Original Paper

Long Non-Coding RNA KCNQ1OT1 Promotes Cataractogenesis via miR-214 and Activation of the Caspase-1 Pathway

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Key Words

Cataract • KCNQ1OT1 • miR-214 • Caspase-1 • Pyroptosis

Abstract

Background/Aims: KCNQ1OT1 regulates the expression of tissue-specific imprinted genes within the Kcnq1 domain. Imprinted genes are positive regulators of apoptosis, one of the forms of cell death related to cataract formation, and thus may provide novel therapeutic targets for cataract treatment. Here, we studied the role of non-coding RNAs(ncRNA) in cataract formation. **Methods:** Human lens epithelium cells (HLECs) were treated with H₂O₂, and the expression of KCNQ1OT1 and miR-214 was detected by qRT-PCR. The expression of caspase-1 was detected using qRT-PCR, western blot and immunostaining. To confirm our findings in cell cultures, we analysed KCNQ1OT1, miR-214, and caspase-1 expression in lens anterior capsules of both cataract patients and normal controls by qRT-PCR and western blot analysis. **Results:** We found that the expression of KCNQ1OT1 was increased in both human cataract lens anterior capsular samples and SRA01/04 cell lines treated with H₂O₂. Knockdown of KCNQ1OT1 expression significantly suppressed H₂O₂-induced SRA01/04 cell pyroptosis in vitro, which is the critical step in cataract formation. The expression of microRNA-214 (miR-214) was also decreased in cataract lens anterior capsular tissues and H₂O₂-induced SRA01/04 cell lines. Knockdown of KCNQ1OT1 significantly increased the expression of miR-214. **Conclusions:** We demonstrated for the first time that caspase-1 is a functional downstream target of miR-214, and knockdown of KCNQ1OT1 reduced the expression of caspase-1. These results provide evidence that the KCNQ1OT1-miR-214-caspase-1 regulatory network is a novel mechanism for promoting cataract formation.

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Introduction

Age-related cataract is a cause of blindness worldwide involving genetic and environmental influences [1]. With the progress of science and technology, the surgical treatment become the most common treatment of cataract, but so far, the risk for the surgical

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complications still could not be avoided completely [2, 3]. Understanding the mechanisms of cataract may help prevent cataract formation, and reduce risks of complications.

Pyroptosis is a pro-inflammatory form of cell death triggered by the inflammasome, a multi-protein complex that assembles in the cytosol to activate caspase-1. Pyroptosis is triggered by various pathological stimuli, such as brain injury [4], heart attack [5] or cancer [6], and is crucial for controlling microbial infections [7]. Our previous study has indicated that H_2O_2 can induce HLECs pyroptosis by the caspase-1 pathway. However, the complete mechanism remains unclear.

A recent study reports that non-coding RNAs are involved in the regulation of pyroptosis [8]. Non-coding RNA is an RNA molecule that is not translated into a protein. Noncoding RNAs include short non-coding RNAs and long non-coding RNAs (lncRNAs). lncRNAs, which are RNA molecules over 200 nucleotides in length, have been implicated in the regulation of a variety of cellular functions, disease processes, and cancer progression [9]. MicroRNAs (miRNAs) are a small non-coding RNA molecules found in plants, animals and some viruses that have functions in RNA silencing and post-transcriptional regulation of gene expression [10]. By binding to specific sequences, usually in the 3' untranslated region (3'UTR) to degrade the transcription of target genes [11], miRNAs have been demonstrated to play an important role in cataract formation [12-15]. Although some studies have implicated lncRNAs and miRNAs in cataract formation, there is no published study to the best of our knowledge revealing the relationships between them.

In the present study, we found that lncRNA KCNQ1OT1 was upregulated in cataract lens anterior capsular samples and that KCNQ1OT1 knockdown inhibited the pyroptosis of HLECs. Furthermore, we demonstrated that KCNQ1OT1 upregulated caspase-1 through inhibition of miR-214 expression. These results provide evidence that the KCNQ1OT1-miR-214-caspase-1 regulatory network promotes cataract formation.

Materials and Methods

Human tissue samples

Twenty pairs of lens anterior capsular samples were obtained from cataract patients (twenty patients, free of other ocular diseases) at the operating room of the Eye Hospital of Harbin Medical University, China. Control lens anterior capsular samples were collected from healthy postmortem eyes (twenty donors, free of ocular diseases) donated to the eye bank of Heilongjiang Province. Samples were immediately snap-frozen and stored at -80°C until RNA extraction. All samples were collected with informed consent obtained from all the patients, and the study was approved by the Research Ethics Committee of Harbin Medical University.

Cell culture and transduction

SV40 T-antigen-transformed human LEC line (SRA01/04 cell) were cultured in Dulbecco's modified DMEM/F12 supplemented with 20% foetal bovine serum (FBS) at 37°C in a 5% CO_2 atmosphere incubator overnight, and the cells were treated with the different concentrations of H_2O_2 for the required time. SiRNAs of human KCNQ1OT1 were constructed by RIBOBIO (Guangzhou, China). The overexpression and knockdown of miR-214 were provided by Invitrogen (Carlsbad, CA, USA). All cell transfections were performed according to the manufacturer's protocol.

RNA extraction and quantitative PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). PCR primers were purchased from Invitrogen (Carlsbad, CA). The concentration of RNA was determined using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). For detection of miR-214 expression level, cDNA was synthesized from 5 ng of total RNA using the TaqMan® miRNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The relative miR-214 expression levels after normalization to U6 small nuclear RNA was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method.

For detection of caspase-1 mRNA expression, cDNAs were synthesized from total RNA using gene specific primers from a cDNA synthesis kit (HIGH-Capacity cDNA Reverse Transcription Kits, USA).

Quantitative real-time PCR was performed using SYBR Green Real-Time PCR MasterMix (ToYoBo, Japan) according to the manufacturer's protocols using an ABI 7500 Sequence Detection System (Life Technologies, NY, USA). PCR amplification was performed in a 20- μ l reaction volume containing 2 μ l cDNA, 6 μ l DEPC, 10 μ l STBR Master Mix, 1 μ l forward primer and 1 μ l reverse primer. PCR conditions were as follows: holding 95°C for 60 s, 40 cycles at 95°C for 15 s, 60°C for 15 s, 72°C for 45 s. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The relative caspase-1 expression level was normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are listed in Table 1.

Western blot analysis

Western blot analysis was used to detect the expression levels of proteins of interest. Protein (50 μ g) per sample was separated using 10% SDS-PAGE and then transferred onto nitrocellulose membranes (USA). The membrane was blocked with 5% non-fat milk (BD Biosciences) and 0.1% Tween 20 in tris-buffered saline, then immunoblotted overnight using appropriate primary antibodies at 4°C with gentle shaking. Fluorochrome-labelled secondary antibody (Alexa Fluor 800, LI-COR, USA) was used to identify the appropriate primary antibody. Immunoreactivity was detected with the Odyssey fluorescent scanning system (LI-COR) and analysed by Image Studio software. GAPDH was used as a loading control.

Cell proliferation assay

Cell proliferation assay kits (CCK-8) were used in accordance with the manufacturer's instructions. HLECs were seeded in 96-well plates at 1×10^4 cells/well and maintained for 24 h. CCK-8 solution (10 μ l) was added to each well, and cells were incubated in 37°C for 2 h. The absorbance (A) at 450 nm was evaluated using a microplate reader. The data are representative of three individual experiments in performed in triplicate.

Immunofluorescence staining

SRA01/04 cells treated with siNC and siKCNQ1OT1 were fixed with 4% paraformaldehyde for 20 minutes at room temperature. After rinsing with PBS, cells were blocked with 5% BSA. The cells were incubated with rabbit caspase-1 (1:200; Sigma) overnight at 37°C. After rinsing three times in PBS, cells were incubated with the corresponding secondary antibody, Cy3-conjugated anti-rabbit IgG (1:100; Sigma). The nuclei were stained with DAPI.

TUNEL staining

SRA01/04 cells were plated onto coverslips in 24-well culture plates, and In Situ Cell Death Detection Kits (TUNEL fluorescence FITC kit, Roche, Indianapolis, IN, USA) were employed to detect DNA fragmentation of individual cells according to the manufacturer's instructions. The nuclei were stained with DAPI. TUNEL staining was assessed via fluorescence microscopy (Eclipse 80i; Nikon Co., Tokyo, Japan). Nuclei double labeled with DAPI and TUNEL were considered positive.

Luciferase assay

The miR-214 binding sites of KCNQ1OT1 and caspase-1 3'-UTR were predicted using the bioinformatics databases (Starbase v2.0, miRcode and RNAhybrid). The CASP1 3'-UTR and KCNQ1OT1 3'-UTR luciferase reporter vector was purchased from RIBOBIO (Guangzhou, China). Restriction sites for XhoI and NotI were added to forward and reverse primers (IDT DNA), respectively. To examine the relationship between miR-214 and CASP1, we constructed wild-type and mutated-type CASP1 3'-UTR reporter plasmid with psiCheck-2 vector. To test whether miR-214 targets KCNQ1OT1 directly, the PCR amplified KCNQ1OT1 3'-UTR(wild-type and mutated-type) were cloned into the pmiR-

Table 1. The primers used for qRT-PCR

Names	Sequence (5'-3')	Length (bp)
GAPDH	AAGAAGGTGGTGAAGCAGGC	20
	TCCACCACCCTGTTGCTGTA	20
CASP1	ACACGTCTTGCCCTCATTATCT	22
	ATAACCTTGGGCTTGCTTTTCA	22
U6	CTCGCTTCGGCAGCACATATACT	23
	ACGCTTCACGAATTTGCGTGTC	22
miR-214	TATACATCAAACAGCAGGCACA	22
	CATTCGATCTTCTCCACAGTCTC	23

RB-Report vector. The luciferase activity was measured 48 hours after transfection using the Dual-Luciferase Reporter Assay System(Promega).

Statistical analysis

Statistical significance was determined using Student's t-test (two-tailed), and one-way ANOVA. Results were expressed as the mean $\pm$ standard deviation (SD). The differences were considered to be statistically significant at $P < 0.05$.

Results

KCNQ10T1 and caspase-1 involved in cataract formation

We first investigated the lncRNA KCNQ10T1 expression level in both cataract and normal capsule tissues by qRT-PCR. The KCNQ10T1 levels were higher in cataract lens anterior capsules than normal controls (Fig. 1A). We also evaluated lncRNA KCNQ10T1 expression level in SRA01/04 cells treated with different concentrations of H_2O_2 (0, 25, 50 and 100 μM). Our results showed that KCNQ10T1 levels were increased in a dose-dependent manner with H_2O_2 concentration (Fig. 1B). We also measured the mRNA and protein expression of caspase-1 both in tissues and in SRA01/04 cells treated with the same concentrations of H_2O_2 as above. The mRNA and protein expression levels of caspase-1 were also significantly upregulated in cataract samples compared to normal controls (Fig. 1C-D). At the same time, caspase-1 levels were higher with the increase of H_2O_2 concentration in SRA01/04 cells (Fig. 1E-F). These findings imply that KCNQ10T1 and caspase-1 levels have a strong correlation with cataract formation.

Validation of pyroptosis in SRA01/04 cells treated with H_2O_2

Caspase-1 is a commonly utilized marker of pyroptosis. To detect whether pyroptosis is involved in cataract formation, SRA01/04 cells were exposed to various concentrations of H_2O_2 (0, 25, 50 and 100 μM) for 24 h. Pyroptosis was detected by CCK-8, immunofluorescence staining and TUNEL staining (Fig. 2A-C). SRA01/04 cell growth was inhibited by 100 and 200 μM H_2O_2 at 24 and 48 h, as shown by the CCK-8 assay (Fig. 2A). Immunofluorescence staining showed much stronger positive staining of caspase-1 in SRA01/04 cells treated with 100 μM H_2O_2 (Fig. 2B). The TUNEL assay also showed that H_2O_2 -induced cell pyroptosis was significantly increased in a dose-dependent manner (Fig. 2C). Based on these results, we chose 100 μM H_2O_2 for pyroptosis induction in our subsequent experiments.

KCNQ10T1 knockdown inhibits the pyroptosis of SRA01/04 cells

We next examined the role of KCNQ10T1 in the pyroptosis of HLECs treated with 100 μM H_2O_2 . After demonstrating the effectiveness of KCNQ10T1 siRNAs (Fig. 3A), pyroptosis in HLECs was determined using CCK-8 and TUNEL staining. The CCK-8 assay results showed that KCNQ10T1 knockdown reduced HLECs pyroptosis compared with cells transfected with control siRNA (Fig. 3B). After successful transfection, pyroptosis was significantly reduced, as shown by TUNEL staining (Fig. 3C). As shown in Fig. 3B-C, both CCK-8 and TUNEL staining showed that KCNQ10T1 siRNAs could reduce pyroptosis in HLECs. These data suggest that KCNQ10T1 contributes to HLECs pyroptosis in vitro. Moreover, we measured caspase-1 expression levels between HLECs transfected with KCNQ10T1 siRNAs and siNC. Our results show that HLECs transfected with KCNQ10T1 siRNAs had lower caspase-1 expression (Fig. 3D-F).

miR-214 as a potential factor in KCNQ10T1-caspase-1 pathway

In the above experiments, we found that KCNQ10T1 expression was correlated with caspase-1. To elucidate this mechanism, we investigated potential miRNAs. We used bioinformatics tools (MicroRNA, Starbase v2.0) to examine the potential complementary base pairing between KCNQ10T1 and miRNAs. We found that miR-214 contains potential

Fig. 1. KCNQ10T1 and caspase-1 involved in cataract formation. A: qRT-PCR results of the relative mRNA levels of KCNQ10T1 in anterior lens capsules of normal donors and cataract patients; B: qRT-PCR results showed that the relative KCNQ10T1 mRNA levels was increased with the increased concentration of H₂O₂ (0, 25, 50, 100 μM); (C and D): The mRNA and protein levels of caspase-1 in anterior lens capsules of normal donors and cataract patients; (E and F): qRT-PCR and western-blot results showed that the relative caspase-1 levels was increased with the increased concentration of H₂O₂ (0, 25, 50, 100 μM); Data are expressed as mean ± SEM. n=3. *P<0.05, ***P<0.001 versus control.

Fig. 2. Validation of pyroptosis in SRA01/04 cells treated with H₂O₂. A: CCK-8 assay results revealed that SRA01/04 cells growth was inhibited when co-cultured with 100, 200 μM H₂O₂ for 24, 48 h; B: Immunofluorescence images showing the expression of caspase-1 in SRA01/04 cells treated with H₂O₂ (0, 25, 50, 100 μM); Blue: nuclear staining (DAPI); Green: caspase-1 staining. Scalebar: 20 μm. C: TUNEL images of SRA01/04 cells. Cells were treated with 0 μM, 25 μM, 50 μM and 100 μM H₂O₂, respectively. Blue, nuclear staining (DAPI); Green, TUNEL staining. Scalebar: 20 μm.

Fig. 3. KCNQ1OT1 knockdown inhibits the pyroptosis of SRA01/04 cells A: Relative mRNA levels of KCNQ1OT1 of SRA01/04 cells in the control, siNC, siKCNQ1OT1 groups. B: Effect of siKCNQ1OT1 on proliferation of SRA01/04 cells by CCK-8 assay. C: TUNEL images of SRA01/04 cells of the control, siNC, siKCNQ1OT1 groups. Blue, nuclear staining (DAPI); Green, TUNEL staining. Scalebar: 20 μ m. (D and E): Relative mRNA and protein levels of caspase-1 of SRA01/04 cells in the control, siNC, siKCNQ1OT1 groups. F: Immunofluorescence images showing the expression of caspase-1 in SRA01/04 cells of the control, siNC, siKCNQ1OT1 groups. Blue: nuclear staining (DAPI); Green: caspase-1 staining. Scalebar: 20 μ m. Data are expressed as mean $\pm$ SEM. n=3. *P<0.05, ***P<0.001 versus control.

Fig. 4. miR-214 as a potential factor in KCNQ1OT1-caspase-1 pathway. A: Bioinformatics predicted miR-214 binding sites in CASP1. Partial sequences of miR-214 and binding sites in the CASP1 3'-UTR are shown. "CUGCUG" in WT CASP1 3'-UTR turn into "UCAUCA" in MUT CASP1 3'-UTR as red line. Bioinformatics predicted miR-214 binding sites in KCNQ1OT1 3'-UTR. The sequences of KCNQ1OT1 WT and mutant are shown below in Fig.4A. B: Co-transfection with WT KCNQ1OT1 3'-UTR, miR-214 mimics, miR-NC or MUT KCNQ1OT1 3'-UTR, miR-214 mimics, miR-NC are showed middle in Fig.4B. C: Luciferase reporter plasmids containing WT or mutant CASP1 3'-UTR were co-transfected into SRA01/04 cells with miR-214 mimics in parallel with miR-NC. The data are presented as the relative luciferase activity.

binding sites for both KCNQ1OT1 and caspase-1 3'-UTR (Fig. 4A). Then, we used a luciferase assay system to test whether these bioinformatics analysis results are correct. As shown in Fig. 4B-C, the relative luciferase activities in cells transfected with WT KCNQ1OT1-3'-UTR were significantly reduced in the presence of miR-214. Co-transfection with WT caspase-1 3'-UTR and miR-214 mimics the significantly reduced luciferase reporter activity compared with cells transfected with MUT caspase-1 3'-UTR and miR-214 mimics.

Fig. 5. miR-214 in cataract A: The miR-214 levels in anterior lens capsules of normal donors and cataract patients; B: qRT-PCR results showed that the relative miR-214 levels was reduced with the increased concentration of H_2O_2 ; C: The miR-214 levels in SRA01/04 cells transfected with control, miR-214, siNC and AMO-miR-214 by qRT-PCR. (D and E): Relative mRNA and protein levels of caspase-1 of SRA01/04 cells in the control, miR-214, siNC, AMO-miR-214 groups. Data are expressed as mean $\pm$ SEM. $n=3$. * $P<0.05$, *** $P<0.001$ versus control.

miR-214 in cataract

Our next experiments were performed to confirm whether miR-214 is involved in cataract formation. We detected miR-214 expression in both cataract lens anterior capsules and in HLECs treated with H_2O_2 . As shown in (Fig. 5A), the relative miR-214 levels were lower in the cataract lens anterior capsule than normal lens capsule tissues. Similarly, relative miR-214 levels decreased in HLECs with the increases in H_2O_2 concentration (Fig. 5B). To further investigate whether miR-214 was a functional target of KCNQ10T1, we first examined miR-214 expression levels in HLECs transfected with KCNQ10T1-siRNA or a negative control (siNC). qRT-PCR results showed that after treatment with 100 μM H_2O_2 , miR-214 expression was elevated in HLECs transfected with KCNQ10T1-siRNA compared with HLECs transfected with siNC (Fig. 6A). To confirm caspase-1 is the downstream target of miR-214, we first demonstrated the effectiveness of miR-214 transfection (Fig. 5C). After pretreatment with 100 μM H_2O_2 , HLECs were transfected with control, miR-214mimic, siNC and AMO-miR-214. We found that overexpression of miR-214 abrogated the increase of caspase-1, and the opposite result occurred in AMO-miR-214 (Fig. 5D-E). Furthermore, inhibition of KCNQ10T1 overcame the increase of caspase-1 by miR-214 inhibitor (Fig. 6A-B). All these results suggest an important role of KCNQ10T1 in regulating caspase-1 by competitively binding miR-214.

miR-214 reverses the accelerative effects of KCNQ10T1 in HLECs

Our results demonstrate that miR-214 is a target of KCNQ10T1 (Fig. 4A, 6A), but the role of miR-214 in KCNQ10T1-mediated effects in HLECs remained unclear. Our next experiments were designed to determine whether KCNQ10T1 might promote pyroptosis in HLECs via the potential KCNQ10T1-miR-214-caspase-1 pathway. qRT-PCR assays revealed that the

Fig. 6. miR-214 reverses the accelerative effects of KCNQ10T1 in SRA01/04 cells. A: miR-214 expression was visibly elevated in SRA01/04 cells transfected with siKCNQ10T1. The caspase-1 mRNA expression levels are reduced by inhibition of KCNQ10T1 and reversed by miR-214 inhibitor. B: The caspase-1 protein expression levels are in accordance with its mRNA expression levels. C: TUNEL images of SRA01/04 cells treated with 100μM H₂O₂ then transfected with NC+AMO-NC, siKCNQ10T1+AMO-NC, siKCNQ10T1+AMO-miR-214 groups. Blue, nuclear staining (DAPI); Green, TUNEL staining. Scalebar: 20μm. Data are expressed as mean ± SEM. n=3. * p < 0.05 when compared with NC+AMO-NC group.

decrease of KCNQ10T1 expression by KCNQ10T1-siRNA could be largely reversed by miR-214 (Fig. 6A). We used TUNEL staining to correlate cell death with caspase-1 expression. H₂O₂-induced cell pyroptosis was reduced by inhibition of KCNQ10T1 and reversed by miR-214 inhibition (Fig. 6C). Taken together, our results suggest that KCNQ10T1 promotes pyroptosis in HLECs through the KCNQ10T1-miR-214-caspase-1 axis and that miR-214 could reverse the influence of KCNQ10T1 on HLECs.

Discussion

Recent studies have demonstrated the functional roles of lncRNAs and provided insights into the molecular mechanisms by which lncRNAs function in a variety of human diseases [16]. Several studies have proposed that mRNAs and lncRNAs can regulate each other through their capacity to compete for miRNA binding [17, 18]. In this study, for the first time, we reported that KCNQ10T1 plays an important role in cataract formation. KCNQ10T1 was highly regulated in H₂O₂-treated HLECs and cataract lens anterior capsule tissues, and its overexpression promotes pyroptosis in HLECs, possibly by targeting and inhibiting miR-214.

KCNQ10T1 encodes a paternally expressed lncRNA that has been shown to regulate imprinting of several genes present at 11p15.5 locus in cis [19]. KCNQ10T1 might inhibit tumour development and/or progression in many diseases, including colorectal cancer [20] and lung cancer [21]. At the same time, KCNQ10T1 regulates the expression of both ubiquitous and tissue-specific imprinted genes within the Kcnq1 domain [22]. Imprinted genes are a positive regulator of apoptosis and tumour suppression [23, 24]. Apoptosis of lens epithelial cells is a common cellular mechanism of initiation and progression for non-congenital cataracts [25]. Apoptosis is the most widely recognized programme of cell death [26], but other cell death programmes, such as autophagy, oncosis and pyroptosis, are also

involved in occurrence and development of diseases [27, 28]. Our previous studies indicate that pyroptosis is implicated in the regulation of cataract formation. However, the role of KCNQ1OT1 in regulating cataracts has remained unknown.

Mir-214 is involved in various physiological processes, such as melanoma tumours [29], uveal melanoma [30], differentiation of stem cells [31], gastric cancer [32], and arterial hypertension [33]. However, the biological functions of miR-214 in cataracts have not been completely elucidated. Our study provides new evidence that highly overexpressed KCNQ1OT1 in cell lines and cataract lens anterior capsule tissues acts in a monogenic role. We also found that KCNQ1OT1 is a specific target of miR-214, and there is an interactive suppression between KCNQ1OT1 and miR-214. Our data suggest that KCNQ1OT1 may promote pyroptosis in cataracts due to its ability to inhibit miR-214 and subsequent activation of caspase-1. Caspase-1 is an important marker in the development of pyroptosis [34]. In this study, we found that KCNQ1OT1 expression in normal lens anterior capsule tissues is remarkably lower than that in cataract lens anterior capsule tissues. Our observations demonstrate KCNQ1OT1 could play a critical role in cataract development and progression. However, the underlying mechanism by which KCNQ1OT1-mediated gene expression remains to be elucidated. Several studies report that lncRNAs can function as competing endogenous RNAs to regulate microRNAs in various cellular processes [35, 36]. We discovered that KCNQ1OT1 may function as endogenous miRNA “sponge” to bind miR-214 and regulate its function. We detected miR-214 expression in HLECs with increased H_2O_2 concentration, and in cataract lens anterior capsule tissues compared to normal controls. KCNQ1OT1 and miR-214 showed an opposite association, suggesting regulatory relationship between miR-214 and KCNQ1OT1 in pyroptosis and cataract formation. We found that miR-214 overexpression decreased caspase-1 levels in HLECs. Furthermore, our data suggests there are miR-214 binding sites on KCNQ1OT1 sequences that miR-214 directly binds.

Taken together, this study supports the hypothesis that KCNQ1OT1 may induce pyroptosis through the miR-214/caspase-1 pathway during cataract formation. This report reveals a novel mechanism of KCNQ1OT1 in cataract formation. KCNQ1OT1 can directly interact with miR-214, lowering its expression and thus modulating caspase-1 downstream. These studies may provide a basis for developing novel therapeutic strategies for cataract prevention and treatment in the future.

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Disclosure Statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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