Original Article

TRPM7 Elicits Proliferation and Differentiation of Human Lens Epithelial Cells through the TGF-β/Smad Pathways

(human lens epithelial cells / proliferation and differentiation / TGF-β/Smad pathway / TRPM7)

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Abstract. Epithelial-mesenchymal transition (EMT) plays a crucial role in the development of cataract. This study aimed to explore the effects of TRPM7 on the proliferation and differentiation of human lens epithelial cells. TRPM7 was over-expressed in LECs treated with TGF-β2. Down-regulation of TRPM7 attenuated the increase in cell viability and cell proliferation induced by TGF-β2. The LEC migration induced by TGF-β2 was also repressed by down-regulation of TRPM7. Epithelial-specific protein E-cadherin was up-regulated through knock-down of TRPM7. EMT-specific proteins, α-SMA, fibronectin and vimentin, were down-regulated through knock-down of TRPM7. Moreover, phosphorylation of Smad2 and Smad3 was also prevented by inhibition of TRPM7. Therefore, TRPM7 elicited LEC proliferation and EMT through enhancing activation of the TGF-β/Smad pathways, implying a new therapeutic target for cataract.

Introduction

Cataract is an age-related eye disease due to the loss of lens transparency, and nearly 50 % of blindness is caused by cataract in middle- and low-income countries (Liu et al., 2017). Introduction of cataract surgery has greatly reduced the rate of blindness in the past 20 years and is the most cost-effective treatment for cataract patients (Liu et al., 2017). Therefore, the standard of care for cataract is removing the cataractous lens by surgery (Liu et al., 2017). Cataract is a fibrotic eye disease mainly caused by myofibroblast transdifferentiation in ocular fibrosis (Shu and Lovicu, 2017). Epithelial-mesenchymal transition (EMT) of lens epithelial cells (LECs) plays an essential role in the formation of cataract, including anterior subcapsular cataract (ASC) and posterior capsule opacification (PCO) (Shu and Lovicu, 2017). During the development of cataract, LECs abnormally proliferate and differentiate into fibroblastic cells (Lovicu et al., 2016). Therefore, inhibition of cell proliferation and EMT will be an effective treatment for the management of cataract.

Transforming growth factor β2 (TGF-β2) is highly activated in the eye and is abundantly present in aqueous humour (de Iongh et al., 2005). TGF-β2 is known to trigger cell proliferation and EMT of LECs, and the Smad signalling pathway is a key regulator in the TGF-β signal transduction (Roberts et al., 2006; Liu and Jiang, 2020). Knock-down of Smad2 and Smad3 blocked TGF-β-induced cell proliferation, migration and differentiation in LECs (Roberts et al., 2006; Liu and Jiang, 2020). Blockade of TGF-β/Smad signal transduction might prevent the development of cataract.

Transient receptor potential melastatin-subfamily member 7 (TRPM7) is a chanzyme that modulates the permeability of ion channels to divalent cations Mg^{2+}, Ca^{2+}, and Zn^{2+} (Xu et al., 2015). TRPM7 has been revealed to be involved in the pathological process of fibrotic diseases (Xu et al., 2015). Suppression of TRPM7 expression reduced fibrotic processes in many organs, including the heart, liver and lung, thereby preventing organ dysfunction induced by fibrosis (Xu et al., 2015). In addition, TRPM7 could increase the extracellular concentration of Ca^{2+}, thus elevating the spontaneous Ca^{2+} influx, resulting in acceleration of cell growth in human retinoblastoma cells (Hanano et al., 2004). However, it is still unclear whether TRPM7 modulates the cataract formation. This study aimed to explore the effects of TRPM7 on the proliferation and differentiation of human lens epithelial cells, which can provide a new insight into the clinical management of cataract.

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Abbreviations: ASC – anterior subcapsular cataract, EMT – epithelial-mesenchymal transition, LEC – lens epithelial cells, POC – posterior capsule opacification, TGF-β – transforming growth factor β, TRPM7 – transient receptor potential melastatin-subfamily member 7.

Material and Methods

Cell culture and transfection

Human LEC line SRA01/04 cells were purchased from Ningbo Mingzhou Biotechnology Co., Ltd. (Ningbo, China). Dulbecco's modified Eagle's medium (DMEM, SenBeiJia Biological Technology, Nanjing, China) was supplemented with 10 % foetal bovine serum (FBS, Invitrogen, Waltham, MA) and 1% penicillin-streptomycin solution (100×, Procell, Wuhan, China). SRA01/04 cells were cultured in DMEM at 37 °C with 5 % CO_2.

TGF-β2 (R&D Systems, Minneapolis, MN) was dissolved into phosphate-buffered solution (PBS) at a stock concentration of 1 mg/ml. SRA01/04 cells were treated with 10 ng/ml of TGF-β2 for 24 h to induce up-regulation of TRPM7 expression.

Silencing RNAs for TRPM7 (si-TRPM7) and its scramble RNAs (si-NC) were obtained from Suzhou Biosyn Biotechnology Co., Ltd. (BiosynTech, Suzhou, China) and dissolved into nano-pure water. One hundred ng/ml of si-TRPM7/si-NC was transfected into SRA01/04 cells using RNAifectin Transfection Reagent (AmyJet, Hubei, China) following the manufacturer's instructions. SRA01/04 cells were then cultured for another 24 h.

CCK-8 and EdU assays

Cell viability was tested using a commercial Cell Counting Kit-8 (CCK-8) kit (Boster Bio, Pleasanton, CA) and the results were measured using a Fluoroskan microplate fluorometer (Thermo Fisher, Waltham, MA). Cell proliferation was detected using an EdU Staining Proliferation Kit (iFluor 647, Abcam, Cambridge, UK) (Beyotime, Shanghai, China). EdU-positive cells were imaged and counted under a fluorescent microscope (Leica, Wetzlar, Germany).

Cell scratch assay

SRA01/04 cells were cultured in 6-well plates. After treatment, a straight line was scratched to disrupt the monolayer of the cells using a sterile 10 μl pipette tip on the bottom of each well. The cells were cultured for 24 h after scratching. The width of the scratch was then observed and measured under the microscope (Leica).

Western blotting

Total protein was extracted using a total protein extraction kit (Solarbio, Beijing, China). NanoOrange™ Protein Quantitation Kit (Thermo Fisher) was used to measure the protein concentration. Five μg of total proteins was loaded and electrophoresed using 10 % NuPAGE Bis-Tris and Bolt Bis-Tris Plus Gels (Thermo Fisher). The proteins were transferred onto nitrocellulose membranes (Invitrogen) followed by blockade of membranes using StartingBlock™ (TBS) Blocking Buffer (Thermo Fisher). The membranes were then probed with primary and secondary antibodies. Finally, the protein signals were developed using the Pierce™ ECL Western Blotting Substrate (Thermo Fisher) and imaged using GelView 6000Plus Imaging Systems (Biolight Biotechnology Co., Guangzhou, China). The primary antibodies (Santa Cruz, Dallas, TX) used in this study were: α-SMA (sc-53412, 1 : 500 dilution), fibronectin (sc-8422, 1 : 1500 dilution), E-cadherin (sc-8426, 1 : 1000 dilution), vimentin (sc-6260, 1 : 500 dilution), p-Smad2 (#3101, 1 : 500 dilution, Cell Signaling, Danvers, MA), Smad2 (sc-393312, 1 : 2000 dilution), p-Smad3 (sc-517575, 1 : 500 dilution), Smad3 (sc-101154, 1 : 1000 dilution) and GAPDH (sc-59540, 1 : 3000 dilution).

Statistical analysis

All results were presented as mean ± SEM. Statistical comparison was performed using GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA). Statistical difference was analysed using the Student's *t*-test (two groups) and one-way ANOVA (more than two groups). Statistical significance was defined as $P < 0.05$.

Results

Knock-down of TRPM7 inhibited TGF-β2-induced cell proliferation and migration in SRA01/04 cells

Treatment with TGF-β2 up-regulated the protein expression of TRPM7 ($P < 0.01$; Fig. 1A). The TGF-β2-induced over-expression of TRPM7 was reduced by si-TRPM7 ($P < 0.01$; Fig. 1B). Cell viability was increased by TGF-β2, and this increase was inhibited by down-regulation of TRPM7 ($P < 0.01$; Fig. 1C). The number of EdU-positive cells was elevated by TGF-β2, and this elevation was prevented by si-TRPM7 ($P < 0.01$; Fig. 1D). The wound width was reduced after the treatment with TGF-β2 ($P < 0.01$; Fig. 2). Transfection of si-TRPM7 into SRA01/04 cells increased the wound width ($P < 0.01$; Fig. 2). These results suggested that knock-down of TRPM7 inhibited TGF-β2-induced cell proliferation and migration in SRA01/04 cells.

Knock-down of TRPM7 suppressed TGF-β2-induced EMT in SRA01/04 cells

Treatment with TGF-β2 up-regulated the protein expression of α-SMA, fibronectin and vimentin ($P < 0.01$; Fig. 3). Transfection of si-TRPM7 into SRA01/04 cells suppressed the TGF-β2-induced up-regulation of α-SMA, fibronectin and vimentin ($P < 0.01$; Fig. 3). The protein expression of E-cadherin was reduced by TGF-β2, and this reduction was reversed by inhibition of TRPM7 ($P < 0.01$; Fig. 3). These results demonstrated that knock-down of TRPM7 suppressed TGF-β2-induced EMT in SRA01/04 cells.

Knock-down of TRPM7 attenuated TGF-β2-induced activation of the TGF-β/Smad pathway in SRA01/04 cells

Treatment with TGF-β2 up-regulated phosphorylation of Smad2 and Smad3 in SRA01/04 cells ($P < 0.01$; Fig. 4). Transfection of si-TRPM7 suppressed the TGF-β2-induced phosphorylation of Smad2 and Smad3 in SRA01/04 cells ($P < 0.01$; Fig. 4). These data suggested that knock-down of TRPM7 attenuated TGF-β2-induced activation of the TGF-β/Smad pathway in SRA01/04 cells.

Discussion

Although cataract surgery is the standard management, almost 30 % of cataract patients will develop PCO, which is the most frequent complication of cataract surgery (Zhang and Xie, 2020). Since both ASC and PCO are caused by abnormal proliferation and differentiation of LECs, inhibition of LEC aberrant growth and EMT might be a potential method for prevention and management of cataract. TGF-β-induced EMT in LECs is an ideal model for cataract in preclinical studies (de Iongh et al., 2005). Therefore, this model was adopted in the present study. Our data demonstrated that TRPM7 was over-expressed in LECs treated with TGF-β2. Down-regulation of TRPM7 attenuated the increase in cell viability and cell proliferation induced by TGF-β2. The LEC migration and EMT were also repressed by down-regulation of TRPM7. Moreover, phosphorylation of Smad2 and Smad3 was also inhibited by down-regulation of TRPM7. Altogether, these results indicated that TRPM7 elicited LEC proliferation and EMT through enhancing activation of TGF-β/Smad pathways, implying a new therapeutic target for cataract.

The results of this study showed that TRPM7 was over-expressed in TGF-β2 LECs, suggesting that TRPM7

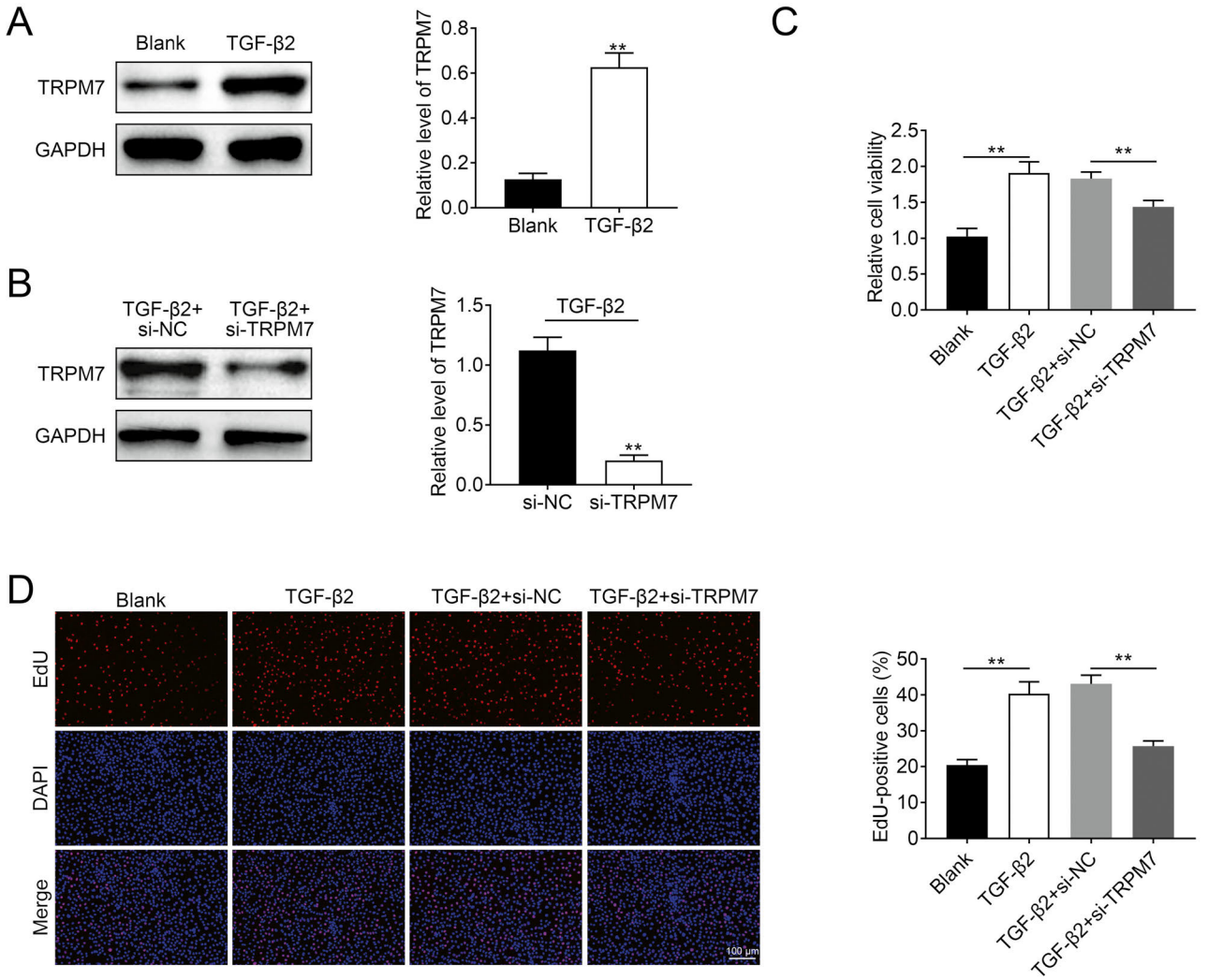

Fig. 1. Knock-down of TRPM7 inhibited TGF-β2-induced cell proliferation in SRA01/04 cells. (**A**) TGF-β2 increased expression of the TRPM7 protein in SRA01/04 cells; (**B**) si-TRPM7 inhibited TGF-β2-induced up-regulation of TRPM7 in SRA01/04 cells; (**C**) si-TRPM7 inhibited TGF-β2-induced increment of EdU-positive cells in SRA01/04 cells. **P < 0.01. si-TRPM7: silencing RNAs for TRPM7; si-NC: scramble of si-TRPM7.

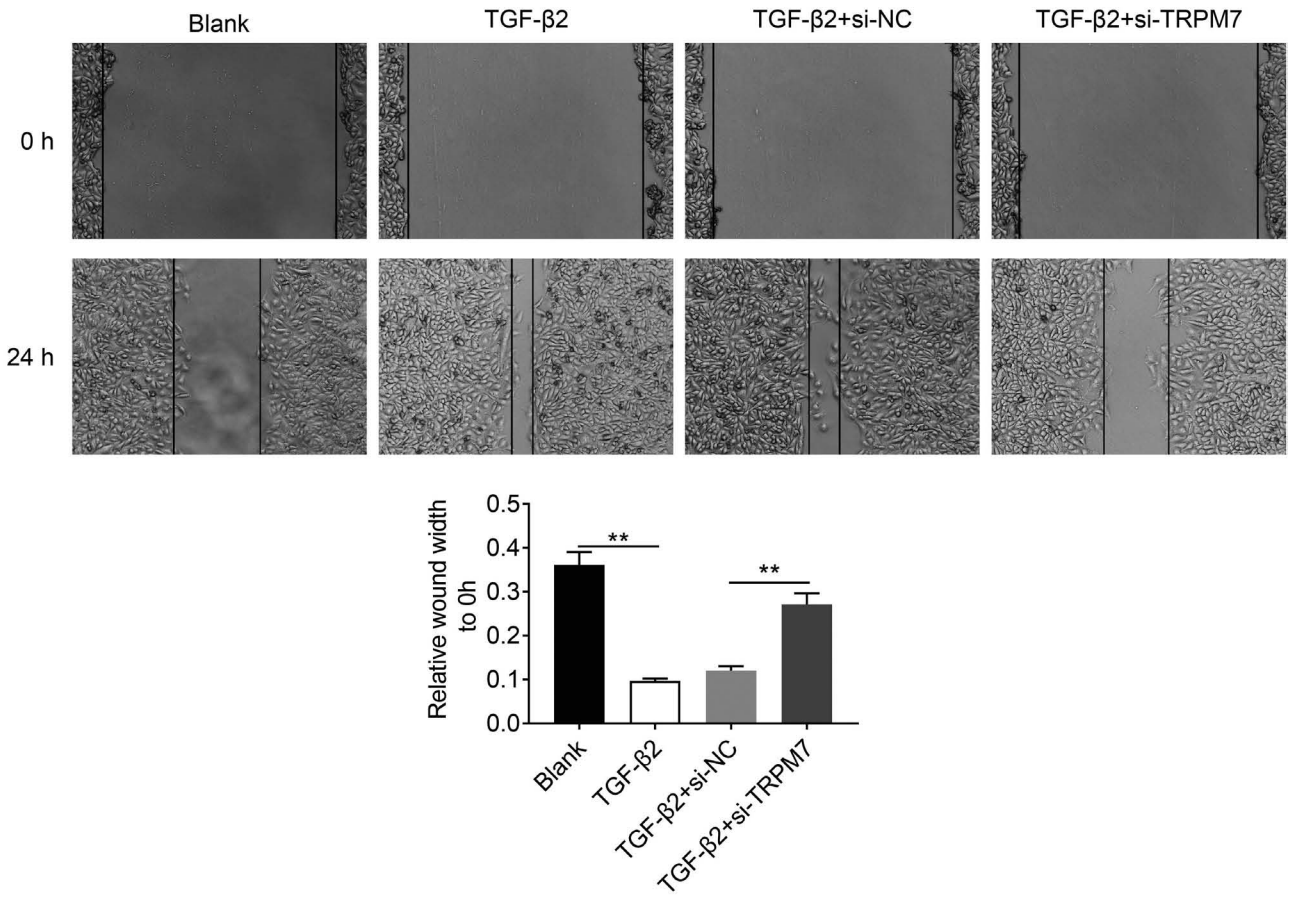

Fig. 2. Knock-down of TRPM7 prevented TGF-β2-induced cell migration in SRA01/04 cells. **P < 0.01.

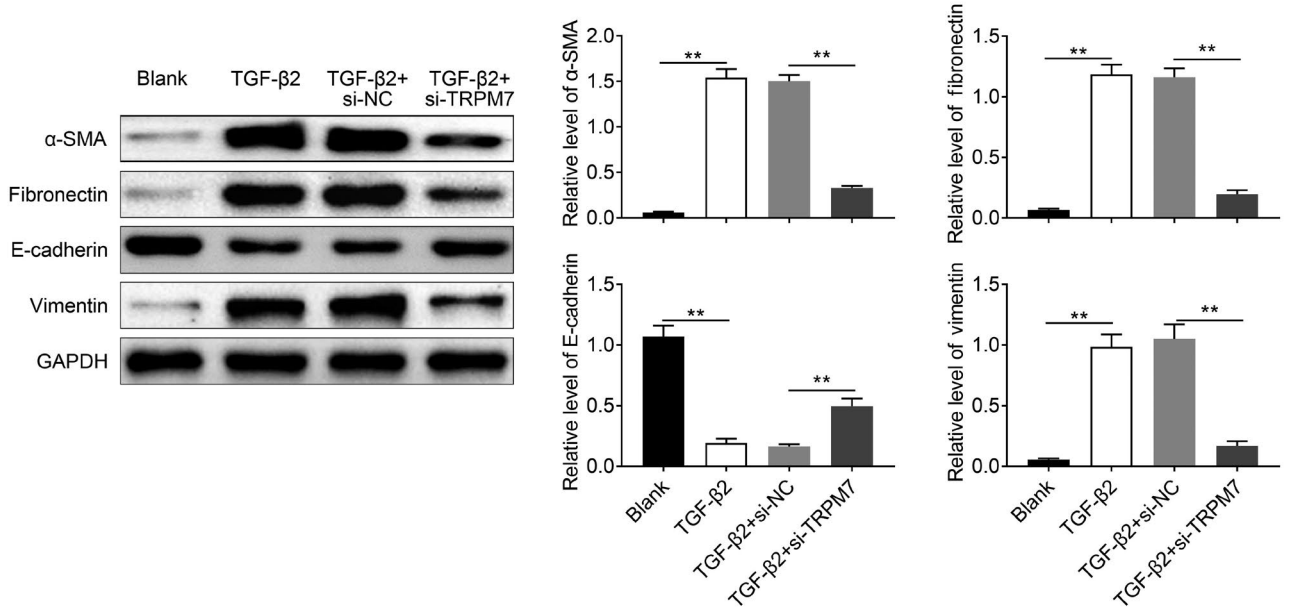

Fig. 3. Knock-down of TRPM7 suppressed TGF-β2-induced EMT in SRA01/04 cells. si-TRPM7 reversed TGF-β2 induced up-regulation of α-SMA, fibronectin and vimentin and down-regulation of E-cadherin in SRA01/04 cells. **P < 0.01.

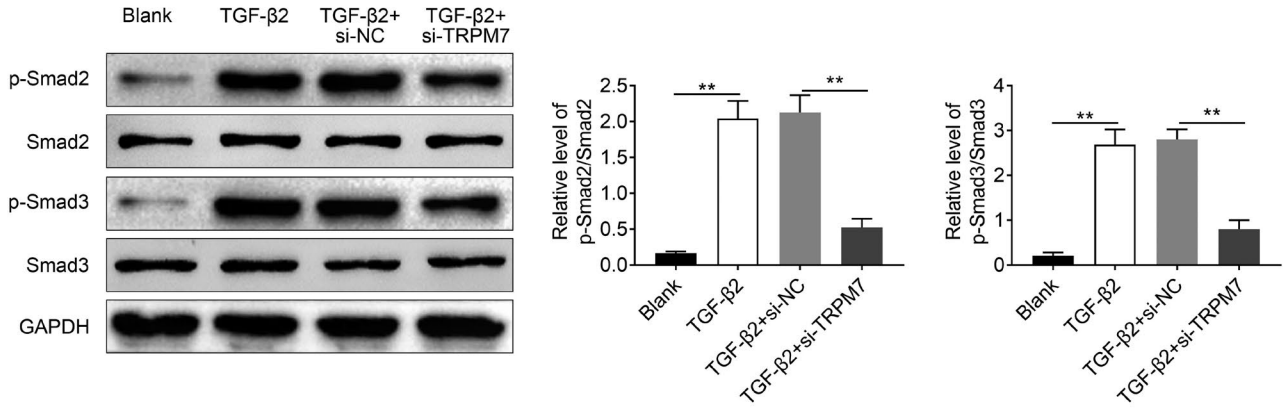

Fig. 4. Knock-down of TRPM7 attenuated TGF-β2-induced activation of the TGF-β/Smad pathway in SRA01/04 cells. si-TRPM7 reversed TGF-β2-induced phosphorylation of Smad2 and Smad3 in SRA01/04 cells. **P < 0.01.

may be involved in the cataract formation. As mentioned above, aberrant LEC proliferation and migration is a key manifestation of cataract formation (Menapace, 2008). Inhibition of TRPM7 repressed the LEC proliferation and migration, demonstrating a potential effect on the progression of cataract. EMT of LECs is another key manifestation of cataract formation, which contributes to the formation of cataract plaques (Liu and Dong, 2008). During EMT, the expression of epithelial markers (E-cadherin, ZO-1, and claudins, etc.) was down-regulated and mesenchymal markers (α-SMA, fibronectin, vimentin and N-cadherin) were over-expressed (Gonzalez and Medici, 2014). In this study, knock-down of TRPM7 attenuated TGF-β2-induced down-regulation of E-cadherin and up-regulation of α-SMA, fibronectin and vimentin, suggesting that TRPM7 is involved in the process of EMT in LECs. Taken together, up-regulation of TRPM7 in LECs promoted TGF-β2-induced cell proliferation, migration and EMT, implying that TRPM7 might contribute to the development and formation of cataract.

Small mother against decapentaplegic (Smad) is a class of transcription factors that could bind to DNA and mediate protein-protein interactions to promote fibrosis (Hu et al., 2018). Smad isoforms Smad2 and Smad3 are key mediators for TGF-β signal transduction (Flanders, 2004). In response to TGF-β, Smad2 and Smad3 are recruited to the TGF-β receptors and then phosphorylated by TGF-β receptor kinases (Flanders, 2004; Massagué et al., 2005). In this study, phosphorylation of Smad2 and Smad3 was induced by TGF-β2, and knock-down of TRPM7 reduced phosphorylation of Smad2 and Smad3, indicating that TRPM7 modulated the signal transduction of TGF-β/Smad pathways in TGF-β2-treated LECs. The data of this study proved that TRPM7 was involved in the regulation of cell proliferation, migration and differentiation in TGF-β2-treated LECs, and these effects were mediated by the TGF-β/Smad pathways.

In conclusion, over-expression of TRPM7 was observed in LECs treated with TGF-β2. Knock-down of TRPM7 attenuated TGF-β2-induced cell proliferation, migration and EMT in LECs. Further experiments proved that inhibition of TRPM7 down-regulated expression of phosphorylated Smad2 and Smad3. Thus, TRPM7 promoted LEC proliferation and differentiation through activation of the TGF-β/Smad pathways. These findings provide a new understanding and target for management of cataract.

Competing interests

The authors state that there are no conflicts of interest to disclose.

Contribution of authors

Guang Yang designed the study and carried it out, Yi Wu supervised the data collection, analysed the data, interpreted the data, Shaohua Tang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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