing some of the positive spots. (Signals at 532 nm were higher in the CRYAA group than in the control group.)

Legend:
- phosphoprotein
- alternative splicing
- acetylation
- non-membrane-bounded organelle
- DNA binding
- nuclear lumen
- ribonucleotide binding
- cell cycle
- WD40 repeat
- protein transport
- transcription factor activity
- GTP binding
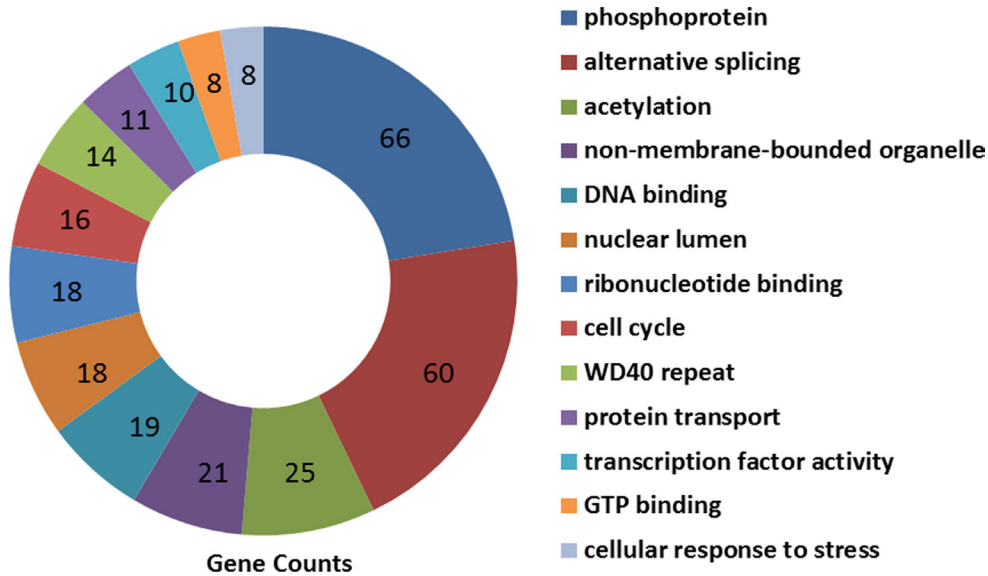- cellular response to stress

Gene Counts

Figure 2. Enrichment of biological processes in terms of gene ontology (GO) categories with respect to differentially alpha A-crystallin (CRYAA)-interacting proteins at a signal-to-noise ratio (SNR) ≥ 1.2. GO categories for each target protein were determined using DAVID Bioinformatics Resources. The number of proteins in each GO category is indicated within the plot.

DISCUSSION

Alpha crystallins are small HSPs that play central roles in maintaining lens transparency and preventing cataract formation [18]. CRYAA can efficiently trap aggregation-prone denatured proteins in vitro, and this is thought to delay the development of age-related cataracts in vivo [19]. Although research into the functions of CRYAA has accelerated in recent years, the precise mechanism underlying their activities is not fully understood. Further research is required to identify CRYAA target substrates and to understand the molecular chaperone role of CRYAA in lens cell biology and cataract pathology.

In this study, we found that CRYAA interacts with 127 proteins. These proteins are associated with phosphoproteins, alternative splicing, acetylation, DNA binding, nuclear lumen, ribonucleotide binding, the cell cycle, WD40 repeats, protein transport, transcription factor activity, GTP binding, and cellular responses to stress. The functional analysis revealed that the proteins are concentrated into four clusters. The first cluster is involved in cell cycle regulation, the second occurs in intracellular organelles or the nuclear lumen, the third participates in protein localization and transport, and the fourth is related to DNA metabolic processes and DNA repair.

We found that CRYAA interacts with various proteins that may contribute to lens transparency in this study. Costello [20] reported that lens transparency relies on the metabolic function of mitochondria. Our results revealed that CRYAA interacts with solute carrier family 25, member 28 (SLC25A28), solute carrier family 25, member 25 (SLC25A25), and electron-transfer-flavoprotein, beta

polypeptide (ETFB), all of which are associated with the metabolic function of mitochondria. SLC25A28 and SLC25A25 belong to the mitochondrial carrier family, which localizes in the mitochondrion. SLC25A28 is a mitochondrial iron transporter that mediates iron uptake and is required for heme synthesis of hemoproteins and Fe-S cluster assembly in nonerythroid cells. SLC25A25 is an ATP-Mg/Pi exchanger. It mediates the transport of Mg-ATP in phosphate exchange and catalyzes the net uptake or efflux of adenine nucleotides into or from the mitochondria. ETFB is an electron transfer flavoprotein, which is involved in oxidation reduction and the generation of precursor metabolites and energy.

Cytoskeletal proteins are important in the establishment and maintenance of transparent lens cell structure [21]. They can maintain the stability of cytoskeletal components, as well as regulating the dynamic assembly of cytoskeletal polymers through sequestration of subunits. Quinlan [22] previously reported that alpha crystallin interacts with cytoskeletal proteins and prevents undesired interactions and aggregation. Brown [23] suggested that CRYAA may have functions related to the cytoskeleton and contribute to cataract formation because the R116C mutation of CRYAA in human cataracts binds less to the cytoskeletal component. In our study, we found that CRYAA interacts with SGCD. SGCD is a component of the sarcoglycan complex, a subcomplex of the dystrophin-glycoprotein complex, which forms a link between the F-actin cytoskeleton and the extracellular matrix.

We also found that CRYAA interacts with various proteins that may contribute to protein stabilization. CRYAA was reported to prevent heat-induced aggregation of actin
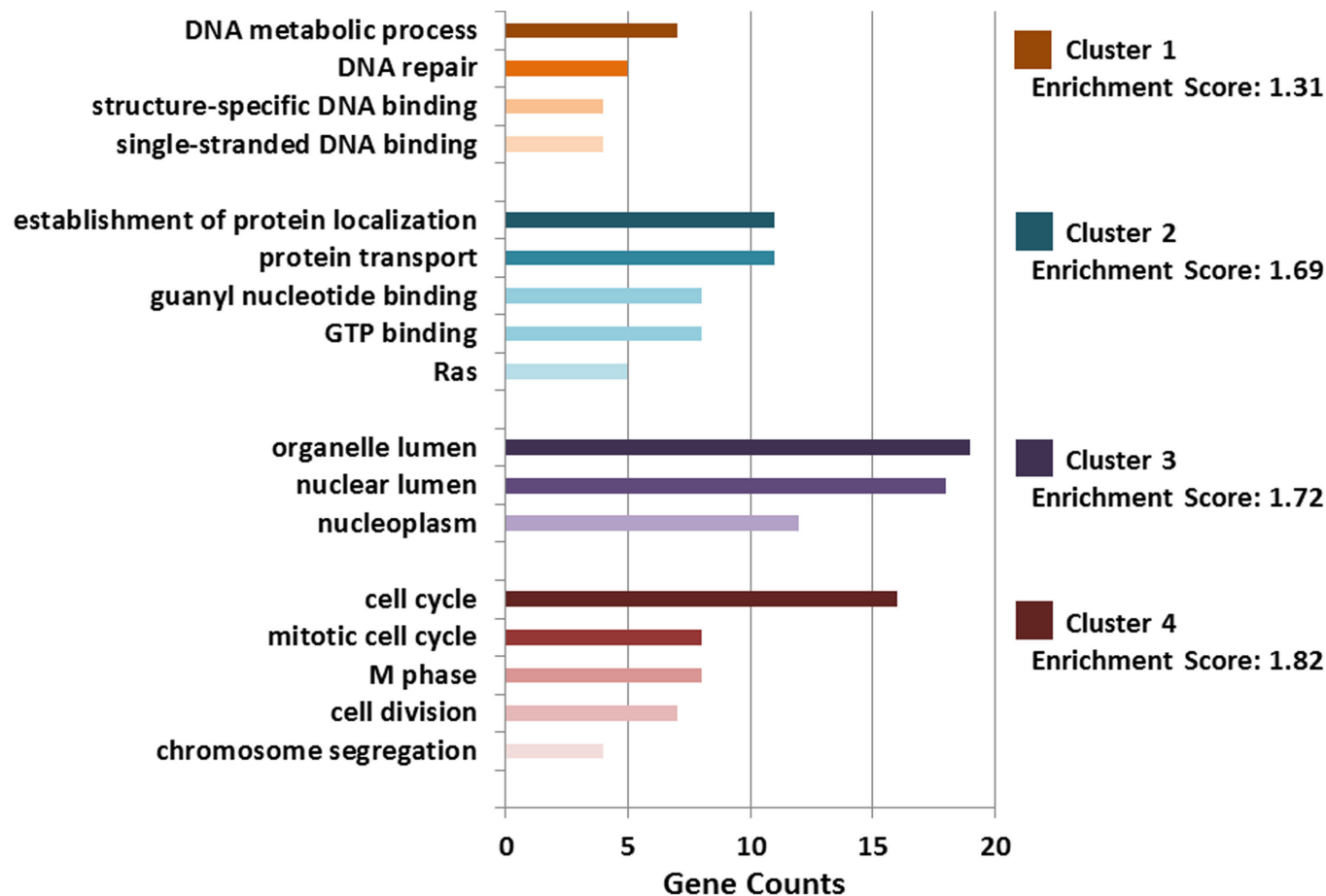
Figure 3. Functional annotation clustering determined using DAVID Bioinformatics Resources with respect to the target proteins. The representative groups with an enrichment score of 1.0 or above are presented. The x-axis represents the number of proteins, while the y-axis represents the ontology categories.

filaments [24]. Our results revealed that CRYAA interacts with the LIM domain and actin binding 1 (LIMA1), which can bind to actin monomers and filaments and inhibit actin filament depolymerization [25]. CRYAA has been shown to prevent heat-induced aggregation of tubulin [26]. As demonstrated in the present study, CRYAA interacted with tubulin, alpha-like 3 (TUBAL3), which is the major constituent of microtubules.

This study indicated a novel and specific function of CRYAA associated with the degradation of proteins. We found that CRYAA does not specifically interact with destabilized, precipitation-bound proteins. Moreover, it promotes proteolysis, which prevents further aggregation and precipitation of target proteins. For example, PLAUR acts as a receptor for the urokinase plasminogen activator and mediates proteolysis-independent signal transduction activation effects of the urokinase-type plasminogen activator (uPA). Proteasome subunit beta type 6 (PSMB6) is a

multicatalytic proteinase complex that is characterized by its ability to cleave peptides at neutral or slightly basic pH. The beta-transducin repeat-containing (BTRC) protein mediates ubiquitination and subsequent proteasomal degradation of target proteins. The interaction of CRYAA with these proteins points to an important role in decreasing the accumulation of denatured proteins.

CRYAA can provide potent antiapoptotic protection against oxidative damage. In our previous study, we also found that CRYAA has high potency in protecting against oxidative stress [5]. One previous study suggested that CRYAA interacts with caspase-3 or Bax and activates the AKT signaling pathway to suppress apoptosis induced by oxidative stress [8]. Other researchers demonstrated that the antiapoptotic function is directly interlinked with the chaperone function [7]. In this study, we found that CRYAA interacts with three groups of proteins that contribute to its protection against oxidative damage. The first group

identified in the functional clustering analysis is involved in DNA metabolism and repair, which are essential for cell proliferation and responses to DNA damage stimulus. This group includes RAD 51 homolog (RAD51), pituitary tumor-transforming 2 (PTTG2), postmeiotic segregation increased 2 (PMS2), and eyes absent homolog 4 (EYA4). RAD51 participates in the common DNA damage response pathway, which is associated with the activation of homologous recombination and double-strand break repair. PTTG2 plays a central role in chromosome stability and DNA repair. PMS2 is a component of the post-replicative DNA mismatch repair system, and EYA4 specifically dephosphorylates Tyr-142 of histone H2AX (H2AXY142ph). Tyr-142 phosphorylation of histone H2AX plays a central role in DNA repair. The second group of proteins regulates antiapoptotic activity by several pathways. For example, HCLS1 is a substrate of the antigen receptor–coupled tyrosine kinase. It positively regulates the JAK-STAT cascade and plays a role in antigen receptor signaling in both clonal expansion and deletion in lymphoid cells. Mitogen-activated protein kinase binding protein 1 (MAPKBP1) is involved in the initiation of the activity of the inactive enzyme JUN kinases (JNKs), and ribosomal protein S6 kinase polypeptide 5 (RPS6KA5) positively regulates the activity of the nuclear factor (NF)-κB transcription factor. The third group of proteins is related to the level of GSH. For example, glutathione transferase zeta 1 (GSTZ1) has been found to play an essential role in protecting cells and tissues against oxidative damage by catalyzing the reduction of hydrogen peroxide by glutathione. It was also shown to have a specific role in the reduction of H_2O_2 and oxidized ascorbate and to participate in the gamma-glutamyl cycle. Solute carrier family 25, member 26 (SLC25A26) specifically mediated the transport of S-adenosylmethionine into mitochondria, which were shown to contain increased glutathione content [27]. Therefore, CRYAA can provide protection against oxidative damage in several ways.

Research with primary lens epithelial cell cultures revealed a role for CRYAA in regulating cell growth. Andley [28] found that CRYAA-knockout lens epithelial cells grow at a 50% slower rate than wild-type cells in primary cultures. Examination of the cellular distribution of CRYAA in the cultured lens epithelial cells showed that it was concentrated in the intercellular microtubules of cells undergoing cytokinesis [29]. Another study found that mouse lens epithelial cell progression through the cell cycle is significantly affected by the expression of alpha-crystallin [30]. This favors the possibility that CRYAA exerts a direct effect on cell cycle kinetics and influences cell proliferation. Proteins involved in the cell cycle that interacted with CRYAA were the first cluster to be identified in the functional clustering analysis

in the present study. They included chromosome 20 open reading frame 19 (C20orf19), platelet-activating factor acetyl-hydrolase isoform Ib (PAFAH1B1), SEH1-like (SEH1L), and tubulin gamma complex associated protein 5 (TUBGCP5). C20orf19 is a centrosomal protein required for establishing a robust mitotic centrosome architecture able to endure the forces that converge on the centrosomes during spindle formation. PAFAH1B1 positively regulates the activity of the minus-end-directed microtubule motor protein dynein and enhances dynein-mediated microtubule sliding. TUBGCP5 is a member of the gamma-tubulin complex, which is necessary for microtubule nucleation at the centrosome. SEH1L is a component of the Nup107–160 subcomplex, which is required for normal kinetochore microtubule attachment. From the above, we can suggest that CRYAA ensures the successful completion of mitosis, as mentioned in the study by Xi [29].

The goal of this paper was to detect proteins that interact with CRYAA. To ensure that the interactions identified are truly specific, further studies should focus on secondary binding assays, including immunoprecipitation, using non-GST-linked counterparts of the identified proteins to confirm these interactions.

In conclusion, we identified 127 proteins that interact with CRYAA by using protein microarrays. The results contribute to a better understanding of the multifaceted functions of CRYAA and provide directions for future research.

APPENDIX 1. DETECTION OF ALPHA-A ANTIBODY SPECIFICITY.

To access the data, click or select the words "Appendix 1." A) Recombinant CRYAA protein was analyzed by 10% SDS–PAGE with Coomassie brilliant blue staining. B) Recombinant CRYAA protein was analyzed by western blotting with anti-alpha A Crystallin antibody. Molecular weight of recombinant CRYAA is 19.9 kDa. M indicates the molecular mass markers. Lanes 1, 2, and 3 indicate 50ng, 25ng, and 12.5ng of CRYAA, respectively.

APPENDIX 2. PROTEINS THAT INTERACTED WITH CRYAA WITH A SNR ≥1.2.

To access the data, click or select the words "Appendix 2."

REFERENCES

1. Kannan R, Sreekumar PG, Hinton DR. Novel roles for alpha-crystallins in retinal function and disease. Prog Retin Eye Res 2012; 31:576-604. [PMID: 22721717].

2. Kato K, Shinohara H, Kurobe N, Goto S, Inaguma Y, Ohshima K. Immunoreactive alpha A crystallin in rat non-lenticular tissues detected with a sensitive immunoassay method. Biochim Biophys Acta 1991; 1080:173-80. [PMID: 1932094].

3. Srinivasan AN, Nagineni CN, Bhat SP. alpha A-crystallin is expressed in non-ocular tissues. J Biol Chem 1992; 267:23337-41. [PMID: 1429679].

4. Zhou P, Luo Y, Liu X, Fan L, Lu Y. Down-regulation and CpG island hypermethylation of CRYAA in age-related nuclear cataract. FASEB J 2012; 26:4897-902. [PMID: 22889833].

5. Zhou P, Ye HF, Jiang YX, Yang J, Zhu XJ, Sun XH, Luo Y, Dou GR, Wang YS, Lu Y. alphaA crystallin may protect against geographic atrophy-meta-analysis of cataract vs. cataract surgery for geographic atrophy and experimental studies. PLoS ONE 2012; 7:e43173-[PMID: 22916220].

6. Sreekumar PG, Spee C, Ryan SJ, Cole SP, Kannan R, Hinton DR. Mechanism of RPE cell death in alpha-crystallin deficient mice: a novel and critical role for MRP1-mediated GSH efflux. PLoS ONE 2012; 7:e33420-[PMID: 22442691].

7. Pasupuleti N, Matsuyama S, Voss O, Doseff AI, Song K, Danielpour D, Nagaraj RH. The anti-apoptotic function of human alphaA-crystallin is directly related to its chaperone activity. Cell Death Dis 2010; 1:e31-[PMID: 21364639].

8. Hu WF, Gong L, Cao Z, Ma H, Ji W, Deng M, Liu M, Hu XH, Chen P, Yan Q, Chen HG, Liu J, Sun S, Zhang L, Liu JP, Wawrousek E, Li DW. alphaA- and alphaB-crystallins interact with caspase-3 and Bax to guard mouse lens development. Curr Mol Med 2012; 12:177-87. [PMID: 22280356].

9. Mao YW, Liu JP, Xiang H, Li DW. Human alphaA- and alphaB-crystallins bind to Bax and Bcl-X(S) to sequester their translocation during staurosporine-induced apoptosis. Cell Death Differ 2004; 11:512-26. [PMID: 14752512].

10. Brennan LA, Lee W, Giblin FJ, David LL, Kantorow M. Methionine sulfoxide reductase A (MsrA) restores alpha-crystallin chaperone activity lost upon methionine oxidation. Biochim Biophys Acta 2009; 1790:1665-72. [PMID: 19733220].

11. Maddala R, Rao VP. alpha-Crystallin localizes to the leading edges of migrating lens epithelial cells. Exp Cell Res 2005; 306:203-15. [PMID: 15878345].

12. Shammas SL, Waudby CA, Wang S, Buell AK, Knowles TP, Ecroyd H, Welland ME, Carver JA, Dobson CM, Meehan S. Binding of the molecular chaperone alphaB-crystallin to Abeta amyloid fibrils inhibits fibril elongation. Biophys J 2011; 101:1681-9. [PMID: 21961594].

13. Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M. Global analysis of protein activities using proteome chips. Science 2001; 293:2101-5. [PMID: 11474067].

14. Jeong JS, Jiang L, Albino E, Marrero J, Rho HS, Hu J, Hu S, Vera C, Bayron-Poueymiroy D, Rivera-Pacheco ZA, Ramos L, Torres-Castro C, Qian J, Bonaventura J, Boeke JD, Yap WY, Pino I, Eichinger DJ, Zhu H, Blackshaw S. Rapid identification of monospecific monoclonal antibodies using a human proteome microarray. Mol Cell Proteomics 2012; 11:016253-[PMID: 22307071].

15. Hu CJ, Song G, Huang W, Liu GZ, Deng CW, Zeng HP, Wang L, Zhang FC, Zhang X, Jeong JS, Blackshaw S, Jiang LZ, Zhu H, Wu L, Li YZ. Identification of new autoantigens for primary biliary cirrhosis using human proteome microarrays. Mol Cell Proteomics 2012; 11:669-80. [PMID: 22647870].

16. . Huang W. Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4:44-57. [PMID: 19131956].

17. Zhu TX, Lan B, Meng LY, Yang YL, Li RX, Li EM, Zheng SY, Xu LY. ECM-related gene expression profile in vascular smooth muscle cells from human saphenous vein and internal thoracic artery. J Cardiothorac Surg 2013; 8:155-[PMID: 23773607].

18. Delaye M, Tardieu A. Short-range order of crystallin proteins accounts for eye lens transparency. Nature 1983; 302:415-7. [PMID: 6835373].

19. Andley UP. Effects of alpha-crystallin on lens cell function and cataract pathology. Curr Mol Med 2009; 9:887-92. [PMID: 19860667].

20. Costello MJ, Brennan LA, Basu S, Chauss D, Mohamed A, Gilliland KO, Johnsen S, Menko AS, Kantorow M. Autophagy and mitophagy participate in ocular lens organelle degradation. Exp Eye Res 2013; 116:141-50. [PMID: 24012988].

21. Clark JI, Matsushima H, David LL, Clark JM. Lens cytoskeleton and transparency: a model. Eye (Lond) 1999; 13:Pt 3b417-24. [PMID: 10627819].

22. Quinlan R. Cytoskeletal competence requires protein chaperones. Prog Mol Subcell Biol 2002; 28:219-33. [PMID: 11908062].

23. Brown Z, Ponce A, Lampi K, Hancock L, Takemoto L. Differential binding of mutant (R116C) and wildtype alphaA crystallin to actin. Curr Eye Res 2007; 32:1051-4. [PMID: 18085469].

24. Wang K, Spector A. alpha-crystallin stabilizes actin filaments and prevents cytochalasin-induced depolymerization in a phosphorylation-dependent manner. Eur J Biochem 1996; 242:56-66. [PMID: 8954153].

25. Abe K, Takeichi M. EPLIN mediates linkage of the cadherin catenin complex to F-actin and stabilizes the circumferential actin belt. Proc Natl Acad Sci USA 2008; 105:13-9. [PMID: 18093941].

26. Xi JH, Bai F, McGaha R, Andley UP. Alpha-crystallin expression affects microtubule assembly and prevents their aggregation. FASEB J 2006; 20:846-57. [PMID: 16675842].

27. Lieber CS. S-adenosyl-L-methionine: its role in the treatment of liver disorders. Am J Clin Nutr 2002; 76:1183S-7S. [PMID: 12418503].

28. Andley UP, Song Z, Wawrousek EF, Bassnett S. The molecular chaperone alphaA-crystallin enhances lens epithelial cell growth and resistance to UVA stress. J Biol Chem 1998; 273:31252-61. [PMID: 9813033].

29. Xi JH, Bai F, Andley UP. Reduced survival of lens epithelial cells in the alphaA-crystallin-knockout mouse. J Cell Sci 2003; 116:1073-85. [PMID: 12584250].

30. Bai F, Xi J, Higashikubo R, Andley UP. Cell kinetic status of mouse lens epithelial cells lacking alphaA- and alphaB-crystallin. Mol Cell Biochem 2004; 265:115-22. [PMID: 15543941].