SUPPLEMENTAL MATERIALS

Materials and Methods

qRT-PCR analysis

In order to validate the reproducibility of the results from miRNA microarray, quantitative qRT-PCR analysis of miR-31, miR-184 and miR-204-5p was performed using the same extracted total RNA used for the microarray analysis. qRT-PCR was performed using SYBR Green protocol on an Rotor-Gene 3000™ multiplex system (Corbett Research, Cambridge, UK) and data analysis was conducted with the corresponding software interface (version 5.0) (Corbett Research). All specific primers were designed using Primer Express version 2.0 (Applied Biosystems, Foster City, CA) (Table 1). Real-time PCR cycling conditions were: initial denaturation at 95°C for 5 min and then 40 cycles of 95°C for 10 s, 60°C for 15 s, 72 °C for 20 s and 79°C for 20 s. Then, dissociation curves were typically generated post-run for analysis of amplicon species. The levels of an endogenous control U6 were used to normalize the expression levels of each miRNA. All reactions were run in triplicate and included no template controls for each miRNA. Fold change in miRNA expression was calculated using comparative Ct method. The normal lens was used as a calibrator and the data were presented as the fold change relative to the calibrator.

For detection of EMT markers, E-cadherin, vimentin and α-SMA, the reagents (TaqMan; Applied Biosystems, Foster City, CA) and sequence detection system (ABI Prism 7500 System; Applied Biosystems) were employed in real-time PCR as
recommended by the manufacturer. Each sample was assayed in duplicate (TaqMan Universal PCR Master Mix; Applied Biosystems). The primers and oligonucleotide probes used are listed in Table 2. Cycling conditions were as follows: 10 minutes at 95°C followed by 40 cycles of amplification for 15 seconds at 95°C and 1 minute at 60°C. Quantification data were analyzed with SDS system software (7500 System; Applied Biosystems). The log-linear portion of the fluorescence versus cycle plot was extended to determine a fractional cycle number at which a threshold fluorescence was obtained (threshold cycle, Ct), and this number was used as a reference for each analyzed gene and for GAPDH.

Transfection of capsular bags and efficiency detection

The transfection of one capsular bag in a 35-mm polymethylmethacrylate Petri dish took place 24 h after starvation of cells in 1.5 ml serum-free medium. Then, the cells were transiently transfected with a mature microRNA mimic or inhibitor, nontargeting negative control, using lipofectamine\textsuperscript{TM} 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. All miRNA mimics, inhibitors, and nontargeting negative control were from Guangzhou RiboBio Co., Ltd (Guangzhou, China). miRNA mimics are small, chemically modified double-stranded RNAs that mimic endogenous miRNAs and enable miRNA functional analysis by up-regulation of miRNA activity. They are more specific than their predecessors due to inactivation of the star strand by proprietary chemical modifications. miRNA inhibitors are small, chemically modified single-stranded RNA molecules designed to specifically bind to and inhibit
endogenous miRNA molecules and enable miRNA functional analysis by down-regulation of miRNA activity. They are more potent than their predecessors.

In brief, dilute miRNA mimic or inhibitor into 250 μL serum-free medium. Dilute 25 μL of lipofectamine™ 2000 into 250 μL serum-free medium and incubate for 5 min at room temperature. Add 250 μl of the diluted Lipofectamine™ 2000 to 250 μL of miRNA mimic or inhibitor, mix gently, and incubate at room temperature for 20 min to allow miRNA- Lipofectamine™ 2000 complexes to form. Then, add the DNA-Lipofectamine™ 2000 complexes (500 μl) directly to the 35-mm dish containing 1.5 ml medium and mix gently. Six hours of incubation, the medium was gently removed and replaced by 2 ml antibiotic-free complete medium. For the transfection of SMAD4 siRNA and miR-204-5p mimic, the cells were transfected with 30 nM siRNA (sc-29484, Santa Cruz Biotechnology, Santa Cruz, CA) with a control siRNA (sc-37007) and 50 nM miR-mimic using lipofectamine™ 2000.

The transfection efficiency detection was performed using siR-Ribo™ Transfection Control (Cy3) (Guangzhou RiboBio Co., Ltd, Guangzhou, China) at different concentration, e.g., 5nM, 50nM, and 500 nM with or without lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) following 24 and 48h hours of transfection (Figure 6A). Imaging was performed using a fluorescence microscope (Eclipse E800; Nikon, Tokyo, Japan). The results were shown in Figure 1.

**Luciferase activity assay**

Human SMAD4 3’-UTR containing the putative target site for miR-204-5p was
amplified from genomic DNA by PCR amplification and inserted into the pGL3 control vector (Promega, Madison, WI, USA) immediately downstream from the stop codon of luciferase (pGL3-SMAD4-3’-UTR). A deletion of 7 bp from the site of perfect complementarity was also generated by the QuikChange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) and following the manufacturer’s instructions. The cells were transiently transfected with wild-type or mutant reporter plasmid and miRNA mimic using lipofectamine™ 2000. Luciferase activity was measured 24 h after transfection using the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA). Firefly luciferase activity was normalized to renilla luciferase activity for each transfected well. Three independent experiments were performed in triplicate.

**Western blot analysis**

Lens epithelia from normal donors and PCO patients or capsular bags were homogenized with a glass pestle in PBS buffer and then sonicated for 30 seconds. 20 μL aliquot of the supernatant was assayed with SDS-polyacrylamide gels (Mini-Protean II system; Bio-Rad Laboratories, Mississauga, ON, Canada) and blotted onto polyvinylidene difluoride membranes, followed by blocking with 5% dry milk in Tris-buffered saline containing 0.1% Tween 20. Membranes were then incubated overnight with primary antibodies, washed for 30 minutes, and stained with specific secondary antibodies at room temperature for 60 minutes. After extensive washing of the membranes, hybridized bands were detected with an enhanced
chemiluminescence kit (PerkinElmer Life Sciences, Boston, MA). The primary antibodies used were listed in Table 3.

**Immunohistochemistry**

Human capsular bag samples were fixed in 10% buffered formalin, and embedded in paraffin. Paraffin sections of 4 μm in thickness were deparaffinized and rehydrated. Then, samples were permeabilized in 1.0% Triton X-100 (Sigma-Aldrich, Shanghai, China) in PBS for 10 min at room temperature, followed by incubation with 5% BSA (Boster Biologic Technology, Ltd., Wuhan, China) in PBS for 10 min at room temperature to block nonspecific binding. Next samples were subjected to staining using the EliVision™ Plus kit (Maxim Corp, Fuzhou, China) according to the manufacturer’s protocol. A color reaction was detected using a diaminobenzidine (DAB) kit (Boster Biologic Technology, Ltd.). The primary antibodies used were listed in Table 3. Additionally, isotype control antibodies were used at the same concentration as the primary antibodies. The isotype control antibodies used were mouse IgG (SC-2025) and rabbit IgG (SC-2027; Santa Cruz Biotechnology). Digital images were obtained using an Eclipse C1si Spectral Imaging Confocal Microscope (Nikon Instruments Inc. Melville, NY).