The impact of IGF1R SNPs on susceptibility to age-related cataract

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Abstract

Background: The insulin-like growth factor 1 receptor (IGF1R) gene is essential for lens development, but the impact of IGF1R on age-related cataract(ARC) has not been investigated. This study explored the association between IGF1R single nucleotide polymorphism (SNP) and ARC susceptibility ,and uncover the underlying mechanism in human lens epithelial (HLE) cells. **Methods:**A total of 1190 unrelated participants ,comprising 690 ARC patients and 550 healthy individuals in Han Chinese population were recruited and genotyped for target SNP. The X²-test was used to detect genotypic distribution between the patient and control groups and the logistic regression was performed to adjust the age and gender. Meanwhile, in the IGF1R knockdown HLE cells, cell proliferation was detected via CCK-8 analysis. Cell cycle and apoptosis were evaluated by flow cytometry,while the expression of cycle- and apoptosis-related molecules were determined via Q-PCR and Western blot. The Caspase-3 activity was measured using its assay kit. **Results:** The rs1546713 in IGF1R gene was identified $P = 0.046$ OR 1.606 CI 1.245,2.071 ,which shown a significant relevance with ARC risk under the dominant model. The results demonstrated that IGF1R knockdown inhibited cell proliferation by inducing cell cycle arrest at S phase and promoting apoptosis. Mechanistically, the cell cycle blocked at S phase was linked with the alterations of cyclinA , cyclinB, cyclinE and P21,while the pro-apoptosis function was related to stimulate the activation of Caspase-3 activities and the alteration of Caspase-3,Bcl-2 and Bax expression levels. **Conclusions:** This study first reported that IGF1R polymorphisms may affect susceptibility to ARCs in Han Chinese population and provided new clues to understanding the pathogenic mechanism of ARCs. Notably, IGF1R is likely a potential target for ARC prevention and treatment.

Background

Cataracts are the leading cause of blindness[1,2], resulting in more than 50% of the vision loss worldwide, including 33.4% of sight-disabled people and 18.4% of people with varying degrees of visual impairments[3,4]. The most common cataract type is age-related cataracts (ARCs), and more than half of elderly people over 65 years old suffer from it[5]. Recently, a growing number of epidemiological studies showed that genetic variation, especially single nucleotide polymorphisms (SNPs), plays a crucial role in the development of ARCs, and studies of monozygotic and dizygotic twins provided the strongest evidence[6,7]. Consequently, many genes and SNPs related to ARCs have been identified [8]. Although genetic variation has become the focus of research, the definite etiology of ARCs is not fully understood.

The insulin-like growth factor-1 receptor (IGF1R) gene, a member of the tyrosine kinase receptor super family[9], is located at 15q26 ([MIM:147370](#)). This gene encodes the IGF1R protein, which is composed of two extracellular alpha subunits and two intracellular beta subunits[10]. It regulates numerous downstream signals and is usually involved in cellular functions, such as cell proliferation and survival. Multiple studies have shown that IGF1R plays a vital role in tumourigenesis, survival and metastasis by regulating the cell proliferation, cell cycle and apoptosis of tumour cells. [11-13] , but the effects of IGF1R on cataracts have not been investigated.

In this study, we first found an association between IGF1R SNPs and ARCs in ARC patients and explored the impact of IGF1R in human lens epithelium (HLE) cells. These data provide new information for understanding the pathogenic mechanism of ARCs, and notably, IGF1R is likely a potential target for ARC prevention and treatment.[4]

Methods

Study participants

The research involved 1190 participants, comprising 690 ARC patients and 550 healthy controls. All subjects were unrelated Han Chinese and were recruited from the Eye Center of the Second Affiliated Hospital, Medical College of Zhejiang University. Each participant received a full explanation of the purpose and procedures of the research and then signed an informed consent form.

All subjects underwent a full ophthalmic examination, including best-corrected visual acuity (BCVA), fundus photography and lens examination with a slit lamp biomicroscope after mydriasis. The diagnoses of ARC patients were performed according to the lens opacity classification system II (Locs II)[14]. Based on the opacity region of the lens, the ARC patients were classified into the following four groups: nuclear cataract (N), cortical cataract (C), posterior subcapsular cataract (PSC), and mixed cataract (M). Patients with secondary cataracts resulting from glaucoma, diabetes, uveitis, trauma, high myopia and other reasons were excluded. The control subjects were healthy individuals whose lenses were transparent and without other diseases.

This study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Second Affiliated Hospital, Medical College of Zhejiang University, Hangzhou, China.

Statistical analysis

The continuous variables of the subject characteristics were analysed by independent sample t-test to detect the correlations between groups, and the discontinuous variables were analysed by χ^2 test. The χ^2 test was performed using SPSS software (SPSS, Chicago, IL, USA) to compare the genotypic distributions between the case and control groups and to estimate odds ratios (ORs) and 95% confidence intervals (CIs). The Hardy-Weinberg equilibrium (HWE) of each SNP was evaluated through the χ^2 test using PLINK (v1.07). A logistic regression analysis was executed to adjust for age and sex. To reduce the rate of type I errors, a Bonferroni correction was conducted (a total of 57 SNPs were detected in the same groups. The target SNP was only a fraction of these; thus, after the correction, the significance level was set to $0.05/57 \times 3$). In addition, the association between ARCs and SNPs was examined under three different genetic models: additive, recessive and dominant. All the continuous variables of the subject characteristics are presented as the mean $\pm$ standard deviation (SD). A two-tailed p value <0.05 was considered statistically significant or is otherwise indicated.

Cell culture and transfection

A human lens epithelium cell line (SRA 01-04) was obtained from the RIKEN Cell Bank in 2018 (Tsukuba, Japan). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Corning, NY, USA) with 10% foetal bovine serum (FBS, Biological Industries, Israel) and a 1% penicillin-streptomycin solution (Gibco, Waltham, MA, USA) in a humidified atmosphere containing 5% CO₂ at 37°C. The cells were seeded in six-well plates and cultured overnight to grow to a 30% cell density. Then, 10 µl IGF1R siRNA (100 nM) and 7.5 µl Lipofectamine™ 3000 reagent (Invitrogen, Carlsbad, CA, USA) were added to 2 ml serum-free medium in six-well plates (IGF1R siRNA groups). The control groups were treated with NC under the same conditions. The cells were cultured for 72 h and then collected for the following experiments.

Cell proliferation assay

Cell proliferation was assessed using a Cell Counting Kit 8 (CCK8) assay (Dojindo, Kyushu, Japan). HLE cells were allowed to adhere to a 96-well plate at a concentration of 1×10^4 cells/ml overnight. The IGF1R siRNA groups and NC groups were transfected with IGF1R siRNA and NC, respectively. Following treatment, the cells were cultured at 37°C for 24 h, 48 h, and 72 h. The medium was replaced, and CCK-8 (10 µl) reagent was added to each well, followed by incubation for another 2 h. The optical density (OD) was read on a microplate reader at 450 nm, and cell viability was calculated according to the absorbance measurements.

Apoptosis assay by flow cytometry

HLE cells were seeded in 6-well plates overnight. After transfection for 72 h, the cells were washed twice with cold PBS and resuspended in 1x Binding Buffer at a density of 1×10^6 cells/ml. Next, 5 µl of FITC Annexin V and 5 µl PI (#556547, BD Pharmingen, San Diego, CA, USA) were added to the solution and incubated for 15 min in the dark at room temperature. Subsequently, the cells were analysed by a flow cytometer.

Cell cycle analysis by flow cytometry

After transfection with siRNA for 72 h, HLE cells were collected by trypsin and washed with PBS. Then, 1 ml DNA staining solution and 5 µl permeabilization solution (CCS012, Multi Sciences, Hangzhou, China) were incubated in the dark at room temperature for 30 min. Finally, the samples were analysed by a flow cytometer.

Quantitative real-time polymerase chain reaction analysis

After transfection, total RNA was extracted using Trizol reagent, and 500 ng total RNA was then transcribed into cDNA using a PrimeScript RT Master Mix kit (Takara, Japan). Quantitative real-time

polymerase chain reaction was conducted with a ChamQ SYBR qPCR Master Mix kit (Q311-02, Vazyme, Nanjing, China) and 7500 Fast Real-time PCR System. The primers used in this study are listed in Table 1. The relative RNA levels were analysed using the $2^{-\Delta\Delta C_t}$ quantification method after normalization to β -actin.

Western blot assay

After transfection for 72 h, the plates were washed with PBS to remove the residual media. The HLE cells were lysed with Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA, USA) on ice. The protein concentrations were determined by BCA protein assay (Beyotime Biotechnology, Nanjing, China), resolved by 12% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and then transferred onto 0.22- μ m PVDF membranes. The blots were blocked with 5% (w/v) non-fat milk in TBST for 2 h and then incubated with primary antibodies at 4°C overnight (Caspase-3 #9665T 1:1000; Bcl-2 #2872T 1:1000; Bax #5023T 1:1000; Cell Signaling Technology) α -Tubulin (ab52866 1:1000; Abcam, Cambridge, UK). Then, the membranes were incubated with the appropriate HRP-conjugated secondary antibody at room temperature for 1 hour and detected with an ECL western blotting system. The protein blots were analysed by ImageJ software.

Caspase 3 activity assay

After transfection, the cells were collected and lysed in lysis buffer, and the extracts were separated by centrifugation at 16,000 g for 10 min at 4°C. The protein concentrations were measured by Bradford Protein Assay. The proteins and Ac-DEVD-pNA (2 mM; Beyotime Biotechnology) were mixed at a certain proportion and then incubated at 37°C for 12 h. The optical density (OD) was measured using a microplate reader at 405 nm. Finally, Caspase-3 activity was calculated as the OD value/ μ g protein.

Results

Characteristics of the participants

This study comprised 690 ARC patients and 500 control subjects. There were no significant differences in age or sex between the two groups ($p > 0.05$). The characteristics of the subjects are shown in Table 2.

Bioinformatics characteristics of target SNP

The target SNP in IGF1R were genotyped, and their bioinformatics characteristics are listed in Table 3. All SNPs in this research were consistent with Hardy-Weinberg equilibrium.

Relevance between the target SNP and risk of ARCs

The IGF1R target SNP (rs1546713) $p = 0.046$ (OR 1.606, CI 1.245-2.071) showed a notable correlation with general type ARC susceptibility under the dominant model (the data are summarized in Table 4). In the subgroup analysis, no SNP showed a significant association with ARC risk under the additive, dominant or recessive models. After Bonferroni correction, the results remained significant.

Regulatory effects of IGF1R knockdown on cell proliferation and the cell cycle in HLE cells

IGF1R siRNA was used to silence gene expression, and the silencing efficiency was measured via Q-PCR. After transfection with 100 nM siRNA for 72 h, the mRNA expression level of IGF1R was decreased by 63% compared to that in the NC groups ($p < 0.0001$, Fig 1A). Next, to explore the effects of IGF1R knockdown on proliferation in the human lens epithelial cell line, the CCK-8 assay was used. As shown in Fig 1B, cell growth was suppressed by IGF1R knockdown compared to that in the NC groups, and a significant difference was observed after IGF1R-siRNA transfection for 48 h ($p < 0.05$). In addition, to determine the mechanism of the anti-proliferation function of IGF1R knockdown, we detected the influence of IGF1R siRNA on cell cycle progression via flow cytometry analysis. As shown in Fig 1C, the percentage of cells in the G0/G1 phase was obviously reduced from $81.05 \pm 0.68\%$ to $71.85 \pm 1.24\%$, and the percentage of cells in the G2/M phase was slightly decreased from $10.24 \pm 1.85\%$ to $9.78 \pm 1.18\%$ for the NC groups compared to the IGF1R siRNA groups. However, a significant accumulation of cells in the S phase was observed for the NC groups ($7.87 \pm 1.04\%$) compared with that in the IGF1R siRNA groups ($17.28 \pm 0.39\%$) ($p < 0.05$, Fig 1D), which indicated that IGF1R knockdown blocked the cell cycle at the S phase. Moreover, to further confirm the above results, we detected the expression of S phase-related molecules using Q-PCR. After IGF1R knockdown, the levels of cyclin A, cyclin E and P21 mRNA were increased, and the levels of cyclin B were decreased ($p < 0.05$, Fig 1E). These findings demonstrated that the knockdown of IGF1R inhibited cell proliferation by inducing S-phase arrest and altered S-phase-related molecules.

Effects of IGF1R on apoptosis in HLE cells

To determine whether IGF1R is associated with ARCs by affecting the apoptosis process, we conducted an Annexin V-FITC/PI staining assay with flow cytometry. Compared to the control groups, the IGF1R siRNA groups had remarkably increased apoptosis, with apoptotic cell rates of 9.13% and 22.24%, respectively. To further verify the above results, we separately measured the expression of apoptosis-related mRNAs and proteins through Q-PCR and western blot assays. As shown in Fig 1C and D, the analysis indicated that Caspase-3 and Bax expression levels were distinctly increased in the IGF1R siRNA groups, while the Bcl-2 expression was decreased ($p < 0.05$). Consistent with the western blot results, the Q-PCR results indicated that the level of Bax mRNA was significantly increased, whereas the level of Bcl-2 mRNA was decreased after IGF1R knockdown ($p < 0.05$, Fig 1E).

Additionally, as shown in Fig 1F, we also detected Caspase-3 activity. In accordance with the western blot results, Caspase-3 activity increased after treatment with IGF1R siRNA for 72 h. The above results revealed that IGF1R knockdown promoted apoptosis in HLE cells. Moreover, the proportion of HLE cells

was obviously increased in the S phase, which indicated that the effects of IGF1R knockdown on inducing cell apoptosis were possibly related to cell cycle arrest at the S phase.

Discussion

IGF1R, a type 2 tyrosine kinase transmembrane receptor[15], consists of two α and two β subunits and has two major ligands, insulin-like growth factor 1 (IGF1) and insulin-like growth factor 2 (IGF2). Once activated, the α subunits induce tyrosine autophosphorylation of the β subunits and then activate many downstream signalling pathways within cells[16]. IGF1R is expressed in numerous cell types and is crucial for controlling proliferation during growth progression. Homozygous *igf1r*^{-/-} mice die at birth and exhibit a serious growth defect, reaching only 45% of the normal size[17,18]. A study of zebrafish embryos stressing the importance of IGF1R in the eyes suggested that IGF1R is indispensable for lens development[19]. Others claimed that the expression of the IGF1R gene in the human lens varies by age, even in the human embryo stage, and is slightly decreased at 65 years and older. IGF1R seems to be age-related, in accordance with the decreased proliferative capacity of HLE cells in elderly persons[20]. These findings may provide new insights into the effects of IGF1R in lenses.

As mentioned above, IGF1R is essential for lens development, but no previous study has linked IGF1R polymorphisms with the risk of ARCs. In the current study, we first identified rs1546713 in the IGF1R gene, which is significantly relevant to an altered risk of ARCs under the dominant model in the Han Chinese population. Generally, variants in lens proteins result in rapid and direct protein aggregation and usually lead to congenital cataracts. Conversely, genetic mutations merely increase susceptibility to environmental hazards and tend to cause age-related cataracts through the accelerated accumulation of damage to lens proteins[21]. Thus, the identification of genetic variants for ARC risk is becoming increasingly important. Finding potentially risky genes and their signalling pathways will provide therapeutic targets for the prevention of ARC and improve the efficiency of screening populations with incipient lens opacity; these targets could even help to directly identify susceptible individuals through genetic testing to start therapeutic measures as early as possible[22]. Additionally, to uncover the underlying mechanism, we investigated the effect of IGF1R on cell proliferation, cell cycle and apoptosis in HLE cells, which resulted in new information for understanding the aetiology of ARC.

In the present study, we found that in IGF1R-knockdown HLE cells, the cell growth rate was significantly decreased after 48 h of treatment; these findings supported that IGF1R promoted cell proliferation in HLE cells, consistent with the study by Haruo Kato et al., who reported the pro-proliferation function of IGF1R in prostate cancer cells[23]. Moreover, cell cycle progression is essential for cell proliferation [24]. Therefore, to explore the underlying mechanism of the anti-proliferation effects induced by IGF1R siRNA, we detected the cell cycle via flow cytometry. As shown in the data, a higher proportion of HLE cells was obviously arrested at the S phase in the IGF1R siRNA groups compared to that in the NC groups. Although our results are different from the data published by Si Wang et al., the blockage of IGF1R by IGF1R knockdown or the addition of picropodophyllin (an IGF1R-specific inhibitor) resulted in cell cycle arrest at the G2/M phase. However, Sell et al. found that IGF1R affected each stage of the cell cycle by

testing the distributions between wild-type and IGF1R KO MEFs[25]. Thus, the different effects of IGF1R on cell cycle progression are likely attributed to different cell types. Furthermore, cell cycle progression is regulated by cyclins[26]. Therefore, we examined S phase-related molecules using Q-PCR. The mRNA expression levels of cyclin A, which is essential for S phase initiation and DNA synthesis[27,28], were increased. In addition, the increased level of cyclin A upon S phase arrest appears to be associated with DNA damage. Several studies suggested that the cyclin A level decreased when the cell cycle arrested at the S phase, but Umma Hafsa Preya et al. noted that α -terthienylmethanol remarkably promoted cyclin A expression when S phase arrest was induced. Similarly, we also found that IGF1R knockdown blocked the cell cycle at the S phase and simultaneously increased cyclin A expression in HLE cells[29]. Cyclin E, responsible for the transition from G0/G1 to S phase, was also increased. Simultaneously, the lower expression of cyclin B mRNA was in response to the fact that HLE cells that could not enter the G2/M phase easily[27,30]. P21^{WAF1/CIP1}, a CDK inhibitor (CKI), and P21^{WAF1/CIP}-overexpression may induce cell cycle arrest at any phase. A 2.5-fold increase was observed after transfection with IGF1R siRNA. Thus, the above data suggested that IGF1R silencing inhibited cell proliferation by inducing the blockage of the cell cycle at the S phase and affecting S phase-related molecule expression.

The cell cycle and apoptosis are two major mechanisms regulating cell proliferation. Interestingly, when the cell cycle arrests at specific checkpoints, apoptotic events occur[31,32]. It has been reported that some anti-cancer drugs act mainly by blocking the cell cycle at the G0/G1, S or G2/M phase to induce apoptosis. There are no data focusing on how IGF1R is related to apoptosis in HLE cells, but apoptosis is a normal [physiological](#) phenomenon and is responsible for cataractogenesis. Several pieces of clinical evidence have revealed that the apoptotic LECs of ARC patients are remarkably higher than those of healthy people[33]. It is widely accepted that the accumulation of damage from UV, oxidative stress and toxic agents triggers apoptosis in HLE cells[34]. Due to HLE cell death via apoptosis, cell growth will slow, leading to lower cell density and a thinner lens[35]. In addition, the breakdown of the lens epithelial cell barrier, which acts as an umbrella to protect the underlying fibre cells from impairment, will contribute to deficiencies in defence systems against stimuli and interfere with the integrity and transparency of fibre cells, thus resulting in lens opacification. These studies demonstrated that the apoptosis of lens epithelial cells is a common cellular basis for the formation of non-congenital cataracts[35], and there may be a potential therapeutic strategy for ARCs. In the present study, we detected apoptosis via an Annexin V-FITC kit in HLE cells. A significantly higher proportion of apoptotic cells was observed after transfection with IGF1R siRNA compared with that in the NC groups. These results are in accordance with the data presented by Mingshi Zhang et al., who reported that the activation of IGF1R is able to protect glioblastoma cancer cells from apoptosis[11]. To confirm the above results, we detected apoptosis-related molecules via western blot and Q-PCR assays. Caspase-3, which is a key executor of cell dismantling that is essential for the development of apoptotic bodies[36] and positively correlates with apoptotic levels, was distinctly increased in the IGF1R siRNA group. Bax, one of the most important proapoptotic genes, is activated at the mitochondrial outer membrane (MOM) and mediates MOM permeabilization, resulting in the release of proapoptotic factors[37,38], is also upregulated. However, lower expression of Bcl-2, which is an anti-apoptotic gene that directly inhibits pro-apoptotic proteins and

blocks upstream pro-apoptotic signals[37], was observed in HLE cells after transfection with IGF1R siRNA. Consistent with the western blot results, the mRNA expression levels of Bax and Bcl-2 were increased and decreased, respectively. Moreover, Caspase-3 activity was also upregulated. These observations suggested that IGF1R knockdown promoted apoptosis by regulating apoptosis-related molecules and altering Caspase-3 activity.

Take into account the limitation of sample size and single population, larger sample researches as well as other ethnic population studies are warranted. Further functional studies are also required to validate the molecular mechanism we have found.

Conclusions

Our study first identified the relationship of rs1546713 in IGF1R with ARCs in a Han Chinese population. Additionally, to better understand the underlying mechanism of its effects on ARC occurrence, we found that IGF1R knockdown inhibited cell proliferation by inducing cell cycle arrest at the S phase and apoptosis in HLE cells, most likely due to the regulation of cell cycle- and apoptosis-related molecules. These results may provide a new basis for IGF1R transmembrane cell signalling in HLE cells for ARC formation. As a result, IGF1R may be a promising target for the treatment of ARCs.

Abbreviations

IGF1R: Insulin-like growth factor-1 receptor; ARC: Age-related cataract; N: Nuclear cataract ; SNP: Single-nucleotide Polymorphism; HLE: Human lens epithelium; C: Cortical cataract ; PSC: Posterior subcapsular cataract; M: mixed cataract; CI: Confidence interval; OR: Odds ratio; HWE: Hardy-Weinberg equilibrium; SD: standard deviation; DMEM: Dulbecco's Modified Eagle Medium; FBS: Fetal bovine serum; LOCS: Lens Opacity Classification System; OD: optical density ; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis ; IGF1: insulin-like growth factor 1 ; IGF2: insulin-like growth factor 2 ; MOM: mitochondrial outer membrane .

Declarations

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CYL performed the experiments and wrote the manuscript. YXN and ZX performed the data analyses. TYL and TXJ participated in the design of the study; YH, PXY and WJ revised the manuscript; YQC, ZJY and XXY collected blood samples; STXC contributed to the conception of the study and provided reagents and materials. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Second Affiliated Hospital, Medical College of Zhejiang University (2019121). Each participant received a full explanation of the purpose and procedures of the research and then signed an informed consent form.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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References

- 1 Bourne RR, Stevens GA, White RA, Smith JL, Flaxman SR, Price H, et al. Causes of vision loss worldwide, 1990–2010: a systematic analysis. *Lancet Glob Health* 2013;1:e339-e349.
- 2 Resnikoff S, Pascolini D, Mariotti SP, Pokharel GP. Global magnitude of visual impairment caused by uncorrected refractive errors in 2004. *Bull World Health Organ* 2008;86:63-70.
- 3 He M, Wang W, Huang W. Variations and Trends in Health Burden of Visual Impairment Due to Cataract: A Global Analysis. *Invest Ophthalmol Vis Sci* 2017;58:4299-4306.

- 4 Khairallah M, Kahloun R, Bourne R, Limburg H, Flaxman SR, Jonas JB, et al. Number of People Blind or Visually Impaired by Cataract Worldwide and in World Regions, 1990 to 2010. *Invest Ophthalmol Vis Sci* 2015;56:6762–6769.
- 5 Klein BE, Klein R, Linton KL. Prevalence of Age-related Lens Opacities in a Population. *Ophthalmology* 1991;99:546-552.
- 6 Hammond CJ, Duncan DD, Snieder H, de Lange M, West SK, Spector TD, et al. The Heritability of Age-Related Cortical Cataract: The Twin Eye Study. *Invest Ophthalmol Vis Sci* 2001;42:601-605.
- 7 Hammond CJ, Snieder H, Spector TD, Gilbert CE. Genetic and environmental factors in age-related nuclear cataracts in monozygotic and dizygotic twins. *N Engl J Med* 2000;342:1786-1790.
- 8 Shiels A, Hejtmancik J F: Genetics of human cataract. *Clin Genet* 2013;84:120-127.
- 9 Liu Q, Guan JZ, Sun Y, Le Z, Zhang P, Yu D, et al. Insulin-like growth factor 1 receptor-mediated cell survival in hypoxia depends on the promotion of autophagy via suppression of the PI3K/Akt/mTOR signaling pathway. *Mol Med Rep* 2017;15:2136-2142.
- 10 Lee JH, Kim JY, Kim SY, Choi SI, Kim KC, Cho EW, et al. APBB1 reinforces cancer stem cell and epithelial-to-mesenchymal transition by regulating the IGF1R signaling pathway in non-small-cell lung cancer cells. *Biochem Biophys Res Commun* 2017;482:35-42.
- 11 Zhang M, Liu J, Li M, Zhang S, Lu Y, Liang Y, et al. Insulin-like growth factor 1/insulin-like growth factor 1 receptor signaling protects against cell apoptosis through the PI3K/AKT pathway in glioblastoma cells. *Exp Ther Med* 2018;16:1477-1482.
- 12 Long L, Rubin R, Baserga R, [Brodt P](#). Loss of the Metastatic Phenotype in Murine Carcinoma Cells Expressing an Antisense RNA to the Insulin-like Growth Factor Receptor. *Cancer Res* 1995;55:1006.
- 13 Basu S, Rajakaruna S, De Arcangelis A, Zhang L, Georges-Labouesse E, Menko A. $\alpha 6$ Integrin Transactivates Insulin-like Growth Factor Receptor-1 (IGF-1R) to Regulate Caspase-3-mediated Lens Epithelial Cell Differentiation Initiation. *J Biol Chem* 2014;289:3842-3855.
- 14 Chylack LT, Leske MC, McCarthy D, Khu P, Kashiwagi T, Sperduto R. lens Opacities Classification System II (LOCS II). *Arch Ophthalmol* 1989;107:991-997.
- 15 Iams WT, [Lovly CM](#). Molecular Pathways: Clinical Applications and Future Direction of Insulin-Like Growth Factor-1 Receptor Pathway Blockade. *Clin Cancer Res* 2015;21:4270-4277.
- 16 Lee J, Hong EM, Jang JA, Park SW, Koh DH, Choi MH, et al. Simvastatin Induces Apoptosis and Suppresses Insulin-Like Growth Factor 1 Receptor in Bile Duct Cancer Cells. *Gut Liver* 2016;10:310-317.

- 17 Wang S, Wang X, Wu Y, Han C. IGF-1R signaling is essential for the proliferation of cultured mouse spermatogonial stem cells by promoting the G2/M progression of the cell cycle. *Stem Cells Dev* 2015;24:471-483.
- 18 Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice Carrying Null Mutations of the Genes Encoding Insulin-like Growth Factor I (Igf.1) and Type 1 IGF Receptor (Igflr). *Cell* 1993;75:59-72.
- 19 Coppola D, Ouban A, Gilbert-Barness E. Expression of the Insulin-Like Growth Factor Receptor 1 during Human Embryogenesis. *Fetal Pediatr Pathol* 2009;28:47-54.
- 20 Bhuyan DK, Reddy PG, Bhuyan KC. Growth factor receptor gene and protein expressions in the human lens. *Mech Ageing Dev* 2000;113:205-218.
- 21 Shiels A, Hejtmancik JF. Mutations and mechanisms in congenital and age-related cataracts. *Exp Eye Res* 2017;156:95-102.
- 22 Ma X, Jiao X, Ma Z, Hejtmancik JF. Polymorphism rs7278468 is associated with Age-related cataract through decreasing transcriptional activity of the CRYAA promoter. *Sci Rep* 2016;6:23206.
- 23 Kato H, Sekine Y, Furuya Y, Miyazawa Y, Koike H, Suzuki K. Metformin inhibits the proliferation of human prostate cancer PC-3 cells via the downregulation of insulin-like growth factor 1 receptor. *Biochem Biophys Res Commun* 2015;461:115-121.
- 24 Zang Q, Zhang L, Gao N, Huang C. Ophiopogonin D inhibits cell proliferation, causes cell cycle arrest at G2/M, and induces apoptosis in human breast carcinoma MCF-7 cells. *J Integr Med* 2016;14:51-59.
- 25 Sell C, Dumenil G, Deveaud C, Miura M, Coppola D, DeAngelis T, et al. Effect of a Null Mutation of the Insulin-Like Growth Factor I Receptor Gene on Growth and Transformation of Mouse Embryo Fibroblasts. *Mol Cell Biol* 1994;14:3604-3612.
- 26 Bendris N, Lemmers B, Blanchard JM. Cell cycle, cytoskeleton dynamics and beyond: the many functions of cyclins and CDK inhibitors. *Cell Cycle* 2015;14:1786-1798.
- 27 Coverley D, Laman H, Laskey R. Distinct roles for cyclins E and A during DNA replication complex assembly and activation. *Nat Cell Biol* 2002;4:523-528.
- 28 Yam CH, Fung TK, Poon RY. Cyclin A in cell cycle control and cancer. *Cell Mol Life Sci* 2002;59:1317-1326.
- 29 Itoh T, Ono A, Kawaguchi K, Teraoka S, Harada M, Sumi K, et al. Phytol isolated from watermelon (*Citrullus lanatus*) sprouts induces cell death in human T-lymphoid cell line Jurkat cells via S-phase cell cycle arrest. *Food Chem Toxicol* 2018;115:425-435.

- 30 Yu J, Zhang L, Ren P, Zhong T, Li Z, Wang Z, et al. Enterovirus 71 mediates cell cycle arrest in S phase through non-structural protein 3D. *Cell Cycle* 2015;14:425-436.
- 31 Li Y, Yu H, Han F, Wang M, Luo Y, [Guo X](#). Biochanin A Induces S Phase Arrest and Apoptosis in Lung Cancer Cells. *Biomed Res Int* 2018;2018:3545376.
- 32 Orren DK, Petersen LN, Bohr VA. Persistent DNA Damage Inhibits S-Phase and G2 Progression, and Results in Apoptosis. *Mol Biol Cell* 1997;8:1129-1142.
- 33 Zhou D, Yin D, Xiao F, Hao J. Expressions of Senescence-Associated b-Galactosidase and Senescence Marker Protein-30 are Associated with Lens Epithelial Cell Apoptosis. *Med Sci Monit* 2015;21:3728-3735.
- 34 Galichanin K. Exposure to subthreshold dose of UVR-B induces apoptosis in the lens epithelial cells and does not in the lens cortical fibre cells. *Acta Ophthalmol* 2017;95:834-838.
- 35 Li WC, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, et al. Lens Epithelial Cell Apoptosis Appears to Be A Common Cellular Basis for Non-Congenital Cataract Development in Humans and Animals. *J Cell Biol* 1995;130:169-181.
- 36 Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 1999;6:99-104.
- 37 Siddiqui WA, Ahad A, Ahsan H. The mystery of BCL2 family: Bcl-2 proteins and apoptosis: an update. *Arch Toxicol* 2015;89:289-317.
- 38 Pena-Blanco A, Garcia-Saez AJ. Bax, Bak and beyond - mitochondrial performance in apoptosis. *FEBS J* 2018;285:416-431.

Tables

Table 1 Sequences of forward and reverse primers used in Q-PCR

RNA species	Primer pairs
IGF1R	Forward:5'-GAGTGCTGTATGCCTCTGTGAAC-3' Reverse:5'-CCTTGGCAACTCCTTCATAGAC-3'
Bcl-2	Forward:5'-GCCACTTACCTGAATGACCACC-3' Reverse: 5'-AACCAGCGGTTGAAGCGTTCCT-3'
Bax	Forward:5'-AGACACCTGAGCTGACCTTGGAG-3' Reverse:5'-GTTGAAGTTGCCATCAGCAAACA-3'
CyclinA	Forward:5'-TAGCTGCTCCAACAGTAAATCAG-3' Reverse:5'-AGGTATGGGTCAGCATCTATCAA-3'
CyclinB	Forward:5'-TGCAGCAGGAGCTTTTTGCT-3' Reverse:5'-CCAGGTGCTGCATAACTGGAA-3'
CyclinE	Forward:5'-ATCAGCACTTTCTTGAGCAACA-3' Reverse:5'-TTGTGCCAAGTAAAAGGTCTCC-3'
P21	Forward:5'-CCTGTCACTGTCTTGTACCCT-3' Reverse:5'-GCGTTTGGAGTGGTAGAAATCT-3'

Table 2 The characteristics of the participants included in this study

Group	N	Gender		Age	
		Male (%)	Female (%)	Mean± SD	Range
Control	500	58.80	41.20	64.306±7.586	49–92
ARC	690	47.83	52.17	66.323±9.781	43–91
N	126	38.10	61.90	68.259±9.856	45–87
C	131	34.35	65.65	67.75±8.383	43–88
PSC	73	45.21	54.79	66.484±10.274	45–90
M	360	56.11	43.89	65.170±9.936	38–91

Table 3 The bioinformatics characteristics of the target SNP

SNP	Minor allele	Call rate	MAF	Test for HWE (p value)	Control(MAF)	ARC(MAF)
rs1546713	A	0.9974	0.367	0.516	0.365	0.369

Table 4 The association between target SNP and ARC risk

SNP	Genetic model	Cataract type	Logistic regression	
			p/pa	OR (95% CI)
rs1546713	Dominant	General	2.69x10 ⁻⁴ /0.046	1.606(1.245-2.071)
		C	-	-
		N	-	-
		PSC	-	-
		M	-	-
	Recessive	General	-	-
		C	-	-
		N	-	-
		PSC	-	-
		M	-	-
	Additive	General	-	-
		C	-	-
		N	-	-
		PSC	-	-
		M	-	-

pa: P value after Bonferroni correction.

Figures

Figure 1

IGF1R-knockdown inhibits the cell proliferation and induced cell cycle arrested in HLE cells .(a)The silence efficiency of IGF1R siRNA was analyzed by Q-PCR. (b)The cell proliferation was measured by CCK-8 assay, after the HLE cells were treated with NC and IGF1R siRNA for 24,48,72h,respectively. (c)The cell cycle analysis was detected by flow cytometry after transfection for 72h in HLE cells. (d)Bar diagram of different cell phase in HLE cells. (e) The mRNA expression of S-phase related molecules was tested via

Q-PCR after transfection for 72h in HLE cells .All data represent the means±SD from three independent experiments, and*p < 0.05, **p < 0.01 , ***p < 0.001, ****p<0.0001 indicted a significant difference ,compared with NC groups.

Figure 2

IGF1R-knockdown induced apoptosis and altered the apoptosis-related molecule expression and Caspase-3 activities in HLE cells.(a) The apoptotic events was detected by Annexin V/PI double-staining assay after transfection with NC and IGF1R siRNA for 72h.(b) The apoptotic proportion of HLE cells after

transfection. (c) The protein expression of Caspase-3, Bax and Bcl-2 was measured via western blot after transfection. (d) Bar diagram of the Caspase-3, Bax, Bcl-2 expression levels from three independent experiments. (e) The mRNA expression levels of Bax and Bcl-2 after transfection, β -actin was used as a control. (f) The Caspase-3 activities was tested by Caspase 3 activity assay. All data are shown as means $\pm$ SD from three independent experiments, and * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicated a significant difference, compared with NC groups.