TLR3 Gene Regulates Cataract-Related Mechanisms Through The Jagged-1/Notch Signaling Pathway

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Research Article

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Abstract

Objective: Epithelial-mesenchymal transition (EMT) is the main cause of organ fibrosis and a common pathogenesis of most cataracts. Therefore, TLR3 may play a vital role in the progress of PCO EMT. This study aims to explore the molecular mechanism of TLR3 in the occurrence and development of post-cataract EMT, and to provide new ideas for the prevention and treatment of posterior capsule opacification (PCO).

Materials and Methods: The expression of TLR3 and EMT-related factors in samples from normal and PCO patients was measured by qPCR. In the presence or absence of TLR3, human lens epithelial cell line SRA01/04 are treated with TGF-β2. The CCK-8 method measures cell proliferation, and the Transwell method detects migration and invasion. The expression of EMT-related molecules and Jagged-1/Notch signaling pathway was analyzed by Western blot and qPC experiments.

Result: In terms of mechanism, the functional gain and loss experiments of human LEC show that TLR3 regulates the proliferation, migration, invasion of LEC and EMT induced by TGF-β2 by targeting the Jagged-1/Notch signaling pathway to affect the development of fibrotic cataract.

Conclusion: These findings indicate that TLR3 prevents the progression of lens fibrosis by targeting the Jagged-1/Notch signaling pathway to regulate LEC proliferation, migration, invasion and TGF-β2-induced EMT. "TLR3-Jagged-1/Notch" signal axis may be a therapeutic target for the treatment of fibrotic cataract.

Introduction

Fibrosis is a common cause of organ dysfunction and organ failure. The survival rate of some fibrotic diseases is even lower than that of cancer[1, 2]. With the development of fibrotic disease research, fibrosis models are constantly improving. Due to its unique biological characteristics, the lens has become an ideal biological tool for the study of fibrosis. The adult lens is separated from other tissues and has no nerve and blood vessel distribution, but draws nutrients from aqueous humor and vitreous humor. Lens epithelial cells are distributed under the anterior capsule of the lens and under the capsule of the equator of the lens. The lens epithelial cells proliferate, differentiate and migrate inward to form lens fibers. Under physiological conditions, the lens fibers are arranged neatly, but some pathological conditions lead to the destruction of the lens structure and lens fibrosis[3]. Although fibrotic diseases can affect many organs, such as liver fibrosis, kidney fibrosis, lung fibrosis and lens fibrosis, there are still some potential common pathogenesis mechanisms for fibrosis in different organs. In recent years, more and more evidence has shown that epithelial-mesenchymal transition (EMT) is the main cause of the progression of organ fibrosis[4], and lens EMT is also a common mechanism of most cataracts[5].

Cataract is a common eye disease that causes visual impairment and is the main cause of blindness. It is characterized by the gradual increase in lens opacity, obstructed vision, and gradual loss of vision[6, 7]. According to reports, 96% of people over 60 years old have varying degrees of lens opacities[8, 9]. Cataracts can be divided into anterior subcapsular cataract (ACS) and posterior capsular opacification

(PCO) according to the location where fibrosis occurs. At present, surgery can effectively restore the vision of patients with cataracts, and may be the most commonly used and most effective treatment for cataracts. However, despite the continuous improvement of cataract surgery methods, postoperative complications such as posterior capsule opacity still severely affect the vision of patients[10–13].

Excessive proliferation and migration of lens epithelial cells and transformation into mesenchymal cells (ie EMT) are common causes of cataracts. In the process of epithelial-mesenchymal transition, epithelial cells secrete excessive extracellular matrix, including collagen type Ⅰ (Col Ⅰ) and fibronectin (Fibronectin, FN), which downregulate E-cadherin (E-Cadherin, E-Cad) deprives cell polarity[14] and weakens intercellular adhesion, which ultimately leads to changes in the phenotype of epithelial cells, which synthesize α-smooth muscle actin (α-SMA), Vimentin, etc. to obtain the interstitial cell phenotype[15, 16].

TLR3 is an important member of the TLRs family. It is a type I transmembrane protein and can recognize double-stranded RNA viruses and Poly I: C. Existing studies have shown that TLR3 has a dual role in tumors. On the one hand, most studies reported that the activation of TLR3 has an inhibitory effect on tumors and can promote tumor cell apoptosis[17], which may be mainly related to the production of type I interferon and natural killer cells, and the activation of immune cells such as natural killer cells, dendritic cells, and macrophages[18, 19], so TLR3 agonists can be used as immune adjuvants to treat tumors. On the other hand, some studies have shown that TLR3 can promote tumor progression. The prognosis of breast cancer patients with high TLR3 expression is relatively poor[20], which may be because the activation of TLR3 triggers the β-catenin and NF-κB signaling pathways, which promotes the transformation of breast cancer cells into cancer stem cells[21]. However, the mechanism of TLR3 in cataract fibrosis is rarely reported.

The Notch signaling pathway is evolutionary and conservative. It controls many cellular processes through the interaction between neighboring cells and the interaction with other signals, including cell differentiation, proliferation, tumor angiogenesis, maintenance of stemness, and apoptosis[22]. Among them, the Notch receptor family is a transmembrane protein, a heterodimer composed of an extracellular domain and an intracellular domain[22]. The ligand Jagged1 is a single-pass transmembrane glycoprotein with a small intracellular domain and a larger extracellular domain. It contains four parts necessary for the normal function of the protein: N-signal peptide, DSL domain, and 16 EGF-like Repeat region and 1 cysteine-rich region. The Notch signaling pathway, especially the ligand Jagged1, is closely related to the occurrence and biological behavior of a variety of tumors, such as tumor angiogenesis, maintenance of stem cell characteristics, and metastasis[23]. At present, the specific role and mechanism of Jagged-1/Notch signal in cataract are not yet clear.

In this study, we identified TLR3 as a key regulator to prevent lens fibrosis. We have shown that TLR3 is significantly elevated in PCO cataracts, and TGF-β2 significantly up-regulates TLR3 expression in fibrotic human lens epithelial cell line SRA01/04. Importantly, by showing the effect of TLR3 upregulation on LEC proliferation, migration, invasion and EMT, we revealed a new mechanism of TGF-β2 induced lens fibrosis by acting on the Jagged-1/Notch signaling pathway.

Methods And Materials

Cell culture

In the study, the fresh postmortem human lenses were used which were collected from the donors dead within 8 hours (20 males and 8 females). The fibrotic lens part came from the PCO tissues of 4 donors who had undergone cataract surgery before death. The fibrotic part of the posterior capsule was separated with capsulotomy scissors. For normal transparent lens, use capsulotomy scissors to cut the anterior capsule with epithelium along the equatorial region of the lens, and peel it from the lens fibers with capsulorhexis to collect the lens epithelial explant. The human lens epithelial cell line SRA 01/04 was purchased from ATCC and cultured in Dulbecco's modified Eagle medium (DMEM, Thermo Fisher Scientific) containing 8% FBS. The lens fibrosis model induced by TGF-β2 is made by treating the LEC cell line SRA01/04 with 5 ng/mL TGF-β2 (302-B2; R&D) for 48-72 hours.

Transfection

The si-NC and si-TLR3 purchased from GenePharma (Shanghai, China) were transfected into LEC cell line SRA01/04 by Lipofectamine 2000 (Invitrogen, CA, USA). The pcDNA 3.1-TLR3 or pcDNA 3.1-NC adenovirus vector was transfected from HanBio Technology Co. Ltd. (Shanghai, China) into human LECs for overexpression of TLR3 or as a control for the following experiments.

CCK-8 assay

The cells were digested into cell suspension with a density of 2×10^4 cells/mL. 100mL of cell suspension was added into a 96-well plate, then placed in a 37°C, 5% CO_2 incubator to culture adherence. After the cells are processed, 10μL of CCK-8 reagent was added into each well and continue to be incubated for 2 hours. Microplate reader was used to detect the OD value at 450 nm for statistical analysis of the data.

Migration and invasion experiment

Migration experiment: adjust the processed cells to 2×10^5 cells/ml, take the pre-prepared Matrigel-free Transwell chamber and place it in a 24-well plate, LEC cells were added in the upper chamber, and 500 μl of 10% serum-containing was added in the lower chamber DMEM medium for 24 h. The chamber was taken out and the upper chamber liquid was discarded. After washed with PBS, the cells were fixed with formaldehyde. The upper cells of the chamber were wiped with a cotton swab. Fix with formaldehyde for 10-15 minutes and stain with crystal violet for 20 minutes. The migrated cells are counted under a microscope.

Invasion experiment: Take 50 μl of Matrigel (Corning) and spread it in an 8 μm Transwell chamber, and place it in a 37 ℃ incubator for 1 h. The logarithmic growth phase LEC cells were seeded into the upper chamber of 8 μm Transwell small wells, 200 μl per well. Add 500 μl of DMEM medium containing 10% serum to the lower chamber and culture for 24 h.

qRT-PCR

The treated LEC cells were collected, the total mRNA was extracted by the Trizol method, and operate according to the instructions of the reverse transcription kit to obtain cDNA. qRT-PCR is as follows: SYBR Premix Ex Taq (Bimake) 5 μl, upstream and downstream primers 1 μl each, cDNA 2 μl, ddH2O 2 μl. Reaction conditions: 95 ℃ 30 s pre-denaturation; 95 ℃ 5 s, 55 ℃ 30 s amplification 45 cycles. Statistical data uses 2-ΔΔCt to calculate the relative expression of the target gene.

Western blot

LEC cells were lysed with radioimmunoprecipitation assay buffer supplemented with protease inhibitors (P0013C; Beyotime). The liquid was aspirated and centrifuged to collect the protein for quantification. A sample of 30 μg of protein was taken and transferred to membrane after SDS-PAGE electrophoresis. Antibody ZO-1 (13663; CST), E-cad (3195; CST), N-cad (13116; CST), Vim (5741; CST), Jagged-1 (70109; CST), Notch-1 (3608; CST), Notch-2 (5732; CST), GAPDH (5174; CST) were diluted 1:1000 and incubated overnight at 4°C. HRP-labeled secondary antibody was added and incubated for 2 h at 37 ℃ in the dark, rinsed with PBS-T repeatedly, and finally added ECL reagent, the chemiluminescence instrument was used to detect the expression of the target protein.

Statistical analysis

The Student's t-test or the one-way ANOVA was used to evaluate the difference between groups. A value of $P < .05$ was considered statistically significant.

Results

FN, α-SMA, Col I, Acan and TLR3 are up-regulated in fibrotic lens tissue

The transparent lens capsule and fibrotic lens capsule from healthy donors and fibrotic cataract patients were collected. Compared with normal lens epithelium, the expression levels of fibrotic lens epithelial mesenchymal markers α-SMA, ECM markers FN, Col I, and Acan increased significantly (Figure 1A), indicating that lens fibrosis involves hyperproliferation and EMT. At the same time, the gene expression level of TLR3 in the fibrotic lens epithelium was significantly increased (Figure 1B), which may be related to the development of lens fibrosis.

TLR3 is upregulated in LEC cell line SRA01/04

In order to further explore the role of TLR3 in lens fibrosis. TGF-β2 was used to stimulate human lens in vitro implants. The increase of α-SMA, FN, Col I and Acan in LEC cell line SRA01/04 treated with TGF-β2 indicated that the cells have morphological and molecular changes similar to fibrotic cataracts (Figure 2A). The expression of occlusion zone tissue (ZO1) and E-cadherin (E-cad) decreased, and the expression of N-cadherin and vimentin (Vim) increased, which transferred from the cell membrane to the cytoplasm, and transformed into mesenchymal-like, lost Epithelial features (Figure 2B). At the same time, the gene

expression level of TLR3 was significantly increased in the LEC cell line SRA01/04 treated with TGF-β2 (Figure 2C).

TLR3 promotes the proliferation, migration, invasion and EMT of human lens epithelial cells

In order to study the function of TLR3 in lens fibrosis, we transfected human lens epithelial cells with si-TLR3 or overexpressed TLR3 (Figure 3A). In human lens epithelial cells treated with TGF-β2, knockdown of TLR3 prevented the expression of mesenchymal transition markers FN, α-SMA, Col I and Acan (Figure 3B), and reduced the cell proliferation rate (Figure 3B). 3C). After transfection with si-TLR3, the migration and invasion of human lens epithelial cells were significantly reduced (Figure 3D-E). At the same time, the results of the WB experiment showed that the epithelial markers ZO-1 and E-cad were retained, and the expression of N-cad and Vim protein was significantly reduced (Figure 3F). The above experimental results indicate that TLR3 has a functional effect on the proliferation, migration, invasion and EMT of human lens epithelial cells.

TLR3 acts directly on the Jagged-1/Notch signaling pathway

In order to confirm the potential role of the Jagged-1/Notch signaling pathway in the process of lens fibrosis, we used Western blot to detect Jagged-1/Notch signaling pathway related proteins in human lens epithelial cells treated with TGF-β2 and transfected with si-TLR3 and overexpressing TLR3. The results showed that compared with the control group, Jagged-1, Notch-1, and Notch-2 proteins in human lens epithelial cells treated with TGF-β2 were significantly increased (Figure 4A). After transfection of si-TLR3 and overexpression of TLR3, Jagged-1, Notch-1, and Notch-2 proteins in the overexpression TLR3 group remained elevated, and the protein expression of si-TLR3 group decreased significantly (Figure 4B). The experimental results show that TLR3 has a direct effect on the Jagged-1/Notch signaling pathway.

TLR3 promotes the proliferation, migration, invasion and EMT of human lens epithelial cells by directly acting on the Jagged-1/Notch signaling pathway

In order to study whether the Jagged-1/Notch signaling pathway mediates the biological function of TLR3 in the process of human lens fibrosis, we used both transfection si-TLR3 and TLR3 activator valproic acid (VA). The results of the qRT-PCR experiment showed that compared with the TGF-β2 group, the FN, α-SMA, Col I and E-cad genes in the si-TLR3 group were significantly reduced, while compared with the si-TLR3 group, the FN and α-SMA in the VA group, Col I and E-cad genes were relatively elevated (Figure 5A). The CCK8 experiment results showed that compared with the TGF-β2 group, the proliferation rate of the si-TLR3 group was significantly decreased, while the VA group was significantly increased compared with the si-TLR3 group (Figure 5B). Compared with the TGF-β2 group, the cell migration and invasion ability of the si-TLR3 group was significantly decreased, and the VA group was significantly improved compared with the si-TLR3 group (Figure 5C-D). The WB experiment results showed that compared with the TGF-β2 group, the ZO-1 and E-cad protein expressions in the si-TLR3 group increased, and the N-cad and Vim proteins decreased. Compared with the si-TLR3 group, the results were opposite in the VA group, compared with the TGF-β2 group, Jagged-1/Notch signaling pathway related proteins

decreased significantly in the si-TLR3 group, and improved in the VA group compared with the si-TLR3 group (Figure 5E). The above results indicate that in the process of human lens epithelial cell fibrosis, TLR3 promotes the proliferation, migration, invasion and EMT of human lens epithelial cells by directly acting on the Jagged-1/Notch signaling pathway.

Discussion

PCO is a common fibrotic disease and the most common complication after cataract surgery. EMT is the common pathological basis of many fibrotic diseases such as PCO[4]. Understanding the pathological mechanism of EMT will not only help the prevention and treatment of PCO, but may also provide a new understanding of the occurrence and development of fibrotic diseases.

When trauma or surgical damage leads to the destruction of the lens structure, the remaining lens epithelial cells proliferate excessively and migrate to the posterior capsule, resulting in EMT, which changes the color of the lens or reduces the transparency, and ultimately leads to the occurrence of cataracts[3]. The typical characteristics of EMT include: E-cadherin, a key molecule that maintains cell polarity and intercellular adhesion, is degraded or lost function, and the abundance of tight junction proteins such as occludin and zonules occlusion 1 (ZO1) is decreased. The expression of keratin decreases. At the same time, the characteristic proteins of mesenchymal cells such as Fibronectin, α-SMA, Vimentin, N-Cadherin (N-Cadherin), and Col □ increase[24, 25]. The connections between cells are dissolved, the cytoskeleton is reconstructed, and the cell morphology is transformed into a mesenchymal-like multidendrite spindle shape, and cell migration and invasion capabilities are enhanced[26]. TGF-β2 is currently known as a potent inducer of EMT. TGF-β2 is not only involved in EMT during growth and development[27], but is also closely related to EMT during wound healing, fibrosis and cancer[28, 29]. In this study, the real-time fluorescent quantitative PCR test results showed that the transparent lens capsule and the fibrotic lens capsule were compared between healthy donors and fibrotic cataract patients, the expression levels of fibrotic lens epithelial mesenchymal marker α-SMA, ECM markers FN, Col I, and Acan were significantly increased. TGF-β2 was used to stimulate human lens in vitro implants. In the LEC cell line SRA01/04 treated with TGF-β2, α-SMA, FN, Col I and Acan increased, and the closed zone tissue (ZO1) and E-cadherin (The expression of E-cad) decreased, and the expression of N-cadherin and vimentin (Vim) increased, confirming that lens fibrosis involves hyperproliferation and EMT, and TGF-β2 can significantly promote the occurrence of EMT in lens epithelial cells.

The human TLR3 gene is located on chromosome 4, and its expression product TLR3 receptor protein can recognize double-stranded RNA associated with viral infection. Studies have shown that TLR3, as an innate immune receptor, plays a role in activating anti-inflammatory signaling pathways during injury and infection. Chronic liver alcohol accumulation can inhibit TLR3-dependent signaling pathways in NK cells during the late stage of liver fibrosis and alcoholic liver disease. The lack of TLR3-mediated killing of NK cells played an important role in accelerating the progression of the disease[30]. However, the expression and function of TLR3 in the lens have not been reported yet. In this study, we detected the expression of TLR3 in human lens epithelial cells. More importantly, we found that TLR3 was significantly increased in

the mouse lens anterior capsule injury model, and in the EMT model of lens epithelial cells induced by TGF-β, 2 TLR3 also increased significantly, suggesting that TLR3 may be involved in the lens EMT process, but its role in the lens epithelial cell EMT process is still unclear.

In order to further study the role of TLR3 in EMT of lens epithelial cells, we constructed siRNA and TLR3 overexpression lentiviral vectors that specifically interfere with TLR3, aiming to observe differences in TLR3 expression on the proliferation, migration, invasion and EMT of lens epithelial cells cultured in vitro. To explore the regulatory effect of TLR3 on the EMT of lens epithelial cells. We found that under the stimulation of TGF-β2, the expression levels of α-SMA, Vimentin, and Col Ⅰ of cells transfected with si-TLR3 increased significantly, while the expression level of E-Cadherin decreased significantly, indicating that both groups of cells had EMT, but the expression of FN, α-SMA, Col I and Acan in the si-TLR3 group decreased, the proliferation rate decreased, and migration and invasion were significantly decreased. The results of WB experiments showed that the epithelial markers ZO-1 and E-cad were retained, and the expression of N-cad and Vim protein was significantly reduced, indicating that TLR3 has a functional effect on the proliferation, migration, invasion and EMT of human lens epithelial cells. The Notch signaling pathway is highly conserved in evolution and is involved in physiological processes such as cell proliferation, differentiation, apoptosis, and maintenance of cell stemness[31]. Notch ligands in mammals are also called DSL proteins. There are five types, namely Jagged1, Jagged2, DLL1, DLL3, and DLL4, all of which are type I transmembrane proteins. TGF-β2 can induce the expression of Notch ligands. In patients with diabetic nephropathy, the expression of Jagged1 and Hey1 induced by TGF-β2 plays a particularly important role, which may be related to the pathological process of the disease[32]. It has been confirmed that Jagged is the target gene of TGF-β2 in a variety of mammalian cells. TGF-β2 relies on Smad3 to regulate the expression of Jagged1 and Hey1 in the process of inducing EMT in cells[33]. In this study, Western blot results showed that Jagged-1, Notch-1, and Notch-2 proteins were significantly increased in human lens epithelial cells treated with TGF-β2. After transfection of si-TLR3 and overexpression of TLR3, Jagged-1, Notch-1, and Notch-2 proteins in the overexpression TLR3 group remained elevated, and the expression of si-TLR3 histone decreased significantly, indicating that TGF-β2 effectively activated Notch signal path. In order to study whether the Jagged-1/Notch signaling pathway mediates the biological function of TLR3 in the process of human lens fibrosis, transfection of si-TLR3 and the use of TLR3 activator valproic acid (VA) were used at the same time. The results showed that compared with the TGF-β2 group, the FN, α-SMA, Col I and E-cad genes in the si-TLR3 group were significantly decreased, the proliferation rate was significantly decreased, and the migration and invasion capabilities were significantly decreased; Compared with the si-TLR3 group, the FN, α-SMA, Col I, and E-cad genes were relatively higher in the VA group, and the proliferation rate increased significantly, and the migration and invasion capabilities increased significantly. The results of WB experiments showed that compared with the TGF-β2 group, the ZO-1 and E-cad protein expressions in the si-TLR3 group increased, and the N-cad and Vim proteins decreased. The VA group and the si-TLR3 group had the opposite results. Compared with the TGF-cβ2 group, Jagged-1/Notch signaling pathway related proteins decreased significantly in the si-TLR3 group, and improved in the VA group compared with the si-TLR3 group. It shows that in the process of human lens epithelial cell fibrosis, TLR3 promotes the proliferation,

migration, invasion and EMT of human lens epithelial cells by directly acting on the Jagged-1/Notch signaling pathway.

Our results provide the first evidence that TLR3 and Jagged-1/Notch pathway play an important role in lens fibrosis. Our data indicate that the blockade of TLR3 and Jagged-1/Notch pathways may be a promising strategy for the prevention and treatment of organ fibrosis. These understandings of the regulatory relationship between TLR3 and Jagged-1/Notch signaling pathway are helpful to understand the pathogenesis of fibrotic diseases.

Declarations

Authors' contributions

Yu Chen and Rui Yang designed the research study. Yu Chen performed the research. Yu Chen wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Data Availability

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors claim that there is no conflict of interests.

Ethics statement

This study was conducted after being approved by the Ethics Committee of the Affiliated Hospital of North Sichuan Medical College.

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Figures

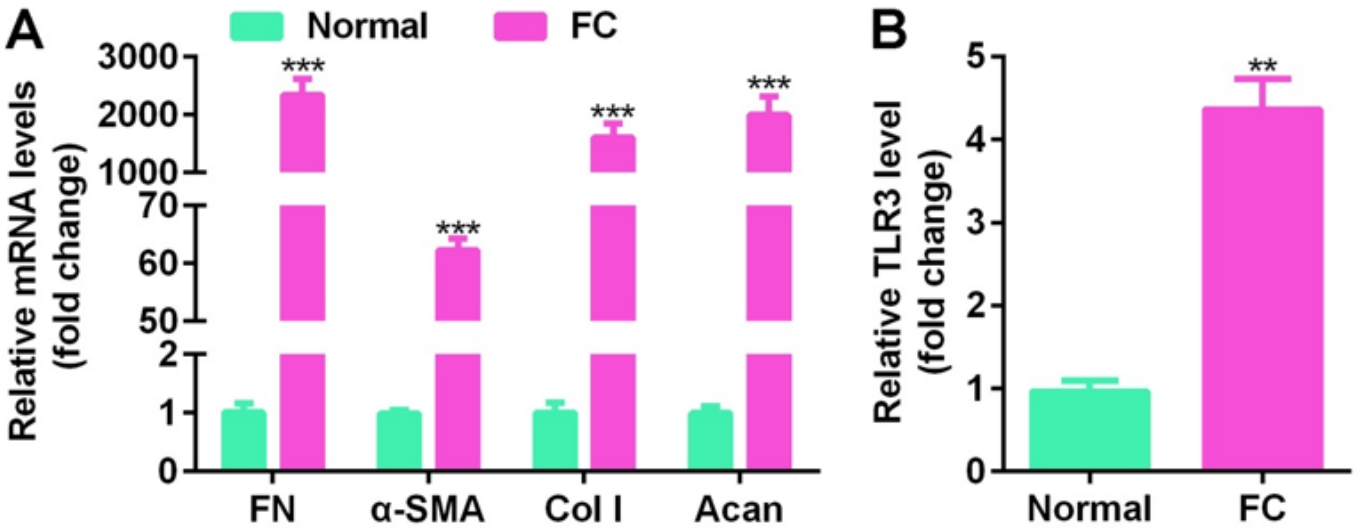

Figure 1

Gene expression levels in normal lens epithelium and fibrotic cataract tissues (FN, α-SMA, Col I, Acan and TLR3). A-B: qRT-PCR is used to detect gene expression levels of FN, α-SMA, Col I, Acan and TLR3. ** $P<0.01$, *** $P<0.001$.

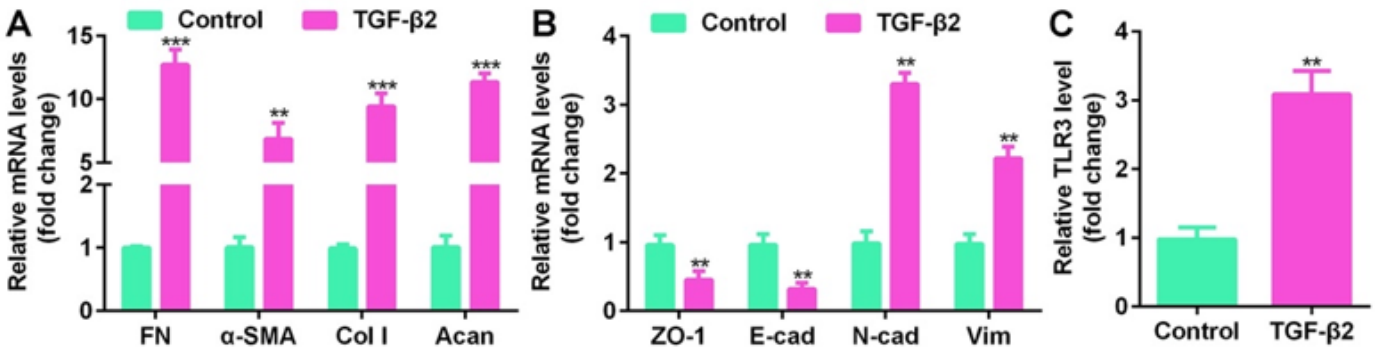

Figure 2

TLR3 is upregulated in LEC cell line SRA01/04. A: Comparison of gene expression levels (FN, α-SMA, Col I and Acan) in lens epithelium treated with or without TGF-β2 (5 ng/mL) for 48 hours by qPCR analysis. B: Comparison of gene expression levels (ZO-1, E-cad, N-cad, Vim) in lens epithelium treated with or without TGF-β2 (5 ng/mL) for 48 hours by qPCR analysis. C: Comparison of TLR3 gene levels in lens epithelium treated with or without TGF-β2 (5 ng/mL) for 48 hours by qPCR analysis. ** $P<0.01$, *** $P<0.001$.

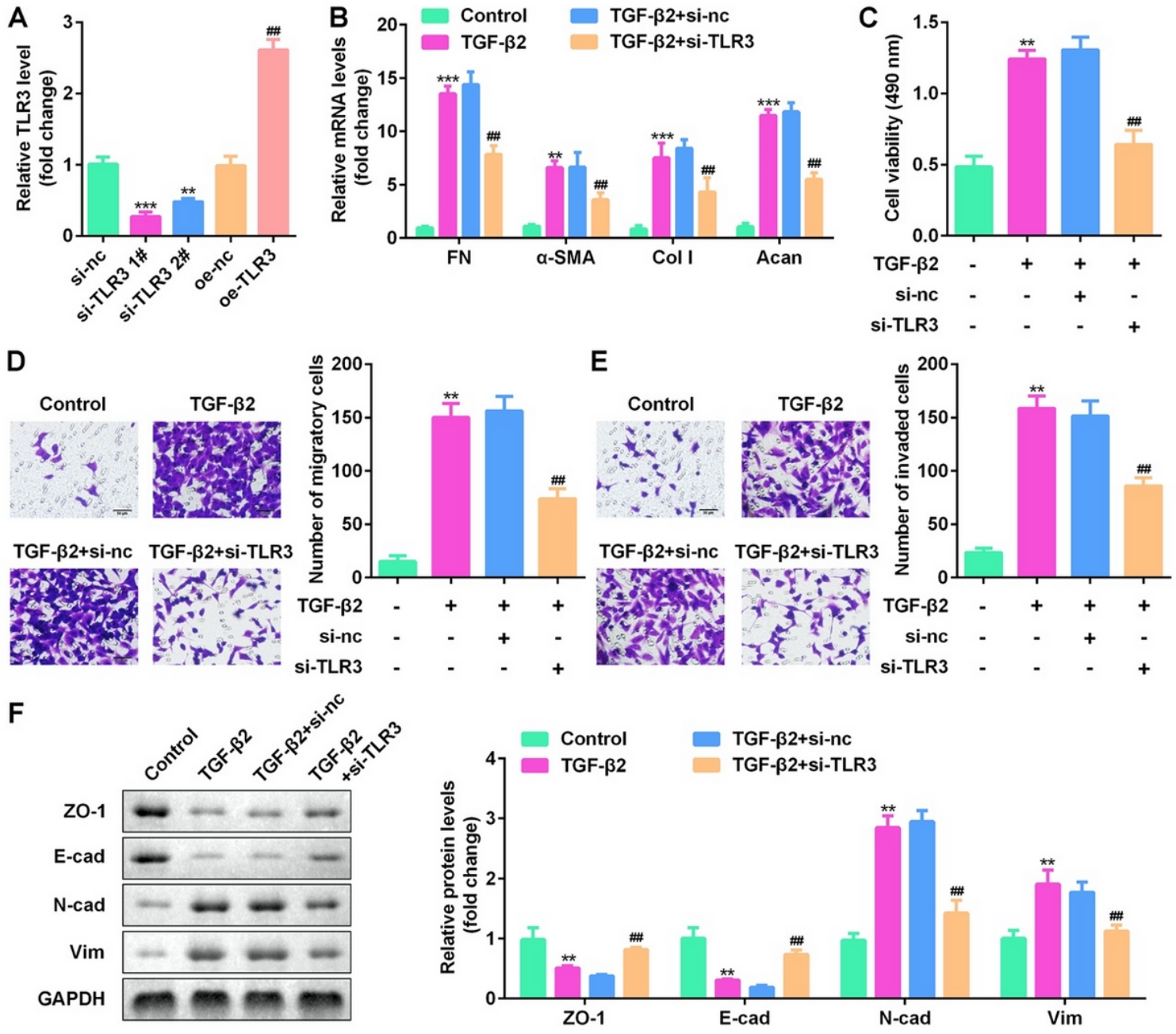

Figure 3

si-TLR3 prevents the proliferation, migration, invasion and EMT of human lens epithelial cells. A: After human lens epithelial cells are transfected with si-TLR3 and overexpressing TLR3, qPCR method is used to detect the level of TLR3 in the cells. B: Analysis of the gene expression levels (FN, α-SMA, Col I and E-cad) after transfection of si-TLR3 in the lens epithelial fibrosis model induced by TGF-β2 by qPCR. C: The CCK8 method is used to detect the cell viability after transfection of si-TLR3 in the lens epithelial fibrosis model induced by TGF-β2. D-E: Transwell method is used to detect the cell migration and invasion ability after transfection of si-TLR3 in the lens epithelial fibrosis model induced by TGF-β2. F: Western blot is used to detect the expression of EMT-related proteins in cells transfected with si-TLR3 in the lens epithelial fibrosis model induced by TGF-β2. ** P<0.01, *** P<0.001 vs Control. $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 vs TGF-β2.

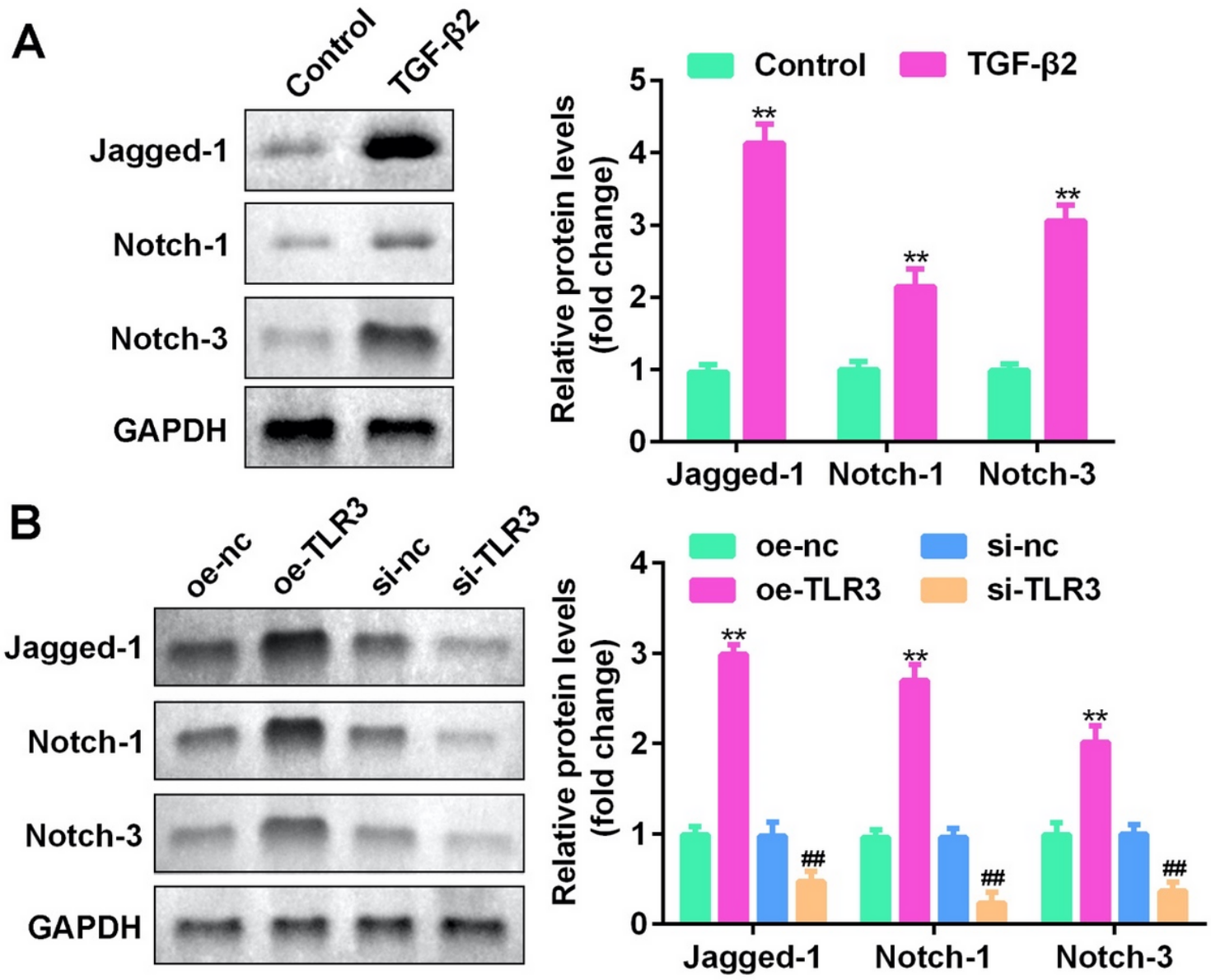

Figure 4

TLR3 directly acts on the Jagged-1/Notch signaling pathway. A: Western blot was used to detect the expression of Jagged-1/Notch pathway protein in lens epithelium treated with or without TGF-β2 (5 ng/mL) for 48 hours. B: Human lens epithelial cells were treated with TGF-β2 (5 ng/mL) and then transfected with si-TLR3 and overexpressed TLR3 for 24 h. The expression of Jagged-1/Notch pathway protein was detected by Western blot. ** $P<0.01$, *** $P<0.001$ vs Control. ## $P<0.01$, ### $P<0.001$ vs TGF-β2.

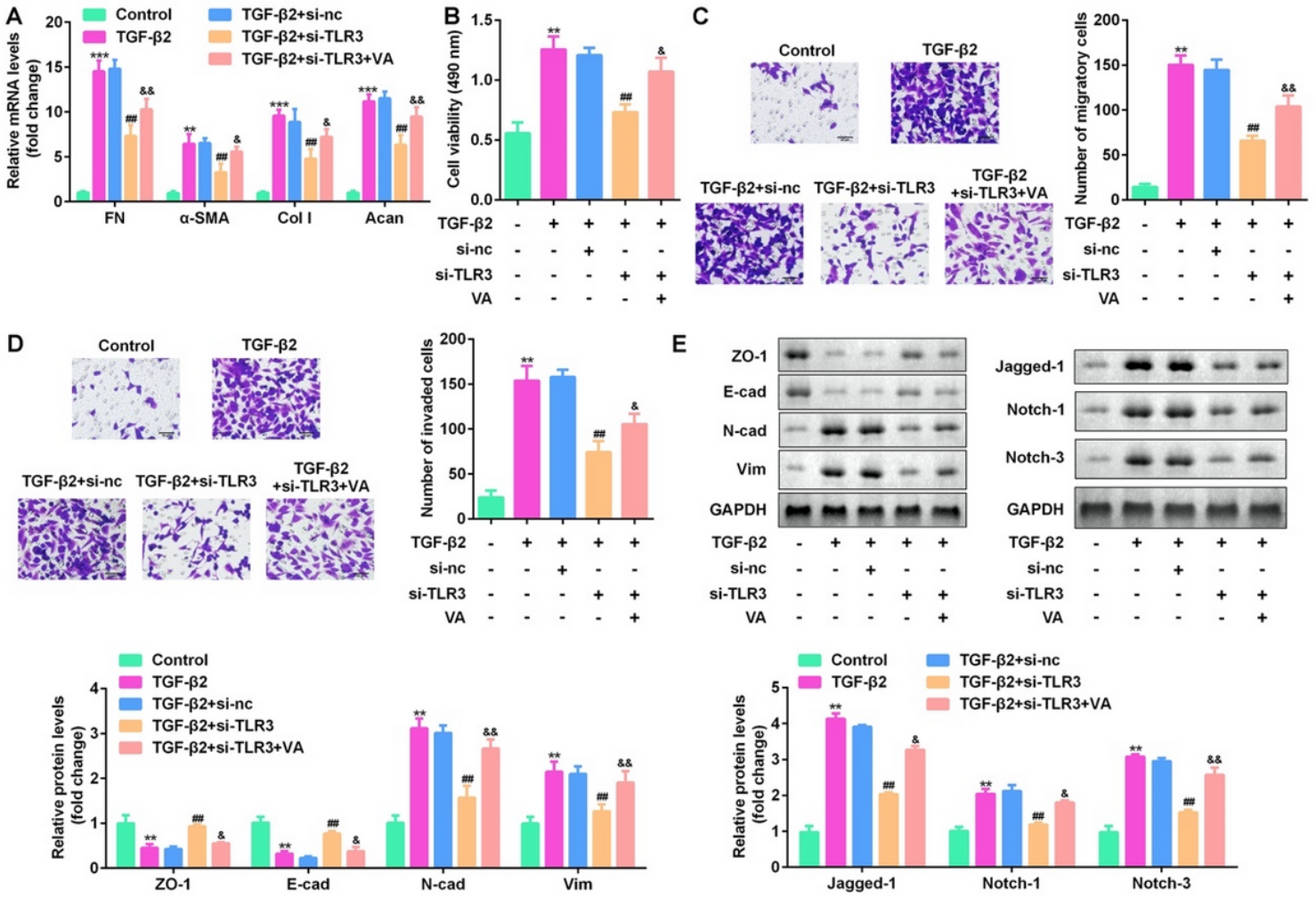

Figure 5

TLR3 promotes the proliferation, migration, invasion and EMT of human lens epithelial cells by directly acting on the Jagged-1/Notch signaling pathway. A: qRT-PCR is used to detect the gene expression levels (FN, α-SMA, Col I and E-cad) after treatment with TGF-β2 (5 ng/mL) and then transfected with si-TLR3 and the TLR3 activator VA. B: The CCK8 method is used to detect the cell viability after treatment with TGF-β2 (5 ng/mL) and then transfected with si-TLR3 and the TLR3 activator VA. C-D: Transwell method is used to detect the cell migration and invasion ability after treatment with TGF-β2 (5 ng/mL) and then transfected with si-TLR3 and the TLR3 activator VA. E: Western blot is used to detect the expression of EMT-related proteins and Jagged-1/Notch signaling pathway-related proteins after treatment with TGF-β2 (5 ng/mL) and then transfected with si-TLR3 and TLR3 activator VA. ** P<0.01, *** P<0.001 vs Control. ## P<0.01, ### P<0.001 vs TGF-β2.