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Lanosterol regulates abnormal amyloid accumulation in LECs through the mediation of cholesterol pathway metabolism

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ABSTRACT

Age-related cataract (ARC) is the predominant cause of global blindness, linked to the progressive aging of the lens, oxidative stress, perturbed calcium homeostasis, hydration irregularities, and modifications in crystallin proteins. Currently, surgical intervention remains the sole efficacious remedy, albeit carrying inherent risks of complications that may culminate in irreversible blindness. It is urgent to explore alternative, cost-effective, and uncomplicated treatment modalities for cataracts. Lanosterol has been widely reported to reverse cataracts, but the mechanism of action is not yet clear. In this study, we elucidated the mechanism through which lanosterol operates in the context of cataract reversal. Through the targeted suppression of sterol regulatory element-binding protein 2 (SREBP2) followed by lanosterol treatment, we observed the restoration of lipid metabolism disorders induced by SREBP2 knockdown in lens epithelial cells (LECs). Notably, lanosterol exhibited the ability to effectively counteract amyloid accumulation and cellular apoptosis triggered by lipid metabolism disorders. In summary, our findings suggest that lanosterol, a pivotal intermediate in lipid metabolism, may exert its therapeutic effects on cataracts by influencing lipid metabolism. This study shed light on the treatment and pharmaceutical development targeting Age-related Cataracts (ARC).

1. Introduction

Cataract is the leading cause of blindness and its incidence is gradually increasing [1], with age-related cataract (ARC) being the most common [1]. A loss of lens transparency is called cataract [2]. Age-related cataract is a clouding of the lens that begins to occur in middle-aged and older adults [3]. The prevalence increases significantly with age. As a transparent tissue, the crystalline lens consists of lens fiber cells and lens epithelial cells (LECs) [4]. LECs are the major cells in the lens throughout its life cycle [5]. By facilitating material transport, synthesis, metabolism, mitosis, and proliferation, LECs contribute to the maintenance of lens homeostasis. If disturbed, this will result in turbidity of the lens [6]. The physiological and pathological status of the lens is largely determined by LECs [7].

The most important factor in cataract formation is abnormal amyloid accumulation, amyloid is directly triggered by crystalline protein aggregates [8,9]. Aging eyes appear to be vulnerable to oxidative stress. Amyloid accumulation can be caused by oxidative stress, which can lead to age-related cataracts [10]. Amyloid of the mutant protein causes stress on LECs [11], leading to disruption of cell viability and cell integrity [12], and leads to the apoptosis of LECs [13]. There are reports that the main cause of cataracts is oxidative stress-mediated apoptosis of LECs [5,14]. So, further research into the mechanisms of LECs steady-state will make it possible to explore new approaches to cataract prevention and treatments.

Lanosterol appears to be an effective molecule in the treatment of cataracts [15]. The amphiphilic molecule lanosterol, which is enriched in the lens, has been reported to reverse amyloid accumulation in cataracts and also reduces intracellular amyloid accumulation of various mutant crystallins, including α-, β-, and γ-crystallins, that contribute to cataracts [16]. Lipid is an essential building block of mammalian cell membranes [17] and plays an important role in multiple cellular functions, such as cell growth [18], intracellular vesicle trafficking [19], and membrane raft signaling transduction [20]. Different tissues are affected

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differently by dysregulated lipid biosynthesis [21]. For example, inadequate cholesterol levels due to various genetic mutations in cholesterol biosynthesis genes preferentially lead to cataracts in the eye lens [22]. Defective cholesterol biosynthesis in eye lens cells is commonly associated with cataracts, and eye lens cells contain more cholesterol than other types of cells [23]. Cataract formation may be partially caused by inhibition of the lipid production pathway [24–26]. However, it has not been reported whether lanosterol, as a key intermediate in lipid metabolism, can be used to treat cataracts by regulating lipid metabolism.

As a key transcription factor, sterol regulatory element binding protein 2 (SREBP2) plays a major role in lipid biosynthesis [27,28], and targeting SREBP2 can inhibit lipid synthesis [29]. Here, we constructed a hydrogen peroxide cataract cell model and found that lanosterol could reverse hydrogen peroxide-induced intracellular amyloid deposition and apoptosis, as well as reverse lipid metabolism. Furthermore, we found that knocking down SREBP2 resulted in apoptosis and intracellular amyloid accumulation in LECs, which was reversed by lanosterol. In summary, we found that lanosterol may exert therapeutic effects on cataracts by reversing intracellular amyloid deposition and cell apoptosis through lipid metabolism pathways.

2. Materials and methods

2.1. Cell lines and cell culture

The Human lens epithelial cell (HLE-B3) cell line was from the American Type Culture Collection (ATCC, VA, USA), HLE-B3 cells were cultured in a humidified 5% CO_2 incubator at 37 °C with MEM (Gibco, Carlsbad, CA, USA) containing 1% PS and 10% FBS.

2.2. Cell transfection

SiRNAs were synthesized by Sangon Biotech (Shanghai, China). HLE-B3 cells with 50% confluence in 6-well plates were transfected with control siRNA or SREBF2 (Sterol Regulatory Element Binding Transcription Factor 2) siRNA using DharmaFECT (T-2001-03, Dharmacon, USA) according to the manufacturer's protocol. A nonspecific oligonucleotide without complementary to any human gene was used as a negative control. The culture medium with or without lanosterol (10 μM) treatment was replaced after 6 h. Table 1 lists the three siRNA sequences targeting SREBP2.

2.3. Oxidative stress cell model

HLE-B3 LECs were cultured in a 6-well plate. When the cells grew to 80%–90%, fresh culture medium was replaced, and the cells were treated with 60 μM H_2O_2 for 8 h. Then the cells were treated with or without lanosterol (10 μM) for 24 h [16].

2.4. Apoptosis assays

The cell apoptosis assay was detected using an Annexin V-FITC Apoptosis Detection Kit I (BB-4101, BestBio, Shanghai, China). Cells were trypsinized to single cell and terminated by culture medium, then cells were washed twice with cold 1 × PBS. The precipitation was resuspended by 300 μL of binding buffer and transferred into 1.5 mL tubes. Then 2.5 μL of Annexin V-FITC and 5 μL of PI were added to the resuspended cells with further incubation at room temperature for 15 min and the apoptotic cells were analyzed by flow cytometer. The analysis was conducted by FlowJo software.

Table 1
The sequences of three sense strands of siRNA targeting SREBP2.

Name	Sequences
SREBF2#1	Sense:5′-GCAACAACAGACGGUAAUGAUTT-3′ Antisense: 5′-AUCAUUACCGUCUGUUGUUGCTT-3′
SREBF2#2	Sense:5′-GACCUGAAGAUCGAGGACUUUTT-3′ Antisense: 5′-AAAGUCCUCGAUCUUCAGGUCTT-3′
SREBF2#3	Sense:5′-GCCAUUGAUUACAUCAAAUAUTT-3′ Antisense: 5′-AUAUUUGAUGUAAUCAAUGGCTT-3′
Negative control	Sense:5′-UUCUCCGAACGUGUCACGUTT-3′ Antisense: 5′-ACGUGACACGUUCGGAGAATT-3′

2.5. RNA extraction and quantitative real-time PCR

Cellular RNA was extracted by using Trizol (YEASEN, Shanghai, China) according to the manufacturer's protocol and cDNAs were obtained from 2 μg total RNA reverse-transcription by using Hifair® II 1st Strand cDNA Synthesis Kit (YEASEN, Shanghai, China) according to the manufacturer's protocol. Quantitative real-time PCR (QPCR) analyses were conducted by a CFX Connect™ real-time system (Bio-Rad, Hercules, CA, USA) with SYBR Green master mix (YEASEN, Shanghai, China). The specific primers used to detect mRNA expression levels of SREBF2, Hmgcr (3-hydroxy-3-methylglutaryl-CoA reductase), Pmvk (Phosphomevalonate kinase), Mvd (Mevalonate diphosphate decarboxylase), Fdps (Farnesyl diphosphate synthase), Fdft1 (Farnesyl-diphosphate farnesyltransferase 1), Sqle (Squalene epoxidase), Lss (Lanosterol synthase), Sc5d (Sterol-C5-desaturase), Dhcr24 (24-dehydrocholesterol reductase), Dhcr7 (7-dehydrocholesterol reductase), Hmgcs (3-hydroxy-3-methylglutaryl-CoA synthase 1), were listed as below (see Table 2). And Rps 18 (Ribosomal protein S18) was used as normalization.

2.6. Total cholesterol content assays

The Total cholesterol was detected using a Total cholesterol (TC) detection kit (BB-47435, BestBio, Shanghai, China) following the manufacturer's protocols. Take appropriate amount of cells, wash twice with 1 × PBS, centrifuge at 1000 rpm for 10 min, discard the supernatant, and resuspend with 200 μL of 1 × PBS. Under the condition of ice bath, sonicate to break the cells, 3–5 s each time, 30 s interval, repeat 5 times. Add the reagents according to the requirements of the kit, mix well and incubate at 37 °C for 10 min. The absorbance of the broken cell solution was measured by enzyme-linked immunosorbent assay (ELISA) at 510

Table 2
The sequences of related genes in qPCR analysis.

Gene	Primer squence
SREBF2 primer	F:5′-GCAACAACAGACGGUAAUGAUTT-3′ R: 5′-AUCAUUACCGUCUGUUGUUGCTT-3′
Hmgcs primer	F: 5′-CTTGTGCCCGAAGGAGGAAA-3′ R: 5′-CTGGCCCAAGCCAATGGTAT-3′
Hmgcr primer	F: 5′-GTTAACTGGAGCCAGGCTGA-3′ R: 5′-GATGGGAGGCCACAAAGAGG-3′
Pmvk primer	F: 5′-GGCAAGAGGAAATCCGGGAA-3′ R: 5′-CCTCCTTGTAGGTGCTGGTG-3′
Mvd primer	F: 5′-ATCAAGTACTGGGGCAAGCG-3′ R: 5′-CAAATCCGGTCCTCGGTGAA-3′
Idi 1 primer	F: 5′-TGGCGAGATTGTGTCGTCAA-3′ R: 5′-TGTTGCTTGTCGAGGTGGTT-3′
Fdps primer	F: 5′-TATCTGGGAACAGGATGCCC-3′ R: 5′-GCACCCTAACGATCTGGGAG-3′
Fdft1 primer	F: 5′-ACGTGGGCGACTTATTGACC-3′ R: 5′-GAAGCGCACCAGGTTGTAGA-3′
Sqle primer	F: 5′-CTCCCAGTTCGCCCTCTTC-3′ R: 5′-TTCCTTTTCTGCGCCTCCTG-3′
Lss primer	F: 5′-TTCGGCATCCTGACATCGAG-3′ R: 5′-CTTGAAGGCCATGGAACGCA-3′
Sc5d primer	F: 5′-ATGGAGAGACTTCAGCGCCT-3′ R: 5′-ATCTTCTGGCCATGTGGCTG-3′
Dhcr24 primer	F: 5′-TGAAGACAAACCGAGAGGGC-3′ R: 5′-CGTTTTGGAAGGTGTGCAGG-3′
Dhcr7 primer	F: 5′-CAGGACTTTAGCCGGTTGAGA-3′ R: 5′-CCCTTGAGATGCGGTTCTGT-3′
rps18 primer	F: 5′-ATTAAGGGTGTGGGCCGAAG-3′ R: 5′-TGGCTAGGACCTGGCTGTAT-3′

nm. TC (mmol/g) = (sample hole absorbance - blank hole absorbance)/(standard hole absorbance - blank hole absorbance) × standard concentration (5.17 mmol/L)/protein concentration.

2.7. Protein aggregation assays

Cells were treated with trypsin digestion, centrifuged to remove the supernatant, and fixed with 1 mL of 4% paraformaldehyde for 30 min at room temperature. The paraformaldehyde was removed, rinsed once with PBS, and resuspended with 1 mL of PBS. After counting, the cell suspension was prepared to 20,000 cells/100 μL, and 100 μL of cell suspension was added to the 96-well plate, and 100 μL of PBS was added to the blank wells, followed by the addition of an equal volume of Thioflavin T (THT, MEC) solution at a concentration of 50 μM. The plate was shaken in the dark for 30 min at room temperature and detected by fluorescent microplate reader at 440 nm excitation and 480 nm emission.

2.8. Confocal assays

Firstly, after treating cells with drugs or siRNA, the cells were placed in a confocal dish. After 12 h, the cells were fixed at room temperature with 4% paraformaldehyde for 15 min, and diluted with 0.5% Triton X-100 (PBS) for 30 min at room temperature. Subsequently, the cells were incubated with 5 mg/mL of ThT staining solution at room temperature in the dark for 1 h, followed by DAPI staining solution and room temperature in the dark for 10 min. Finally, observation was performed using a laser confocal microscope.

2.9. Western blotting

The cells were lysed with RIPA lysis buffer (Beyotime, Shanghai, China) supplemented with protease inhibitors (Beyotime, Shanghai, China) and phosphatase inhibitors cocktail (Bimake, Houston, TX, USA). Whole cell lysates were measured using a BCA Protein Assay Kit (Thermo fisher, Waltham, MA, USA) and denatured for 5 min by heating in 99 °C. The protein samples were electrophoresed through a 5%–12% SDS-PAGE gel and transferred to polyvinylidene fluoride (PVDF) membranes (Merck millipore, Darmstadt, Germany), and the membranes were blocked and probed by primary Anti-SREBP2(ab30682, Abcam, USA) and secondary goat anti-rabbit IgG (ab6721, Abcam, USA). After that, the membranes were imaged by chemiluminescence instrument (Bio-Rad, Hercules, CA, USA).

2.10. Statistical analysis

Statistical analysis was performed on mean values using GraphPad Software. The significance of differences between groups was determined via the unpaired *t*-test as *P < 0.05, **P < 0.01, ***P < 0.001.

3. Result

3.1. Lanosterol can reverse amyloid protein accumulation and apoptosis in cataract cell models

Oxidative stress-induced cell apoptosis and amyloid protein aggregation are the main causes of age-related cataracts [14,30]. Detection of amyloid protein accumulation in cellular models is a key indicator of cataract [31]. In the early stage, we tested the toxicity of hydrogen peroxide at different concentrations (Fig. S1A). At the optimal concentration, compared with the control group, the accumulation of amyloid protein in the model group significantly increased, and after treatment with lanosterol, the accumulation of amyloid protein was significantly reduced (Fig. 1A). Fluorescence confocal display yields the same results

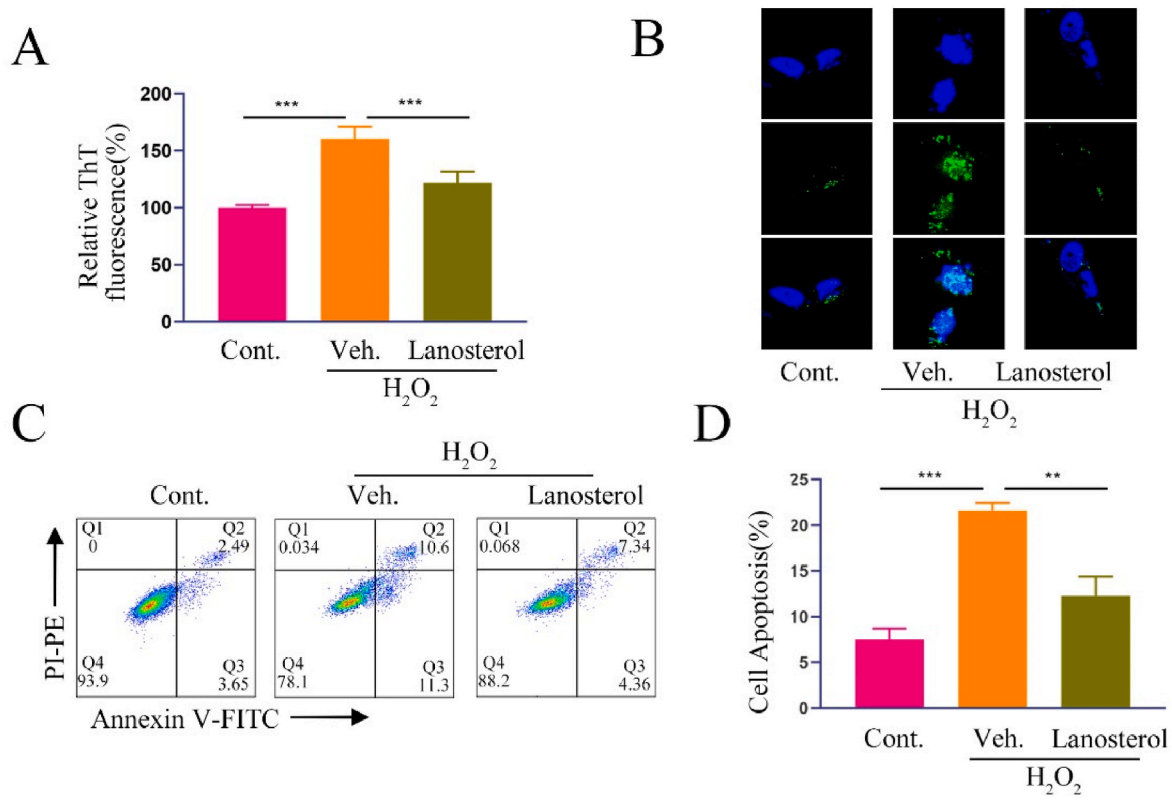

Fig. 1. | Lanosterol can reverse amyloid protein accumulation and apoptosis in LECs. Enzyme reader (A) and confocal detection (B) for amyloid in oxidative stress cell model, blue represents DAPI, green represents THT. (C) Flow cytometry detection of LECs apoptosis in oxidative stress cell model. (D) Statistics of LECs apoptosis. Data were presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

(Fig. 1B). Apoptosis can cause the density of LECs to reach a threshold where it can no longer sustain lens circulation and substance metabolism, which can impact the differentiation of succeeding LECs and cause cataract formation [32]. To verify whether lanosterol could inhibit H_2O_2-induced apoptosis in HLE-B3, we performed flow cytometry experiments to detect apoptotic cells. A relatively low rate of apoptosis was observed after treatment with lanosterol compared to treatment with H_2O_2 alone (Fig. 1C–D). The above results suggest that lanosterol can reverse amyloid protein accumulation and apoptosis in cataract cell models.

3.2. Lanosterol can rescue the inhibited lipid pathway in a cataract cell model

Lipid is important for the health of the lens of the eye, and disorders of lipid metabolism may lead to cataracts [33]. Lanosterol is a major intermediate in lipid metabolism [15,34], so we would explore whether lanosterol can affect lipid metabolism in LECs. Studies have shown that under conditions of severe oxidative stress, the total content of SREBP2 protein in HLE-B3 cells is reduced [35]. We found that genes related to cholesterol synthesis and metabolism including Hmgcs, Hmgcr, and Pmvk [36], were downregulated in LECs after hydrogen peroxide treatment (Fig. 2B). To verify the changes in lipid metabolism, we also tested the changes of cholesterol, which is the end products of this pathway. We found that the total cholesterol content in the model group also significantly decreased, but lanosterol can restore changes in metabolic genes and metabolites (Fig. 2C). We also verified that hydrogen peroxide treatment can reduce the content of SREBP2 protein (Fig. 2D).

3.3. Disturbing cholesterol pathways can cause amyloid protein accumulation and apoptosis in LECs

SREBP2 is a principal transcription factor that regulates cholesterol biosynthesis [27]. To verify whether the accumulation of intracellular amyloid proteins acts through the lipid metabolic pathway, we first constructed three Si RNAs of SREBP2 and verified the knockdown effect. We found that Si #1 and #2 had the best knockdown effect (Fig. 3A–B). Then, we used these two siRNAs to knock down the SREBP2 gene and found that knocking down SREBP2 can reduce the expression of cholesterol metabolism pathway genes (Fig. 3C) and the production of metabolites (Fig. 3D).

In addition, we tested the accumulation of intracellular amyloid protein and cell apoptosis. The amyloid deposition (Fig. 3E–F) and cell apoptosis (Fig. 3G–H) significantly increased after knocking down SREBP2. And cell counting result also suggested that the knockdown of SREBP2 significantly inhibited the proliferation of LECs (Fig. 3I). The above results indicate that interference of lipid metabolism leads to amyloid protein accumulation and apoptosis in LECs, resulting in cataract development.

3.4. Lanosterol can rescue LECs apoptosis and intracellular amyloid protein accumulation caused by disturbing cholesterol pathways

Next, we explored whether lanosterol, as a key intermediate in lipid metabolism, can reverse phenotype of cataract cells caused by lipid metabolism disorders. We added lanosterol to the LECs model of SREBP2 knockdown for treatment. It was found that lanosterol can reverse the decrease of lipid metabolism gene expression (Fig. 4A) and metabolite content (Fig. 4B) caused by knocking down SREBP2. Similarly, lanosterol could reduce the accumulation of amyloid (Fig. 4C–D) and cell apoptosis (Fig. 4E–F) caused by lipid metabolism disorders, and restored cell proliferation (Fig. 4G). In conclusion, these data indicate that lanosterol reverses LECs apoptosis and decreases intracellular amyloid protein accumulation caused by disturbing cholesterol pathway.

4. Discussion

LECs lesions are the main cause of cataracts [37]. Cataract occur due to a loss of transparency in the crystalline lens of the eye, which is the leading cause of blindness in the world [38]. Currently, surgery is the only effective solution, but cataract surgeries are a kind of burden for healthcare costs owing to the sheer prevalence of the disease among aging populations [39,40]. Currently, there is an urgent need for drug assisted treatment to reduce the cost and difficulty of cataract treatment.

Past studies have demonstrated that cholesterol is present in large amounts in the normal lens [41,42]. And cataractous lenses contain more cholesterol and sphingomyelin than normal lenses [43]. Disorders

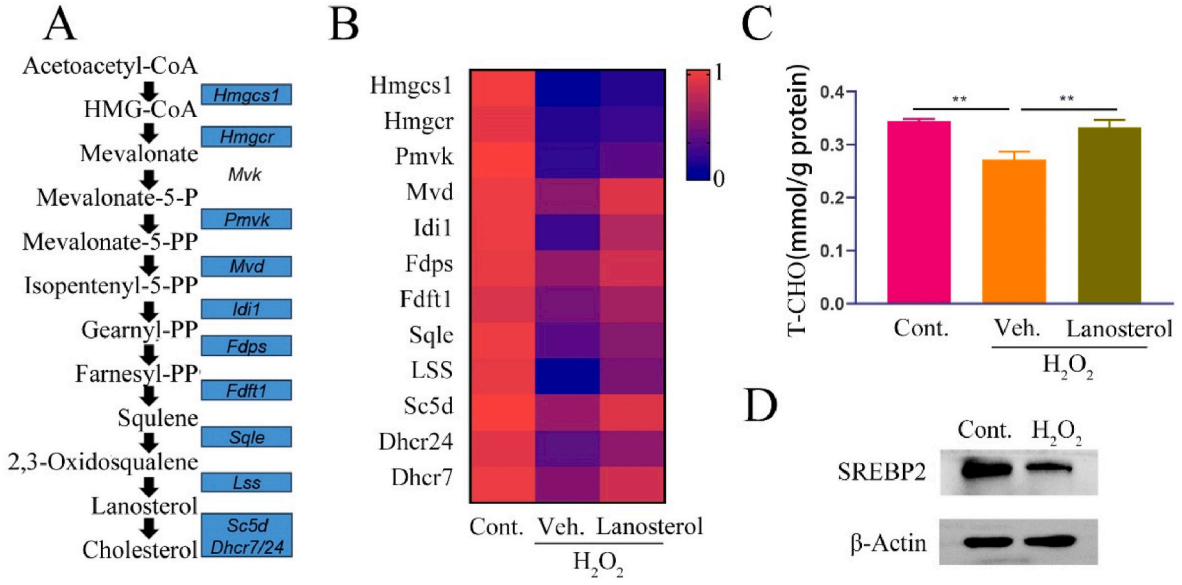

Fig. 2. | Lanosterol can rescue the inhibited cholesterol synthesis pathway gene expression and product production in LECs. (A) Schematic diagram of cholesterol synthesis-related genes. (B) RT-PCR analysis of cholesterol synthesis-related genes in oxidative stress cell model. (C) Total cholesterol content in oxidative stress cell model. (D) Western blot analysis of the SREBP2 protein in LECs treated with hydrogen peroxide for 24 h. Data were presented as mean ± SEM. *P < 0.05, **P < 0.01.

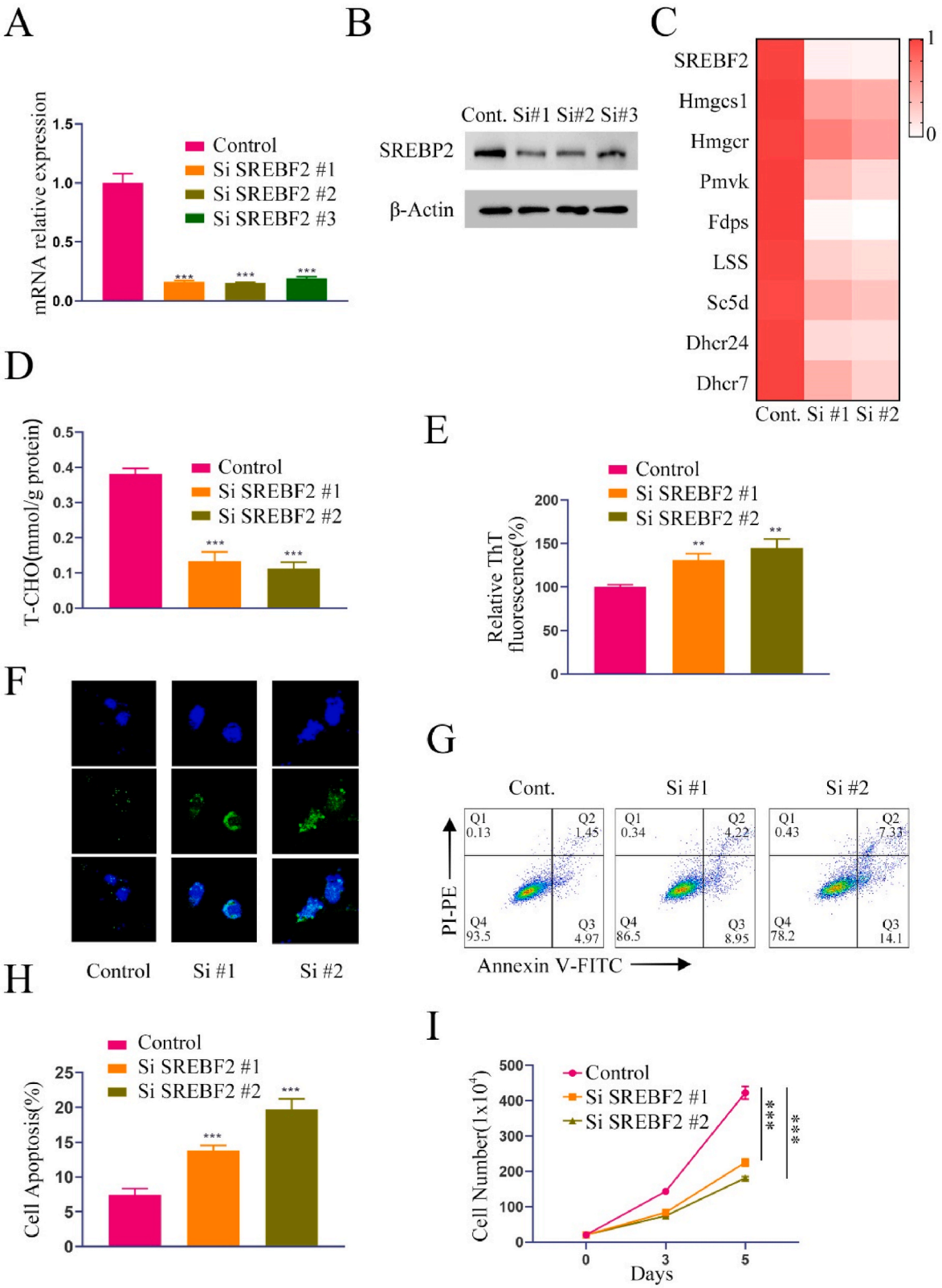

Fig. 3. | Knocking down SREBP2 can cause amyloid protein accumulation and apoptosis in LECs. (A) RT-PCR and Western blot (B) analysis of the SREBF2 gene and SREBP2 protein in LECs transfected with different Si SREBF2 for 48 h. (C) RT-PCR analysis of cholesterol-related synthesis genes in LECs transfected with Si #1 or Si #2 for 48 h. (D) Total cholesterol content in LECs transfected with Si #1 or Si #2 for 72 h. Microplate reader (E) and confocal detection (F) for amyloid in LECs after being transfected with different Si SREBF2 for 72 h, blue represents DAPI, green represents THT. (G) Flow cytometry detection of apoptosis in LECs transfected with different Si SREBF2 for 48 h. (H) Statistics of LECs apoptosis. (I) Number of LECs transfected with different Si SREBF2 for 3 and 5 days. Data were presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

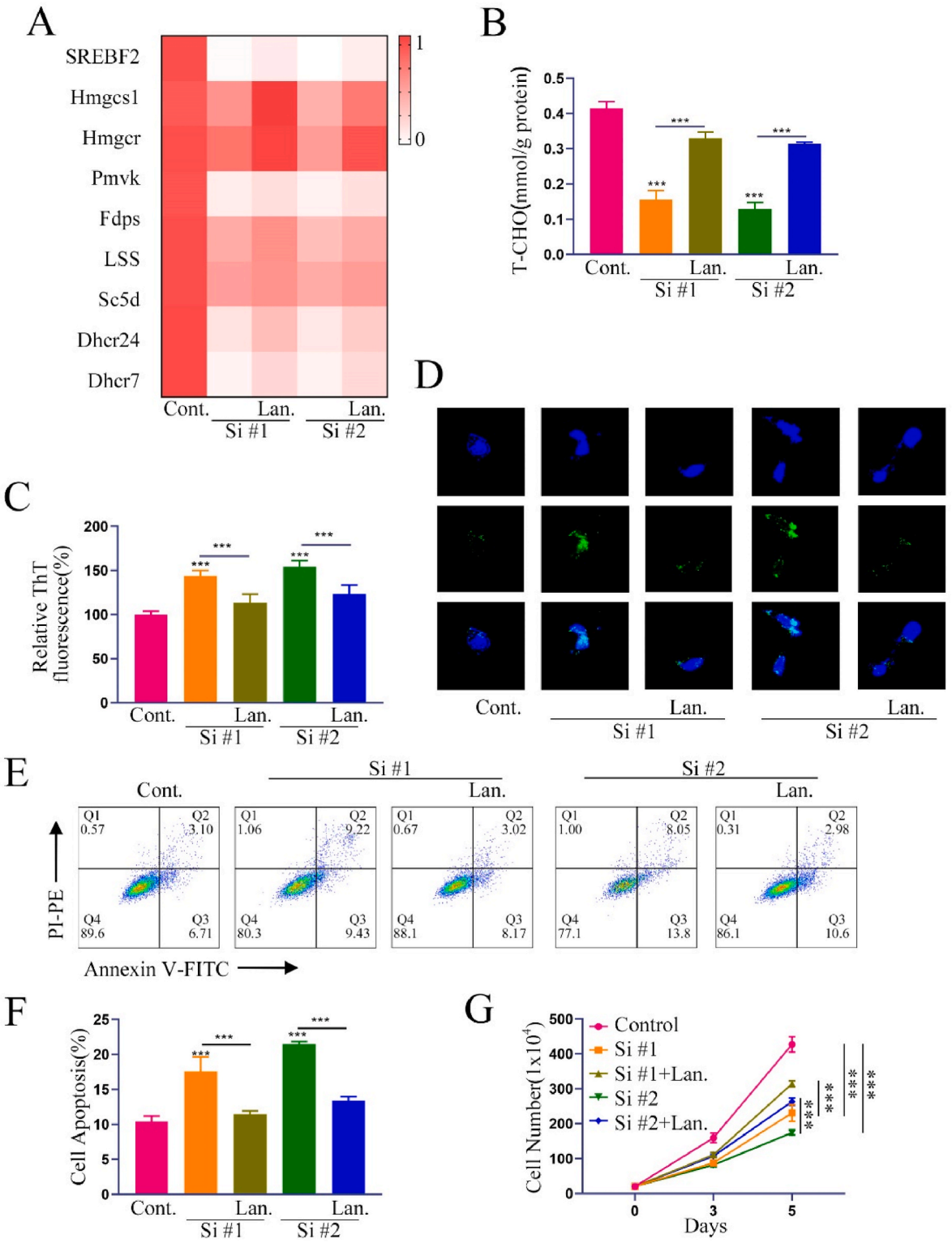

Fig. 4. | Lanosterol can rescue LECs apoptosis and intracellular amyloid protein accumulation caused by knocking down SREBP2. (A) RT-PCR analysis of cholesterol-related synthesis genes in LECs After transfection with different Si SREBF2, followed by treatment with or without lanosterol (10 μM) for 48 h. (B) Total cholesterol content in LECs after transfection with different Si SREBF2, followed by treatment with or without lanosterol (10 μM) for 72 h. Microplate reader (C) and confocal detection (D) for amyloid proteins in LECs, blue represents DAPI, green represents THT. First transfected with different Si SREBF2, and then treated with or without lanosterol (10 μM) for 72 h to observe amyloid in LECs. (E) Flow cytometry detection of LECs apoptosis after transfection with different Si SREBF2, followed by treatment with or without lanosterol (10 μM) for 48 h. (F) Statistics of LECs apoptosis. (G) Number of LECs After transfection with different Si SREBF2, followed by treatment with or without lanosterol (10 μM) for 3 and 5 days. Data were presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

of sterol metabolism leads to an increase in oxidized derivatives of cholesterol, which further cause oxidative stress, inflammation and apoptosis leading to age-related cataracts [44]. Lanosterol is a major intermediate in lipid metabolism. Mutations in genes that block lanosterol synthase have been reported in children with congenital cataracts [45]. It has been widely reported that lanosterol could reverse cataracts, particularly in multiple animal models where its reversal effect has been demonstrated [15,16]. However, the pharmacological mechanism of how lanosterol reverses cataracts needs to be explored. Abnormal amyloid accumulation in LECs and apoptosis of LECs are important causes of cataracts [46]. We induced a cellular model of cataract with hydrogen peroxide and found that lanosterol reversed amyloid accumulation and apoptosis in this model. Meanwhile, lanosterol reversed lipid metabolism disorders induced by oxidative stress. One limitation of this study is that it only conducted validation at the cellular level and lacked in vivo pharmacological support. Therefore, further investigation is needed to determine whether lanosterol can reverse oxidative stress-induced lipid metabolism disorders in vivo.

Mammals have two SREBP-encoding genes that express three SREBPs, SREBP1a, SREBP1c, and SREBP2. Of these, SREBP2 predominantly regulates genes involved in cholesterol metabolism [27]. SREBP2 is one of the key transcription factors in lipid metabolism [47]. SREBP2 acts by binding to SREBP cleavage activating protein (SCAP), and blocking SREBP2 prevents cholesterol biosynthesis [27]. Next, we knocked down SREBP2 and found that reducing lipid synthesis metabolism from the source leaded to an increase in abnormal amyloid in LECs and apoptosis of LECs. There was no clear report whether lanosterol, as a key intermediate in lipid metabolism, could treat cataracts by affecting lipid metabolism. This study found that lanosterol could restore lipid metabolism disorders caused by SREBP2 knockdown in LECs, and reverse amyloid accumulation and cell apoptosis caused by lipid metabolism disorders. The result suggested that the effect of lanosterol to revert hydrogen peroxide-induced amyloid aggregation and apoptosis may be mediated by increased cholesterol biosynthesis.

Amyloid accumulation and apoptosis represent crucial phenotypes in the context of cataracts. Amyloid beta protein, particularly in the form of aggregated plaques, exhibits toxicity and has been implicated in inducing apoptosis [48]. However, not all apoptosis in the process of cataract is caused by amyloid accumulation. From our data, targeting the key rate-limiting enzyme of cholesterol synthesis can indeed induce amyloid accumulation and apoptosis. And some evidence have shown that 33.7% of patients receiving statin treatment, which is a Hmgcr inhibitor and inhibits the biosynthesis of cholesterol, could cause cataract as a side effect [49,50].

In 2015, Zhao et al. made an interesting discovery, establishing that exogenous lanosterol, rather than cholesterol, possesses the capability to reverse lens protein aggregation [16]. Our present findings align with this paradigm, revealing that augmenting cholesterol pathway gene expression and intracellular cholesterol levels can effectively mitigate protein aggregation at the cellular level. This observation is likely linked to the lens's pronounced reliance on de novo cholesterol biosynthesis, as documented in cholesterol-rich tissues like the lens and brain, which predominantly fulfill their cholesterol requirements through endogenous synthesis rather than relying on plasma lipoprotein supply [24,51].

Treatment with hydrogen peroxide induces oxidative stress, precipitating apoptosis. Lanosterol's ability to depolymerize protein aggregates may contribute to an increase in soluble lens protein content. Notably, the protein CRYAA has been reported to impede the release of reactive oxygen species (ROS) from mitochondria, thereby inhibiting apoptosis through potential involvement in the caspase-3 pathway [52,53]. Consequently, we hypothesize that lanosterol, by depolymerizing CRYAA aggregates, may partially attenuate apoptosis induced by hydrogen peroxide, or alternatively, it may directly diminish ROS levels to mitigate apoptosis. Additionally, cholesterol, a vital component of mammalian cell membranes, plays a crucial role in cell growth. In the context of the eye lens, cholesterol, a major constituent of the cell plasma membrane, ensures membrane fluidity, orderliness, and lens transparency [54]. As one of the major components of the eye lens's cell plasma membrane, cholesterol ensures the fluidity and orderliness of eye lens's cell plasma membrane and maintains the transparency of the lens [55]. The level of cholesterol decreases in lens tissue of cataract [56]. Consistent with these observations, our results demonstrate that lanosterol, by upregulating the expression of cholesterol synthesis genes and increasing cholesterol levels, effectively hinders aggregation and cell death induced by hydrogen peroxide. Consequently, we guess that lanosterol's inhibition of hydrogen peroxide-induced cell death may be attributed, in part, to its facilitation of cholesterol biosynthesis. However, the precise mechanistic underpinnings of this hypothesis warrant further experimental validation, and we remain committed to conducting in-depth research in this regard in future investigations.

In summary, we found that lack of lipid synthesis could trigger amyloid accumulation and apoptosis in LECs, finally leading to cataract, and such a process could be reversed by lanosterol. This study may open up new avenues for the treatment and drug development of cataract.

CRediT authorship contribution statement

Yingxue Su: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation. **Danyuan Sun:** Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Chen Cao:** Resources, Project administration, Investigation, Conceptualization. **Yandong Wang:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

All authors disclosed no relevant relationships.The author(s) declared no potential conflicts of interest with respect to the research, author-ship, and/or publication of this article.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101679.

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