Long noncoding RNA expression profile in HLE B-3 cells during TGF-β₂-induced epithelial-mesenchymal transition

Bingyu Zhang¹, Yang Chen¹, Meiyuan Qiu² and Zhixiang Ding*²

Abstract

Background: Recent evidence has shown that long noncoding RNAs (lncRNAs) are involved in the process of epithelial-mesenchymal transition (EMT). However, little research has focused on the expression profile of lncRNAs during EMT in human lens epithelial cells (LECs) and their functions have not yet been described.

Methods: Dysregulated lncRNAs and mRNAs in normal human lens epithelial B-3 (HLE B-3) cells and during transforming growth factor β₂ (TGF-β₂)-induced EMT were analyzed via lncRNA microarray. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway analyses of differentially expressed mRNAs were performed to identify their functions and pathologic pathways. Six candidate lncRNAs were validated via quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) to confirm the microarray data.

Results: A total of 775 lncRNAs (325 up-regulated and 450 down-regulated) and 935 mRNAs (329 up-regulated and 606 down-regulated) were differentially expressed in HLE B-3 cells during TGF-β₂-induced EMT compared to normal HLE B-3 cells. GO and KEGG Pathway analyses indicated the functions of differentially expressed mRNAs in the TGF-β₂-induced EMT in HLE B-3 cells. qRT-PCR confirmed the trends indicated in microarray analysis for all 6 candidate lncRNAs.

Conclusion: Our study lays the foundation for future research in lncRNAs related to EMT in HLE B-3 cells and could provide new avenues for the prevention and treatment of posterior capsule opacification (PCO).

Keywords: LncRNA, Microarray, HLE B-3 cells, Epithelial-mesenchymal transition, Posterior capsule opacification

Background

Cataracts are the leading cause of blindness, and account for 51% of blindness, about 20 million people, and 33% of visual impairment worldwide, according to the latest assessment (2010) [1]. Surgery is an effective treatment for cataracts, however, complications can arise [2]. Posterior capsule opacification (PCO) is the most common complication after cataract surgery [3]. The residual lens epithelial cells (LECs) in the anterior capsule after cataract surgery, over proliferate, and migrate to the posterior capsule, and then epithelial-mesenchymal transition (EMT) takes place. This dynamic process is the main mechanism of PCO [4–6]. EMT is an abnormal differentiation of epithelioid cells to myofibroblast cells and is central to the development of PCO [7]. Inhibition of EMT has emerged as an option for treating PCO.

Transforming growth factor β₂ (TGF-β₂) is a signaling molecule, that plays a crucial role in activating and promoting EMT in LECs. Recent studies have shown that TGF-β₂ can induce EMT in LECs through the Smads pathway, PI3K/Akt pathway, and other pathways [8, 9]. TGF-β₂-induced EMT is a proper model for studying PCO [10].

Long noncoding RNAs (lncRNAs) are transcripts of more than 200 nucleotides, that do not have the ability to encode proteins, but participate in transcriptional, post-transcriptional and epigenetic regulation of gene expression [11, 12]. The expression, mechanism, and function of lncRNAs have attracted attention in recent years [13]. However, it's not clear how lncRNAs are related to the TGF-β₂-induced EMT in LECs.

To understand the functions of lncRNAs in TGF-β₂-induced EMT in LECs, we performed lncRNA and
mRNA microarray analysis in normal LECs and those in TGF-β2-induced EMT. Gene Ontology (GO) analysis [14, 15] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis [16, 17] were performed to identify the functions of differentially expressed genes. The results of our study indicate that dysregulated expression of lncRNAs may influence the development and progression of EMT in LECs and that studying these lncRNAs may introduce new avenues for the prevention and treatment of PCO.

Methods

Cell culture and treatment

The human lens epithelial B-3 (HLE B-3) cell line, a type of LEC, was purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS (Shanghai, China). It was cultured in Dulbecco’s modified Eagle’s medium (Gibco, Grandisland, NY, USA) supplemented with 15% fetal bovine serum (Gemini, West Sacramento, CA, USA). Cells were maintained in humidified air with 5% CO2 at 37 °C before use. For further experiments, cells were trypsinized and seeded in 6 wells plates. When cell cultures reached 70% confluence, 3 wells of cells were stimulated with 10 ng/ml recombinant human TGF-β2 (Peprotech, Inc., Rocky Hill, NJ, USA) for 24 h, while the other 3 wells of cells were incubated in DMEM for 24 h. Following treatment, phase-contrast micrographs were used to observe morphology change of cells. Then the cells were collected for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), western blot analysis, and immunofluorescence assay.

qRT-PCR of cells

Total RNA was extracted using TRIZOL Reagent (Cat#15596-018, Life Technologies, Carlsbad, CA, US), and then reverse transcribed using Prime Script RT Master Mix (TaKaRa, Dalian, China) according to the manufacturer’s instructions. qRT-PCR was performed using SYBR Green PCR Master Mix (ABI Applied Biosystems, Foster City, CA, USA) in the ABI 7900HT sequence detection system (ABI Applied Biosystems, Foster City, CA, USA) following the manufacturer’s instructions, and beta-actin was used as an internal control. All the genes were amplified in separate wells in triplicate. The following primer pairs were used: E-cadherin (F 5’- AGCAGAACTAAACACACGGGG -3’, R 5’- ACCCA CCTCTAAGGCCATCT -3’), vimentin (F 5’- GACGCCATCAACACCG AGTT-3’, R 5’- GTTTTGCTG TTTGTTAGCTGTT -3’), α-SMA (F 5’- GTGTTGCCCC TGAAAGACAT -3’, R 5’- GCTGGAACATTGAAAGT CT CA -3’), and beta-actin (F 5’- CTGGAACGGTGA AGGTGA CA -3’, R 5’- CGGCCACATTGTAACCTTTG -3’). Gene expression was calculated using the 2ΔΔCT method [18].

Western blot

The cells were collected and lysed in lysis buffer on ice, and the proteins were quantified using a Pierce BCA Protein Assay kit (Thermos Fisher Scientific, Inc., Waltham, MA, USA). Cell lysates were separated by 8-12% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and proteins were transferred to polyvinylidene difluoride membranes. The membranes were subsequently incubated with the following primary antibodies overnight at 4 °C: E-cadherin (1:1000; cat. no. 3195; Cell Signaling Technology, Inc.), α-SMA (1:1000; cat. no. 4691; Cell Signaling Technology, Inc.), and vimentin (1:1000; cat. no. 5741; Cell Signaling Technology, Inc.). The membranes were washed three times with TBS/T and then incubated for 1 h in IRDye®680RD goat anti-rabbit immunoglobulin G (H + L) diluted at 1:5000 in TBST. Protein levels were visualized and quantified using the LI-COR Odyssey scanner and software (LI-COR Biosciences).

Immunofluorescence assay

After specific treatment, HLE B-3 cells were fixed with ice-cold methanol for 10 min. After washed with PBS for three times, non-specific binding sites were blocked with 4% bovine serum albumin for 30 min at room temperature. Cells were incubated with the primary antibodies (E-cadherin, 1:100; cat. no. 3195; Cell Signaling Technology, Inc. α-SMA, 1:100; cat. no. 4691; Cell Signaling Technology, Inc. vimentin, 1:100; cat. no. 5741; Cell Signaling Technology, Inc.) overnight at 4 °C, and then incubated with the FITC-conjugated secondary antibody for 1 h at room temperature. After counterstained with 4,6-diamidino-2-phenylin-dole (DAPI) for 3 min, these cells were subsequently observed under a confocal microscope(Carl Zeiss, LSM710, Jena, Germany).

Microarray

The microarray (SBC Human lncRNA microarray v6.0, Shanghai Biotechnology Corporation, Shanghai, China) used in this study detects approximately 77,103 lncRNAs and 18,853 coding transcripts. The lncRNAs were carefully constructed using the most highly respected public transcriptome databases including Ensembl (http://www.ensem bl.org/index.html), LNCipedia (http://www.lncipedia.org/), Lncrnadb (http://lncrnadb.org/), Noncoder (http://www.non code.org/), and UCSC (http://genome.ucsc.edu/index. html). 6 sets of microarray assay were done, including 3 for the control group, and 3 for the TGF-β2 group.

RNA extraction

Total RNAs were extracted using TRIZOL Reagent (cat. No. 15596-018, Life Technologies, Carlsbad, CA, US) following the manufacturer’s instructions, and the RNA
used: NR-015410 (F 5′- CTGTCTAATTTTCCA
GAGCCCCCT -3′, R 5′- GTCATCTCTCCCCCA CAT ACC -3′), ENST00000618591 (F 5′- GGGAGAGCCA TTCTTTCCAG GT -3′, R 5′- GGACACTGTGAA CGGA- GACA -3′), ENST00000512323 (F 5′- ATCTGCCG TTGGTGTGAGGT -TTT -3′, R 5′- GTACTGCTTCTC TGTTGCTG -3′), ENST00000528717 (F 5′- GTTT CTTGGAATGTGAAGTG CTG -3′, R 5′- CCATAGGCA GTAGTAGCCC AAC -3′), Inc-PF4-1:1 (F 5′- AAC TGCCCTTGCA GTTGCTT -3′, R 5′- A GGGACTT CAGGTTCAC -3′), NR-034138 (F 5′- TTTGGA GAAT CTTGGAAGCA -3′, R 5′- CAGAAAGAAAGA GACCCCTATGG -3′), and beta-actin (F 5′- CTGG AACCGTGAAAGGTGACA -3′, R 5′- CGGCCA CATT GTGAACTTTG -3′). Gene expression was calculated using the 2^ΔΔCT method.

RNA labeling and array hybridization
Total RNA was amplified and labeled using a Low Input Quick Amp WT Labeling Kit (cat. no. 5190-2943, Agilent Technologies, Santa Clara, CA, US), according to the manufacturer’s instructions. Labeled cRNAs were purified using an RNeasy mini kit (cat. no. 74106, QIAGEN, GmBH, Germany).

Each slide was hybridized with 1.65 μg Cy3-labeled cRNA using a Gene Expression Hybridization Kit (cat. no. 5188-5242, Agilent Technologies, Santa Clara, CA, US) in a Hybridization Oven (cat. no. G2545A, Agilent Technologies, Santa Clara, CA, US). After 17 h of hybridization, slides were washed in staining dishes (cat. no. 121, Thermos Shandon, Waltham, MA, US) with a Gene Expression Wash Buffer Kit (cat. no. 5188-5327, Agilent Technologies, Santa Clara, CA, US).

Data analysis
Slides were scanned with an Agilent Microarray Scanner (cat. no. G2565CA, Agilent Technologies, Santa Clara, CA, US). Data were extracted with Feature Extraction software v10.7 (Agilent Technologies, Santa Clara, CA, US). Raw data were normalized using the Quantile algorithm, GeneSpring software v12.6.1 (Agilent Technologies, Santa Clara, CA, US). Fold Change filtering and Student’s t test were used to identify differentially expressed lncRNAs and mRNAs (fold change ≥2.0, P < 0.05).

Gene function analysis
To identify the functions of lncRNAs, target genes of differentially expressed lncRNAs were predicted via cis- or trans-regulatory effects. GO analysis was performed to analyze the functions of differentially expressed mRNAs by using the Database for Annotation, Visualization, and Integrated Discovery (http://david.abcc.ncifcrf.gov/) [14, 15]. Pathway analysis was used to determine the significant biological pathways of these differentially expressed mRNAs according to KEGG, (http://www.genome.jp/eg/) [16, 17]. The threshold of significance was defined by the P-value (recommended P < 0.05).

qRT-PCR validation
qRT-PCR was used to validate the microarray data. The method was the same as that described in section "qRT-PCR of cells". The following primer pairs were used: NR-015410 (F 5′- CTGTCTAATTTTCCA
GAGCCCCCT -3′, R 5′- GTCATCTCTCCCCCA CAT ACC -3′), ENST00000618591 (F 5′- GGGAGAGCCA TTCTTTCCAG GT -3′, R 5′- GGACACTGTGAA CGGA- GACA -3′), ENST00000512323 (F 5′- ATCTGCCG TTGGTGTGAGGT -TTT -3′, R 5′- GTACTGCTTCTC TGTTGCTG -3′), ENST00000528717 (F 5′- GTTT CTTGGAATGTGAAGTG CTG -3′, R 5′- CCATAGGCA GTAGTAGCCC AAC -3′), Inc-PF4-1:1 (F 5′- AAC TGCCCTTGCA GTTGCTT -3′, R 5′- A GGGACTT CAGGTTCAC -3′), NR-034138 (F 5′- TTTGGA GAAT CTTGGAAGCA -3′, R 5′- CAGAAAGAAAGA GACCCCTATGG -3′), and beta-actin (F 5′- CTGG AACCGTGAAAGGTGACA -3′, R 5′- CGGCCA CATT GTGAACTTTG -3′). Gene expression was calculated using the 2^ΔΔCT method.

Statistical methods
All statistical data were analyzed using SPSS software v18.0 s (SPSS Inc., Chicago, IL, USA). The threshold value we used to screen differentially expressed lncRNAs and mRNAs was a fold change ≥2.0 (P < 0.05). LncRNAs and mRNAs expressed differentially in the TGF-β2 group compared to the control group were analyzed using Student’s t tests. P < 0.05 was considered statistically significant. The false discovery rate was calculated to correct the P-value.

Results
qRT-PCR/Western blot/Immunofluorescence of cells
Phase-contrast micrographs showed, after stimulated with 10 ng/ml TGF-β2, cells were transformed from single polygonal to long and spindle-shaped (Fig. 1). To confirm EMT in TGF-β2 group, we detected 3 typical EMT-related biomarkers including E-cadherin, α-SMA, and vimentin [19] by qRT-PCR, Western blot, and immunofluorescence. All experiments showed that E-cadherin was reduced, while α-SMA and vimentin expression was increased (Fig. 1c-e). In conclusion, HLE B-3 cells in TGF-β2 group were undergoing EMT.

Differentially expressed lncRNAs and mRNAs
To screen the differentially expressed lncRNAs and mRNAs with statistical significance (fold change ≥2.0, P < 0.05) between TGF-β2 group and control group, volcano plots were constructed (Fig. 2a, b). Furthermore, the IncRNA and mRNA expression patterns across the samples were distinguishable in the heat map generated by hierarchical clustering (Fig. 2c, d).

Based on the microarray data, 775 lncRNAs were differentially expressed (fold change ≥2.0, P < 0.05), including 325 upregulated lncRNAs and 450 downregulated lncRNAs. The top 20 upregulated lncRNAs and top 20 downregulated lncRNAs between the two
groups are listed in Table 1. Lnc-PMEPA1-2:1 (fold change: 15.830) was the most significantly upregulated lncRNA, and NR-033931 (fold change: 13.756) was the most significantly downregulated lncRNA. Downregulated lncRNAs were found to be more common than upregulated lncRNAs in these microarray data.

Using the same criteria for lncRNAs, we identified 935 mRNAs that were differentially expressed (fold change ≥2.0, P < 0.05), including 329 upregulated mRNAs and 606 downregulated mRNAs. The top 20 upregulated and top 20 downregulated mRNAs between the two groups are listed in Table 2. C4orf26 (fold change: 37.775) was the most significantly upregulated mRNA, and KRTAP1-5 (fold change: 21.691) was the most significantly downregulated mRNA.

**LncRNA target prediction**

To identify the potential function of differentially expressed lncRNAs, we predicted the target genes by target prediction programs. 565 lncRNAs had cis target genes, and 213 lncRNAs had trans genes.

**GO and KEGG pathway analysis of differentially expressed mRNAs**

To determine the potential roles of differentially expressed lncRNAs, GO analysis and KEGG pathway analysis were applied to the differentially expressed mRNAs. The GO categories comprised 3 structured networks: biological processes, cellular components and molecular function. We found the most enriched GO terms associated with differentially expressed mRNAs were “single-organism process” (biological process) (Fig. 3a), “extracellular space” (cellular component) (Fig. 3b), and “binding” (molecular function) (Fig. 3c).

KEGG Pathway analysis indicated that 36 pathways were significantly enriched among the differentially expressed mRNAs (P < 0.05) (Fig. 3d). The most enriched pathway was “Staphylococcus aureus infection”, which was associated with 13 differentially expressed genes. Many of these pathways were linked to EMT, such as the PI3K-Akt signaling pathway (associated with 32 genes), TGF-β signaling pathway (associated with 11 genes), ECM-receptor interaction (associated with 15
genes), regulation of actin cytoskeleton (associated with 17 genes), and cell adhesion molecules (associated with 15 genes).

qRT-PCR validation
To confirm the validity of the microarray data, we randomly selected 6 differentially expressed lncRNAs for qRT-PCR. These included 3 upregulated lncRNAs (NR-015410, ENST00000618591, and ENST00000512323) and 3 downregulated lncRNAs (ENST00000528717, Inc-PF4-1:1, NR-034138). qRT-PCR was carried out to confirm the expression of the selected lncRNAs in LECs during TGF-β2-induced EMT. qRT-PCR showed the same trend for the 6 lncRNAs that were shown in the microarray analysis (Fig. 4a). The changes were statistically different for only 4 of the 6 lncRNAs (Fig. 4b). ENST00000618591 was upregulated, while ENST00000528717, Inc-PF4-1:1, and NR-034138 were downregulated (P < 0.05).

Discussion
LncRNAs are among the least well-understood of nonprotein-coding RNAs. They were previously considered merely transcriptional “noise” [11] but have increasingly garnered attention in recent years. Newer studies have shown that lncRNAs are involved in EMT. For example, several lncRNAs can be involved in the regulation or activation of the WNT signaling pathway in the Twist-induced EMT process [20]. H19 can promote pancreatic cancer metastasis by derepressing let-7’s suppression on its target HMGA2-mediated EMT [21]. However, to our knowledge, no previous study has focused on the microarray expression profile of lncRNAs in LECs during EMT. Thus, we conducted the current study to assess the role of lncRNAs in the development and progression of EMT in LECs from the perspective of lncRNA.

In this study, we chose the HLE B-3 cell line. It is a primary cell line of LECs that is immortalized via infection with an adenovirus 12-SV40 virus, and can be used to
### Table 1 Top 20 differentially expressed lncRNAs in TGF-β2 group compared to control group

| LncRNA Source | p-values | Fold change |
|---------------|----------|-------------|
| Inc-PMEPA1-2:1 Lncipedia | 0.00455517 | 15.830 |
| ENST00000597865 GENCODE | 0.0020187 | 12.737 |
| Inc-ZNF2-1:1 Lncipedia | 0.01371849 | 12.647 |
| Inc-RCK2-5:1 Lncipedia | 4.6625E-06 | 10.882 |
| Inc-ABCA2-5:2 Lncipedia | 0.0043109 | 10.147 |
| Inc-SMADS-7:1 Lncipedia | 1.89988E-05 | 9.649 |
| ENST00000597865 GENCODE | 0.00020187 | 12.737 |
| ENST00000591217 GENCODE | 0.00195645 | 8.503 |
| ENST00000605588 GENCODE | 0.0016452 | 8.302 |
| ENST00000605588 GENCODE | 0.00071386 | 8.289 |
| ENST00000605588 GENCODE | 0.00049138 | 8.302 |
| ENST00000605588 GENCODE | 0.00016452 | 8.302 |
| ENST00000605588 GENCODE | 0.00071386 | 8.289 |
| ENST00000605588 GENCODE | 0.00049138 | 8.302 |

### Table 2 Top 20 differentially expressed mRNAs in TGF-β2 group compared to control group

| mRNA | p-values | Fold change |
|------|----------|-------------|
| NM_001206981(C4orf26) | 0.00682992 | 37.775 |
| NM_004118(FOXS1) | 2.01224E-05 | 26.221 |
| NM_0020182(PMEPA1) | 0.00070997 | 23.537 |
| NM_0020182(PMEPA1) | 0.00105858 | 23.038 |
| NM_001001557(GDF6) | 0.00070997 | 23.038 |
| NM_001855(COL15A1) | 0.00199402 | 17.745 |
| NM_001855(COL15A1) | 0.00199402 | 17.745 |
| NM_00121584(DHRS2) | 0.00296753 | 13.614 |
| NM_00114320(ADAMTS2) | 0.00044198 | 12.692 |
| NM_001142393(NEDD9) | 0.00109819 | 8.843 |
| NM_00120400(LPAP5) | 0.0020825 | 8.816 |
| NM_001135057(LRRC15) | 0.0014638 | 8.510 |
| NM_0014433(LI17B) | 0.00166941 | 8.333 |
| NM_001781(CD69) | 0.0029504 | 8.139 |
| NM_0008884(TG68) | 0.00518906 | 8.100 |
| NM_0047177(DG3) | 0.00994008 | 7.453 |
| NM_001955(EDN1) | 0.0198286 | 7.174 |
| NM_0021666(XYL1T1) | 0.0008475 | 7.043 |
| NM_00023(SGCA) | 0.00022045 | 6.844 |
investigate HLE physiology and cataracts [22]. We chose 3 typical biomarkers: E-cadherin, \( \alpha \)-SMA, and vimentin to confirm EMT in LECs [19]. E-cadherin is the prototypical epithelial cell marker of EMT. It is expressed in epithelial cells, and its expression is decreased during EMT. Vimentin and \( \alpha \)-SMA are mesenchymal markers, and their expression is increased during EMT [6, 7]. The results of qRT-PCR, western blot and immunofluorescence suggested that the cells in the TGF-\( \beta \)_2 group were undergoing EMT.

The findings of our study indicate that lncRNAs play a potential role in the EMT pathogenesis of PCO. A total of 325 upregulated lncRNAs and 450 downregulated lncRNAs were differentially expressed in the TGF-\( \beta \)_2 group compared to the control group. Furthermore, we used GO and KEGG pathway analyses to identify potential functions of the differentially expressed mRNAs. GO analysis revealed the dysregulation of 1165 mRNAs involved in biological processes, 72 mRNAs involved in cellular components, and 112 mRNAs involved in molecular functions. Many items were involved in critical processes in EMT, such as protein binding, growth factor binding, cell proliferation. KEGG Pathway analysis indicated that 36 pathways were significantly enriched, many of which are related to EMT, including the PI3K-Akt signaling pathway [8, 23], TGF-\( \beta \) signaling pathway, and ECM-receptor...
interaction [24, 25]. These results indicated the general functional roles of the differentially expressed IncRNAs and also confirmed the reliability of our microarray data.

Although huge numbers of IncRNAs have been found, the functions of most IncRNAs remain unknown. Previous reports have suggested that IncRNAs can guide gene expression either in cis (on neighboring genes) or in trans (on distantly located genes) manner [26, 27]. We can predict the functions of IncRNA via the cis/trans genes. We chose the top 4 up and down IncRNAs to analyze. The upregulated Inc-PMEPA1-2:1 is a 361 bp sense-overlapping IncRNA and was predicted to have a cis target gene, prostate transmembrane protein, androgen-induced 1(PMEPA1), which is important in cancer development. PMEPA1 encodes a transmembrane protein that contains a Smad-interacting motif [28]. PMEPA1 expression is induced by androgens and TGF-β, and can suppress the androgen receptor and TGF-β signaling pathways by interacting with Smad proteins. In breast cancer cells, PMEPA1 could be upregulated by classical TGF-β/Smad signaling pathway, and silencing of PMEPA1 significantly could inhibit the migration ability of MDA-MB-231 cells and promoted the process of EMT. Previous studies also showed that PMEPA1 can regulate EMT in lung cancer cells by modulating the ROS and IRS-1 signaling pathways [28]. Lnc-PMEPA1-2:1 may regulate EMT in PCO by PMEPA1 via the signaling pathways. NR033931, also known as linc01085, is a 1984 bp intergenic IncRNA, of which little is known. ENST00000597865 is a 520 bp antisense IncRNA, and have a cis genes Neurotrophin 4(NTF4), which is a member of the nerve growth factor family of neurotrophins, that control survival and differentiation of mammalian neurons. Kim et al. reported that NTF4 receptor TrkB could induce EMT through activation of the JAK2/STAT3 pathway and PI3K/AKT pathway in breast cancer [29]. NTF4 may have the similar function in EMT of PCO. ENST00000582120 is a 673 bp sense lncRNA. It had a cis gene: collectin subfamily member 12 (COLEC12). COLEC12 is a scavenger receptor, a cell surface glycoprotein that displays several functions associated with host defense. According to the GO analysis, COLEC12 plays roles in biological process(single-organism process, response to stimulus, and positive regulation of biological process) and cellular component(extracellular region, cell periphery, and plasma membrane). ENST00000582120 may play roles in EMT via regulating COLEC12. Further researches are needed to confirm the functions of these differentially expressed IncRNAs and their potential target genes.

Despite these promising findings, the current study did have some limitations. First, the sample size in the microarray analysis was small, as was the number of candidate IncRNAs, and these might limit the validity of the array results. Second, the results were not validated in animal or tissue experiments. Moreover, more experiments should be performed in future work to confirm and illustrate the detailed functional roles of the dysregulated IncRNAs by using RNA interference approaches in vitro and in vivo.

Conclusions
In conclusion, this is the first report of microarray analysis of IncRNA and mRNA differential expression in LECs during TGF-β2-induced EMT compared to normal LECs. These differentially expressed IncRNAs and mRNAs likely play important roles in the development and progression of EMT in LECs. Our study lays the foundation for future research on IncRNAs related to EMT in LECs and could introduce new avenues for the prevention and treatment of PCO.

Abbreviations
EMT: Epithelial-mesenchymal transition; GO: Gene ontology; HLE B-3: Human lens epithelial B-3; KEGG: Kyoto Encyclopedia of Genes and Genomes; LECs: Lens epithelial cells; IncRNAs: Long noncoding RNAs; PCO: Posterior capsule opacification; qRT-PCR: Quantitative real-time reverse transcription polymerase chain reaction; TGF-β2: Transforming growth factor β2

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Availability of data and materials
The microarray data discussed in this article have been deposited in National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) and are accessible through GEO Series accession number GSE98225.

Authors’ contributions
2XD and MYQ were responsible for the conception and design of this study. BYZ and YC acquired the data. BYZ analyzed and interpreted the data, and drafted the manuscript. 2XD revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

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Author details
1Guillin Medical University, Guangxi Zhuang Autonomous Region, Guillin 541000, China. 2Department of Ophthalmology, Guillin Medical University Affiliated Hospital, Guangxi Zhuang Autonomous Region, Guillin 541001, China.
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References
1. Global Data On Visual Impairments 2010. Geneva: World Health Organization; 2012.
2. Facts About Cataract. National Eye Institute: National Eye Institute, 2015.
3. Awasthi N, Guo S, Wagner BJ. Posterior capsular opacification: a problem reduced but not yet eradicated. Arch Ophthalmol. 2009;127:555–62.
4. Greenburg G, Hay ED. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. J Cell Biol. 1982;95:333–9.
5. Wormstone IM. Posterior capsule opacification: a cell biological perspective. Exp Eye Res. 2002;74(3):337–47.
6. Wormstone IM, Wang L, Liu CS. Posterior capsule opacification. Exp Eye Res. 2009;88(2):257–69.
7. Raj SM, Vasanava AR, Johar SR, Vasanava VA, Vasanava VA. Post-Operative Capsular Opacification: A Review. Int J Biomed Sci. 2007;3(4):237–50.
8. Yao K, Ye PP, Tan J, Tang XJ, Shen T. X. C. Involvement of PI3K/Akt pathway target HMGA2-mediated epithelial mesenchymal transition in human lens epithelial cells. Ophthalmic Res. 2008;40(2):69–76.
9. Saika S, Kono-Saika S, Ohnishi Y, Sato M, Muragaki Y, Ooshima A, et al. Smad3 signaling is required for epithelial-mesenchymal transition of lens epithelium after injury. Am J Pathol. 2004;164(2):651–63.
10. de Jongh RJ, Wederell E, Lovicu FJ, McAvoy JW. Transforming growth factor-beta-induced epithelial-mesenchymal transition in the lens: a model for cataract formation. Cells Tissues Organs. 2005;179(1-2):43–55.
11. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136(4):629–41.
12. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 2009;23(13):1494–504.
13. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem. 2013;81:145–66.
14. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000;25(1):25–9.
15. Gene Ontology C. The Gene Ontology (GO) project in 2006. Nucleic Acids Res. 2006;34(Database issue):D322–6.
16. Draghici S, Khatri P, Tarca AL, Amin K, Dono A, Voichita C, et al. A systems biology approach for pathway level analysis. Genome Res. 2007;17(10):1537–45.
17. Kaneshia M, Goto S, Kawashima S, Okuno Y, Hattori M. The KEGG resource for deciphering the genome. Nucleic Acids Res. 2004;32(Database issue):D277–80.
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.
19. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest. 2009;119(6):1429–37.
20. Hu P, Yang J, Hou Y, Zhang H, Zeng Z, Zhao L, et al. LncRNA expression signatures of twist-induced epithelial-to-mesenchymal transition in MCF10A cells. Cell Signal. 2014;26(1):83–9.
21. Ma C, Norg K, Zhou H, Wang W, Huang X, Yuan Z, et al. H19 promotes pancreatic cancer metastasis by derepressing let-7s suppression on its target HMGA2-mediated EMT. Tumour Biol. 2014;35(3):9163–9.
22. Andley UP, Rhim JS, Chylack LT Jr, Fleming TP. Propagation and immortalization of human lens epithelial cells in culture. Invest Ophthalmol Vis Sci. 1994;35(7):3094–102.
23. Guo R, Meng Q, Guo H, Xiao L, Yang X, Cui Y, et al. TGF-B2 induces epithelial-mesenchymal transition in cultured human lens epithelial cells through activation of the PI3K/Akt/mTOR signaling pathway. Mol Med Rep. 2016;13(2):1105–10.
24. Macias WR, Salton DJ, Stanford MR. Role of cytokines in the pathogenesis of posterior capsular opacification. Br J Ophthalmol. 2000;84(3):332–6.
25. Saika S, Ohmni S, Kanagawa R, Tanaka S, Ohnishi Y, Ooshima A, et al. Lens epithelial cell outgrowth and matrix formation on intraocular lenses in rabbit eyes. J Cataract Refract Surg. 1996;22(Suppl 1):835–40.
26. Roberts TC, Morris KV, Weinberg MS. Perspectives on the mechanism of transcriptional regulation by long non-coding RNAs. Epigenetics. 2014;9(1):13–20.
27. Prensner JR, Chinnaïyan AM. The emergence of IncRNAs in cancer biology. Cancer discovery. 2011;1(5):391–407.
28. Hu Y, He K, Wang D, Yuan X, Liu Y, Ji H, et al. TMEPAI regulates EMT in lung cancer cells by modulating the ROS and IRS-1 signaling pathways. Carcinogenesis. 2013;34(8):1764–72.
29. Kim MS, Lee WS, Jeong J, Kim SJ, Jin W. Induction of metastatic potential by TAF via activation of IL6/STAT3 and PI3K/AKT signaling in breast cancer. Oncotarget. 2015;6(37):40158–71.

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