LncRNA NEAT1 promotes proliferation, migration, invasion and epithelial-mesenchymal transition process in TGF-β2-stimulated lens epithelial cells through regulating the miR-486-5p/SMAD4 axis

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Abstract

Background: Abnormal proliferation, metastasis and epithelial-mesenchymal transformation (EMT) of lens epithelial cells (LECs) are direct factors of posterior capsular opacification (PCO). Nuclear enriched abundant transcript 1 (NEAT1) has been shown to promote cell proliferation, metastasis and EMT, but whether it affects the progression of PCO is unclear.

Methods: The expression of NEAT1, microRNA-486-5p (miR-486-5p) and Drosophila mothers against decapentaplegic 4 (SMAD4) was determined using quantitative real-time polymerase chain reaction (qRT-PCR). The proliferation of cells was measured via 3-(4, 5-dimethyl-2 thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay. Transwell assay was employed to detect the migration and invasion of cells. The levels of EMT marker proteins, SMAD4 protein and transforming growth factor-β (TGF-β)/SMAD signaling pathway-related proteins were assessed by western blot (WB) analysis. Further, the relationship between miR-486-5p and NEAT1 or SMAD4 was confirmed by dual-luciferase reporter assay, RNA immunoprecipitation (RIP) assay and biotin-labeled RNA pull-down assay.

Results: NEAT1 is upregulated and miR-486-5p is downregulated in the posterior capsular tissues of PCO patients and TGF-β2-induced LECs. Interference of NEAT1 reverses the promoting effect of TGF-β2 on the proliferation, migration, invasion and EMT of LECs. MiR-486-5p can be sponged by NEAT1, and its inhibitor reverses the suppression effect of NEAT1 silencing on the progression of TGF-β2-induced LECs. SMAD4 functions as a target of miR-486-5p, and its overexpression recovers the inhibition effect of miR-486-5p overexpression on the progression of TGF-β2-induced LECs. The activity of the TGF-β/SMAD signaling pathway is regulated by the NEAT1/miR-486-5p/SMAD4 axis.

Conclusion: Our study shows that NEAT1 has a positive effect on the progression of PCO and is expected to become a new target for PCO treatment.

Keywords: Posterior capsular opacification, TGF-β2, NEAT1, miR-486-5p, SMAD4

Highlights

1. Depletion of NEAT1 suppresses the biological functions of TGF-β2-stimulated LECs;
2. NEAT1 directly interacts with miR-486-5p;

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3. MiR-486-5p targets SMAD4;
4. The NEAT1/miR-486-5p/SMAD4 axis regulates the activity of the TGF-β/SMAD signaling pathway.

**Background**

After extracapsular cataract extraction or after ocular trauma crystal rupture, the remaining cortex and capsular membrane become cloudy are called after cataract, also known as secondary cataract [1]. Modern secondary cataract mainly refers to posterior capsular opacification (PCO) [2, 3]. Current studies suggest that the proliferation, migration and epithelial-mesenchymal transformation (EMT) of lens epithelial cells (LECs) are the key factors for PCO formation [4, 5]. The EMT process of LECs plays a central role in the formation of PCO, which can lead to cell adhesion and loss of the apical-basal polarity of the mesenchymal phenotype, leading to the production of fibroblasts [6, 7]. Although the cause of PCO has been clarified, the molecular targets that influence its occurrence still need to be further explored. At present, transforming growth factor-β2 (TGF-β2)-induced LECs are considered to be an effective way to construct an in vitro model of PCO [8, 9], which provides a convenient experimental model for us to carry out relevant research on PCO.

Long non-coding RNA (lncRNA) is a non-coding protein that participates in the regulation of various processes in cells [10]. Research has confirmed that lncRNA can regulate cell growth, differentiation and apoptosis, and is related to many diseases progression [11, 12]. More importantly, many lncRNAs have been shown to regulate LECs proliferation, migration and EMT, such as FEZF1-AS1, HOTAIR and MIAT [13–15]. Nuclear enriched abundant transcript 1 (NEAT1) is a nuclear-restricted lncRNA that is abnormally expressed in many diseases and is thought to be associated with disease progression. Xiong et al. report that NEAT1 promotes proliferation, migration and invasion of cells to enhance the progression of breast cancer [16]. And Wang et al. show that NEAT1 regulates the EMT process of diabetic nephropathy [17]. Therefore, NEAT1 may play a vital function in cell proliferation, migration and EMT process. In PCO, Dong et al. conducts microarray analysis on the LECs of PCO patients and normal humans and reveals that the expression of NEAT1 in PCO is significantly increased [18]. However, it is unclear whether NEAT1 participates in the regulation of PCO progress.

Studies on the functions of microRNAs (miRNAs) have been largely confirmed by many researchers. In the regulatory network of lncRNA-miRNA-messenger RNA (mRNA), the role of miRNA as a bridge between lncRNA and target genes has also become the key to elucidate the molecular mechanism of lncRNA [19, 20]. MiR-486-5p is involved in the mediation of proliferation, metastasis and EMT of many diseases, including breast cancer and hepatocellular carcinoma [21, 22]. The study confirms that miR-486-5p shows a low expression trend in TGF-β2-induced LECs and can participate in the proliferation, invasion and EMT of TGF-β2-induced LECs [23].

**Materials and methods**

**Sample tissues collection**

This study was approved by the ethics committee of The First Affiliated Hospital of Zhengzhou University and was performed in accordance with the Declaration of Helsinki. Posterior capsular tissues were obtained from 30 PCO patients (30 eyes, age range was 50–75, free of other ocular diseases) in The First Affiliated Hospital of Zhengzhou University. All patients were diagnosed with PCO and were graded: 6 cases of grade I, 21 cases of grade II, and 3 cases of grade III. Similarly, we also obtained normal posterior capsular tissues from 30 organ donors (age range was 45–72). Written informed consent was signed from each patient and donor.

**Cell culture, TGF-β2 treatment and cell transfection**

Human LECs (SRA01/04) were bought from Biovector (Beijing, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; 12,100-046, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; 10437028, Invitrogen) and 1% penicillin/streptomycin (15140148, Invitrogen). SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 50% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 60% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 70% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 80% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 90% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 100% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 110% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 120% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 130% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 140% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 150% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 160% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 170% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 180% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 190% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 200% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 210% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 220% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 230% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 240% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 250% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 260% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 270% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 280% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 290% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 300% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 310% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 320% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 330% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 340% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 350% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 360% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 370% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 380% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 390% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 400% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 410% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 420% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 430% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 440% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 450% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incubator. When cells reached 460% confluence, SRA01/04 cells were incubated at 37 °C with 5% CO₂ incu
interfering RNA and pcDNA overexpression vector (si-NEAT: 5'-GAGCAATTGACCCCGGTGACG-3' and NEAT1: F 5'-TTGGGACAGTFFACGTTGCG-3', R 5'-TCAAGTCCAGCAGAGCA-3') or their negative controls (si-NC: 5'-TAGATACCCCCAGGCCCTAC-3' and pcDNA: 5'-TAGAGGACAGTCGGGAGA-3'), miR-486-5p mimic and inhibitor (miR-486-5p: 5'-UCCUGUACUGACUCGCGCCAG-3' and anti-miR-486-5p: 5'-CUCCGGACUCUGACAGGAG-3') or their negative controls (miR-NC: 5'-UUUCUCCGAAAGCUGUCACGUTT-3' and anti-miR-NC: 5'-CAGACUUCUUGUGUAGUACAA-3').

Luciferase activities were evaluated using the Dual-luciferase reporter assay. The fragments of NEAT1 and SMAD4 3' UTR containing the predicted miR-486-5p binding sites or mutant binding sites were amplified and cloned into pGL3 reporter vector (Promega, Madison, WI, USA), recorded as wild-type and mutant-type NEAT1 or SMAD4 3' UTR reporter vectors (NEAT1-WT/MUT or SMAD4 3'UTR-WT/MUT). SRA01/04 cells were co-transfected with the reporter vectors and miR-486-5p mimic or miR-NC, and the luciferase activities were evaluated using the Dual-Luciferase Assay Kit (D0023A, Beyotime). 30 μg protein samples were subjected to SDS-PAGE (10% separating gel and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Next, the membranes were blocked with 5% non-fat milk, incubated with primary antibodies and probed with secondary antibody (bs-0295G, 1:20,000). Chemiluminescent Western Blotting Substrate (180-501, Tanon, USA). A red laser (785 nm, stained using 0.1% crystal violet. The cells were counted using a microscope (DM500, Leica, Wetzlar, Germany). Non-treated and non-transfected cells were used as Control.
**RNA immunoprecipitation (RIP) assay**

Magna RIP Kit (17-700) was bought from Millipore. Magnetic beads were pre-coated with antibodies against immunoglobulin G (IgG) or argonaute2 (Ago2) overnight at 4 °C. SRA01/04 cells were lysed and then incubated with magnetic beads. After washed with RIP buffer, total RNA was isolated and the abundances of NEAT1, miR-486-5p and SMAD4 were measured by qRT-PCR.

**Biotin-labeled RNA pull-down assay**

The biotinylated miR-486-5p (bio-miR-486-5p) probe and negative control (bio-miR-NC) probe were synthesized by Sangon (Shanghai, China) and transfected into SRA01/04 cells. After incubated for 48 h, the cells were harvested and then incubated with Dynabeads M-280 Streptavidin (11205D, Invitrogen). The enrichment of NEAT1 and SMAD4 was determined by qRT-PCR.

**Statistical analysis**

Each experiment was carried out at least three times. All statistical analyses were performed using GraphPad Prism 6.0 (GraphPad, La Jolla, CA, USA). Data were represented as mean ± standard deviation. Student’s t-test or one-way analysis of variance was used for statistical analysis. The correlation between miR-486-5p and NEAT1 or SMAD4 was determined using Pearson correlation analysis. P < 0.05 indicated statistical significance.

**Results**

The expression of NEAT1 and miR-486-5p in PCO patients and TGF-β2-stimulated SRA01/04 cells

To identify the function of NEAT1 in PCO, we first detect its expression in posterior capsular tissues. As shown in Fig. 1a, NEAT1 expression is elevated in the posterior capsular tissues of PCO patients compared to the normal posterior capsular tissues. Moreover, in TGF-β2-stimulated SRA01/04 cells, NEAT1 expression is increased in a dose-dependent manner (Fig. 1b). At the same time, we also measure the miR-486-5p expression and discover that miR-486-5p is lower expressed in PCO patients compared with that in normal humans (Fig. 1c). Besides, miR-486-5p also has decreased expression in TGF-β2-stimulated SRA01/04 cells in a dose-dependent manner (Fig. 1d). Through the correlation analysis, we discover that miR-486-5p expression is negatively correlated with NEAT1 (Fig. 1e). Therefore, we speculate that
NEAT1 and miR-486-5p may have important roles in the development of PCO.

**Knockdown of NEAT1 suppresses proliferation, migration, invasion and EMT in TGF-β2-stimulated SRA01/04 cells**

Subsequently, we use the si-NEAT1 to explore the effect of NEAT1 knockdown on the proliferation, migration, invasion and EMT of TGF-β2-stimulated SRA01/04 cells. The concentration of TGF-β2 used is 10 ng/mL. The reversing effect of si-NEAT1 on TGF-β2-induced NEAT1 expression confirms its high transfection efficiency (Fig. 2a). Further, MTT assay results suggest that TGF-β2 promotes the proliferation of SRA01/04 cells, while NEAT1 silencing can invert this effect (Fig. 2b). Also, the acceleration effect of TGF-β2 on the numbers of migrated and invaded SRA01/04 cells also can be recovered by NEAT1 knockdown, indicating that silenced NEAT1 represses the migration and invasion of SRA01/04 cells (Fig. 2c, d). Meanwhile, we also determine the protein levels of EMT marker proteins. As presented in Fig. 2e, TGF-β2 hinders the E-cadherin protein level and enhances the Vimentin and α-SMA protein levels, showing that TGF-β2 can induce the EMT process of SRA01/04 cells. However, si-NEAT1 can change the effect of TGF-β2 on the E-cadherin, Vimentin and α-SMA protein expression, thus reversing the promoting effect of TGF-β2 on the EMT process of SRA01/04 cells. Our results suggest that NEAT1 may have an essential function in regulating the proliferation, metastasis and EMT of LECs.

**NEAT1 interacts with miR-486-5p**

Through the StarBase v2.0 tool, we found that miR-486-5p has binding sites with NEAT1 (Fig. 3a). Besides, we also use the dual-luciferase reporter assay, RIP assay and biotin-labeled RNA pull-down assay to verify the interaction between NEAT1 and miR-486-5p. The results reveal that miR-486-5p overexpression inhibits the luciferase activity of NEAT1-WT vector without affecting that of the NEAT1-MUT vector (Fig. 3b). RIP assay results suggest that the enrichment of miR-486-5p and NEAT1 is significantly increased in Ago2 compared to IgG (Fig. 3c), and biotin-labeled RNA pull down assay results show that NEAT1 enrichment is markedly enhanced in the bio-miR-486-5p probe compared to the bio-miR-NC probe (Fig. 3d). The above data illuminate that NEAT1 can interact with miR-486-5p. Next, we investigate the effect of NEAT1 expression on miR-486-5p expression. The expression of NEAT1 is markedly inhibited by si-NEAT1 and remarkably increased by NEAT1 overexpression vector, indicating that the transfection efficiency of both is good (Fig. 3e). By measuring the miR-486-5p expression, we found that silenced NEAT1 can improve miR-486-5p expression, while overexpressed NEAT1 can hinder its expression (Fig. 3f).

**NEAT1 sponges miR-486-5p to regulate LECs progression**

To determine whether NEAT1 regulates LECs progression through miR-486-5p, we co-transfect si-NEAT1 and anti-miR-486-5p into TGF-β2-stimulated SRA01/04 cells. Through detecting the expression of miR-486-5p, we confirm that miR-486-5p inhibitor can reverse the promoting effect of NEAT1 knockdown on miR-486-5p expression, suggesting that the transfection of both is effective (Fig. 4a). MTT assay results determine that miR-486-5p inhibitor reverses the inhibitory effect of NEAT1 silencing on the proliferation of TGF-β2-stimulated SRA01/04 cells (Fig. 4b), and transwell assay results also show that the suppression effect of NEAT1 knockdown on the migration and invasion of TGF-β2-stimulated SRA01/04 cells can be inverted by miR-486-5p inhibitor (Fig. 4c, d). In addition, the miR-486-5p inhibitor also reverses the promoting effect of silenced NEAT1 on the E-cadherin expression and the inhibiting effect on the Vimentin and α-SMA expression, thus restoring the EMT process of TGF-β2-stimulated SRA01/04 cells (Fig. 4e). Hence, our results demonstrate that NEAT1 regulates the progression of PCO through miR-486-5p.

**SMAD4 serves as a target of miR-486-5p**

For perfecting the mechanism of the NEAT1/miR-486-5p axis, we also use the StarBase v2.0 tool to predict the targets of miR-486-5p. And we discover that SMAD4 has a targeting sequence of miR-486-5p (Fig. 5a). Dual-luciferase reporter assay results indicate that the luciferase activity of SMAD4 3′UTR-WT instead of SMAD4 3′UTR-MUT can be restrained by miR-486-5p overexpression (Fig. 5b). Moreover, RIP assay results show that the levels of miR-486-5p and SMAD4 are enriched in Ago2 compared with IgG (Fig. 5c). Furthermore, we also found that SMAD4 enrichment is remarkably increased in the bio-miR-486-5p probe compared to the bio-miR-NC probe (Fig. 5d). By measuring the mRNA and protein expression of SMAD4, we found that it is highly expressed in the posterior capsular tissues of PCO patients compared with normal posterior capsular tissues (Fig. 5e, f), and negatively correlated with the expression of miR-486-5p (Fig. 5g). Further, SMAD4 is also upregulated in TGF-β2-stimulated SRA01/04 cells with a dose-dependent manner (Fig. 5h, i). Meanwhile, we determine the influence of miR-486-5p expression on SMAD4 expression. The expression of miR-486-5p can be elevated by miR-486-5p mimic and reduced by its inhibitor, confirming the effectiveness of mimic and inhibitor (Fig. 5j). WB analysis results show that
miR-486-5p overexpression inhibits the protein level of SMAD4, while miR-486-5p inhibitor increases its protein level (Fig. 5k). These data reveal that miR-486-5p directly targets SMAD4.

MiR-486-5p regulates the progression of TGF-β2-stimulated LECs through SMAD4
To confirm the role of SMAD4 in miR-486-5p regulates the progression of LECs, we perform the rescue
experiments using miR-486-5p mimic and SMAD4 overexpression vector. The increased protein level of SMAD4 confirms the effectiveness of SMAD4 overexpression vector (Fig. 6a). The results of cell viability show that miR-486-5p has a suppressed effect on the proliferation of TGF-β2-stimulated SRA01/04 cells, whereas SMAD4 overexpression reverses this effect (Fig. 6b). Furthermore, overexpressed SMAD4 also reverses the suppression effect of miR-486-5p overexpression on the migration and invasion of TGF-β2-stimulated SRA01/04 cells (Fig. 6c, d). Similarly, the increasing effect of overexpressed miR-486-5p on the E-cadherin level and the decreasing effect on the Vimentin and α-SMA levels can be reversed by SMAD4 overexpression (Fig. 6e). In addition, we also found that knockdown of NEAT1 inhibits SMAD4 expression, and this effect can be recovered by the miR-486-5p inhibitor (Fig. 6f). In a word, our results reveal that the regulation effect of the NEAT1/miR-486-5p axis on the progression of LECs is achieved by SMAD4.

NEAT1 regulates the TGF-β/SMAD signaling pathway by affecting SMAD4 and miR-486-5p in TGF-β2-stimulated SRA01/04 cells

For determining whether TGF-β/SMAD signaling pathway is involved in the development of NEAT1 regulating the progression of LECs, we detect the expression of p-SMAD2 and p-SMAD3. Through WB analysis, we first confirm that TGF-β2 induces the expression of p-SMAD2 and p-SMAD3 proteins, indicating that TGF-β2 can activate the TGF-β/SMAD signaling pathway (Fig. 7a). Also, we found that interfering of NEAT1 inhibits the expression of p-SMAD2 and p-SMAD3 in TGF-β2-stimulated SRA01/04 cells, and this inhibition
is partially restored after the addition of miR-486-5p inhibitor and SMAD4 overexpression vector in TGF-β2-stimulated SRA01/04 cells (Fig. 7b). Therefore, our study confirm that the NEAT1/miR-486-5p/SMAD4 axis mediates the development of LECs by regulating the activity of the TGF-β/SMAD signaling pathway (Fig. 7c).

**Discussion**

PCO often leads to progressive loss of vision in patients and can cause vision loss in severe cases, so it brings a lot of inconvenience to the life of patients [27]. At present, Nd-YAG laser capsulotomy is often used for the treatment of PCO, but postoperative complications are still possible [28]. Therefore, a better understanding of the
factors affecting the pathogenesis of PCO is conducive to the development of new strategies to prevent and alleviate the development of PCO. NEAT1 is often considered an oncogene in cancer because of its role in promoting proliferation, metastasis, and EMT [29, 30]. Dong et al. found that NEAT1 and MALAT1 were highly expressed in PCO, and confirmed that MALAT1 could promote EMT of TGF-β2-induced LECs by regulating the miR-26a/SMAD4 axis [18]. However, the role of NEAT1 in TGF-β2-induced LECs is unclear. Similarly with the previous study, our study found that NEAT1 was highly expressed in posterior capsular tissues of PCO patients and TGF-β2-stimulated LECs. But not only the EMT of cells, our research also explored the effect of lncRNA on cell proliferation and metastasis. The inhibitory effect of NEAT1 on the proliferation, metastasis and EMT of TGF-β2-stimulated LECs indicated that NEAT1 was a key factor for LECs to maintain normal biological function. Consistent with previous findings [18], our results provide new evidence for NEAT1 as a target for the treatment of PCO.

The involvement of miR-486-5p in disease progression in the form of low expression had been well documented. For example, miR-486-5p was believed to interact with IncRNA DLGAP1-AS1 to participate in the regulation of hepatocellular carcinoma cell proliferation by DLGAP1-AS1 [31]. Also, miR-486-5p had been reported to modulate the EMT process in papillary thyroid cancer by regulating KIAA1199 expression [32]. Therefore, the negative influence of miR-486-5p on cell proliferation, metastasis and EMT has been widely confirmed. In our study, we found that the expression trend of miR-486-5p in posterior capsular tissues of PCO patients and TGF-β2-stimulated LECs was opposite to NEAT1, and verified the interaction between the two through bioinformatics. At the same time, the reversing effect of anti-miR-486-5p on the function of si-NEAT1 also confirmed the negative regulatory effect of miR-486-5p on the proliferation,
Fig. 6 Effects of miR-486-5p overexpression and SMAD4 overexpression on the progression of TGF-β2-stimulated SRA01/04 cells. a SRA01/04 cells were transfected with SMAD4 overexpression vector or pcDNA, and then treated with 10 ng/ml TGF-β2. WB analysis showed that the protein level of SMAD4 was promoted by SMAD4 overexpression vector (P < 0.0001) in TGF-β2-treated SRA01/04 cells. b–f SRA01/04 cells were co-transfected with miR-486-5p mimic and SMAD4 overexpression vector or pcDNA, and then treated with 10 ng/ml TGF-β2. MTT assay (b) and transwell assay (c, d) suggested that overexpressed SMAD4 reversed the inhibition effect of miR-486-5p on the proliferation (P < 0.0001), migration (P < 0.01, P < 0.001) and invasion (P < 0.0001) (100 μM) of TGF-β2-stimulated SRA01/04 cells. e WB analysis revealed that the regulation of miR-486-5p on the protein levels of E-cadherin, Vimentin and α-SMA could be reversed by SMAD4 overexpression (P < 0.0001) in TGF-β2-stimulated SRA01/04 cells. f WB analysis showed that miR-486-5p inhibitor reversed the decreasing effect of NEAT1 silencing on SMAD4 protein expression (P < 0.0001). **P < 0.01, ***P < 0.001, ****P < 0.0001
migration, invasion and EMT of TGF-β2-stimulated LECs. The anti-proliferation, anti-metastasis and anti-EMT effects of miR-486-5p on LECs also were verified by our data, which was consistent with the results of Liu et al. [23]. Hence, miR-486-5p might also be an effective molecular target to prevent PCO progression.

SMAD has been a focus of research because it is a key protein in the TGF-β/SMAD signaling pathway that mediates cell growth and differentiation [25]. Some studies have shown that the low expression of SMAD4 is believed to inhibit cell proliferation, metastasis and EMT, such as in colon cancer and esophageal squamous cell carcinoma [33, 34]. Herein, we suggested that SMAD4 had an increased expression in posterior capsule tissues of PCO patients and TGF-β2-stimulated LECs. The inverting effect of SMAD4 on the function of miR-486-5p mimic confirmed that SMAD4 was the target of miR-486-5p. More importantly, the pro-proliferation, pro-metastasis and pro-EMT effects of SMAD4 on LECs also was demonstrated by this study, which was consistent with previous research [18, 26]. In addition, we also indicated the regulatory effect of the NEAT1/miR-486-5p/SMAD4 axis on p-SMAD2 and p-SMAD3 expression, which confirmed that TGF-β/SMAD signaling pathway was the downstream pathway of the NEAT1/miR-486-5p/SMAD4 axis. These results provided a perfect molecular mechanism for NEAT1 to regulate the progress of PCO.

Of course, there are still some deficiencies in our current research. In the rescue experiment, we found that the reversal effect of miR-486-5p inhibitor on NEAT1 silencing function is partial, so this indicates that there may be other miRNAs involved in the regulation of NEAT1 on the biological functions of LECs. Similarly, the reversal effect of SMAD4 on miR-486-5p function is also partial, which indicates that there may be other targets involved in the regulation of miR-486-5p on the biological functions of LECs. In future research, we will focus on exploring more mechanisms by which NEAT1 regulates the biological functions of LECs, in order to provide new ideas for the treatment of PCO.

Conclusion

Taken together, the current study showed that NEAT1 might have a promoting effect on the development of PCO. Our results concluded that NEAT1 regulated the proliferation, migration, invasion and EMT of TGF-β2-induced LECs through the miR-486-5p/SMAD4 axis. These findings might provide a new target and theoretical basis for molecular targeted therapy of PCO, and had great clinical significance.

Abbreviations

LECs: Lens epithelial cells; EMT: Epithelial-mesenchymal transformation; PCO: Posterior capsular opacification; SMAD4: Drosophila mothers against decapentaplegic 4; qRT-PCR: quantitative real-time polymerase chain reaction; NEAT1:
References

1. Whitman MC, Vanderveen DK. Complications of pediatric cataract surgery. Seminars in ophthalmology. 2014;29(5–6):414–20.

2. Milazzo S, Grenot M, Benzerroug M. Posterior capsule opacification. J Fr Ophthomol. 2014;37(10):825–30.

3. Wormstone IM, Wang L, Liu CS. Posterior capsule opacification. Exp Eye Res. 2009;88(2):257–69.

4. Wormstone IM, Eldred JA. Experimental models for posterior capsule opacification research. Exp Eye Res. 2016;142:2–12.

5. Apple DJ, Solomon KD, Tetz MR, Assia E, Holland EY, Legler UF, Tsai JC, Canestanda VE, Hoggatt JP, Kostick AM. Posterior capsule opacity. Surv Ophthomol. 1999;43(2):7–13.

6. Zheng D, Song T, Zhonglou X, Wu M, Liang J, Liu Y. Downregulation of transforming growth factor-beta type II receptor inhibit epithelial-to-mesenchymal transition in lens epithelium. Mol Vis. 2012;18:1238–46.

7. Kim JT, Lee EH, Chung KH, Kang IC, Lee DH, Joo OK. Transdifferentiation of cultured bovine lens epithelial cells into myofibroblast-like cells by serum modulation. Yonsei Med J. 2004;45(3):380–91.

8. de Jongh RU, Werdelin E, Lovisio 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.

9. Yang Y, Ye Y, Lin X, Wu K, Yu M. Inhibition of periferidone on TGF-beta2 induced proliferation, migration and epithelial-mesenchymal transition of human lens epithelial cells SRA01/04. PLoS ONE. 2013;8(2):e56837.

10. Aikhade VS, Pal D, Kandurci C. Long noncoding RNA: genome organization and mechanism of action. Adv Exp Med Biol. 2017;1008:47–74.

11. Zhang X, Wang W, Zhu W, Dong J, Cheng Y, Yin Z, Shen F. Mechanisms and functions of long non-coding RNAs at multiple regulatory levels. Int J Mol Sci. 2019;20(22):5573.

12. Peng H, Liang Y, Liang J, Zhang YQ, Ai WB, Wu JF. The role of lncRNA in hepatic fibrosis. Cell Biosci. 2018;8:63.

13. Wang Y, Chen L, Gu Y, Wang Y, Yuan Y, Zhu Q, Bi M, Gu S. LncRNA FEFZ1-AS1 promotes TGF-beta2-mediated proliferation and migration in human lens epithelial cells SRA01/04. J Ophthomol. 2019;2019:4736203.

14. Zhang Z, Zhu H, Liu Y, Qian F, Zhang X, Yu L. LncRNA HOTAIR mediates TGF-beta2-induced cell growth and epithelial-mesenchymal transition in human lens epithelial cells. Acta Biochim Biophys Sin. 2018;50(10):1028–37.

15. Shen Y, Dong LF, Zhou RM, Yao J, Song YC, Yang H, Jiang Q, Yan B. Role of long non-coding RNA WAT in proliferation, apoptosis and migration of lens epithelial cells: a clinical and in vitro study. J Cell Mol Med. 2016;20(3):537–48.

16. Xiong Y, Liu Z, Liu Z, Wang S, Shen N, Xing H, Huang T. Long noncoding RNA nuclear paraseptacle assembly transcript 1 interacts with microRNA-107 to modulate breast cancer growth and metastasis by targeting carcinoma palmitoyltransferase1. Int J Oncol. 2019;55(5):1125–36.

17. Wang X, Yu Y, Zhu YC, Wang YK, Li J, Li XY, Ji T, Bai SJ. LncRNA NEAT1 promotes extracellular matrix accumulation and epithelial-to-mesenchymal transition by targeting miR-27b-3p and ZEB1 in diabetic nephropathy. J Cell Physiol. 2019;234(8):12926–33.

18. Dong N. Long noncoding RNA MALAT1 acts as a competing endogenous RNA to regulate TGF-beta2 induced epithelial-mesenchymal transition of lens epithelial cells by a microRNA-26a-dependent mechanism. Biomed Res Int. 2019;2019:1569638.

19. Ma XY, Ma Y, Zhang H, Zhang HJ, Sun MJ. Identification of the lncRNA-miRNA-mRNA network associated with gastric cancer via integrative bioinformatics analysis. Oncol Lett. 2019;18(6):5769–84.

20. Tao L, Yang L, Huang X, Hua F, Yang X. Reconstruction and analysis of the IncRNA-miRNA-mRNA network based on competitive endogenous RNA reveal functional IncRNAs in dilated cardiomyopathy. Front Genet. 2019;10:1149.

21. Li H, Mou Q, Li P, Yang Z, Wang Z, Niu L, Liu Y, Sun Z, Lv S, Zhang B, et al. MiR-486-5p inhibits IL-22-induced epithelial-mesenchymal transition of breast cancer by repressing Dock1. J Cancer. 2019;10(19):4695–706.

22. Gao J, Dai C, Yu X, Yin XB, Zhou F. circ-TFCF4-85 silencing inhibits cancer progression through microRNA-486-5p-targeted inhibition of ABCF2 in hepatocellular carcinoma. Mol Oncol. 2019;14:447–61.

23. Liu B, Sun J, Lei X, Zhu Z, Pei G, Qin L. MicroRNA-486-5p suppresses TGF-beta2-induced proliferation, invasion and epithelial-mesenchymal transition of lens epithelial cells by targeting Smad2. J Biosci. 2017;42(4):575–84.

24. McCarthy AJ, Chetty R, Smaad/DPC4 J. Clin Pathol. 2018;71(8):661–4.

25. Hu HH, Chen DQ, Wang YN, Peng YL, Cao G, Yazini ND, Zhao YY. New insights into TGF-beta/Smad signaling in tissue fibrosis. Chem Biol Interact. 2019;292:76–83.

26. Chen B, Ma J, Li C, Wang Y. Long noncoding RNA KCNQ1OT1 promotes proliferation and epithelialmesenchymal transition by regulation of SMAD4 expression in lens epithelial cells. Mol Med Rep. 2018;18(1):16–24.

27. Nibourg LM, Geelen E, Kuijer R, Hooymans JM, van Kooten TG, Koopmans SA. Prevention of posterior capsular opacification. Exp Eye Res. 2015;136:100–15.

28. Zornatiene R. Posterior capsule opacification: incidence and pathogenesis. Medicina. 2003;39(9):830–7.

29. Xia TF, Chen J, Wu K, Zhang J, Yan Q. Long noncoding RNA NEAT1 promotes the growth of gastric cancer cells by regulating miR-497-5p/PIK3R1 axis. Eur Rev Med Pharmacol Sci. 2019;23(16):6914–26.

30. Shan G, Tang T, Xia Y, Qian HJ. Long non-coding RNA NEAT1 promotes bladder progression through regulating miR-410 mediated HMGB1. Biomed Pharmacother. 2020;121:109248.

31. Peng X, Wei F, Hu X. Long noncoding RNA DLGAP1-AS1 promotes cell proliferation in hepatocellular carcinoma via sequestering miR-486-5p. J Cell Biochem. 2019;121(2):1953–62.

32. Jiao X, Ye J, Wang X, Yin X, Zhang G, Cheng X. KIAA1199, a target of microRNA-486-5p, promotes papillary thyroid cancer invasion by influencing miR-27b and miR-107. Cell Biosci. 2019;9:10.

33. Shen Y, Dong LF, Zhou RM, Yao J, Song YC, Yang H, Jiang Q, Yan B. Role of long non-coding RNA WAT in proliferation, apoptosis and migration of lens epithelial cells: a clinical and in vitro study. J Cell Mol Med. 2016;20(3):537–48.

34. Xiong Y, Liu Z, Liu Z, Wang S, Shen N, Xing H, Huang T. Long noncoding RNA nuclear paraseptacle assembly transcript 1 interacts with microRNA-107 to modulate breast cancer growth and metastasis by targeting carcinoma palmitoyltransferase1. Int J Oncol. 2019;55(5):1125–36.

35. Wang X, Yu Y, Zhu YC, Wang YK, Li J, Li XY, Ji T, Bai SJ. LncRNA NEAT1 promotes extracellular matrix accumulation and epithelial-to-mesenchymal transition by targeting miR-27b-3p and ZEB1 in diabetic nephropathy. J Cell Physiol. 2019;234(8):12926–33.