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MicroRNA-486-5p suppresses TGF-β_2-induced proliferation, invasion and epithelial–mesenchymal transition of lens epithelial cells by targeting Smad2

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The pathological development of lens epithelial cells (LECs) leads to posterior capsular opacification (PCO). This study was undertaken to investigate the effects of microRNA-486-5p (miR-486-5p) on TGF-β_2-induced proliferation, invasion and epithelial-mesenchymal transition (EMT) in the lens epithelial cell line SRA01/04, and to explore the underlying molecular mechanisms. The expression of miR-486-5p in TGF-β_2-induced SRA01/04 cells was down-regulated, and the expression of Smad2, p-Smad2 and p-Smad3 was up-regulated. A dual-luciferase reporter assay revealed that miR-486-5p directly targets the 3′-UTR of Smad2. MiR-486-5p mimic transfection markedly down-regulated the expression levels of Smad2, thus inhibiting the expression of p-Smad2 and p-Smad3. MiR-486-5p overexpression in SRA01/04 cells markedly suppressed TGF-β_2-induced proliferation and invasion, inhibited protein expression of CDK2 and CDK4, down-regulated fibronectin, α-SMA and vimentin and up-regulated E-cadherin; these effects were partly reversed by Smad2 overexpression. In short, these data show that miR-486-5p overexpression can inhibit TGF-β_2-induced proliferation, invasion and EMT in SRA01/04 cells by repressing Smad2/Smad3 signalling, implying that miR-486-5p may be an effective target to interfere in the progression of PCO.

Keywords. Cell invasion; cell proliferation; epithelial–mesenchymal transition; lens epithelial cells; miR-486-5p

1. Introduction

Posterior capsular opacification (PCO), also called secondary cataract, is a common complication of cataract surgery (Milazzo *et al.* 2014). After cataract surgery, the residual lens epithelial cells (LECs) undergo a pathological process, leading to PCO (Wormstone *et al.* 2009). Residual LECs rapidly proliferate, migrate and invade, resulting in contraction and wrinkling of the normally smooth posterior capsule (De Iongh *et al.* 2005) and leading to severely degraded visual acuity (Wormstone 2002; Awasthi *et al.* 2009). Epithelial-mesenchymal transition (EMT) always occurs during this process, resulting in the accumulation of fibroblasts. Therefore, the suppression of LEC proliferation, invasion and EMT may be considered important points at which to intervene in PCO pathogenesis.

It has been reported that cytokines exert vital functions in PCO, which have been found to be up-regulated after cataract surgery (Wormstone *et al.* 2009; Huang *et al.* 2011).Transforming growth factor-β_2 (TGF-β_2), one functional factor, plays a crucial role in wound healing (Wormstone *et al.* 2002; Dawes *et al.* 2007). TGF-β binds with its receptor to activate Smad2 and Smad3, the receptor-related Smads, which further cooperate with Smad4; this signalling complex is translocated to the nucleus to modulate the expression of TGF-β-dependent genes (Yang *et al.* 2013). So, TGF-β_2/Smad silencing may be an effective PCO treatment.

MicroRNAs (miRNAs) are small RNAs that can target the 3′-untranslated regions (UTRs) of mRNAs to modulate the transcription of genes (Bartel 2004). Previous studies have demonstrated that miRNAs are involved in many physiological and pathological events, including cell proliferation, invasion and apoptosis (Alvarez-Garcia and Miska 2005;

Filipowicz *et al.* 2008). The abnormal expression of miRNAs is closely related to a variety of diseases. Recent advances have demonstrated that miRNAs are involved in lens differentiation (Wolf *et al.* 2013), cataractgenesis (Varma *et al.* 2012) and PCO (Hoffmann *et al.* 2012). Given the importance of TGF-β signalling in stimulating PCO, in this study, the 3′-UTR of Smad2 was found to be directly targeted by miR-486-5p by a bioinformatics analysis. Recent studies have shown that miR-486-5p is a multifunctional miRNA involved in many cancers, such as prostate cancer, esophageal squamous cell carcinoma and colorectal carcinoma (Liu *et al.* 2016; Yi *et al.* 2016; Zhang *et al.* 2016). However, functional roles of miR-486-5p in PCO have not been reported to date.

Therefore, the aim of the present study was to investigate the effects of miR-486-5p in SRA01/04 cells. We hypothesized that miR-486-5p would suppress TGF-β_2-induced proliferation, invasion and EMT of lens epithelial cells by targeting Smad2.

2. Materials and methods

2.1 *Cell culture*

The human lens epithelial cell line was purchased from ATCC. The cells were cultured in DMEM supplemented with 10% FBS, 100 IU/mL penicillin and 100 mg/mL streptomycin in a 5% CO_2 atmosphere at 37°C. Cells were passaged at approximately 90% confluence.

2.2 *Cell transfection*

MiR-486-5p mimics and negative control mimics were obtained from GenePharma (Shanghai, China), and the sequences are shown in table 1. The coding sequences of Smad2 were amplified by PCR form human cDNA, and were cloned into pcDNA3.1 overexpression plasmids. The mimics or plasmids were transfected into SRA01/04 cells by Lipofectamine^{TM} 2000 reagent (Invitrogen, CA) according to the manufacturers' protocols.

2.3 *Cell groups*

SRA01/04 cells were mainly divided into six groups: control group, the normal cultures SRA01/04 cells; TGF-β_2 group, SRA01/04 cells treated with 5 ng/mL TGF-β_2 for 48 h; miR-486-5p + TGF-β_2 group, cells transfected with miR-486-5p mimics in the presence of 5 ng/mL TGF-β_2 for 48 h; miR-NC + TGF-β_2 group, cells transfected with miR-NC mimics in the presence of 5 ng/mL TGF-β_2 for 48 h; miR-486-5p + Smad2 + TGF-β_2 group, cells co-transfected with miR-486-5p mimics and pcDNA3.1-Smad2 plasmids in the presence of 5 ng/mL TGF-β_2 for 48 h; miR-486-5p + pcDNA3.1 + TGF-β_2 group, cells co-transfected with miR-486-5p mimics and pcDNA3.1 empty plasmids in the presence of 5 ng/mL TGF-β_2 for 48 h.

2.4 *MTT assay*

SRA01/04 cells were seeded in 96-well plates. The proliferation rate of the treated cells was determined using the MTT assay. Briefly, the cells were incubated with 100 μg/mL MTT for 4 h in a 37°C incubator, following dissolving in 150 μL of DMSO. The absorbance value was determined at a wavelength of 490 nm.

2.5 *Cell count*

SRA01/04 cells were seeded in 24-well plates. For cell counting at different time points, the cells were removed by trypsinization, and the number of viable cells was counted in a hemocytometer with the use of trypan blue staining.

2.6 *Wound-healing assay*

SRA01/04 cells were seeded in 24-well plates. After 24 h of different treatments in the six groups, 20 μL pipette tips were used to produce a scratch, and the detached cells were removed by rinsing with PBS. Images of each well were obtained at 0 and 24 h. The width of wound at 0 h was regarded as 100% and the rate of wound healing = (0 h width of the wound−24 h width of the wound)/(0 h width of the wound).

Table 1. Sequences of oligonucleotides

Name	Sequences (5′-3′)
miR-486-5p mimic	Sense: CGGGGCAGCUCAGUACAGGAUU Anti-sense: UCCUGUACUGAGCUGCCCCGAG
negative control mimic	Sense: UUCUCCGAACGUGUCACUUTT Anti-sense: ACGUGACACGUUCGGAGAAATT
miR-486-5p Forward primer	ACACTCCAGCTGGGTCCTGTACTGAGCTGCCC
miR-486-5p Reverse primer	CTCAACTGGTGTCGTGGA
U6 Forward primer	CTCGGGCTCGCTTCGGCAGCACA
U6 Reverse primer	AACGCTTCACGAATTTGCGT

2.7 *Transwell assay*

Cell invasion was determined by the Transwell assay according to the manufacturers' protocol. After 24 h of different treatments in the six groups, the cells at a density of 5×10^5 cells/mL were seeded in the upper compartment and then migrate through 8 μm pores. In the lower well, 400 μL DMEM with 20% FBS was added. After incubating for 24 h at 37°C, the cells were fixed with 4% formaldehyde and stained with crystal violet. The migrated cells in the lower well were counted in five high power fields.

2.8 *Quantitative real-time PCR (qRT-PCR)*

The total RNA was extracted using TRIzol reagent (Invitrogen, CA) according to the manufacturer's guidelines. cDNA was reverse transcribed using the PrimeScript RT Reagent Kit (TaKaRa, China). The level of miR-486-5p was determined using the TaqMan MiRNA Assay Kit (Applied Biosystems, USA) and normalized to U6 small nuclear RNA. Relative expression was analysed by the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers are shown in table 1.

2.9 *Western blot*

The cells were lysed in M-PER reagent (Bioteke, China) and denatured at 100°C for 10 min. The protein concentrations were quantified using BCA protein assay kit (Pierce, USA). SDS-PAGE was performed to separate the proteins and then blotted onto PVDF membranes (Millipore, USA). Non-specific binding was blocked using 5% non-fat milk for 1 hours and subjected to immunolabeling using primary antibodies for p-Smad2 (1:500), Smad2 (1:500), p-Smad3 (1:500), Smad3(1:500), CDK2 (1:1000), CDK4 (1:1000), E-cadherin (1:500), fibronectin (1:500), α-SMA (1:500), vimentin (1:500) and GAPDH (1:1000). The membranes were probed with secondary antibodies conjugated to horseradish peroxidase (Millipore). The blots were visualized by detecting chemiluminescence using a Bio-Rad ChemiDoc XRSþ imaging system.

2.10 *Luciferase assay*

The 3′-UTR sequences of Smad2 were amplified by PCR and were cloned into pGL3 vector. The sequence for the mutation within the miR-486-5p binding site was amplified by the point mutation method using the KOD-Plus mutagenesis Kit (TOYOBO, Japan) according to the protocols, and then cloned into pGL3 vector. The pGL3-Smad2 3′-UTR or pGL3-Smad2 3′-UTR mutant reporter plasmids were co-transfected with the miR-486-5p mimics or negative control mimics, along with pRL-TK renilla plasmid into SRA01/04 cells by Lipofectamine 2000 reagent. After 48 h, the firefly and renilla luciferase activities were measured by Dual Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. The relative firefly luciferase activity was determined by normalization with Renilla luciferase activity.

2.11 *Statistical analysis*

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). The results are shown as mean±SEM of triplicate experiments. Data were analysed by one-way ANOVA with Bonferroni test or Student's t-test. $P<0.05$ was considered statistically significant.

3. Results

3.1 *MiR-486-5p was decreased and TGF-β_2/Smad signalling was activated in TGF-β_2-induced SRA01/04 cells*

As shown in figure 1, qRT-PCR showed that the expression of miR-486-5p was downregulated in TGF-β_2-induced SRA01/04 cells, suggesting that it may be involved in the regulation of LECs. Western blot showed that, compared with the control group, the expression levels of Smad2 in the TGF-β_2 group were increased (2.54-fold), but the expression of Smad3 in the two groups showed no statistically significant differences. These results suggested that there may be some relationship between miR-486-5p down-regulation and Smad2 up-regulation. In addition, the levels of p-Smad2 and p-Smad3 were obviously up-regulated (3.45-fold and 3.12-fold) in TGF-β_2-induced SRA01/04 cells, showing that TGF-β_2 activated downstream Smad2/ Smad3 signalling.

3.2 *MiR-486-5p directly targeted the 3′-UTR of Smad2 mRNA*

The predicted targeting sequences of miR-486-5p and Smad2 are shown in figure 2A. The dual-luciferase reporter assay confirmed that miR-486-5p directly targets the 3′-UTR of Smad2 mRNA. The activity of the WT reporter was markedly decreased (0.51-fold) by miR-486-5p overexpression. However, the activity of the mutated reporter was not inhibited by miR-486-5p mimic transfection. As shown in figure 2B–D, the expression level of

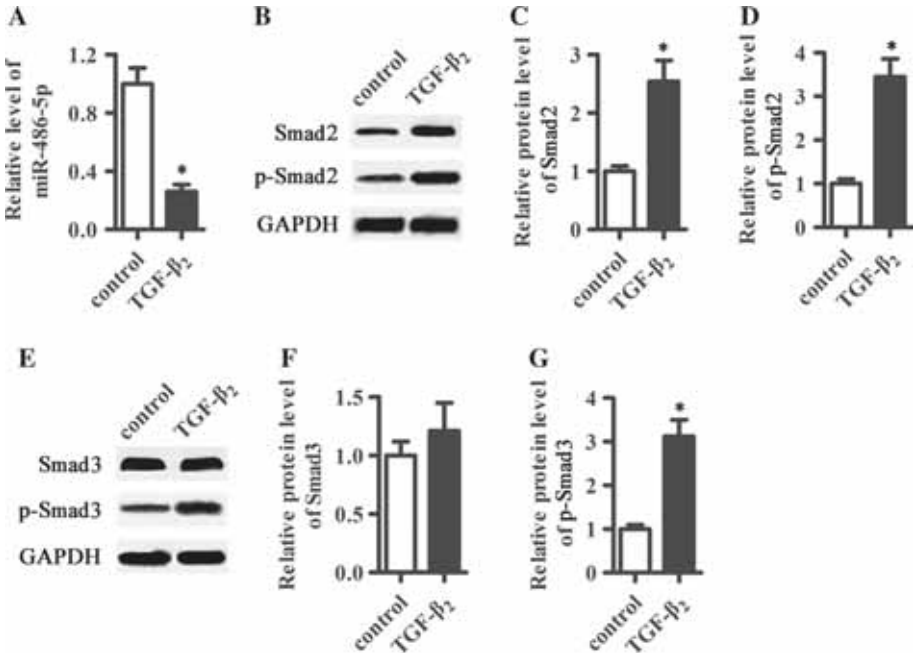

Figure 1. MiR-486-5p expression was decreased and TGF-β/Smad signalling was activated in TGF-β_2-induced SRA01/04 cells. (**A**) MiR-486-5p levels were assessed using RT-PCR and normalized to U6. (**B**) The protein expression levels of Smad2 and p-Smad2 were detected by Western blot and normalized to GAPDH. (**C**) The relative expression of Smad2 is shown in the bar graph. (**D**) The relative expression of p-Smad2 is shown in the bar graph. (**E**) The protein expression levels of Smad3 and p-Smad3 were detected by Western blot and normalized to GAPDH. (**F**) The relative expression of Smad3 is shown in the bar graph. (**G**) The relative expression of p-Smad3 is shown in the bar graph. *P<0.05 vs. control group.

Smad2 was markedly down-regulated (0.32-fold) by miR-486-5p overexpression, further confirming that Smad2 is a target gene of miR-486-5p. Moreover, the protein levels of p-Smad2 and p-Smad3 were significantly down-regulated (0.43-fold and 0.42-fold) in the miR-486-5p group as compared with the control group. These results suggest that miR-486-5p directly targets the 3′-UTR of Smad2 mRNA to regulate Smad signalling.

3.3 *MiR-486-5p overexpression inhibited TGF-β2-induced cell proliferation by down-regulating Smad2 expression in SRA01/04 cells*

The previous results showed that miR-486-5p is down-regulated in TGF-β_2-induced SRA01/04 cells and that miR-486-5p directly targets Smad2, a downstream molecule of TGF-β_2 signalling. We further investigated the effects of miR-486-5p on the proliferation of TGF-β_2-induced SRA01/04 cells. We transfected miR-486-5p or miR-NC mimic into TGF-β_2-induced SRA01/04 cells, and cell viability was measured by the MTT assay. The results showed that, compared with the TGF-β_2 group, cell viability was markedly inhibited (0.65-fold) in the miR-486-5p + TGF-β_2 group, suggesting that miR-486-5p overexpression inhibited TGF-β_2-induced cell viability. To investigate whether the effect was mediated by down-regulating Smad2, pcDNA3.1-Smad2 or the control empty plasmid pcDNA3.1 were introduced into cells transfected with miR-486-5p mimics. The MTT results show that the effect of miR-486-5p in terms of inhibiting cell viability was partially reversed in pcDNA3.1-Smad2-transfected SRA01/04 cells (figure 3A). Furthermore, we performed cell count assay at 4, 12, 24, 36 h and 48 h. As shown in figure 3B, in the presence of TGF-β_2, the cell number increased progressively. Compared with the TGF-β_2 group, the cell number in miR-486-5p + TGF-β_2 group was decreased at all the time points, and decreased to 0.57-fold at 48 h. Compared with the miR-486-5p + TGF-β_2 group, the decrease in cell number was partly reversed in miR-486-5p + Smad2 + TGF-β_2 group. These data suggest that miR-486-5p overexpression inhibits TGF-β_2-induced cell proliferation by down-regulating Smad2 expression in SRA01/04 cells.

Cell cycle-related proteins have been found to be related to the proliferation of LECs (Awasthi and Wagner 2006). Thus, we evaluated the protein expression levels of CDK2 and CDK4. Western blot showed that, compared with the TGF-β_2 group, the expression of CDK2 and CDK4 was significantly decreased (0.56-fold and 0,46-fold) in the miR-486-5p + TGF-β_2 group; and, as with the miR-486-5p + TGF-β_2 group, the levels of CDK2 and CDK4 were

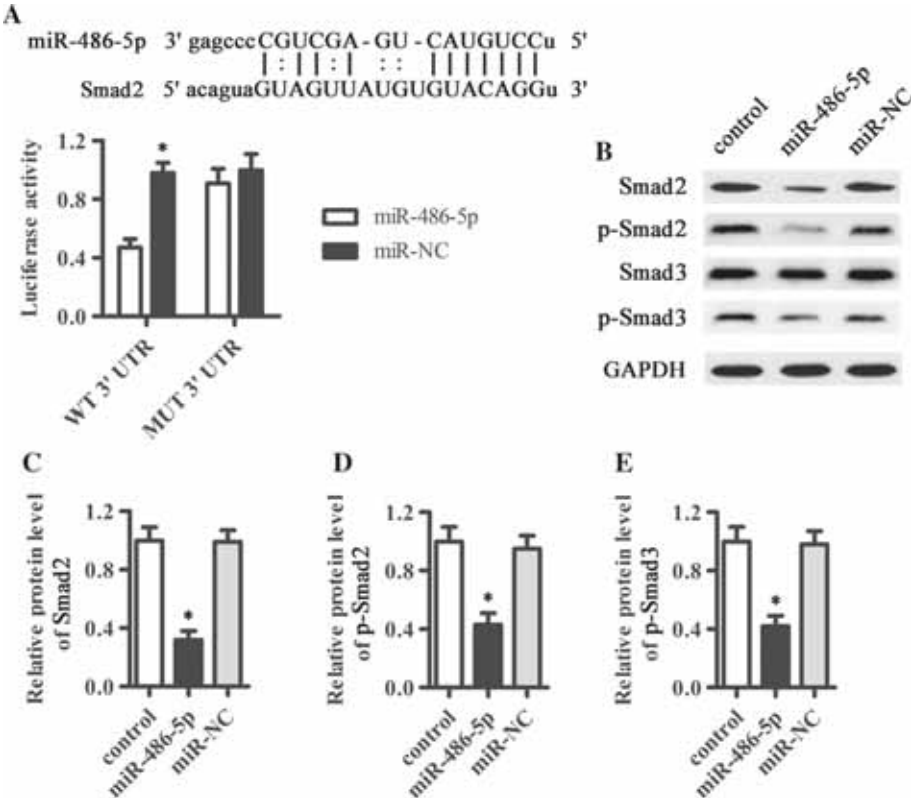

Figure 2. MiR-486-5p directly targeted Smad2 in SRA01/04 cells. (**A**) The 3′-UTR of Smad2 mRNA predicted to be targeted by miR-486-5p. MiR-486-5p or miR-NC mimics were co-transfected with Smad2 3′-UTR or Smad2 3′-UTR mut reporter plasmids, along with a control Renilla luciferase pRL-TK vector, into SRA01/04 cells. After 48 h, the relative luciferase activity was analysed. (**B**) The protein expression levels of Smad2, p-Smad2, Smad3 and p-Smad3 were determined by Western blot and normalized to GAPDH. (**C**) The relative expression of Smad2 is shown in the bar graph. (**D**) The relative expression of p-Smad2 is shown in the bar graph. (**E**) The relative expression of p-Smad3 is shown in the bar graph. *$P<0.05$ vs. control group.

significantly increased (1.46-fold and 1.89-fold) in the miR-486-5p + Smad2 + TGF-β_2 group (figure 3C-D).

3.4 *MiR-486-5p overexpression inhibited TGF-β2-induced migration and invasion by down-regulating Smad2 expression in SRA01/04 cells*

As shown in figure 4, the effects of miR-486-5p on cell migration and invasion were assessed by the wound healing and Transwell assays. The results showed that compare with the TGF-β_2 group, the wound healing rate in the miR-486-5p + TGF-β_2 group was lower (0.65-fold), suggesting that cells in the miR-486-5p + TGF-β_2 group migrated slower than those in the TGF-β_2 group. Compared with the TGF-β_2 group, the miR-486-5p + TGF-β_2 group showed decreased invasion (0.39-fold). Moreover, the miR-486-5p-mediated inhibition of migration and invasion was partly reversed when Smad2 was overexpressed. These data provide evidence that miR-486-5p overexpression inhibits TGF-β_2-induced migration and invasion by down-regulating Smad2 expression in SRA01/04 cells.

3.5 *MiR-486-5p overexpression inhibited TGF-β2-induced EMT via down-regulating Smad2 expression in SRA01/04 cells*

We further determined the effects of miR-486-5p on TGF-β_2-induced EMT; levels of EMT-related proteins were measured by Western blot. As shown in figure 5, compared with the TGF-β_2 group, the expression of E-cadherin was up-regulated (1.78-fold), while the expression of fibronectin, vimentin and α-SMA was down-regulated (0.52-fold, 0.51-fold and 0.54-fold) in the miR-486-5p + TGF-β_2 group. When cells were transfected with pcDNA3.1-Smad2 plasmids, the effects of miR-486-5p on EMT-related proteins were partly reversed. Compared with the miR-486-5p + TGF-β_2 group, the level of E-cadherin was down-regulated (0.66-fold), while the levels of fibronectin, vimentin and

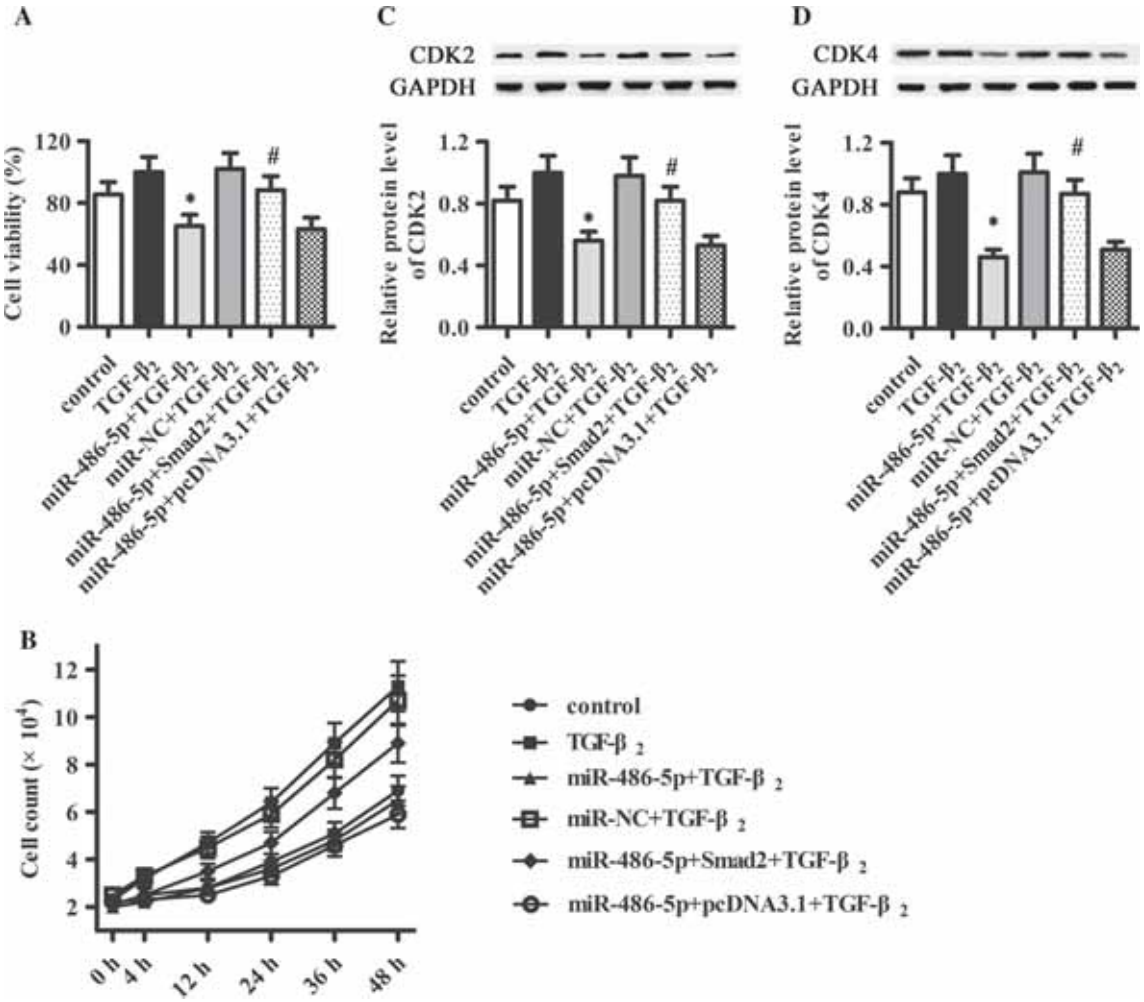

Figure 3. MiR-486-5p overexpression inhibited TGF-β_2-induced cell proliferation via down-regulating Smad2 expression in SRA01/04 cells. SRA01/04 cells were transfected with miR-486-5p or miR-NC mimics, or together with pcDNA3.1-Smad2 or pcDNA3.1 empty plasmids in the presence of TGF-β_2. (**A**) Cell viability was assessed using the MTT assay. (**B**) Cell count of viable cells was measured in a hemocytometer. (**C**) The protein levels of CDK2 were detected by Western blot and the relative expression of CDK2 is shown in the bar graph. (**D**) The protein levels of CDK4 were detected by Western blot and the relative expression of CDK4 is shown in the bar graph. *P<0.05 vs. TGF-β_2 group. $^{\#}P$<0.05 vs. miR-486-5p + TGF-β_2 group.

α-SMA were up-regulated (1.60-fold, 1.55-fold and 1.63-fold) in miR-486-5p + Smad2 +TGF-β_2 group. These data demonstrate that miR-486-5p overexpression inhibits TGF-β_2-induced EMT by down-regulating Smad2 expression in SRA01/04 cells.

4. Discussion

PCO is the most common postoperative complication of cataract surgery. The incidence of PCO is 30–50% in adults and 100% in children after surgery (Awasthi *et al.* 2009). It often causes vision loss. Although it is known that PCO is closely related to the pathological progression of postoperative residual LECs, including proliferation, migration, invasion and EMT, there is considerable need to better understand the mechanisms underlying this process. The activation of TGF-β/Smad signalling is a major cause of poor prognosis for PCO patients. Transgenic mice overexpressing TGF-β in the lens develop morphological changes that closely mimic PCO in humans (Hales *et al.* 1994). Thus, targeting TGF-β/Smad signalling is important for the development of effective therapeutic approaches in PCO. It is now generally accepted that miRNAs are involved in the regulation of various physiological activities by regulating the expression of multiple target genes (Bartel 2004; Hou *et al.* 2013). In this study, we discovered that miR-486-5p expression was decreased in TGF-β_2-induced SRA01/04

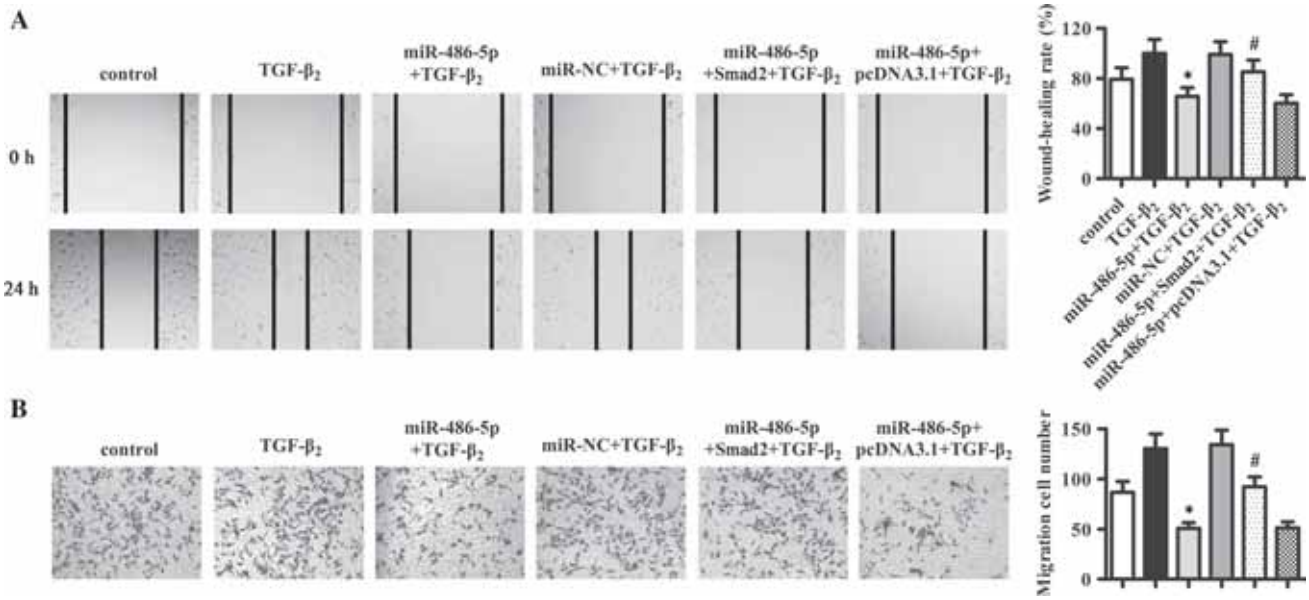

Figure 4. MiR-486-5p overexpression inhibited TGF-β_2-induced migration and invasion by down-regulating Smad2 expression in SRA01/04 cells. (**A**) The capacity of cell migration was assessed by the wound healing assay; the wound healing rate is shown in the bar graph. (**B**) The capacity of cell invasion was assessed by the Transwell assay; the number of migrating cells is shown in the bar graph. *$P<0.05$ vs. TGF-β_2 group. $^{\#}P<0.05$ vs. miR-486-5p + TGF-β_2 group.

cells, suggesting miR-486-5p may have a potential role in PCO.

It has been reported that TGF-β signalling regulates various cellular processes (Massagué 2000). TGF-β can act on Smad2 and Smad3 and promote their translocation to the nucleus in association with Smad4, leading to the transcription of genes (Massagué and Wotton 2000; Shi and Massagué 2003). In the present study, Western blot showed that the protein levels of Smad2, p-Smad2 and p-Smad3 were obviously up-regulated in TGF-β_2-induced SRA01/04 cells, while the expression of Smad3 was not different between the control and TGF-β_2 groups. These data suggest that treatment with TGF-β2 activated downstream Smad signalling, indicating that there may be some relationship between miR-486-5p down-regulation and Smad2 up-regulation. We predicted that miR-486-5p would bind directly to the 3′-UTR of Smad2 by bioinformatics analysis. This prediction was further confirmed by the dual-luciferase reporter assay. MiR-486-5p mimic transfection induced a marked reduction in Smad2 3′-UTR reporter activity, while this reduction was abolished after mutated 3′-UTR reporter transfection. In addition, overexpression of miR-486-5p caused a decrease in Smad2 expression, further confirming that Smad2 is a target gene of miR-486-5p. Moreover, the protein levels of p-Smad2 and p-Smad3 were markedly down-regulated in the miR-486-5p group. These data suggest that miR-486-5p directly targets the 3′-UTR of Smad2 mRNA to regulate Smad signalling, so we speculated that miR-486-5p may be involved in the regulation of TGF-β_2-induced SRA01/04 cells by regulating TGF-β/Smad signalling.

After cataract surgery, the remaining LECs start to proliferate and migrate within a few hours. Inhibiting the proliferation and migration of LECs is an effective strategy for the treatment of PCO. The MTT and cell count assay showed that miR-486-5p overexpression inhibits the TGF-β_2-induced proliferation of SRA01/04 cells. Cyclin-dependent kinases (CDK) has been reported to been involved in the regulation of cell cycle and transcription (Malumbres and Barbacid 2005). CDK2 and CDK4, members of CDK family, are important for modulating the G1/S phase transition (Malumbres and Barbacid 2005). The expression levels of CDK2 and CDK4 were determined by Western blot. The results reveal that the levels of CDK2 and CDK4 in miR-486-5p + TGF-β_2 group were lower as compared with those in the TGF-β_2 group. These findings suggest that miR-486-5p overexpression inhibits the TGF-β_2-induced proliferation of SRA01/04 cells. We further investigated whether the effect of miR-486-5p on the proliferation was mediated by regulating Smad2 signalling; the results show that the inhibition of proliferation induced by miR-486-5p overexpression in the TGF-β_2 group was partially reversed when Smad2 was overexpressed. These data suggest that miR-486-5p overexpression inhibited TGF-β_2-induced cell proliferation by down-regulating Smad2 expression in SRA01/04 cells. Similarly, the wound healing and Transwell assays showed that miR-486-5p overexpression inhibited

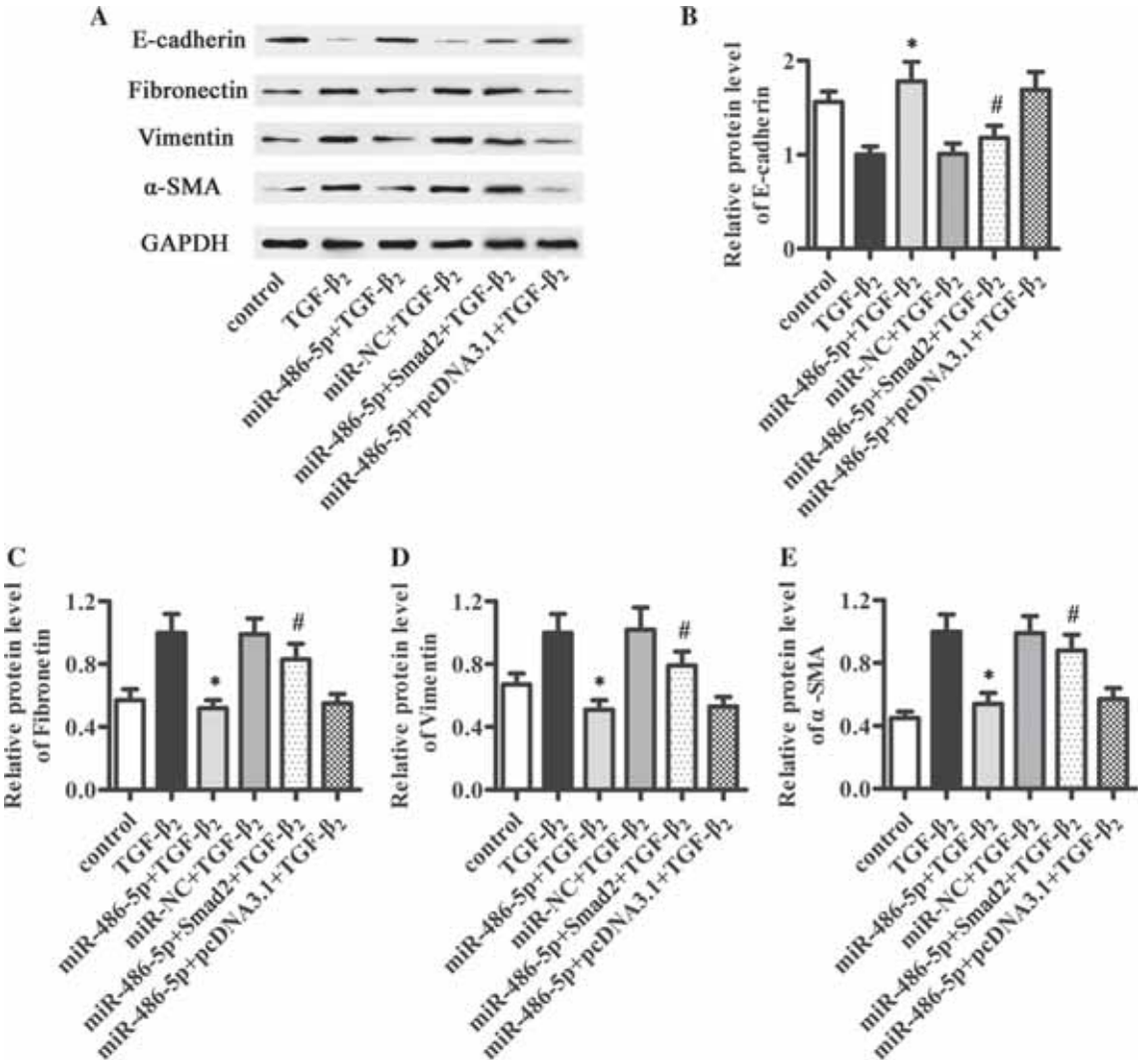

Figure 5. MiR-486-5p overexpression inhibited TGF-β_2-induced EMT via down-regulating Smad2 expression in SRA01/04 cells. (**A**) The expression of E-cadherin, fibronectin, vimentin and α-SMA was detected by Western blot and normalized to GAPDH. The relative expression levels of (**B**) E-cadherin, (**C**) fibronectin, (**D**) vimentin, and (**E**) α-SMA are shown in the bar graphs. *P<0.05 vs. TGF-β_2 group. $^{\#}P$<0.05 vs. miR-486-5p + TGF-β_2 group.

TGF-β_2-induced cell migration and invasion by down-regulating Smad2 expression in SRA01/04 cells.

A variety of investigations have revealed that the residual LECs after cataract surgery differentiate into fibroblast-like cells, leading to the abnormal production of many extracellular molecules (Pau *et al.* 1986; Lee *et al.* 2000). EMT has been reported to be involved in the postoperative course of PCO (Awasthi *et al.* 2009; Wormstone *et al.* 2009). Proteins such as E-cadherin that build junctional complexes are suppressed during the progression of EMT, which may result in the dysregulation of epithelial cell-cell adhesion. In addition, these fibroblast-like cells are characterized by mesenchymal features, including the production of extracellular molecules (like α-SMA and fibronectin) (Ignotz and Massague 1986; Hales *et al.* 1994; Saika *et al.* 2002). TGF-β_2 is considered to be the key cytokine that regulates EMT (Xu *et al.* 2011). It has been reported that interfering with Smad expression leads to the suppression of EMT markers associated with PCO (Saika *et al.* 2004; Wang *et al.* 2013). In the present study, compared with the TGF-β_2 group, the expression of E-cadherin was up-regulated, while the expression of fibronectin, vimentin and α-SMA was down-regulated in the miR-486-5p + TGF-β_2 group. As compared with the miR-486-5p + TGF-β_2 group, the level of E-cadherin was down-regulated, while the levels of fibronectin, vimentin and α-SMA were up-regulated in the miR-486-5p + Smad2 +TGF-β_2 group. These data demonstrate that miR-486-5p overexpression inhibits TGF-β_2-induced EMT by down-regulating Smad2 expression in SRA01/04 cells.

Taken together, these results demonstrate that miR-486-5p overexpression inhibits TGF-β_2-induced proliferation, invasion and EMT of SRA01/04 cells by suppressing Smad2 expression. These findings suggest that miR-486-5p overexpression may be utilized as a novel strategy in the treatment of PCO. However, the detailed molecular mechanisms of miR-486-5p in SRA01/04 cells should be further studied.

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