·Basic Research·

Effects of lentiviral RNA interference-mediated downregulation of integrin-linked kinase on biological behaviors of human lens epithelial cells

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Abstract

• AIM: To investigate the effects of lentivirus (LV) mediated integrin-linked kinase (ILK) RNA interference (RNAi) on biological behaviors of human lens epithelial cells (LECs).

• METHODS: Human cataract LECs and immortalized human LEC line, human lens epithelial (HLE) B-3 cells were transfected by lentiviral vector expressing ILK-specific short hairpin RNA (shRNA) and then stimulated by transforming growth factor-β (TGF-β), the silencing of ILK gene and protein was identified by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot methods; biological behaviors including cell cycle and apoptosis, cell morphology, α-smooth muscle actin (SMA) stress fiber formation and cell migration were examined.

• RESULTS: Remarkable decreases of ILK protein expression were detected in LECs carrying lentiviral ILK-shRNA vector; flow cytometry revealed arresting of cell cycle progression through the G1/S transition and higher apoptosis rate in ILK-RNAi-LV transfected cells. Less α-SMA stress fiber formation and migration was observed in ILK-RNAi-LV transfected LECs.

• CONCLUSION: The present study demonstrated that ILK was an important regulator for LECs proliferation and migration. LV mediated ILK RNAi is an effective way to decrease ILK-regulated cell growth by arresting cell cycle progression and increasing cell apoptosis, as well as, to prevent cell migration by inhibiting TGF-β induced α-SMA stress fiber formation. Thus, LV mediated ILK RNAi might be useful to prevent posterior capsular opacification.

• **KEYWORDS:** human lens epithelial cells; integrin-linked kinase; RNA interference; Lentivirus; posterior capsular opacification
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INTRODUCTION

Cataract is the leading cause of reversible blindness worldwide [1]. Improvement in surgical technique and lens implant design has provided effective visual restoration for cataract patients [2-3]. However, a significant number of patients gradually develop posterior capsule opacification (PCO) after any type of extracapsular cataract surgery, which remains a common clinical problem because it causes secondary loss of vision and needs corrective laser surgery[4-5]. PCO is a fibroproliferative disease. Aberrant proliferation and migration of lens epithelial cells (LECs) are two important pathological factors in PCO formation [6-7]. The normal lens is composed of an anterior monolayer of epithelial cells and an underlying fiber cell population, LEC proliferation is only limited in the germinative zone, a region just anterior to the lens equator [8]. While in PCO, residual LECs within the capsule after cataract surgery are triggered to undergo a transdifferetiation into myofibroblasts, through a phenomenon known as epithelial-to-mesenchymal transition (EMT). These transdifferentiated cells acquire ability to migrate, and move onto the previously cell-free posterior capsule, as the migrating cells proliferate and accumulate, capsular wrinkling and opacities occur, leading to vision impairment [9-10]. Because EMT of LECs plays such an important role in the pathogenesis of PCO, constraining of

EMT process might prove an effective means of inhibiting of PCO. Recent studies have proved that EMT can be regulated by numerous growth factors and hormones in different ways [11-12], in which transforming growth factor-β (TGF-β) is the most principal inducer [13-14]. However, other factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF) and connective tissue growth factor (CTGF), which are present in the ocular environment and promote LECs proliferation, can augment effects of TGF-β in the progression of PCO [15-17], so more attention should be paid to the network of signaling pathways that affect the EMT and proliferation of LECs, with the aim of identifying potential therapeutic targets to inhibit PCO.

Integrin-linked kinase (ILK) is an important molecule in TGF-β induced EMT, and also couples integrins and growth factor receptors to downstream signaling components [18-19]. In murine lens, ILK expression is found being confined to the differentiating LEC of the equatorial region and to fiber cells, which is consistent with its role in EMT of LECs *in vitro*[20]. In a previous study, we examined ILK expression in rat traumatic cataract, and found that uniform and extensive ILK positive staining existed in proliferating and migrating cells, the changed ILK expression pattern implied that ILK might involve in all the procedures of fibrotic tissue formation in lens [21]. Thus we constructed lentiviral vector expressing ILK-specific short hairpin RNA (shRNA) and introduced it into cultured human LECs to make a long-term silencing of ILK. Then the transduced cells were examined for cell cycle, apoptosis, α -smooth muscle actin (SMA) stress fiber formation, and cell migration to further elucidate the therapeutic role of ILK inhibition in PCO.

SUBJECTS AND METHODS

Surgical Procedure The protocol for research involving human tissue was approved by the Xi'an Jiaotong University Ethics Committee, and complied with the guidelines set forth by the Declaration of Helsinki. Ten anterior capsules with LECs attached were obtained by anterior capsulotomy from cataract patients <3 years old during their first extracapsular cataract surgery were used for human LEC (hLEC) culture.

Construction of Lentiviral Vector Expressing Integrin-linked Kinase-specific Short Hairpin RNA Lentiviral vector expressing ILK-specific shRNA was constructed as described previously [22]. The lentivirus (LV) containing the human ILK shRNA-expressing cassette (pGCSIL/ILK) was used as a positive control for LV production and named as ILK-RNA interference (RNAi)-LV in the next experiments. The LV only containing pGCSIL/U6 mock vector (Gikai gene company, Shanghai, China) was also packaged and used as negative control, which named negative control-green fluorescent protein-LV (NC-GFP-LV). Viral concentrations were determined by serial dilutions of the concentrated vector stocks on 293 cells in 96-well plates. The number of GFP-positive cells was measured 4d post-transduction under microscopy. The titers were averaged and typically ranged 8×10^9 TU/mL.

Cell Culture and Lentiviral Transduction Human cataract LECs were cultured as described previously [23]. Briefly, a circular section of the anterior capsule with LECs attached was obtained by capsulotomy from patients younger than 3 years old during cataract surgery and cultured directly without dispersion of the cells in Dulbecco's modified Eagle's medium/F-12 medium (DMEM /F12; Gibco, Grand Island, NY, USA) containing 15% fetal calf serum (FCS; Invitrogen, Carlsbad, CA, USA), 5 μg/mL insulin, 0.5 μg/mL hydrocomrtisone and 50 μg/mL gentamicin. After two weeks, hLECs on the capsules and cells that had migrated onto the bottom of the culture well were issociated by 0.05% trypsin and 0.02 mmol/L EDTA solution, and seeded in 12 well or 24 well culture plates.

Immortalized human LEC line, human lens epithelial (HLE) B-3 cells (ATCC, Manassas, VA, USA) was maintained in Dulbecco's modified Eagle's Minimum Essential Medium (DMEM, Gibco) with 10% FCS. For lentiviral transduction, HLE B-3 cells and primary hLECs were passaged into 12 well plates precoated with laminin (Sigma-Aldrich, St. Louis, MO, USA) at a density of 1×10^5 cells/well. When cells got 30% confluence, the medium was replaced with 1 mL of fresh medium containing LV at an MOI of 10 and 6 μg/mL polybrene (Gikai gene company, Shanghai, China). The medium was replaced with fresh medium on the following day.

Real-time Reverse Transcription-polymerase Chain Reaction Transduced cells were trypsinized and harvested 5d after transduction. Total RNA was isolated using Trizol reagent (Invitrogen-Gibco, Grand Island, NY, USA), and cDNA was acquired according to the M-MLV procedures (Promega) with 2 μg of total RNA. Two-step real-time reverse transcription-polymerase chain reaction (RT-PCR) reactions were performed using the TP800 real-time PCR system (Takara), which included cycle 1 (1X): 95℃, 15s; and cycle 2 (45X): 95℃, 5s; 60℃, 30s; absorbance data were collected at every end of extension (60℃) and graphed by GraphPad Prism 4.0 software, the real-time PCR data were analyzed by $2^{-\Delta\Delta Ct}$.

The specific PCR primer sequences of these genes designed by Beacon designer 2 software were as follow: ILK forward 5'-TCC ACC TGC TCC TCA TCC-3'; ILK reverse 5'-CCT CAT CAA TCA TTA CAC TACGG-3'; actin forward: 5'-GGC GGC ACC ACCA TGTACCCT-3'; actin reverse: 5'-AGG GGC CGG ACT CGT CAT ACT-3'.

Western Blot Analysis Cells were washed 3 times with PBS, homogenized in cell lysis buffer (50 mmol/L Tris, pH7.8, 150 mmol/L NaCl, 1% nonidet-40) containing 10 μL/mL protease inhibitor (Sigma-Aldrich), incubated on ice for 30min, and then centrifuged 30min at 10 000 rpm. The

aqueous supernatants were collected and quantified using Bradford reagent (Sigma-Aldrich). Individual samples, each containing 30 μg protein, were separated on a pre-cast SDS/12.5% polyacrylamide gel in a Tris/HCl buffer (pH 7.4) and blotted onto a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA). The membrane was incubated at room temperature for 1h in phosphate-buffered saline Tween (PBST) buffer [phosphate-buffered saline (PBS) pH 7.4, 0.05% Triton-100], containing 1% (w/v) bovine serum albumin to block nonspecific protein binding sites. After blocking, the blots were probed with anti-ILK or anti-α-SMA antibody (Santa Cruz Biotechnology Inc., CA, USA) overnight at 4℃ followed by 5 washes with PBST. They were then incubated with an anti-rabbit IgG (1:5000, Santa Cruz) for 1h at room temperature. After washing, the immunoreactive bands were detected using a chemiluminescent substrate (NEN Life Science, Boston, MA, USA). Subsequently, the blots were re-probed with a mouse anti-GAPDH control antibody (1:5000, Santa cruz).

Analysis of Cell Cycle and Apoptosis The HLE B-3 cells were trypsinized after 5d lentivral transfection, centrifuged for 5min at 800 rpm, washed with PBS, and fixed with ice cold 70% (vol/vol) ethanol. Cells were stained with propidium iodide (Sigma-Aldrich) and detected using FACSort (Becton Dickson, San Diego, USA), and the percentage of cells in different phases was analyzed using CELLQuest software (Becton Dickson, San Jose, CA, USA). The cell apoptosis was examined by flow cytometry using the Annexin V-FITC apoptosis detection kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions.

Immunofluorescence Staining for α -smooth Muscle Actin Stress Fiber Formation in Transformed Human Lens Epithelial B -3 Cell Line and Human Lens Epithelial Cells HLE B-3 and the first passage hLEC cells with or without lentiviral transduction were seeded into 24-well plates containing glass coverslips pre-coated with laminin (50 μg/mL) at 0.5×10^5 cells per well. Cultures were washed by PBS after cells being attached, and then fed with 1% FCS culture medium containing 10 ng/mL TGF-β (PeproTech, Rocky Hill, NJ, USA) for 2d. Control cells were not exposed to TGF-β treatment. The identification α-SMA stress fiber formation was performed using immunohistochemistry techniques with monoclonal anti-α-SMA (Santa Cruz). The secondary antibody was conjugated with Cy3 (Sigma Saint Louis, USA). Slides were examined by fluorescence microscope (Olympus BX50F, Olympus Optical Co. Ltd., Tokyo, Japan). Control coverslips, treated with either primary or secondary antibody only, were included in each experiment.

Migration Assay Migration assays were performed in Boyden microchemotaxis chambers as previously described[24]. Subconfluent human LECs at passage 1, with or without ILK-RNAi-LV treatment, were released with trypsin and resuspended to a final concentration of 5×10^5 cells/mL in DMEM /F12 containing 2% FCS, cells of TGF-β group were suspended in 2% FCS with 10 ng/mL TGF-β (R&D Systems, Minneapolis, MN, USA). Microchemotaxis chambers (Transwell; Corning Costar, Acton, MA, USA) with polycarbonate membrane filter (8.0 μm pore size, 0.33 cm^2 growth area) were precoated with laminin (50 μg/mL) at 37℃ for 1h and air dried. Medium containing 10% FCS was added to the lower chambers as chemoattractant, the upper wells received 50 μL of the final cell suspension and the chambers were incubated at 37℃ in a humid atmosphere for 48h. The filters were removed and the upper surface of the membrane filters was scraped with a cotton-tripped stick to remove nonmigrated cells and then fixed with 4% paraformaldehyde for 15min at room temperature. Migrated cells which pass through the transwell filter pores and attached on the lower surface of the filters were counted in five no overlapping fields after nuclear staining with hematoxylin. The experiments were carried out in triplicate cultures.

Statistical Analysis Experiments were analyzed statistically using the SPSS statistical package, version 11.0. *t* -test was used for statistical analysis. A *P* value of <0.05 was considered to be statistically significant.

RESULTS

Integrin -linked Kinase -RNA Interference -lentivirus Transfection Inhibited the Expression of Integrin-linked Kinase mRNA and Protein in Cultured Human Lens Epithelial Cells Positive expression of ILK protein was identified in cultured human LECs and immortalized LEC line HLE B-3 by Western blot. Levels of ILK expression in both cells could satisfy the following RNAi experiment (data not shown). The LV containing the human ILK shRNA-expressing cassette (sequence: 5'-CGAAGCTCAAC GAGAATCA-3') showed the most ILK gene silencing efficacy among the four candidate target sequences by screening in 293 cells, it's named ILK-RNAi-LV, the negative control containing pGCSIL/U6 mock vector only was named NC-GFP-LV, as we have constructed previously[22]. Cells were trypsinized and harvested 5d after ILK-RNAi-LV construct was transfected into HLE B-3 cells. ILK mRNA expression in the cells was compared to that in untransfected cells and negative transfection (NC-GFP-LV) by quantitative RT-PCR. As a result, cells with ILK-RNAi-LV transfection showed decreased level of ILK mRNA expression for about 82% (Figure 1A). To further confirm the specificity of ILK-RNAi-LV-mediated silencing of ILK, the detection of ILK protein expression of these cells was determined by Western blot. As shown in Figure 1B, ILK protein expression of cells with ILK-RNA-LV transfection, decreased

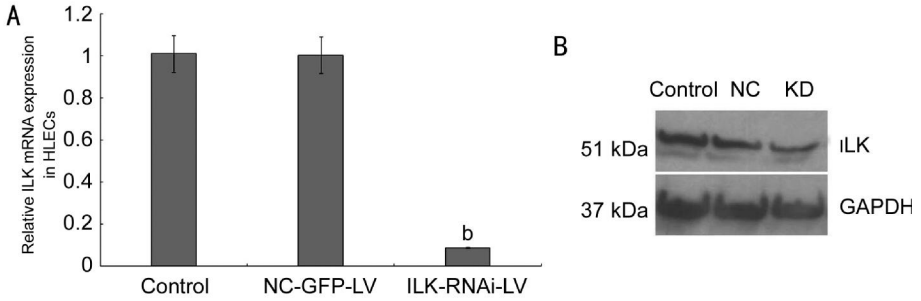

Figure 1 ILK-RNAi-LV transfection silenced the mRNA and protein expression of ILK in HLE B-3 cells A: Real-time RT-PCR data were analyzed by $2^{-\Delta\Delta Ct}$. ILK mRNA expression decreased significantly in cells transfected with ILK-RNAi-LV, as compared to that in untransfected cells (*vs* control group, t=17.616) or negative transfection (*vs* NC-GFP-LV group, t=18.838). Data were expressed as means±SEM value (n=3, asterisks indicated ^{b}P<0.01). B: ILK protein expression detected by Western blot. NC stands for NC-GFP-LV group, while KD represented cells with ILK knockdown through ILK-RNAi-LV transfection.

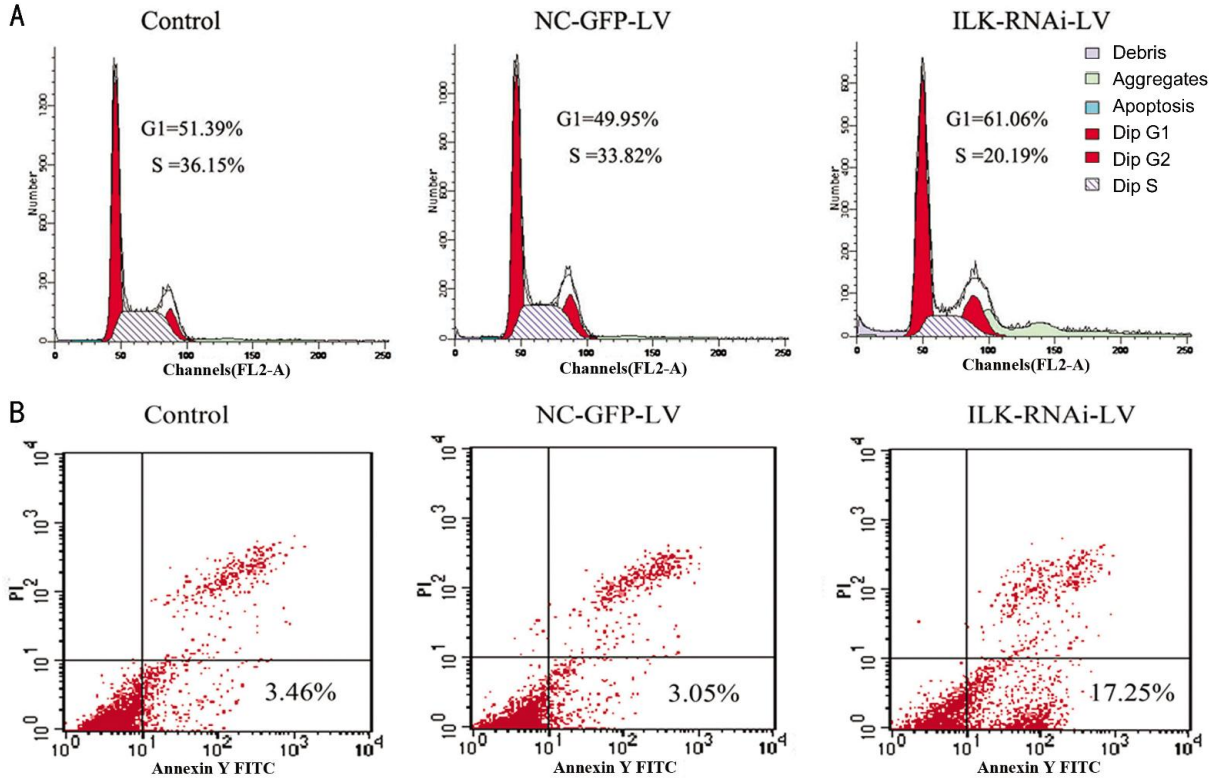

Figure 2 Effect of ILK-RNAi-LV transfection on human LEC cycle and apoptosis A: ILK-RNAi-LV transfection inhibited transition of HLE B-3 cells from G1 to S phase. B: Flow cytometric analyses showed effect of ILK-RNAi-LV transfection on apoptosis of LECs. Cells of high reactivity with FITC and low reactivity with PI (right lower quadrant) are the apoptotic cells.

significantly than that of control cells. The results of quantitative RT-PCR and Western blot assays revealed that expression of ILK in HLE B-3 cells was markedly decreased, which demonstrated that RNAi technique mediated by LV was an effective way to modulate the ILK expression in cultured LECs.

Effect of Integrin-linked Kinase-RNA Interference-lentivirus Transfection on Human Lens Epithelial Cell Cycle and Apoptosis We examined the effect of ILK-RNAi-LV transfection on the cell cycle and apoptosis of LECs using HLE B-3, which remained constant proliferation rate in passage cells. Flow cytometry showed that ILK-RNAi-LV transfection inhibited cell proliferation. Compared with control cells, the percentage of the cell population in the S-phases in ILK-RNAi-LV transfected cells decreased significantly, and percent of the cell population in the G1 phase increased (Figure 2A). Flow cytometry also revealed 17.25% apoptosis rate in ILK-RNAi-LV transfected cells, while apoptosis rate of control and NC-GFP-LV group was 3.46% and 3.05% respectively, much lower than that of ILK-RNAi-LV group. Thus, ILK silencing induced inhibition of LEC proliferation resulted from arresting of cell cycle progression through the G1/S transition and increased apoptosis (Figure 2B).

Integrin-linked Kinase-RNA Interference-lentivirus Transfection Inhibited α-smooth Muscle Actin Expression Stimulated by Transforming Growth Factor-β in Cultured Human Lens Epithelial Cells

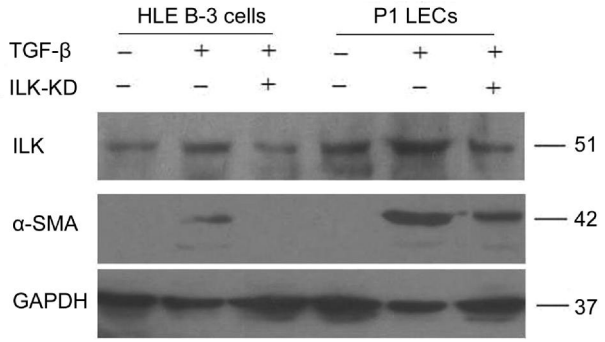

Figure 3 Downregulation of α-SMA protein by ILK-RNAi-LV transfection in LECs Western blot analysis showed that TGF-β stimulated ILK expression higher and induced α-SMA expression in HLE B-3 cells and the first passage hLECs. ILK-RNAi-LV transfection caused synchronous decrease of ILK and α-SMA protein expression. ILK-KD represented cells with ILK knockdown through ILK-RNAi-LV transfection.

Western blot analysis revealed changes of ILK and a-SMA expression stimulated by TGF-β in cells with or without ILK-RNAi-LV transfection (Figure 3). Basic amount of ILK expression was detected in immortalized HLE B-3 cells and the first passage hLECs, while that of the latter was slightly higher. When cells were treated with TGF-β ILK expression got stronger, accompanied with the expression of α-SMA. However, in cells with ILK-RNAi-LV transfection, ILK expression were inhibited, in parallel with downregulation of ILK, α-SMA expression were also decreased, which indicated that TGF-β might induce α-SMA expression through ILK.

Effect of Integrin-linked Kinase-RNA Interference-lentivirus Transfection on α-smooth Muscle Actin Stress Fiber Formation in Human Lens Epithelial Cells α-SMA stress fiber formation in cultured hLECs and HLE B-3 cells were observed by indirect immunofluorescence staining under microscope. The primary hLECs and immortalized HLE B-3 cells exhibited typical epithelial cellular appearance and both types of cells could be stimulated by TGF-β to transform. However, cellular morphology and α-SMA expression pattern were different between the two transformed cells as shown in Figure 4A. Transformed HLE B-3 cells were spindle shaped and showed diffuse granular α-SMA expression in the cytoplasm. The primary cultured hLECs elongated when stimulated by TGF-β with apparent filamentous α-SMA staining in the peripheral cells which spread out and got large and irregular shape. Compared with the immortalized HLE B-3 cells, the naturally cultured hLECs were easier to transform to myofibroblasts with α-SMA microfilaments forming stress fibers, so they were more suitable for the follow experiments about cell motility.

Effect of ILK-RNAi-LV transfection on α-SMA stress fiber formation was identified in the first passage hLECs and showed in Figure 4B. Cultures attached to the coverslips and

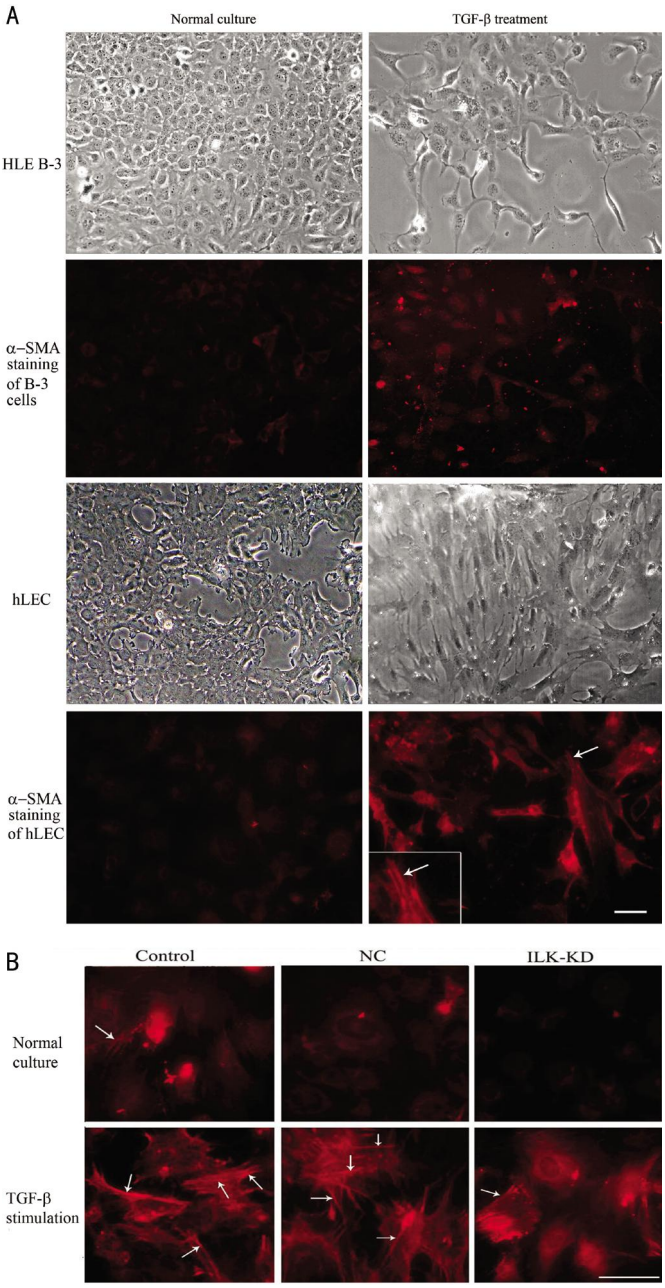

Figure 4 Effect of ILK-RNAi-LV transfection on α-SMA stress fiber formation in the first passage hLECs A: Cell morphologic change and α-SMA stress fiber formation stimulated by TGF-β in immortalized HLE B-3 cells and cultured hLECs. Transformed HLE B-3 cells were spindle shaped and showed diffuse granular α-SMA expression in the cytoplasm. The primary cultured hLECs elongated when treated by TGF-β with apparent filamentous α-SMA staining in the peripheral cells which spread out and got large and irregular shape. Arrows indicate α-SMA-positive stress fibers (scale bar=50 μm, insets depict expansion of the area indicated by arrows). B: ILK-RNAi-LV transfection showed inhibitory effect on TGF-β induced cell phenotype transition, as epithelial cellular feature was mainly remained in the transduced cells, with slight diffuse granular α-SMA staining and minute α-SMA microfilament; arrows indicate α-SMA-positive stress fibers (scale bar=100 μm).

maintained in different culture conditions were fixed and stained. The first passage hLECs were still negative for

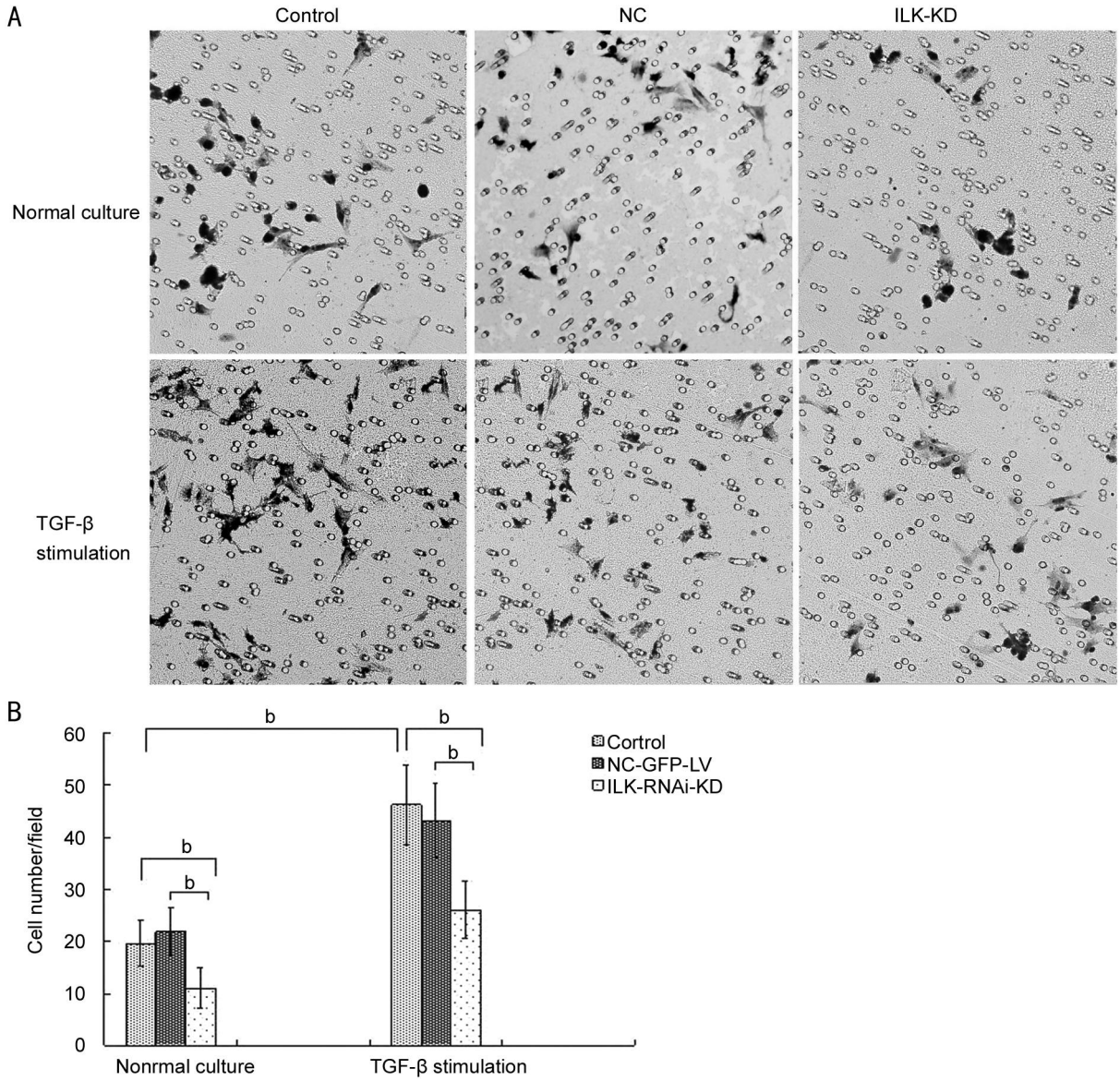

Figure 5 Effect of ILK silencing on the migration of hLECs A: Cell migration assay by Boyden microchemotaxis chambers; B: Statistical analyses revealed that TGF-β treatment significantly promoted cell migration, and ILK knock down by ILK-RNAi-LV transfection (ILK-KD groups) robustly inhibited cell motility. $^{b}P<0.01$, $n=5$.

α-SMA staining under normal condition, when stimulated by TGF-β cells lost their typical epithelial morphology, got large and showed more processes which were easily recognized by strong positive filamentous α-SMA staining, the α-SMA microfilaments forming stress fibers also appeared in the cytoplasm. ILK-RNAi-LV transfection showed inhibitory effect on TGF-β induced cell phenotype transition, as epithelial cellular feature was mainly remained in the transduced cells, with slight diffuse granular α-SMA staining and minute α-SMA microfilament; negative transfection couldn't prevent TGF-β induced cell phenotype change, while α-SMA stress fiber formation appeared in cells transfected with NC-GFP-LV.

Integrin –linked Kinase –RNA Interference –lentivirus Transfection Inhibited Human Lens Epithelial Cell Migration Cultured hLECs at the first passage were used to analyze cell migration by Boyden technique, as described in the methods section and showed in Figure 5. TGF-β treatment stimulated cell migration, with about two-fold increases in the number of migrating cells ($t=19.221$, $P<0.001$). Cells transfected with ILK-RNAi-LV displayed less migration compared with non-transfected control cells, no matter under normal culture($t=23.533$, $P<0.001$) or TGF-β stimulation ($t=18.611$, $P<0.001$). Cells transfected with NC-GFP-LV control vector didn't exhibited apparent difference of cell motility under normal culture or TGF-β stimulation, comparing to cells without virus transfection, which indicated decreased hLEC motility due to downregulation of ILK by LV-mediated RNAi, other than LV vector transfection.

DISCUSSION

PCO is a common clinical problem after any type of extracapsular cataract surgery, caused by aberrant proliferation and migration of LECs after lens capsule

opening. The reason that LECs migrate along the capsule has been searched by many studies and accumulating evidence suggests EMT, a phenomenon by which epithelial cells lose their epithelial-specific markers, undergo cytoskeletal remodeling and acquire α-SMA expression, is the cause of the abnormal cellular behavior [25-27]. As an orchestrated process, EMT is regulated by multiple regulators such as growth factors, cytokines, hormone, adhesion molecules, ECM and intracellular signaling molecules[28]. Recent studies demonstrate that TGF-β is important in the pathogenesis of PCO because it is a sole factor that can initiate and complete the entire EMT course [29]. However, TGF-β also inhibits cell growth [30] and its up-regulation cannot explain the exuberant cell proliferation in PCO. In fact, other factors such as FGF and integrins, also modulate the progression of PCO by their direct influence on LECs growth, or by augmenting effects of TGF-β[15-18]. So, optimal pharmacological targets should direct to the crossroads connecting these causative agents to constrain both EMT process and abnormal cell proliferation, to prevent PCO formation.

ILK is a serine/threonine kinase, which interacts with the cytoplasmic tails of the integrin β1/β3 subunits and plays a significant role in cell adhesion, motility, differentiation and survival [31]. ILK is involved in the regulation of integrin binding properties as well as the in TGF-β induced EMT, whose multiple functions profit from its special position coupling integins and growth factor receptors[32]. ILK activity can be modulated by cellular interaction with extracellular matrix components and growth factors, or integrin clustering[33]. In a previous study, up-regulation of ILK was found to be accompanied with proliferating and migrating LECs in rat traumatic cataract model, which indicated the important controlling role of ILK in lens fibroproliferative disorders[21].

We examined effects of ILK on LECs with LV mediated ILK RNAi technique *in vitro*. RNAi is a useful genetic tool for silencing gene expression, but direct transfection of double-stranded small interfering RNA (siRNA) achieves only a transient reduction in target gene expression in cell culture models [34-35], which is difficult to explore the real influence of target gene in an evolutionary event. LV is a good mediator to get lasting RNAi due to its high transfection efficiency in a wide variety of primary human cells and permanent integration into the genomic DNA of transduced cells [36-37]. The effect of ILK on cell proliferation was identified in HLE B-3 cells, an immortalized human LEC line with stable growth rate. Decreased cell growth appeared in cells with ILK RNAi and the reason for the inhibited growth was explained by further flow cytometry examinations, which demonstrated that silencing of ILK gene lead to holdup of cell cycle progression through the G1/S transition and increased apoptosis, these data supported the previous finding by Weaver *et al* [38] that ILK had pro-survival activity, and also indicated that LV mediated ILK RNAi was an effective method to prohibit ILK-regulated cell proliferation.

Naturally cultured hLECs were used to detect change of cell migration because the immortalized HLE B-3 cells were weakly responding to TGF-β to generate α-SMA stress fiber, which is a sensitive marker of epithelial cell phenotype change and major component of microfilaments controlling cell morphology and motility[39-40]. The primary and 1st passage cultured hLECs displayed the same morphology as that of B-3 cells, both without filamentous α-SMA expression under normal culture, but TGF-β induced α-SMA stress fiber formation appeared in the primary hLECs and got more apparent in the first passage cells. Silencing of LIK gene inhibited the effect of TGF-β stimulation, compared with cells without or with negative transfection, cells with lentiviral vectors expressing ILK-specific shRNA had much less positive α-SMA staining under TGF-β treatment, in parallel with the decreased α-SMA stress fiber formation, marked decrease of cell migration was also observed in cells carrying the lentiviral shRNA vector.

Taken these data together, our study demonstrated that ILK was an important regulator for LECs proliferation and migration. Lentiviral ILK-specific shRNA mediated ILK gene silencing is an effective way to decrease ILK-regulated cell growth by arresting cell cycle progression and increasing cell apoptosis; it is also effective to prevent cell migration by inhibiting TGF-β induced α-SMA stress fiber formation. So, LV mediated ILK RNAi might be useful to treat lens fibroproliferative diseases.

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