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The E156K mutation in the *CRYAA* gene affects the epithelial–mesenchymal transition and migration of human lens epithelial cells

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ABSTRACT

Purpose: To investigated the biological effects of E156K-mutated αA-crystallin (CRYAA) in human lens epithelial cells (HLECs).
Methods: FLAG-tagged, human, full-length, wild-type (WT), or E156K-mutated CRYAA was expressed in HLECs under *CRYAA* knockdown. CRYAA expression was determined by quantitative reverse transcription polymerase chain reaction and western blotting (WB). Rhodamine cytoskeleton staining was used to observe the changes in cell morphology following transfection with WT or E156K-mutated *CRYAA* plasmids. WB was performed to assess the expression of markers related to epithelial–mesenchymal transition (EMT) and migration.
Results: Rhodamine cytoskeleton staining revealed changes in the morphology of cells transfected with E156K-mutated *CRYAA* and opposite responses occurred after treatment with a β-catenin inhibitor. Cells transfected with E156K-mutated *CRYAA* expressed remarkably higher levels of the mesenchymal biomarkers N-cadherin and vimentin but decreased levels of the epithelial biomarker E-cadherin, whereas opposite trends were observed in cells treated with the β-catenin inhibitor, ICG001. The migratory capability of E156K-mutated *CRYAA* cells was significantly greater than that of WT cells ($P < 0.001$). This effect was accompanied by significantly increased expression levels of phosphorylated (p)-focal adhesion kinase (FAK) and *p*-Src. These changes were decreased significantly by treatment with FAK and Src inhibitors.
Conclusion: E156K-mutated CRYAA induced EMT, in which the HLECs lost cell polarity, and acquired a mesenchymal phenotype with greater migratory capability. These biological effects may be associated with activation of the Wnt/β-Catenin and FAK/Src signaling pathways.

1. Introduction

Cataract is a leading cause of blindness worldwide, and the aggregation of crystallin in the lens owing to genetic mutations is an

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important contributor to cataractogenesis [1,2]. αA-crystalline (CRYAA), a major structural protein in the lens, has a strong molecular chaperone activity by which it promotes the dissolution of denatured protein, and makes it easy to renature proteins after denaturation to maintain lens transparency [3,4]. Mutations in the *CRYAA* gene can cause different types of congenital cataract, which seriously interfere with the function of CRYAA at birth or during adolescence [5–7]. Age-related cataract (ARC) is also characterized by a genetic component, which leads to significant changes in the function of CRYAA. In this context, sequence variations associated with ARC tend to increase the risk of disease when exposed to environmental insults that all individuals face. This could be due to an increase in the individual's vulnerability to the triggering factors, which accumulate over an extended period [8,9].

Some mutations in the *CRYAA* gene are unambiguously associated with increased risk of ARC, although the complex inheritance pattern and late age of onset make them more difficult to study. Validandi et al. [10] found that the missense mutation F71L, which is associated with age-related cortical cataract, does not disrupt the secondary or tertiary structure of CRYAA protein, but leads to a decrease in its molecular chaperone activity. Kanade et al. [11] reported that mutations of the amino acid residues at positions 21, 103, 131, and 142 of CRYAA alter its tertiary structure and enhance its molecular chaperone activity. Those studies either investigated the correlation between mutations and cataract or verified the changes in protein function caused by mutations using in vitro experiments. Pasupuleti et al. [12] induced the R21A mutation in the *CRYAA* gene and found that the mutated CRYAA inhibited apoptosis by enhancing phosphoinositide 3-kinase (PI3K) activity and inactivating phosphatase and tensin homolog (PTEN). The antiapoptotic function of CRYAA is directly related to its chaperone activity. Currently, however, few studies have examined the effects of specific mutations in *CRYAA* on cellular functions.

In our previous studies, we found that the single nucleotide polymorphism (SNP) rs76740365 G > A in exon 3 of the *CRYAA* gene increases susceptibility to posterior subcapsular cataract (PSC) [13]. This SNP is a missense mutation that changes the amino acid at position 156 of αA-crystallin from glutamic acid (E) to lysine (K). This substitution alters the tertiary structure of CRYAA and enhances its surface hydrophobicity, which increases its molecular chaperone activity and antiapoptotic capability [14]. Despite these findings, it remains unclear whether this change in protein structure directly contributes to the development of PSC or changes in cell functions. Epithelial–mesenchymal transition (EMT) is believed to play a key role in the proliferation and migration of lens epithelial cells during the formation during posterior capsule opacification (PCO). Similar to the pathogenesis of PCO, PSC exhibits one of its pathological mechanisms through the migration of lens epithelial cells from the equatorial region to the posterior pole. These cells then undergo differentiation into fibroblast-like cells that, in turn, promote the development of posterior subcapsular opacity [15–18]. Therefore, in this study, we investigated the possible mechanism of EMT and migration, including underlying signaling pathways, by designing a plasmid containing wild-type (WT) or E156K-mutated *CRYAA* to transfect cell lines having undergone knockdown of background CRYAA expression.

2. Materials and methods

The use of cultured human lens epithelial cells (HLECs) was approved by the institutional review board of the Eye and ENT Hospital of Fudan University.

2.1. Establishment of stable CRYAA knockdown cell lines

Four *CRYAA* short hairpin RNA (shRNA) sequences (*CRYAA*-sh1: GGGACAAGTTCGTCATCTTCC; *CRYAA*-sh2: GCAGGAC-GACTTTGTGGAGAT; *CRYAA*-sh3: CCGGCATCTCTGAGGTTCGAT; and *CRYAA*-sh4: CCTCGTCCTAAGCAGGCATTG) were cloned into the hU6-MCS-CMV-ZsGreen 1-PGK-Puro plasmid to knock down endogenous *CRYAA*. Quantitative polymerase chain reaction was performed to confirm the knockdown efficiency of the shRNA candidates.

2.2. Plasmid construction

The coding sequence of human *CRYAA* (NM_000394.3) was synthesized with primers contained the *Xho*I and *BamH*I restriction enzyme cutting sites and then integrated into CMV-MCS-FLAG-PGK-Puro to upregulate CRYAA expression, as follows: WT-CRYAA-F, 5′-CCGCTCGAGGCCACCATGGACGTGACCATCCAGCAC-3' (*Xho*I); WT-CRYAA-R, 5′-CGGGATCCGGACGAGGGAGCCGAGGTGG-3' (*BamH*I); E156K-CRYAA-F, 5′-CCGCTCGAGGCCACCATGGACGTGACCATCCAGCAC-3' (*Xho*I); and E156K-CRYAA-R, 5′-CGGGATCCggacgagggagccgaggtgggcttctcctcccgcgacacggggatggctcgctTggcgtgggtggcatc-3' (*BamH*I).

2.3. Cell culture and transfection

The HLEC line SRA01/04 was obtained from the Cancer Institute of the Chinese Academy of Medical Science (Beijing, China) and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, NY, USA), supplemented with 10 % fetal bovine serum (FBS, Gibco) at 37 °C in a humidified 5 % CO_2 atmosphere. Before transfection, the cells were seeded in six-well plates and grown overnight to a density of approximately 70 %–90 %. A transfection mixture comprising 2.5 μg of plasmid DNA and 7.5 μl of Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) reagent in 2 mL of serum-free medium was added to each well for 8 h. The cells were collected after 48 or 72 h for subsequent analyses.

2.4. Rhodamine cytoskeleton staining

HLECs were fixed in 4 % paraformaldehyde and permeabilized in 0.5 % Triton X for 20 min before blocking in 5 % bovine serum albumin (BSA) for 30 min at room temperature. After washing extensively, rhodamine phalloidin probes (Yisheng, Shanghai, China) were added, simultaneously with rhodamine (TRITC) AffiniPure Goat anti-rabbit IgG (H + L) (Yisheng, Shanghai, China). After washing three times, the coverslips were mounted onto glass slides with antifade reagent and 4′,6-diamidino-2-phenylindole (DAPI) to label cell nuclei. Fluorescent images were obtained using a confocal laser microscope (TCSSP8, Leica Microsystems GmbH, Wetzlar, Germany).

2.5. Quantitative real-time PCR (qRT-PCR)

The mRNA expression of *CRYAA* was measured by qRT-PCR. Total RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized using a Primescript RT reagent kit (Takara, Japan). qRT-PCR was performed in 10-μL reaction mixtures comprising 5 μL of SYBR Mix, 0.4 μL of the forward and reverse primers, 4.4 μL of RNase-free water, and 0.2 μL of cDNA. The PCR primers for amplification of CRYAA were: GCGAGGGCCTTTTTGAGTATG (forward) and GGTCGGATCGAACCTCAGAGA (reverse). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as internal control and amplified using the primers TTGTTACAGGAAGTCCCTTGCC (forward) and primer ATGCTATCACCTCCCCTGTGTG (reverse).

qRT-PCR reactions were performed using SYBR Green PCR master mix (Roche, Basel, Switzerland) and the following conditions: DNA denaturing at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and then 60 °C for 60 s. Quantification analysis of *CRYAA* mRNA was normalized against *GAPDH* as the internal control. Relative multiples of changes in mRNA expression were determined by calculating $2^{-\Delta\Delta Ct}$.

2.6. Western blotting

Western blotting was performed using standard methods. After the specified treatment, whole-cell extracts were prepared using RIPA lysis buffer (Beyotime Biotechnology, China) with 1 mM phenylmethanesulfonyl fluoride (Beyotime Biotechnology). The lysates were centrifuged at 12,000×*g* for 15 min at 4 °C. The protein concentration of each supernatant was determined using a bicinchoninic acid protein assay kit (Thermo Scientific, USA). Equal amounts (20 μg) of protein were resolved on 10 % sodium dodecyl sulfate–polyacrylamide electrophoresis gels and transferred to nitrocellulose membranes (Millipore, USA). The membranes were blocked in 5 % BSA for 1 h at room temperature to reduce nonspecific binding and then incubated with the specified primary antibody at 4 °C overnight. The following primary antibodies were used: CRYAA (ab181866, Abcam, Cambridge, UK), E-cadherin (#3195, CST, Ipswich, MA), N-cadherin (#4061, CST), Vimentin (5741, CST), β-catenin (#8480, CST), focal adhesion kinase (FAK; BS3583, Bioworld Technology, USA), phosphorylated (p)-FAK (BS4617, Bioworld Technology), *p*-Src (Ab32078, Abcam), Src (Ab109381, Abcam), and β-actin (A3854; Sigma). After washing, the blot was incubated with goat anti-mouse IgG (H + L) or anti-rabbit IgG (H + L; Yeasen, China) for 1 h at room temperature. Finally, the membranes were scanned. Representative blots from several experiments are shown.

2.7. Transwell migration assay

Cell migration was determined using Transwell cell migration assays. After transfection, 1×10^4 cells/100 μl were transferred to

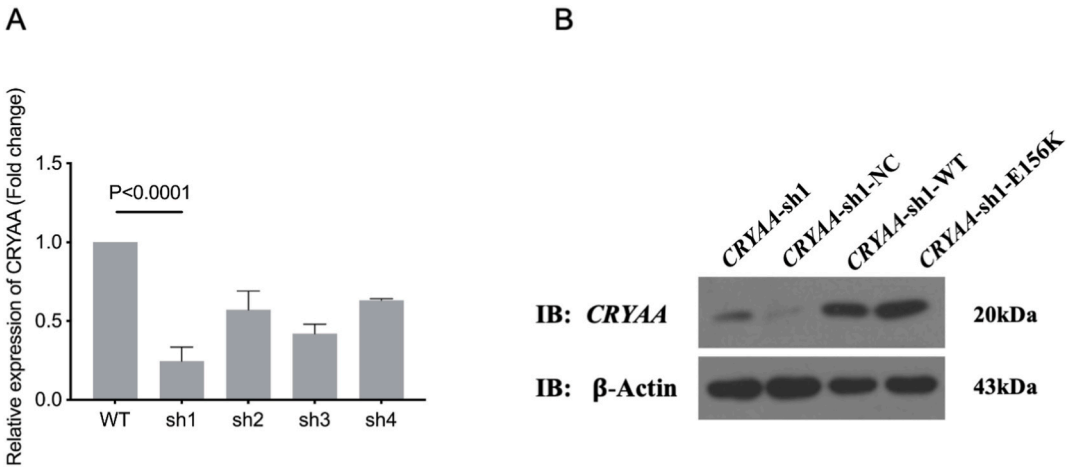

Fig. 1. Knockdown of *CRYAA* and transfection of E156K-mutated *CRYAA* into HLECs. (A) The mRNA expression of *CRYAA* was significantly decreased by *CRYAA*-sh1. (B) Western blotting confirmed the transient transfection with the corresponding plasmid induced the expression of WT or E156K-mutated CRYAA protein in the *CRYAA*-sh1-knockdown HLECs.

the upper chamber of 8 μm hanging inserts placed in 24-well plates (Corning, USA) with 100 μL of serum-free medium. Then, 800 μl of DMEM containing 10 % FBS was added to the lower chamber. After incubation for 48 h, the noninvaded cells were removed with cotton swabs. The invaded cells were fixed with 4 % paraformaldehyde for 15 min and stained with 1 % crystal violet for 30 min and photographed. Cells were counted under three different microscopic fields, and the average number was calculated.

2.8. Statistical analysis

All experimental data are presented as the mean ± standard deviation. Each result was obtained from at least three replicated experiments. Figures were drawn with GraphPad Prism version 7.0 (GraphPad, La Jolla, CA, USA). Statistical significance was analyzed by two-tailed Student's *t*-test or one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. $P < 0.05$ was considered to indicate statistically significant differences. The statistical analyses were performed using SPSS 23.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Knockdown of CRYAA and transfection of E156K-mutated CRYAA into HLECs

Reducing the endogenous expression of WT *CRYAA* in HLECs is the first step to evaluate the effect of E156K-mutated CRYAA on the function of HLECs. CRYAA expression in HLECs was knocked down using four shRNAs (*CRYAA*-sh1, *CRAYY*-sh2, *CRYAA*-sh3, and *CRYAA*-sh4), with knockdown efficiency of more than 70 %. The mRNA expression of *CRYAA* was significantly decreased by *CRYAA*-sh1 with an efficiency of 85.44 % ($P < 0.0001$, Fig. 1A), achieving the desired effect. Therefore, the *CRYAA*-sh1-knockdown HLECs were used in the subsequent experiments.

WT and E156K-mutated *CRYAA* were transfected into the *CRYAA*-sh1-knockdown HLECs, and western blotting was performed to confirm the induction of CRYAA (Fig. 1B). Transient transfection with the corresponding plasmid induced the expression of WT or E156K-mutated CRYAA protein in the *CRYAA*-sh1-knockdown HLECs.

3.2. E156K-mutated CRYAA promoted EMT in HLECs

We first performed rhodamine cytoskeleton staining to determine the changes in cell morphology due to E156K-mutated CRYAA. Rhodamine cytoskeleton staining showed that the cells remained normal with a flat polygon shape in the WT group, whereas HLECs transfected with E156K-mutated *CRYAA* formed narrow bands of fibrous-like cells accompanied by the growth of pseudopodia synapse-like structures, indicative of increased EMT. Exposure to a β-catenin inhibitor suppressed EMT in HLECs transfected with the E156K-mutated CRYAA, and the cells had polygonal morphologies (Fig. 2).

To evaluate how transfection with E156K-mutated *CRYAA* regulated EMT in HLECs, we examined the expression of EMT-related markers, including E-cadherin, β-catenin, N-cadherin, and vimentin. As shown in Fig. 3, E156K-mutated CRYAA significantly increased the protein expression levels of the mesenchymal biomarkers N-cadherin and vimentin but reduced the protein expression levels of the

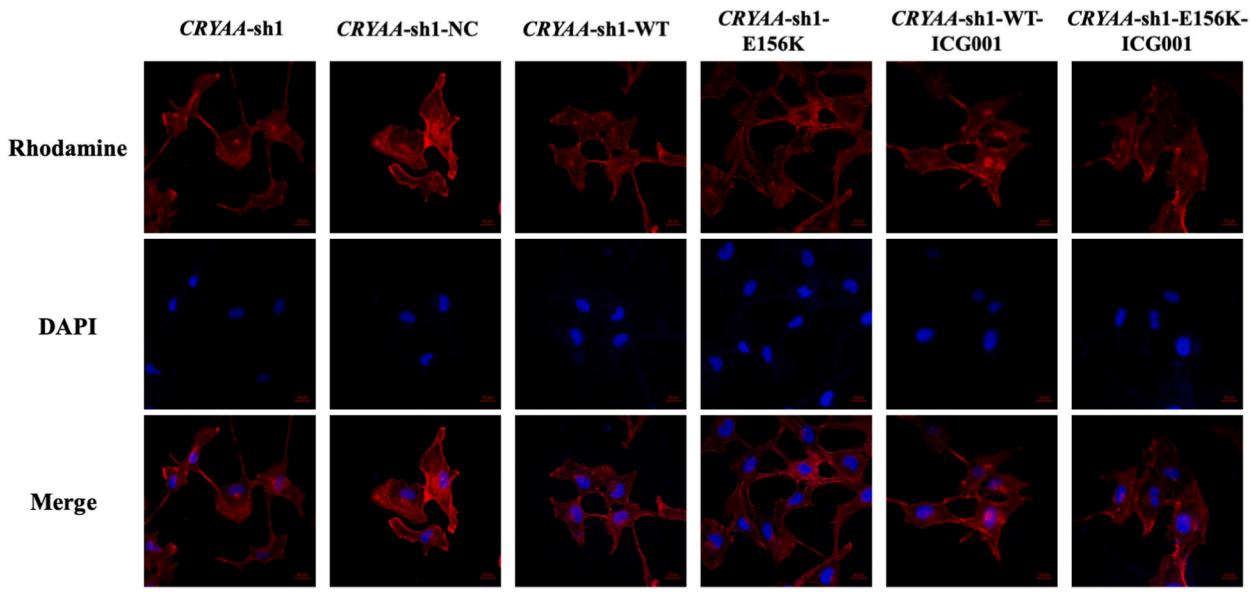

Fig. 2. Changes in cell morphology HLECs transfected with the E156K-mutated *CRYAA* with or without exposure to β-Catenin inhibitor (ICG001) by Rhodamine cytoskeleton staining.

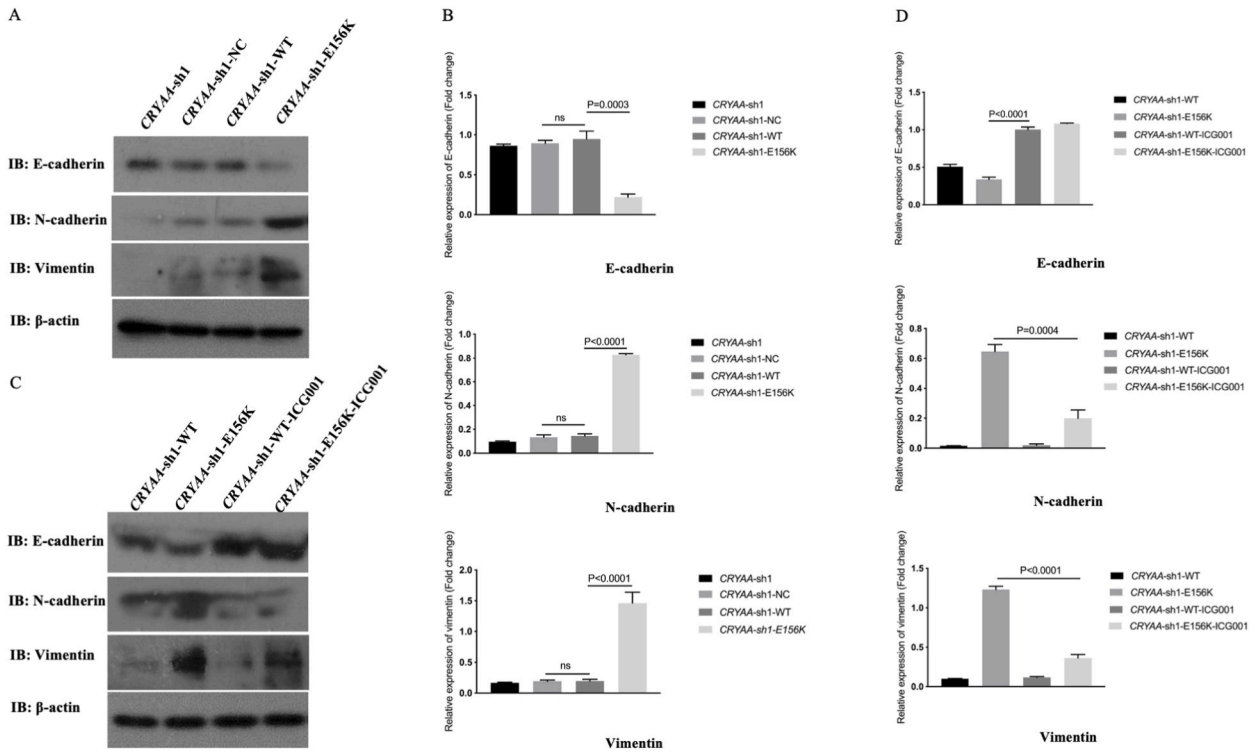

Fig. 3. E156K-mutated *CRYAA* promoted epithelial–mesenchymal transition (EMT) in HLECs. **(**A–B) Western blotting was used to determine the expression of E-cadherin, N-cadherin and Vimentin. **(**C–D) Western blotting was used to determine the expression of E-cadherin, N-cadherin and Vimentin treated or not treated with the β-catenin inhibitor, ICG001.

epithelial biomarker E-cadherin (all $P < 0.001$). Opposite trends were observed in cells treated with the β-catenin inhibitor, ICG001 (S2662, Selleck, Houston, USA, all $P < 0.0001$, Fig. 3A–D). The expression of β-catenin in the nuclei of HLECs was greater in *CRYAA*-sh1-knockdown HLECs transfected with E156K-mutated *CRYAA* than cells transfected with WT *CRYAA* ($P = 0.0026$, Fig. 4), and treatment with ICG001 significantly inhibited the nuclear expression of β-catenin in the cells transfected with E156K-mutated *CRYAA* (Fig. 4). Taken together, these results imply that E156K-mutated CRYAA caused pronounced morphological changes in HLECs, including enhanced EMT, and that the Wnt/β-catenin signaling pathway is involved in this process.

3.3. E156K-mutated CRYAA increased the migratory capability of HLECs

We investigated the effects of E156K-mutated CRYAA on the migration capability of HLECs, as induced by EMT, and examined whether the FAK/Src signaling pathway is involved in the process. In this experiment, we used *CRYAA*-sh1-knockdown HLECs, *CRYAA*-sh1-NC HLECs, *CRYAA*-sh1-knockdown HLECs transfected with WT *CYRAA*, and CRYAA-sh1-knockdown HLECs transfected with E156K-mutated *CRYAA*. Under a light microscope, the density of HLECs in each field of view appeared to be greater in the *CRYAA*-sh1-knowdown HLECs transfected with either WT or E156K-mutated *CRYAA* compared with *CRYAA*-sh1-knockdown or *CRYAA*-sh1-NC cells (Fig. 5A). This suggests that CRYAA could promote cell migration. The cell counts confirmed this finding ($P < 0.001$). Further analysis showed that the migratory capability of the *CRYAA*-sh1-knockdown HLECs was significantly greater in those transfected with E156K-mutated *CRYAA* than those transfected with WT *CRYAA* ($P < 0.001$, Fig. 5B).

We then examined the role of the FAK/Src signaling pathway in regulating the migratory capability of E156K-mutated CRYAA in HLECs by treating the above groups of cells with FAK (PF562271, S2890, Selleck) and Src (SKI-606, S1014, Selleck) inhibitors prior to the Transwell assay. The expression levels of FAK, *p*-FAK, Src, and *p*-Src were determined by western blotting. The protein expression levels of *p*-FAK and *p*-Src were significantly greater in the *CRYAA*-sh1-knockdown HLECs transfected with E156K-mutated *CRYAA* compared with the other groups ($P < 0.05$, Fig. 6A–C). Treating the CRYAA-sh1-knockdown WT and *CRYAA*-sh1-knockdown E156K-mutated CRYAA cells with FAK and Src inhibitors significantly decreased the expression levels of *p*-FAK and *p*-Src (Fig. 6D–F) as well as the migratory capacity, as determined assessed using the Transwell assay ($P < 0.0001$, Fig. 7A–D).

4. Discussion

EMT is a process by which epithelial cells transform into mesenchymal cells under certain pathological or biological conditions [19]. This process involves disappearance of the cell polarity, cytoskeletal rearrangement, increased production of extracellular

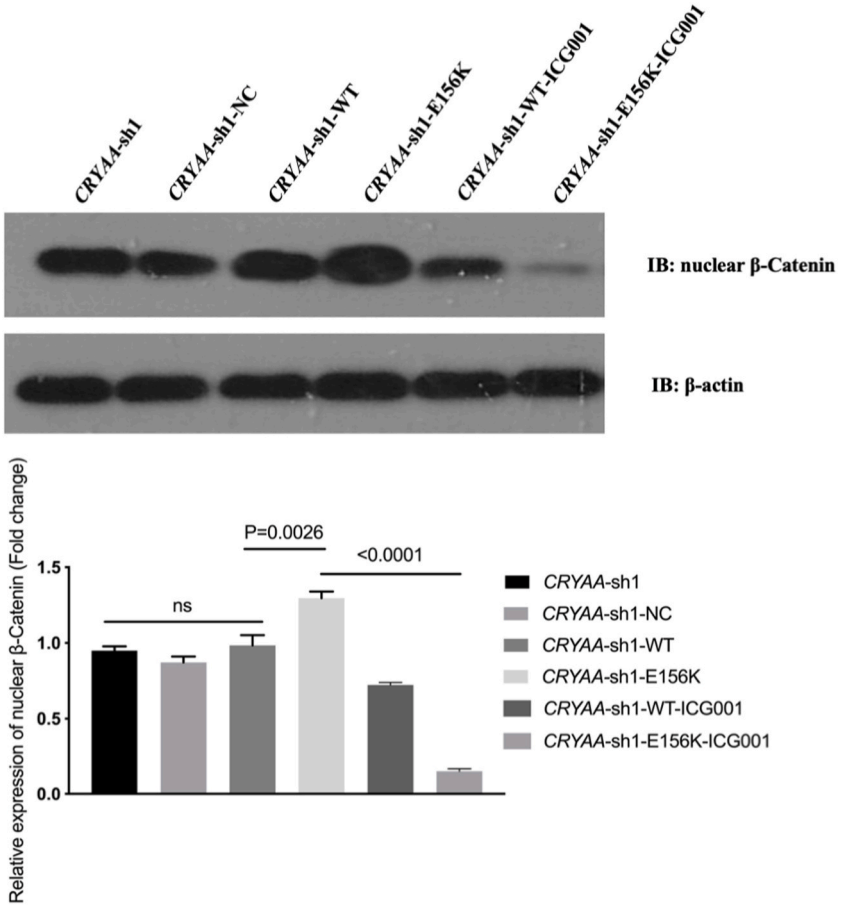

Fig. 4. Western blotting was used to detect the expression of β-Catenin in the nucleus of HLECs treated or not treated with the β-catenin inhibitor, ICG001.

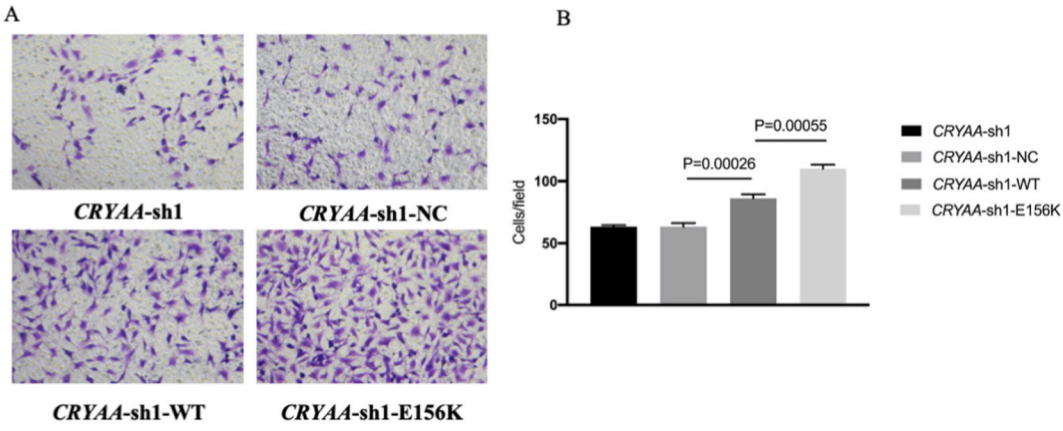

Fig. 5. E156K-mutated *CRYAA* increased the migratory capability of HLECs. (A) The density of human lens epithelial cells in each field of view under a light microscope. (B) The cell counts of human lens epithelial cells in each field of view.

matrix, changes in cellular the adhesion, altered cell migration and invasion, and enhanced antiapoptotic capability. EMT-associated changes include concurrent downregulation of the intercellular adhesion molecule E-cadherin and upregulation of mesenchymal-associated genes, such as N-cadherin, vimentin, fibronectin, and α-smooth muscle actin [20–22]. EMT occurs during PCO and multiple studies have examined the induction and regulation of EMT in PCO [17,18]. The posterior subcapsular opacity caused by PSC is due to the migration and incomplete differentiation of lens epithelial cells from the equator of the lens [15,16].

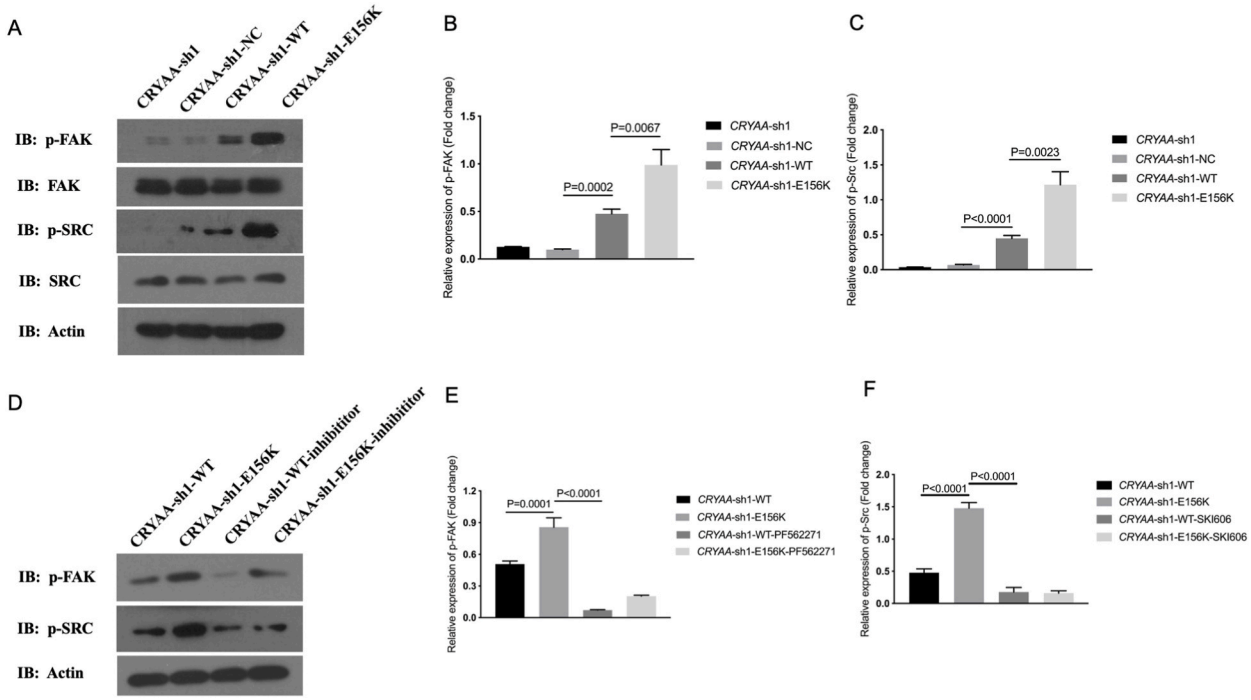

Fig. 6. E156K-mutated *CRYAA* increased the migratory capability of HLECs by activating the FAK/Src signaling pathway. (A–C) Western blotting was used to determine the expression of FAK, *p*-FAK, Src and *p*-Src without FAK and Src inhibitors. (D–F) Western blotting was used to determine the expression of *p*-FAK and *p*-Src with FAK and Src inhibitors.

Similarly, EMT of Lens epithelial cells in PSC is speculated to contribute to the formation of posterior subcapsular opacities. In this study, we observed the morphological changes of HLECs transfected with WT or E156K-mutated *CRYAA* by performing rhodamine cytoskeleton staining. Cells transfected with WT *CRYAA* had normal polygonal morphology and were generally flat, whereas cells transfected with E156K-mutated *CRYAA* formed narrow, long, cord-like fibrous cells with a foot synapse-like structure. This demonstrated that the E156K mutation in *CRYAA* promotes EMT in HLECs, mimicking the pathogenesis of PCO. To confirm the involvement of EMT, we also analyzed the expression of EMT-related markers, and the results indicated that EMT was activated in E156K mutant HLECs.

The Wnt/β-catenin signaling pathway is an important activator of EMT, regulating cell proliferation, invasion, and differentiation [23,24]. Activation of the Wnt pathway causes β-catenin to accumulate in the cytoplasm and translocate to the nucleus. To investigate whether the Wnt/β-catenin signaling pathway regulates EMT in HLECs transfected with E156K-mutated *CRYAA*, we performed western blotting to determine the nuclear expression of β-catenin. This experiment revealed a significant increase in β-catenin expression in the nuclei of HLECs transfected with E156K-mutated *CRYAA*. The expression of β-catenin in the nuclei and EMT decreased after treatment with the β-catenin inhibitor ICG001, as demonstrated by rhodamine cytoskeleton staining, which also restored the normal morphology of these HLECs. Bao et al. reported that transfected HLECs with Wnt plasmids enhanced the proliferation and migration of HLECs, downregulated E-cadherin expression, and upregulated the expression of mesenchymal cell markers such as β-catenin and c-Myc [25]. Although the precise mechanism by which the Wnt/β-catenin pathway induces EMT remains unclear, β-catenin fulfills two key functions: as a structural protein in conjunction with E-cadherin, it plays a role in cellular adhesion junctions, and as an intermediary in the Wnt/β-catenin pathway. The loss of the E-cadherin/β-catenin adhesion complex triggers the disassembly of adherens junctions, which leads to cell migration, a critical process in EMT. Cells transfected with E156K-mutated CRYAA expressed decreased levels of the epithelial biomarker E-cadherin and a significant increase in β-catenin expression in the nuclei of HLECs, whereas opposite trends were observed in cells treated with the β-catenin inhibitor, ICG001. Therefore, we believe that E156K-mutated CRYAA enhanced EMT by activating the Wnt/β-catenin signaling pathway in the fibrotic process, which can be suppressed by inhibiting β-catenin.

It is well known that the cell polarity disappears and cell migration is enhanced during EMT. Cell migration, a cornerstone process in PCO, is mediated by a variety of growth factors and cytokines, such as transforming growth factor-β [26–28]. As we expected, the Transwell assays showed that E156K-mutated CRYAA increased cell migration. To our knowledge, this is the first report showing that the E156K mutation in CRYAA is associated with enhanced migratory capacity of HLECs.

FAK, a cytoplasmic non-receptor tyrosine kinase, acts as a substrate for Src and is a key element of integrin subsignaling [29,30]. Initially, *p*-FAK is bound by the SH2 domains of Src, and the activated FAK–Src complex plays a crucial role in facilitating the intracellular signaling pathways involved in cell proliferation, migration, and invasion by inducing EMT [31–34]. In this study, western blotting showed that E156K-mutated CRYAA increased the expression levels of *p*-FAK and *p*-Src, and administration of FAK

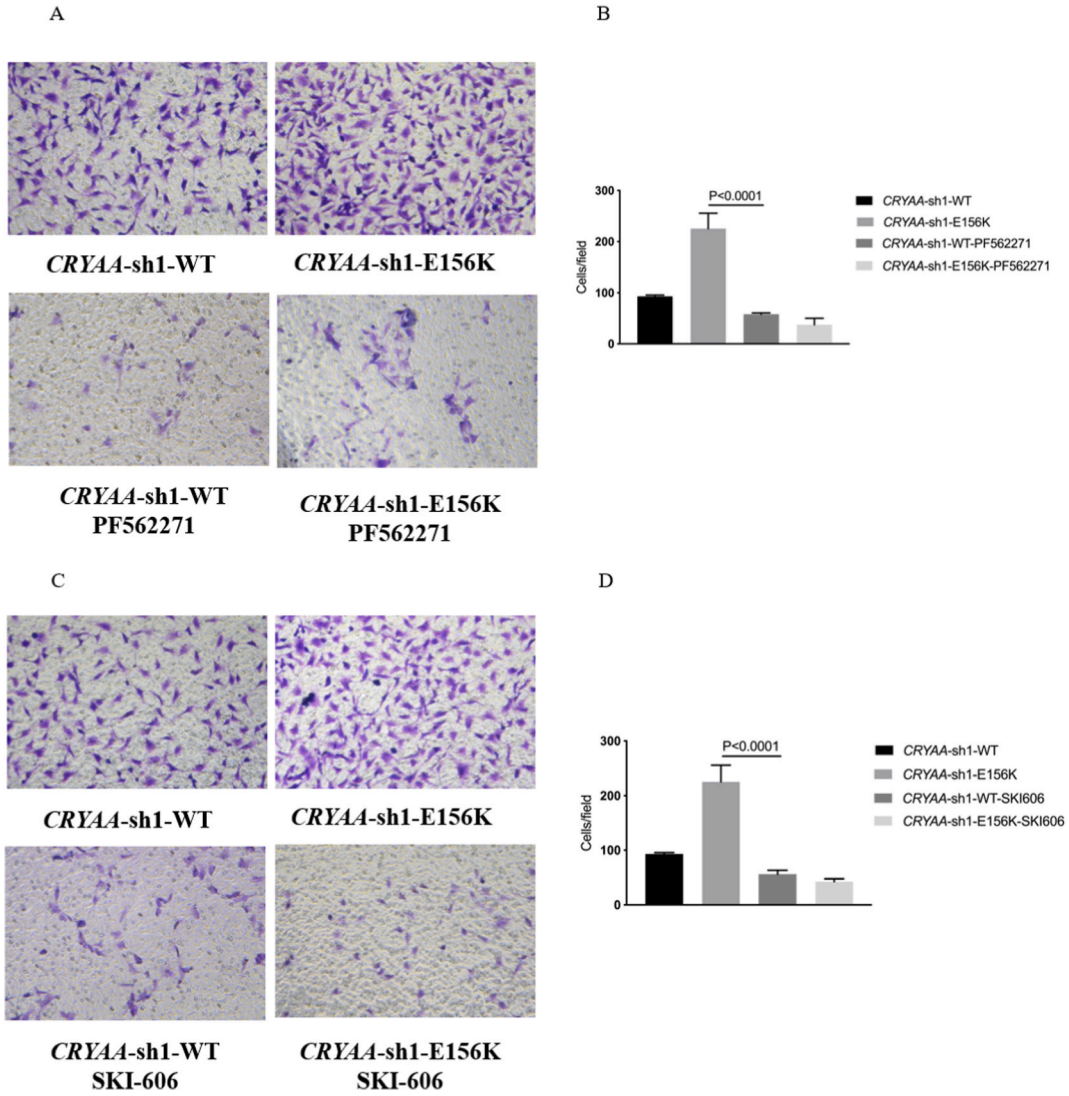

Fig. 7. Changes in cell migration capability of HLECs with FAK and Src inhibitors by Transwell assay. (A) The density of human lens epithelial cells in each field of view under a light microscope with FAK inhibitor. (B) The cell counts of human lens epithelial cells in each field of view with FAK inhibitor. (C) The density of human lens epithelial cells in each field of view under a light microscope with Src inhibitor. (D) The cell counts of human lens epithelial cells in each field of view with Src inhibitor.

and Src inhibitors reduced the migration of HLECs. The FAK–Src complex activates p130CAS or paxillin to cause the formation of silk feet or lamellipodia, which are responsible for cell movement [29]. Rhodamine cytoskeleton staining revealed that the HLECs transfected with E156K-mutated *CRYAA* had silk foot/pseudopod-like structures. These findings indicate that E156K-mutated CRYAA enhance the migration of HLECs via the FAK/Src signaling pathway. We speculate that the E156K-mutated CRYAA activates the FAK/Src signaling pathway through an increase in the molecular chaperone activity of CRYAA, which inhibits the phosphorylase and aggregatory functions of FAK/Src, leading to increased phosphorylation. A change in the substrate-binding ability of E156-mutated CRYAA may also contribute to this process. Further studies are needed to explore the mechanism in more detail.

Very few studies had investigated the pathogenesis of PSC. One of the pathological mechanisms of PSC involves migration of the lens epithelial cells from the equatorial region to the posterior pole where they differentiate into fibroblast-like cells which induce posterior subcapsular opacity [15]. Microscopic examinations revealed these cells had a cytoplasmic eosinophilic structure, suggesting a failure of fibroblast differentiation [35]. In patients with PSC, the increased migration of HLECs owing to the E156K mutation in CRYAA cannot fully explain the occurrence of PSC, and the FAK/Src signaling pathway may play an important regulatory role. Similar to the onset of PCO, the pathological process of PSC is often accompanied by morphological changes of HLECs.

This study has several limitations. First, FAK is an upstream factor that mediates multiple signal pathways and complicated functions in cells. In this study, we only detected changes in cell migration mediated by the E156K-mutated CRYAA, and the interactions with downstream factors need to be studied further. Second, the cellular function of the E156K mutation in CRYAA was

examined in HLECs. Although we constructed a plasmid to stably knockdown and eliminate background CYRAA expression, some unknown factors may exist in immortalized cell lines and primary HLECs. Finally, the mechanism by which the E156K mutation in CRYAA mainly induces cells to migrate to the posterior capsule instead of simultaneously migrating to the anterior capsule needs to be explored in the future.

In conclusion, the E156K mutation in CRYAA induced EMT, which caused the HLECs to lose cell polarity and acquire mesenchymal phenotypes, with enhanced migratory capability. These biological changes may be mediated by activation of the Wnt/β-catenin and FAK/Src signaling pathways. These findings provide insight into the pathogenesis of PSC and reveal a novel target for gene-targeted therapy.

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Data availability statement

Data included in article/supp. Material/referenced in article.

CRediT authorship contribution statement

Zhennan Zhao: Data curation, Project administration, Writing - review & editing, Methodology. **Jiahui Chen:** Data curation, Writing - original draft, Writing - review & editing, Validation. **Yongxiang Jiang:** Conceptualization, Project administration, Resources, Writing - review & editing. **Yi Lu:** Conceptualization, Project administration, Writing - review & editing, Resources.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23690.

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