

Expression of miR-181a and TGF- β 2 in lens epithelial cells of patients with cataractous retinal detachment and its clinical significance

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Abstract. Expression and clinical significance of miR-181a and TGF- β 2 in lens epithelial cells of patients with cataract-induced retinal detachment were investigated. Forty patients with rhegmatogenous retinal detachment combined with age-related cataract (cast-off group) and another 40 patients with simple age-related cataract (non-exfoliated group) in Tongren Hospital were enrolled between January 2017 and June 2018. Lens epithelial cells were collected, and expression levels of miR-181a and TGF- β 2 mRNA in lens epithelial cells were measured by RT-qPCR. Expression of protein was detected by western blot analysis. miR-181a overexpression vector (miR-181a-mimic group) was constructed and transfected into lens epithelial cells isolated from patients with rhegmatogenous retinal detachment combined with age-related cataract. Empty vector (miR-control group) transfection was performed. Untransfected lens epithelial cells were the control group. Independent sample t-test was performed for comparison between groups. Correlation between miR-181a and TGF- β 2 protein expression levels was analyzed by Pearson's correlation analysis. Relative expression level of miR-181a in the non-exfoliated group was significantly higher than that in the cast-off group ($P < 0.05$). Relative expression levels of TGF- β 2 mRNA and TGF- β 2 protein in the cast-off group were significantly higher than those in the non-exfoliated group ($P < 0.05$). Relative expression level of E-cadherin in the miR-control group was significantly lower than that in the miR-181a-mimic group ($P < 0.05$). Relative expression level of vimentin in the miR-control group was significantly higher

than that in the miR-181a-mimic group ($P < 0.05$). Relative expression level of TGF- β 2 protein in the miR-control group was significantly higher than that in the miR-181a-mimic group ($P < 0.05$). The expression level of miR-181a was negatively correlated with the expression level of TGF- β 2 protein ($r = -0.875$, $P < 0.001$). miR-181a expression is decreased, while TGF- β 2 expression is increased in lens epithelial cells of patients with rhegmatogenous retinal detachment combined with age-related cataract. Over-expression of miR-181a may inhibit epithelial cell epithelial-inhibition by inhibiting TGF- β 2 expression. Our findings may provide guidance for future postoperative prevention and treatment of rhegmatogenous retinal detachment in cataract patients.

Introduction

Cataract is a disorder of lens metabolism caused by aging, heredity, malnutrition, abnormal immune function and so on. The lens protein becomes denatured and cloudy, causing the diminution of vision and even the lose of vision. Cataract is an important cause of blindness, especially in the elderly. With the growth of aging population, incidence of cataract is increasing (1-3). At present, the most effective means of treating cataract is cataract extraction combined with intraocular lens implantation, but ~0.32-1.00% of patients will have rhegmatogenous retinal detachment after cataract surgery. Although success rate of treatment for rhegmatogenous retinal detachment has increased significantly during past several years, some patients are still incurable, and irreversible visual impairment occurs (4,5).

At present, mechanism of the occurrence of postoperative rhegmatogenous retinal detachment in cataract patients has not been fully clarified. Studies have reported that most of the lens epithelial cells in the anterior lens capsule of cataract patients migrate to posterior capsule and undergo epithelial-mesenchymal transition after surgery, and the secretion of collagen and extracellular matrix is the main cause of rhegmatogenous retinal detachment (6,7). Inhibition of epithelial-mesenchymal transition in lens epithelial cells may be an effective strategy to prevent rhegmatogenous retinal detachment. miRNA (21-25 bp) is a kind of small biological molecule closely related to cell

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Table I. Primer sequences.

Genes	Forward	Reverse
miR-181a	5'-GCGGTAACATTCAACGCTGTCG-3'	5'-GTGCAGGGTCCGAGGT-3'
TGF- β 2 mRNA	5'-GCTTTGGATGCGGCCTATTG-3'	5'-CCAGCACAGAAGTTGGCATTGTA-3'
GAPDH	5'-CGGAGTCAACGGATTTGGTCGTAT-3'	5'-AGCCTTCTCCATGGTGGTGAAGAC-3'

epithelial-mesenchymal transition. A previous study found that miR-181a can inhibit epithelial-mesenchymal transition of lens epithelial cells (8), and has been reported that TGF- β 2 induces epithelial-mesenchymal transition of human lens epithelial cells through PI3K/Akt signaling pathway (9). The interactions between miR-181a and TGF- β 2 in regulating epithelial-mesenchymal transition of lens epithelial cells are unknown, while research has found that miR-181a can promote TGF- β expression and mediate epithelial-mesenchymal transition in ovarian cancer cells (10).

In this study, we analyzed the expression of miR-181a and TGF- β 2 in lens epithelial cells of patients with cataract combined with rhegmatogenous retinal detachment, and explored the effect of miR-181a and TGF- β 2 on epithelial-mesenchymal transition in lens epithelial cells.

Patients and methods

Research subjects. Forty patients with rhegmatogenous retinal detachment combined with age-related cataract (cast-off group) and another 40 patients with simple age-related cataract (non-exfoliated group) in Tongren Hospital (Shanghai, China) were enrolled between January 2017 and June 2018. Lens epithelial cells were collected. Inclusion criteria: All patients met the relevant diagnostic criteria for retinopathy established by the American Retina Association. Lens was opaque, visual acuity was ≤ 0.6 , and double lacrimal passage was unobstructed. Patients in the cast-off group were confirmed with round and horseshoe-shaped holes by round-eye ultrasonography. Medical records were complete. Exclusion criteria: Patients with other eye diseases, patients with severe ocular trauma or history of eye surgery, acute metabolic disorders such as diabetic ketoacidosis within the previous 1 month, patients who had used tear film stabilization drug, patients with severe acute infection, patients with liver and kidney dysfunction or severe medical problems, and women during pregnancy or lactation were excluded.

The present study was approved by the Ethics committee of Tongren Hospital. Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients or the guardians.

Materials. Primary rabbit anti-human TGF- β 2, E-cadherin, vimentin polyclonal antibodies and secondary goat anti-rabbit IgG polyclonal antibody (cat. nos. 19999-1-AP, 20874-1-AP, 10366-1-AP and SA00001-2) were purchased from Wuhan Sanying Biotechnology (Wuhan, China). Polyacrylamide gel electrophoresis buffer (cat. no. orb154330) was purchased from Xiamen Huijia Biotechnology Co., Ltd. (Xiamen, China). Western blot analysis kit (cat. no. UFC04948) was purchased

from Shanghai Junrui Biotechnology Co., Ltd. (Shanghai, China).

Construction and transfection of miR-181a expression vector. miR-181a over-expression vector (miR-181a-mimic group) and empty vector (miR-control group) were designed and synthesized by Thermo Fisher Scientific, Inc. (Waltham, MA, USA). The lens epithelial cells of 20 patients in the cast-off group were selected by random number table method. Lens epithelial cells of patients were subjected to trypsinization 24 h before transfection. Transfection was performed when 80% confluence was reached. Cells were incubated at 37°C and 5% CO₂ incubator for 12 h, then cells were washed with normal medium (DMEM + 20% fetal bovine serum) and cultivated for additional 48 h. Cell culture medium was changed every 6 h, and transfection was confirmed by RT-qPCR. Untransfected lens epithelial cells were the blank control cells. Lipofectamine™ 2000 (cat. no. 11668019) transfection kit was purchased from Thermo Fisher Scientific, Inc.

RT-qPCR. Single lens epithelial cell suspension (1×10^7 /ml) was prepared and TRIzol lysate was added in a ratio of 3:1. TRIzol™ reagent (cat. no. R0016) was purchased from Beyotime Institute of Biotechnology (Shanghai, China). After extraction, integrity of the RNA was analyzed by 1.5% agarose gel electrophoresis, and purity of extracted RNA was detected by a micro nucleic acid analyzer. Only RNA samples with A260/A280 between 1.8 and 2.1 were used in reverse transcription, which was performed using the following system: Oligo (dT) primer (50 μ M) 0.5 μ l, random 6 mers (100 μ M) 0.5 μ l, 5X PrimeScript Buffer 2.0 μ l, PrimeScript RT Enzyme Mix 0.5 μ l, total RNA 2 μ l and 4.5 μ l RNase Free ddH₂O was added to 10 μ l in total. Reaction conditions were: 37°C for 15 min and 85°C for 5 sec. Reverse transcription kit was purchased from Beyotime Institute of Biotechnology. PCR amplification was performed after transcription. PCR amplification system consists of 2 μ l of cDNA template, 25 μ l of 2X bare SYBR mixture, and 1 μ l of upstream and downstream primer. Double distilled water was added to 50 μ l volume. Reaction conditions were: 95°C for 5 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. GAPDH was used as endogenous control. Three replicate wells were set and results were analyzed by 2^{- Δ C_q} method (11). RT-qPCR kit (cat. no. WE0140-UIM) was purchased from BioLab Inc. (Lawrenceville, USA). Primers were designed and synthesized by Thermo Fisher Scientific, Inc. (Table I).

Western blot analysis. Protein in the lens epithelial cells was extracted with RIPA lysis buffer. A total of 10 μ l protein per lane was separated by 12% polyacrylamide gel electrophoresis.

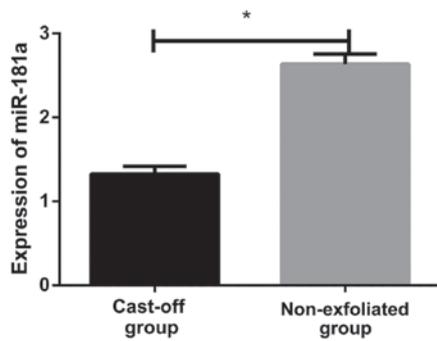


Figure 1. Relative expression levels of miR-181a in lens epithelial cells. Results of RT-qPCR showed that relative expression level of miR-181a in the non-exfoliated group was significantly higher than that in the cast-off group ($P < 0.05$).

The initial voltage was 90 V. After the electrophoresis, the transmembrane to PVDF membrane was performed, with 100 V constant pressure for 100 min. Membranes were blocked with 5% BSA at 37°C for 60 min. After incubating with primary antibodies at 4°C overnight, membranes were washed twice with PBS and incubated with secondary antibody at 37°C for 2 h. Signal development was performed using ECL. Gray scale was measured using Quantity One software (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Protein relative expression level = gray value of target gene / gray value of endogenous control.

Statistical analysis. SPSS 19.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Measurement data were expressed in %, and ratios were compared using the χ^2 test. Count data were expressed as mean \pm standard deviation (SD). t-test was used for comparison between two groups. Analysis of variance was used for comparison among groups with Dunnett's post hoc test, and repeated variance measurement was used for comparison at different time points within the group. Pearson's correlation was used to analyze the correlation between the relative expression level of miR-181a and TGF- β 2 in lens epithelial cells in the miR-181a-mimic group. Differences were statistically significant at $P < 0.05$.

Results

Relative expression levels of miR-181a in lens epithelial cells.

Results of RT-qPCR showed that relative expression level of miR-181a in lens epithelial cells of the cast-off group was (1.325 ± 0.094) , and relative expression level in lens epithelial cells of the non-exfoliated group was (2.634 ± 0.121) . There was a statistically significant difference between the two groups. Relative expression level of miR-181a in the non-exfoliated group was significantly higher than that in the cast-off group ($P < 0.05$; Fig. 1).

Relative expression levels of TGF- β 2 in lens epithelial cells. Results of qRT-PCR showed that relative expression level of TGF- β 2 mRNA in lens epithelial cells of the non-exfoliated group was (0.351 ± 0.066) , and relative expression level in lens epithelial cells of the cast-off group was (0.712 ± 0.073) . There was a statistically significant difference between the two groups. Relative expression level of TGF- β 2 mRNA in

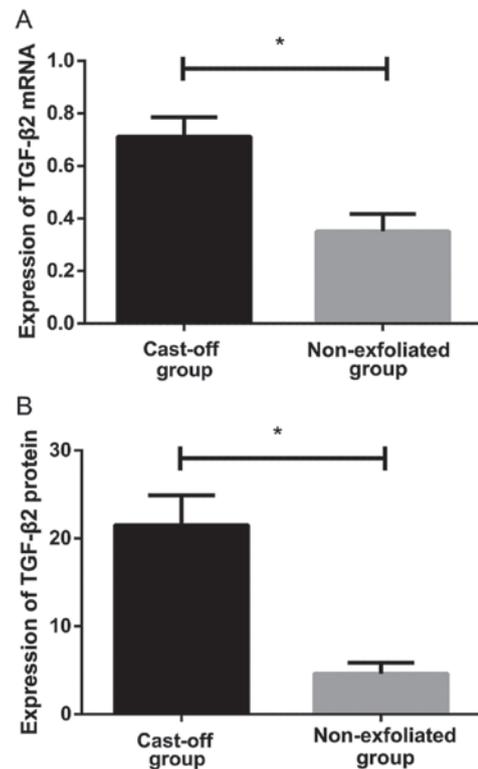


Figure 2. (A) Relative expression levels of TGF- β 2 mRNA in lens epithelial cells. Results of RT-qPCR showed that relative expression level of TGF- β 2 mRNA in the cast-off group was significantly higher than that in the non-exfoliated group. (B) Relative expression level of TGF- β 2 in lens epithelial cells. Western blot analysis showed that relative expression level of TGF- β 2 protein in the cast-off group was significantly higher than that in the non-exfoliated group ($P < 0.05$).

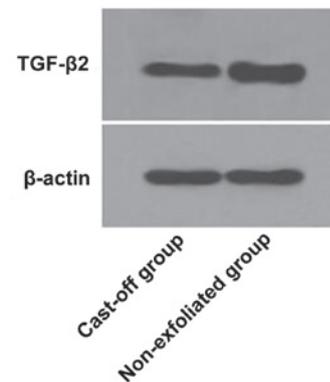


Figure 3. A western blotting image of TGF- β 2 protein in lens epithelial cells. Expression level of TGF- β 2 protein in the cast-off group was significantly higher than that in the non-exfoliated group. ($P < 0.05$).

the cast-off group was significantly higher than that in the non-exfoliated group ($P < 0.05$; Fig. 2A). Western blot analysis showed that relative expression level of TGF- β 2 protein in lens epithelial cells of the non-exfoliated group was (4.621 ± 1.247) , and relative expression level in lens epithelial cells of the cast-off group was (21.522 ± 3.364) . There was a statistically significant difference between the two groups. Relative expression level of TGF- β 2 protein in the cast-off group was significantly higher than that in the non-exfoliated group ($P < 0.05$; Fig. 2B and 3).

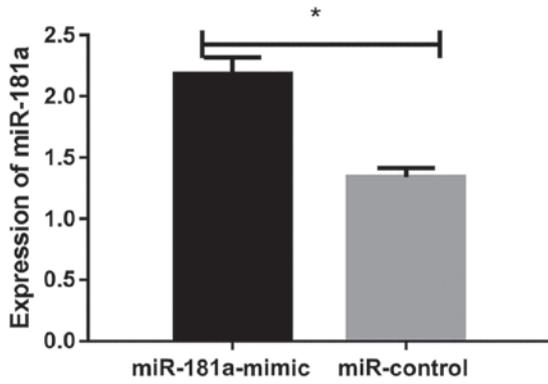


Figure 4. Results of lens epithelial cell transfection. Relative expression level of miR-181a in the miR-181a-mimic group was significantly higher than that in the miR-control group ($P < 0.05$).

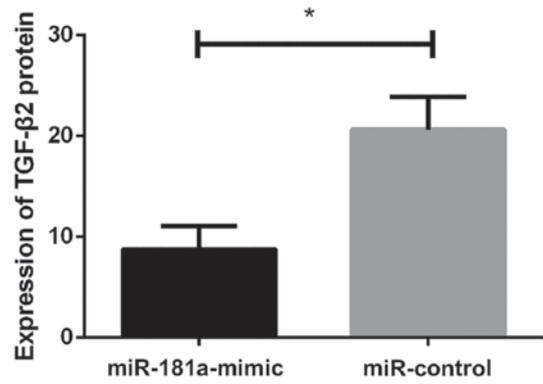


Figure 5. Effects of miR-181a over-expression on TGF- β 2 protein. Relative expression level of TGF- β 2 protein in the miR-control group was significantly higher than that in the miR-181a-mimic group. Upregulation of miR-181a expression decreased the expression of TGF- β 2 protein in lens epithelial cells ($P < 0.05$).

Results of lens epithelial cell transfection. Results of RT-qPCR showed that relative expression level of miR-181a in the miR-181a-mimic group was (2.183±0.134) after transfection, and relative expression level of miR-181a in the miR-control group was (1.342±0.074). There was a statistically significant difference between the two groups. After transfection of miR-181a overexpression vector, relative expression level of miR-181a in lens epithelial cells was significantly increased ($P < 0.05$), suggesting successful transfection ($P < 0.05$; Fig. 4).

Effects of miR-181a overexpression on TGF- β 2 protein. Western blot analysis showed that relative expression level of TGF- β 2 protein in lens epithelial cells of the miR-control group was (20.631±3.245), and relative expression level in lens epithelial cells of the miR-181a-mimic group was (8.752±2.283). There was a statistically significant difference between the two groups. Relative expression level of TGF- β 2 protein in the miR-control group was significantly higher than that in the miR-181a-mimic group ($P < 0.05$). Upregulation of miR-181a expression decreased the expression of TGF- β 2 protein in lens epithelial cells ($P < 0.05$; Fig. 5 and Fig. 6).

Correlation analysis between miR-181a and TGF- β 2 protein expression. Pearson's correlation analysis showed that the expression level of miR-181a was negatively correlated with the expression of TGF- β 2 protein in miR-181a-mimic group. The higher the expression level of miR-181a, the lower the expression level of TGF- β 2 protein ($r = -0.875$, $P < 0.001$) (Fig. 7).

Effect of transfection of miR-181a overexpression vector on epithelial-mesenchymal transition in lens epithelial cells. Western blot analysis showed that relative expression level of E-cadherin in lens epithelial cells of the miR-control group was (0.983±0.023), and relative expression level of E-cadherin in lens epithelial cells of the miR-181a-mimic group was (1.345±0.042). There was a statistically significant difference between the two groups. Relative expression level of E-cadherin in the miR-control group was significantly lower than that in the miR-181a-mimic group ($P < 0.05$; Fig. 8A). Relative expression level of vimentin in the miR-control group was (1.465±0.038), and relative expression level of vimentin in the miR-181a-mimic group was (1.121±0.013). There was

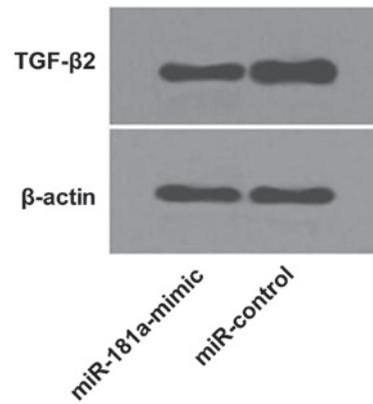


Figure 6. A western blotting image of TGF- β 2 protein in lens epithelial cells with effects of miR-181a overexpression. Relative expression level of TGF- β 2 protein in the miR-control group was significantly higher than that in the miR-181a-mimic group ($P < 0.05$).

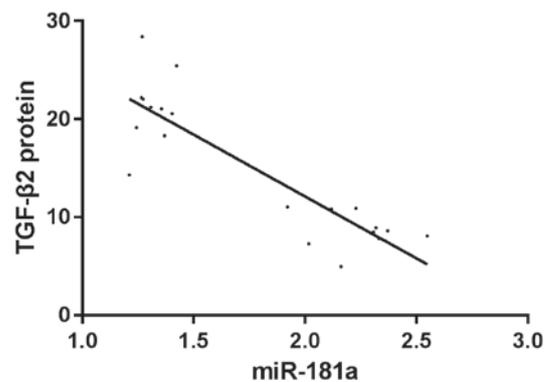


Figure 7. Analysis of the correlation between miR-181a and TGF- β 2 protein expression. Pearson's correlation analysis showed that expression level of miR-181a was negatively correlated with expression levels of TGF- β 2 protein. ($r = -0.875$, $P < 0.001$).

significant statistical difference between the two groups. Relative expression level of vimentin in the non-exfoliated group was significantly higher than that in the miR-181a-mimic group ($P < 0.05$; Fig. 8B and 9).

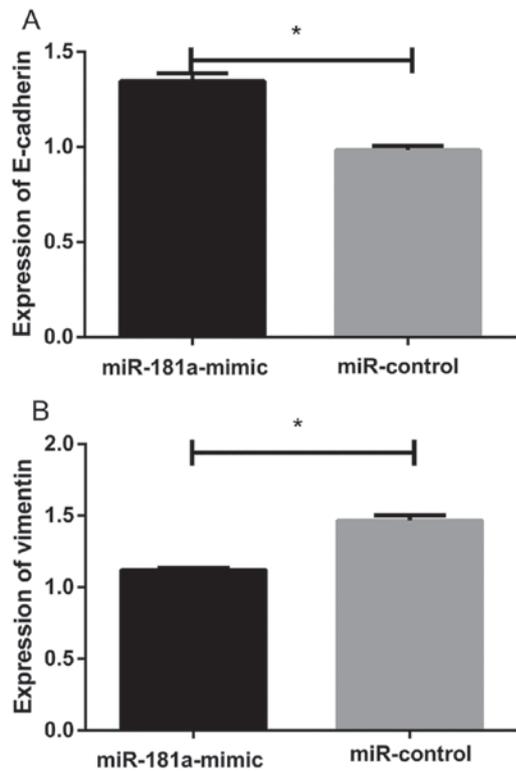


Figure 8. (A) Relative expression levels of E-cadherin and vimentin in lens epithelial cells. Results of RT-qPCR showed that the relative expression level of E-cadherin in the miR-control group was significantly lower than that in the miR-181a-mimic group. (B) Relative expression level of vimentin in lens epithelial cells. Western blot analysis showed that relative expression level of vimentin in the miR-control group was significantly higher than that in the miR-181a-mimic group. * $P < 0.05$.

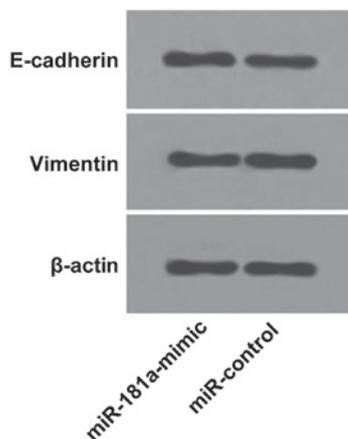


Figure 9. A western blotting image of E-cadherin and vimentin in lens epithelial cells. Relative expression level of E-cadherin was significantly lower and expression level of vimentin was significantly higher in the miR-control group than that in the miR-181a-mimic group ($P < 0.05$).

Discussion

Rhegmatogenous retinal detachment is the most common type of clinical retinal detachment. The prevention of rhegmatogenous retinal detachment is important to improve the postoperative prognosis of cataract patients. Many biological factors have been shown to be involved in the development

of rhegmatogenous retinal detachment, and TGF- β , especially TGF- β 2, plays a key role in epithelial-mesenchymal transition of lens epithelial cells (12,13). miRNAs also play an important role in epithelial-mesenchymal transition, and many miRNAs are expressed in the eye. miR-181a has also been shown to be expressed in lens epithelial cells (14,15). miR-181a was found to inhibit epithelial-mesenchymal transition in lens epithelial cells (8), while the specific mechanism remains unknown. The role of miR-181a in patients with rhegmatogenous retinal detachment after cataract surgery has not been reported.

We first detected the difference of miR-181a and TGF- β 2 in lens epithelial cells of cataract patients with rhegmatogenous retinal detachment and simple cataract patients by RT-qPCR. Results showed that the expression level of miR-181a was decreased, while expression levels of TGF- β 2 mRNA and TGF- β 2 protein were increased in lens epithelial cells of cataract patients with rhegmatogenous retinal detachment. One of the important causes of rhegmatogenous retinal detachment is epithelial-mesenchymal transition in residual lens epithelial cells in cataract patients (16). Previous studies have found that miR-181a inhibits epithelial-mesenchymal transition of lens epithelial cells (8), while TGF- β 2 can promote epithelial-mesenchymal transition of lens epithelial cells (9), indicating the potential interactions between miR-181a and TGF- β 2. This should be an important reason for the decreased or increased expression of miR-181a and TGF- β 2 in lens epithelial cells of cataract patients with rhegmatogenous retinal detachment.

To further investigate the role of miR-181a in epithelial-mesenchymal transition of lens epithelial cells in cataract patients with rhegmatogenous retinal detachment and the mechanism, we constructed a miR-181a overexpression vector. RT-qPCR confirmed the significant increase in the expression level of miR-181a in lens epithelial cells and successful transfection. We also measured the expression levels of two factors closely related to epithelial-mesenchymal transition-intracellular epithelial marker E-cadherin and interstitial marker Vimentin. Studies have shown that upregulation of Vimentin expression and inhibition of E-cadherin expression promote epithelial-mesenchymal transition (17,18). In our study it was found that after over-expression of miR-181a, expression level of E-cadherin was significantly increased, while expression level of Vimentin was significantly decreased in lens epithelial cells, suggesting inhibition of epithelial-mesenchymal transition. This is consistent with the findings of Dong *et al* (8), which showed that miR-181a inhibits epithelial-mesenchymal transition of lens epithelial cells. miR-181a has been reported to promote epithelial-mesenchymal transition in tumor cells (19,20), which is completely contrary to our findings and may be due to the existence of multiple downstream targets of miR-181a. After over-expression of miR-181a, we found that the expression level of TGF- β 2 protein in lens epithelial cells decreased, and the results of Pearson's correlation analysis showed that the expression level of miR-181a was negatively correlated with the expression level of TGF- β 2 protein. Therefore, we speculate that miR-181a and TGF- β 2 may interact with each other to participate in the epithelial-mesenchymal transition of lens epithelial cells. miR-181a may play a role in inhibiting epithelial-mesenchymal transition of lens epithelial cells by

downregulating TGF- β 2. Previous studies have reported that miR-181a is involved in cell epithelial-mesenchymal transition by regulating TGF- β (10,19-21), which is consistent with our study.

In conclusion, expression level of miR-181a was decreased, while expression level of TGF- β 2 protein was increased in lens epithelial cells of cataract patients with rhegmatogenous retinal detachment. Overexpression of miR-181a may inhibit epithelial-mesenchymal transition in lens epithelial cells by inhibiting TGF- β 2 expression. Our findings provide new insights into the treatment of cataract patients with rhegmatogenous retinal detachment.

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Availability of data and materials

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

Authors' contributions

YJ wrote the manuscript. YJ and YG performed PCR and western blot analysis. NL and WQ were responsible for construction and transfection of miR-181a expression vector. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics committee of Tongren Hospital (Shanghai, China). Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients or the guardians.

Patinet consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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