miR-630 can regulate the proliferation and apoptosis of human lens epithelial cells through targeting B-cell lymphoma-2

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Abstract: miR-630 was significantly up-regulated in cataract, however, the related mechanism remains unclear. The aim of this study was to examine the expression of miR-630 in cataract and the related mechanism. The expressions of miR-630 in the anterior lens capsules of patients with age-related cataracts and the healthy controls were determined by RT-qPCR methods; moreover, human lens epithelial cells were transfected with miR-630 mimics, and the effects of miR-630 on the proliferation and apoptosis of human lens epithelial cells were examined by real time q-PCR and flow cytometry methods, and the expression of Bcl-2 as well as apoptosis related proteins were examined by WB methods. We observed that miR-630 was significantly up-regulated in the anterior lens capsules of patients with age-related cataracts; moreover, transfection of miR-630 mimics induced significant increase in apoptosis and decrease in the proliferation of lens epithelial cells; furthermore, transfection of miR-630 mimics also induced marked decrease in the expression of Bcl-2, and Bcl-2 has been proved to be a direct target of miR-630. In conclusion, we reported for the first time that miR-630 can regulate the proliferation and apoptosis of human lens epithelial cells through targeting Bcl-2, suggesting that miR-630 may has the potential to become a novel therapeutic target for the treatment of cataract.

Keywords: miR-630, cataract, Bcl-2, lens epithelial cell

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Introduction

Cataract is a common aging related disease. With the aging of the world population, the incidence of cataract is increasing rapidly (1). The pathogenesis of cataract is still unknown, and the only consensus on the mechanism of cataract is that the aberrant apoptosis of lens epithelial cells (2), but the specific mechanism is not clear yet. Therefore, it is of great academic significance and social value to explore the regulation mechanism of cataract, especially the age-related cataract, and explore novel and effective methods to prevent and control the progress of the disease without surgery.

microRNAs (miRNA, miR) are a group of single chain small molecule noncoding RNA with 25 nucleotides long, which specifically bind to the 3 end non-translation region of the target gene mRNA, thereby negatively regulating the expression of target gene and function (3,4), and regulating 30% of the whole genome of human genome at post transcriptional level. MicroRNA participates in a series of important activities in the life process, including embryo development, organogenesis, cell proliferation, apoptosis and so on (5,6). In recent years, studies have shown that microRNA is closely related to the occurrence and development of diseases such as cataract (7,8).

miR-630 is a widely studied microRNA in human cells. It has been reported that miR-630 can regulate the apoptosis of tumor cells and play the roles either as tumor suppressor gene or oncogene in the occurrence and development of multiple tumors, (9-11). It was observed that miR-630 was significantly up-regulated in cataract (12), however, the related mechanism remains unclear. Therefore, in this study, the effects of miR-630 on the apoptosis of human lens epithelial cells were investigated. The effects of miR-630 on the apoptosis of human lens epithelial cells were investigated by cell transfection, Real-time q-PCR and flow cytometry, and the expression of apoptosis related proteins were examined by WB methods. Our data may provide novel evidence to determine whether miR-630 can become a new target for the diagnosis and non-surgical treatment of cataract.
Material and methods

Specimens

A total of 30 fresh anterior lens capsules were collected between Sep 2016 and May 2017 at Chengdu First People's Hospital from age-related cataract patients. Fifteen transparent (healthy) anterior lens capsules were served as the control group. The specimens were immediately stored in liquid nitrogen when collecting. This study has been approved by the Ethics Committee of Chengdu First People's Hospital, and the informed consent was obtained from every patient.

Cell Culture and transfection

A human lens epithelial cell line (SRA01/04) was purchased from cellbank (Shanghai, China). The cells were cultured in DMEM containing 10% fetal bovine serum (FBS, Gibco, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (Thermo, USA) at a 37°C with 5% CO2. The miR-630 mimics and miR-630 mimics negative control (NC) oligonucleotides were synthesised by GenePharma (Shanghai, China). At confluence, cells were seeded at the density of 100,000 cells/well and transfected with of miR-630 mimics or NC with Lipofectamine 2000 (Thermo Fisher Scientific, Massachusetts, USA) for 48h.

Cell proliferation analysis

MTT was performed at 24, 48 and 72h after transfection to determine the cell viability using MTT proliferation assay kit (Sigma-Aldrich, St. Louis, MO, USA) according to manufacturer’s instruction.

Cell apoptosis analysis

48h after transfection, the apoptosis of the cells was determined by with PI/Annexin V-FITC apoptosis detection kit (BD Biosciences, California, USA) and analyzed with BD FACSVerse flow cytometer (BD Biosciences, California, USA).

Real-time quantitative PCR

The total RNAs were extracted from the cells and tissues samples with TRIzol (Invitrogen, Massachusetts, USA). The expression of miR-490-3p was determined by
using the Hairpin-it™ miRNAs qPCR Quantitation Kit (GenePharma, Shanghai, China). U6 (RNU6B; GenePharma) served as the internal control. The expression of Bcl-2, Bax and Caspase-3 was determined by YBR ExScript RT-PCR kit (TaKaRa, Dalian, China), and GAPDH was used as the internal control. Real-time quantitative PCR(qRT-PCR) was performed with ABI 7500 Real-Time PCR System (Applied Biosystems, CA, USA).

**Western blot analysis**

Cells were lysed by RIPA Lysis Buffer, and the concentration of the protein was determined by the BCA Protein Assay Kit (Beyotime, Shanghai, China). The electrophoresis was performed to separate the proteins, and the proteins were then transferred onto PVDF membranes and blocked with 5% non-fat milk. The membranes were then incubated with primary antibodies (anti-human Bcl-2, anti-human Bax, anti-human-Caspase-3 and anti-human-GAPDH, all purchased from Abcam, MA, USA) at 4°C overnight; in the following day, the membranes were washed and then incubated with the HRP-conjugated secondary antibodies (Abcam, MA, USA), and the enhanced chemiluminescent reagent (Beyotime, Shanghai, China). Finally, the signals were detected by ChemiDoc™XRS+ imaging system (Bio-Rad, CA, USA).

**Dual luciferase reporter assay**

The fragments of wild-type Bcl-2 3-UTR (Bcl-2-3’UTR) or mutant Bcl-2 3-UTR (Bcl-2-MUT) containing miR-630 binding site were cloned into the pGL6-TA-reporter plasmid (Beyotime, Shanghai, China), and transfected into 293 cells with Lipofectamine® 2000. 48h later, and the activities of the luciferases were examined by dual-luciferase reporter system (Beyotime, Shanghai, China).

**Statistical analysis**

Statistical analysis was performed with SPSS 17.0 software. Data were presented as the means±standard deviation, and the two independent sample t-test was performed to draw a comparison between groups. ANOVA has been performed for the comparison among multiple groups. P<0.05 was considered as statistically significant.
difference.

Results

Increased expression of miR-630 in the anterior lens capsules of patients with age-related cataracts

First of all, the expressions of miR-630 in the anterior lens capsules of patients with age-related cataracts and the healthy controls were determined by RT-qPCR methods. As shown in Figure 1, compared with the healthy control, the expression of miR-630 in anterior capsules of patients with age-related cataracts were significantly increased (P<0.01).

![Figure 1. Comparison of the expressions of miR-630 in the anterior lens capsules of patients with age-related cataracts and the healthy controls.](image)

**P<0.01 vs. the control group.

Effect of miR-630 on the proliferation of human lens epithelial cell line SRA01/04

To further explore the potential roles of miR-630 in the pathogenesis of cataract, the effect of miR-630 on the proliferation of human lens epithelial cell line SRA01/04 was evaluated by MTT methods. As shown in Figure 2, transfection of miR-630 mimics induced significant decrease in the proliferation of SRA01/04 cells in vitro.

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(P<0.01).

Figure 2. Effect of miR-630 on the proliferation of human lens epithelial cell line SRA01/04. Control, un-transfected cells; mimics, miR-630 mimics transfected cells; NC, miR-630 mimics negative control transfected cells. **P<0.01 vs. the control group.

Effect of miR-630 on the apoptosis of human lens epithelial cell line SRA01/04

Next, the effect of miR-630 on the apoptosis of SRA01/04 cells were examined by flow cytometry methods. It was observed that transfection of miR-630 mimics induced significant increase in the apoptosis of SRA01/04 cells in vitro (P<0.01).
Figure 3. Effect of miR-630 on the apoptosis of human lens epithelial cell line SRA01/04.
Control, un-transfected cells; mimics, miR-630 mimics transfected cells; NC, miR-630 mimics negative control transfected cells.**P<0.01 vs. the control group.

Figure 4. mRNA expression of Bcl-2, Bax and Caspase-3 in different groups.
Control, un-transfected cells; Control, un-transfected cells; mimics, miR-630 mimics transfected cells; NC, miR-630 mimics negative control transfected cells.**P<0.01 vs. the control group.

**Bcl-2 is a direct target of miR-630 in SRA01/04 cells in vitro**

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Finally, the underlying mechanism of miR-630 in the pathogenesis of cataract was explored. Using bioinformatic tools, Bcl-2 has been predicted as a direct target of miR-630. We observed that transfection of miR-630 mimics induced significant decrease in the expression of Bcl-2 on both mRNA and protein levels (Figure 4 and 5, p<0.05); moreover, transfection of miR-630 mimics also lead to significant increase in the expression of pro-apoptotic proteins Bax and Caspase-3; furthermore, dual luciferase reporter assay has been performed to determine that Bcl-2 is a direct target of miR-630. As shown in Figure 6, transfection of miR-630 mimics and Bcl-2-WT significantly decrease the activities of the luciferases, while transfection of miR-630 mimics and Bcl-2-MUT did not affect the activities of the luciferases, indicating that Bcl-2 is a direct target of miR-630.

![Figure 5](image_url)  
Figure 5. Protein expression of Bcl-2, Bax and Caspase-3 in different groups.

Control, un-transfected cells; Control, un-transfected cells; mimics, miR-630 mimics transfected cells; NC, miR-630 mimics negative control transfected cells. **P<0.01 vs. the control group.
Figure 6. Bcl-2 is direct target of miR-630. (A) Sequence alignment of the paired 3’-UTR of miR-630 and Bcl-2; (B) Results of dual luciferase reporter assay. Control, un-transfected cells; mimics, miR-630 mimics transfected cells; NC, miR-630 mimics negative control transfected cells. **P<0.01.

Discussion

MicroRNAs are a group of stable non-coding small molecules, which control multiple biological processes such as apoptosis, proliferation, differentiation, development and metabolism. Single microRNA can act on multiple target genes, while multiple microRNA can act on the same target gene, thus forming a complex network of microRNA regulation. So far, there are about one thousand mature microRNA that have been discovered (13).

Studies have shown that microRNA is closely related to the occurrence and development of many diseases (14,15). MicroRNA plays an important role in the growth, development and functional regulation of the eye (16-18). Studies have shown that microRNA plays an important role in the pathogenesis of cataract, in which miR-125b controls the apoptosis of endothelial cells and affects the process of cataract (19); moreover, the levels of let-7 has been proved to be correlated with the the severity of the age related cataract(20); furthermore, miR-31, miR-99 may also participate in the pathogenesis of cataract; The seed region mutation of miR-184 may lead to the anterior polar cataract, and the differential expression of microRNA may be involved in the apoptosis of lens epithelial cells, leading to the occurrence and
development of cataract (21). The expression of some microRNAs are restricted to ocular tissues, including lens, cornea and retina. (22,23). In a recent report, it was observed that miR-630 was significantly up-regulated in cataract, however, the underlying mechanism remains unclear.

In the present study, we observed that the expression of miR-630 in anterior capsules of patients with age-related cataracts were significantly increased compared with the healthy controls, which was consistent with previous reports; more importantly, we also reported for the first time that transfection of miR-630 mimics can induce significant decrease in the proliferation and increase in the apoptosis of SRA01/04 cells in vitro. Taken together, these results suggested that miR-630 was up-regulated in cataract, and miR-630 can regulate the proliferation and apoptosis of lens epithelial cells in vitro.

Using biomiformatic tools, B-cell lymphoma-2 (Bcl-2) has been predict as a direct target of miR-630. Bcl-2 was known as an onco-gene in cancer related studies (24). It has been proved in many cases that Bcl-2 was up-regulated in different type of cancers, and knockdown of Bcl-2 induced significant increase in the apoptosis of cancer cells. However, in the case of cataract, Bcl-2 may play a different role. Li et al proved that miR-34a can induce the apoptosis of human lens epithelial cells through down-regulation of Bcl-2 (25); Fang et al observed that over-expression of BAX and down-regulation of Bcl-2 gene can induce apoptosis in human lens epithelial cells and participate in the pathogenesis of cataract (26). In the present study, we observed that transfection of miR-630 significantly decreased the expression of Bcl-2, and also marked increase in the expression of BAX and Caspase-3, respectively; moreover, results of dual luciferase reporter assay confirmed that Bcl-2 is a direct target of miR-630. In conclusion, our results indicated that miR-630 can regulate the proliferation and apoptosis ofSRA01/04 trough targeting Bcl-2.

Our study has limitations. First of all, the number of the tissue samples included in this study were relative small. This is because of the ethic issue, especially for the eye samples from the control group. Second, our results should also be verified with
animal models.

To sum up, we proved that miR-630 was up-regulated in cataract, and miR-630 can regulate the proliferation and apoptosis of SRA01/04 trough targeting Bcl-2. Our data has provided novel evidence that to target miR-630 may be potential methods for the treatment of cataract.

References


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