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Original Research Article

Catalpol represses the migration, proliferation and epithelial-mesenchymal transition of TGF-β2-stimulated lens epithelial cells via TGF-β/Smad and Notch1 signaling pathways

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Abstract

Purpose: *To investigate the role of catalpol in posterior capsule opacification (PCO).*
Methods: *Human lens epithelial cells (SRA01/04) were treated with TGF-β2 or co-treated with TGF-β2 and different concentrations of catalpol. Cell migration and viability were assessed via wound healing and CCK8, respectively. Epithelial-mesenchymal transition and the underlying mechanism of action were evaluated using western blot.*
Results: *Treatment with TGF-β2 significantly increased cell viability and promoted the migration of SRA01/04 ($p < 0.001$). However, catalpol significantly reduced cell viability and repressed the migration of TGF-β2-stimulated SRA01/04 ($p < 0.001$). Moreover, TGF-β2-stimulated increases in fibronectin, α-smooth muscle actin (α-SMA), snail and vimentin. Decreases of E-cadherin and connexin-43 in SRA01/04 were reversed by catalpol. Moreover, TGF-β2-stimulated the up-regulation of p-smad2/3, while SRA01/04 was down-regulated by catalpol, but attenuated TGF-β2-stimulated increases in Notch1 and Jagged1 in SRA01/04.*
Conclusion: *Catalpol inhibits TGF-β2-stimulated migration, proliferation and epithelial-mesenchymal transition of SRA01/04 through the inactivation of TGF-β/Smad and Notch1 signaling. Catalpol might be a novel preventive agent for PCO. However, the effect of catalpol on animal models of PCO should be investigated further.*

Keywords: *Catalpol, TGF-β2, Lens epithelial cells migration, Proliferation, Epithelial-mesenchymal transition*

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INTRODUCTION

Posterior capsular opacification (PCO) is caused by the migration and proliferation of residual lens epithelial cells (LECs) in equatorial lens capsules after cataract surgery [1]. About 30 % of adults and 100 % of children suffer vision loss due to posterior cataract [2]. Although a variety of

pharmacological and surgical strategies, such as anterior vitrectomy, posterior continuous curvilinear capsulorhexis, laser capsulotomy, and lens capsule polishing have been assessed for the prevention of PCO, implantation of intraocular lens is the most effective method for now [2]. However, design and materials of the intraocular lens are known as risk factors for PCO [3]. Therefore, effective strategies for the prevention of PCO are still urgently needed.

Previous studies have shown that transforming growth factor-β2 (TGF-β2) stimulated capsular fibrosis, cell migration, differentiation, proliferation, and epithelial-mesenchymal transition (EMT) of LECs, also contribute to the development of PCO [4]. The EMT of LECs, characterized by gain of mesenchymal cell features and loss of epithelial cell features, contributes to the loss of cell adhesion and gives rise to myofibroblasts and fibroblasts, leading to PCO [5]. Blockage of TGF-β2-stimulated abnormal proliferation and EMT is considered to be an effective strategy for PCO [5].

Catalpol inhibited cell proliferation and migration, and blocked the EMT of osteosarcoma [6]. Moreover, the application of catalpol reduced the cell migration of human umbilical vein endothelial cells, and exerted anti-inflammatory as well as antiangiogenic effects against corneal neovascularization [7]. Catalpol reduced secretion of lactate dehydrogenase, and suppressed oxidative stress and inflammation in retinal ganglion cells, which attenuated diabetic retinopathy [8]. Catalpol suppressed the activation of TGF-β/smad2/3 pathway so as to exhibit a nephroprotective effect against diabetes mellitus-associated complications [9]. Therefore, catalpol was hypothesized to suppress TGF-β2-stimulated proliferation and EMT of LECs through the inactivation of TGF-β/smad2/3 pathway.

METHODS

Cell culture and treatment

Human LECs (SRA01/04) were acquired from Biovector (Beijing, China), and cultured in DMEM (Life Technologies, Auckland, New Zealand) with 10 % fetal bovine serum (Life Technologies). Catalpol (Sigma-Aldrich, Milwaukee, WI, USA) was dissolved in physiological saline, and SRA01/04 was incubated with 5, 10, or 20 μM catalpol for 48 h. SRA01/04 was treated with 10 ng/mL TGF-β2 (Sigma-Aldrich) for 48 h, and SRA01/04 was also co-treated with 10 ng/mL TGF-β2 and 5, 10, or 20 μM catalpol for 48 h.

Cell viability and migration assays

The SRA01/04 was seeded into 96-well plates, and treated with TGF-β2 or catalpol. A total of 10 μL CCK8 (Beyotime, Beijing, China) was added into each well for 2 h. Absorbance at 450 nm was measured using microplate reader (Bio-Rad, Hercules, CA, USA). For wound healing assay, SRA01/04 was seeded into 6-well plates, and treated with TGF-β2 or catalpol. A p20 pipette tip was used to make a straight line scratch in the middle of the well. The suspended cells were removed, and the scratch gap was photographed under a light microscope (Olympus, Tokyo, Japan) 24 h later. The wound width was analyzed with Image J v.1.46 (National Institutes of Health, Bethesda, MD, USA).

Western blot

The SRA01/04 was lysed in RIPA buffer (Beyotime), and proteins were then collected using centrifugation. Protein concentration of samples were analyzed by BCA kit (Thermo Fisher Scientific, Waltham, MA, USA), and then separated using 10 % SDS-PAGE. Protein samples were transferred onto nitrocellulose membranes, and the membranes were blocked in 5 % bovine serum albumin. The membranes were probed with specific antibodies: anti-fibronectin and anti-α-SMA (1:2000), anti-snail and anti-vimentin (1:2500), anti-E-cadherin and anti-connexin-43 (1:3000), anti-p-smad2/3 and anti-smad2/3 (1:3500), anti-Jagged1 and anti-Notch1 (1:4000), anti-GAPDH (1:4500), overnight, and then washed using Phosphate-buffered saline-Tween-20. The membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:5000, Abcam), and the immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich). All the antibodies were purchased from Abcam (Cambridge, USA).

Statistical analysis

All the data were obtained from at least three independent experiments and expressed as mean ± SEM, and analyzed via student's t test or one-way analysis of variance using SPSS software. $P < 0.05$ was considered statistically significant.

RESULTS

Catalpol reduced cell viability

To investigate the effect of catalpol in PCO, TGF-β2-stimulated SRA01/04 was treated with catalpol (Figure 1 A). Catalpol showed no

cytotoxicity on SRA01 / 04 (Figure 1 B). The TGF-β2 treatment increased cell viability of SRA01/04 (Figure 1 C), while treatment with catalpol reduced the cell viability of TGF-β2-stimulated SRA01 / 04 (Figure 1 C), demonstrating the anti-proliferative effect of catalpol against TGF-β2-stimulated LECs.

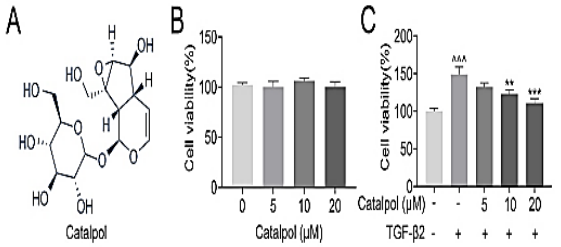

Figure 1: Catalpol reduced cell viability of TGF-β2-stimulated SRA01/04. (A) Chemical structure of catalpol, (B) Catalpol did not reduce cell viability of SRA01/04, (C) Catalpol attenuated TGF-β2-stimulated increase of cell viability in SRA01/04; ** $p < 0.01$, *** $p < 0.001$. vs. control ^^^ $p < 0.001$

Catalpol reduced cell migration

Cell migration of SRA01/04 was promoted by TGF-β2 treatment (Figure 2). However, incubation with catalpol repressed the migration (Figure 2), suggesting the anti-invasive effect of catalpol against TGF-β2-stimulated LECs.

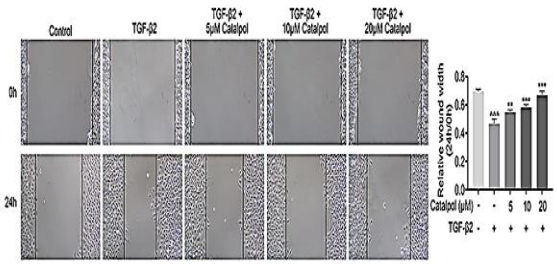

Figure 2: Catalpol reduced cell migration of TGF-β2-induced SRA01/04, vs. TGF-β2, ** $p < 0.01$, *** $p < 0.001$. vs. control ^^^ $p < 0.001$

Catalpol reduced EMT

Treatment with TGF-β2 increased the expressions of fibronectin, α-SMA, snail and vimentin, and decreased E-cadherin and connexin-43 in SRA01/04 (Figure 3). However, catalpol suppressed EMT of TGF-β2-stimulated SRA01/04 through the down-regulation of fibronectin, α-SMA, snail and vimentin, up-regulation of E-cadherin and connexin-43 (Figure 3).

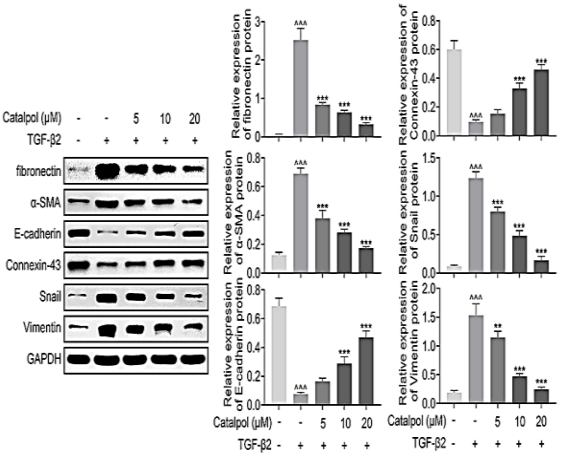

Figure 3: Catalpol reduced EMT of TGF-β2-induced SRA01/04 and attenuated TGF-β2 induced increases in fibronectin, α-SMA, snail and vimentin, and decreases the E-cadherin and connexin-43; in SR vs. TGF-β2, ** $p < 0.01$, *** $p < 0.001$. vs. control, ^^^ $p < 0.001$

Catalpol inhibited TGF-β/Smad and Notch1 signalings

TGF-β2 induced the up-regulation of p-smad2/3 in SRA01/04 (Figure 4), while the expression of p-smad2/3 in TGF-β2-stimulated SRA01/04 was reduced by catalpol (Figure 4). Moreover, catalpol attenuated TGF-β2-induced increase of Notch1 and Jagged1 expression in SRA01/04 (Figure 4), revealing that catalpol reduced EMT of TGF-β2-stimulated LECs through the inactivation of TGF-β/Smad and Notch1 signaling.

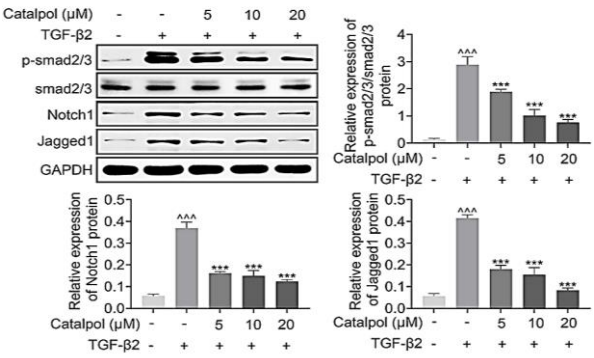

Figure 4: Catalpol inhibited activation of TGF-β/Smad and Notch1 signaling. Catalpol attenuated TGF-β2 induced increase of p-smad2/3, Notch1 and Jagged1 in SRA01/04; vs. TGF-β2, *** $p < 0.001$. vs. control ^^^ $p < 0.001$

DISCUSSION

Posterior capsule opacification (PCO) is related to the pathological progression in residual LECs [4]. Catalpol has been shown to suppress cell

proliferation and metastasis of various tumors [6]. In this study, catalpol suppressed cell proliferation, migration and EMT of TGF-β2-stimulated SRA01/04, suggesting the possibility that catalpol might be a potential agent for PCO.

The TGF-β pathway is an inducer of EMT, and TGF-β2-stimulated LECs have been widely used as model of PCO [10,11]. In this study, TGF-β2 was used to induce an increase of cell proliferation and migration in SRA01/04. This TGF-β2 induced the activation of Smad2/3 signaling during the development of PCO [12]. Protein expressions of mesenchymal biomarkers, including fibronectin, α-SMA, Snail and vimentin, was up-regulated in SRA01/04 followed by TGF-β2. However, the protein expression of epithelial biomarkers, E-cadherin and connexin-43, were down-regulated. Moreover, phosphorylation of smad2/3 was increased in TGF-β2-stimulated SRA01/04, suggesting that TGF-β2 induced EMT of LECs through the activation of smad2/3 signaling.

A previous study has shown that a decrease in p-smad2/3 in TGF-β2-stimulated SRA01/04 inhibited the EMT [13]. Catalpol reduced TGF-β1 expression and exerted anti-fibrotic effects through the inactivation of TGF-β1 signaling [14]. Moreover, the EMT of non-small-cell lung cancer induced by TGF-β1 was suppressed by catalpol through the inactivation of Smad2/3 signaling [15]. Catalpol reduced cell viability and migration of TGF-β2-stimulated SRA01/04. In addition, catalpol attenuated TGF-β2-stimulated increase of fibronectin, α-SMA, snail and vimentin, decrease in E-cadherin and connexin-43 in SRA01/04, thereby suppressing the EMT. Protein expression of p-smad2/3 in TGF-β2-stimulated SRA01/04 was also reduced by catalpol treatment, indicating that catalpol inhibited TGF-β2-stimulated proliferation, migration and EMT of LECs through the inactivation of TGF-β/Smad signaling.

TGF-β2 activates Jagged1/Notch signaling, and contributes to the EMT of LECs during the development of PCO [16]. Down-regulation of Notch1 and Jagged1 inhibited TGF-β2-stimulated EMT in the LECs [17]. Catalpol reduced expressions of Notch1 signaling-related proteins, including Jagged1 and Notch1, and stimulated the differentiation of oligodendrocyte precursor cells into oligodendrocytes [18]. In this study, TGF-β2 treatment enhanced protein expressions of Notch1 and Jagged1 in SRA01/04, while catalpol decreased the Notch1 and Jagged1 in TGF-β2-stimulated SRA01/04, revealing that catalpol might suppress the activation of Notch1/Jagged1 signaling, and repress TGF-β2-stimulated EMT of LECs.

CONCLUSION

Catalpol inhibits TGF-β2-stimulated proliferation, migration and EMT of LECs through inactivation of TGF-β/Smad and Notch1 signaling. Therefore, catalpol might be a novel preventive agent for PCO. However, the effect of catalpol in animal models of PCO should be investigated in further research.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiaoyu Li and Honglei Ma designed the study and carried them out, supervised the data collection, analyzed the data, interpreted the data, prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

REFERENCES

1. *Agarwalla I, Bhattacherjee H, Chiraniya P, Garg M. Fish-net posterior capsular opacification: A newer variant. Indian J Ophthalmol 2020; 68(10): 2258-2258.*
2. *Nibourg LM, Gelens E, Kuijer R, Hooymans JMM, van Kooten TG, Koopmans SA. Prevention of posterior capsular opacification. Exp Eye Res 2015; 136: 100-115.*
3. *Chen H-C, Lee C-Y, Sun C-C, Huang J-Y, Lin H-Y, Yang S-F. Risk factors for the occurrence of visual-threatening posterior capsule opacification. J Transl Med 2019; 17(1): 209-209.*
4. *de Iongh RU, Wederell E, Lovicu FJ, McAvoy JW. Transforming growth factor-β-induced epithelial-mesenchymal transition in the lens: A model for cataract formation. Cells Tissues Organs 2005; 179(1-2): 43-55.*
5. *Wang H, Zheng G. 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. Cancer Cell Int 2020; 20(1): 529-529.*
6. *Wang L, Xue GB. Catalpol suppresses osteosarcoma cell proliferation through blocking epithelial-mesenchymal transition (EMT) and inducing apoptosis. Biochem Bioph Res Co 2018; 495(1): 27-34.*
7. *Han Y, Shen M, Tang LY, Tan G, Yang QC, Ye L, Ye LH, Jiang N, Gao GP, Shao Y. Antiangiogenic effects of catalpol on rat corneal neovascularization. Mol Med Rep 2018; 17(2): 2187-2194.*
8. *Yi Shao YZ, Yao Yu, Ting-Ting Xu, Rong Wei, Qiong Zhou. Impact of catalpol on retinal ganglion cells in diabetic retinopathy. Int J Clin Exp Med 2016; 9(9): 17274-17280.*
9. *Bhattamisra SK, Koh HM, Lim SY, Choudhury H, Pandey M. Molecular and biochemical pathways of catalpol in alleviating diabetes mellitus and its complications. Biomolecules 2021; 11(2): 323.*
10. *Yang Y, Ye Y, Lin X, Wu K, Yu M. Inhibition of pirfenidone on TGF-beta2 induced proliferation, migration and epithelial-mesenchymal transition of human lens epithelial cells line SRA01/04. PloS one 2013; 8(2): e56837-e56837.*
11. *Apriasari ML, Pramitha SR, Puspitasari D, Ernawati DS. Expression of fibroblast growth factor-β and transforming growth factor-β in mauli banana stem (Musa acuminate) extract gel-treated traumatic ulcer. Trop J Pharm Res 2019; 18(3): 527-531.*
12. *Li H, Yuan X, Li J, Tang X. Implication of smad2 and smad3 in transforming growth factor-β-induced posterior capsular opacification of human lens epithelial cells. Curr Eye Res 2015; 40(4): 386-397.*
13. *Zhang Z, Zhu H, Liu Y, Quan F, Zhang X, Yu L. LncRNA HOTAIR mediates TGF-β2-induced cell growth and epithelial–mesenchymal transition in human lens epithelial cells. Acta Bioch Bioph Sin 2018; 50(10): 1028-1037.*
14. *Xu DQ, Zhao L, Li SJ, Huang XF, Li CJ, Sun LX, Li XH, Zhang LY, Jiang ZZ. Catalpol counteracts the pathology in a mouse model of Duchenne muscular dystrophy by inhibiting the TGF-β1/TAK1 signaling pathway. Acta Pharmacol Sin 2021; 42(7): 1080-1089.*
15. *Wang Z, Lu Y, Sheng B, Ding Y, Cheng X. Catalpol inhibits TGF-β1-induced epithelial-mesenchymal transition in human non–small-cell lung cancer cells through the inactivation of Smad2/3 and NF-κB signaling pathways. J Cell Biochem 2019; 120(2): 2251-2258.*
16. *Lovicu FJ, Shin EH, McAvoy JW. Fibrosis in the lens. Sprouty regulation of TGFβ-signaling prevents lens EMT leading to cataract. Exp Eye Res 2016; 142: 92-101.*
17. *Zhang G, Kang L, Chen J, Xue Y, Yang M, Qin B, Yang L, Zhang J, Lu H, Guan H. CtBP2 regulates TGFβ2-induced epithelial–mesenchymal transition through notch signaling pathway in lens epithelial cells. Curr Eye Res 2016; 41(8): 1057-1063.*
18. *Sun Y, Ji J, Zha Z, Zhao H, Xue B, Jin L, Wang L. Effect and mechanism of catalpol on remyelination via regulation of the NOTCH1 signaling pathway. Front Pharmacol 2021; 12: 628209-628209.*