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Cassia polysaccharides can regulate the effect of low PAX6 expression on the function of ARPE-19 cells through the Wnt/β-catenin pathway

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Purpose: To explore the protective effects of cassia polysaccharides on myopia by examining their influence on ARPE-19 cells with reduced PAX6 expression.

Methods: The ARPE-19 cell line with diminished PAX6 expression was established using a lentiviral approach and the addition of XAV-939, an inhibitor of Wnt/β-catenin. We assessed the expression of genes and proteins involved in Wnt/β-catenin, scleral remodeling, and cell cycle regulation following treatment with cassia polysaccharides. Gene and protein expression were quantified using reverse transcription PCR and western blot analyses, respectively. Additionally, the migratory capabilities of these cells were evaluated using a scratch assay.

Results: Optimal transduction was achieved with a multiplicity of infection of 20, successfully generating a stable ARPE-19 cell line with low PAX6 expression. Cassia polysaccharides did not significantly alter the expression of Wnt2 compared to control groups. Similarly, when treated with XAV-939, β-catenin levels were modified in PAX6-shRNA and XAV-939 but remained unchanged in the cassia polysaccharides. Scleral remodeling markers, including MMP-2 and TGF-β, were elevated, and COL1A1 was decreased in PAX6-shRNA, with no significant changes observed in the cassia polysaccharides. Cell cycle analysis indicated reduced cyclin-dependent kinase 1 and proliferating cell nuclear antigen levels in PAX6-shRNA, with cassia polysaccharides showing no significant effect. Scratch assay results demonstrated slower wound healing in PAX6-shRNA compared to controls over 72 h, with no significant differences observed in the cassia polysaccharides.

Conclusions: Cassia polysaccharides may mitigate ARPE-19 cell damage induced by low PAX6 expression through modulation of Wnt/β-catenin, potentially slowing the progression of myopia and offering a protective effect on vision.

Myopia is one of the most common eye diseases and is a frequent concern worldwide [1,2]. The prevalence of myopia among high school students, especially in some parts of East and Southeast Asia, reaches 80% to 90% [3]. Myopia imposes a public health burden of refractive error correction on patients and society and affects quality of life [4]. Myopia that develops at an earlier age is more likely to progress to high myopia [5], increasing the risk of potential vision pathologies such as macular degeneration [6]. Therefore, it is important to prevent the development of myopia.

The Wnt/β-catenin signaling pathway is highly conserved during species evolution and plays a crucial role in animal embryonic development and adult tissue homeostasis [7], and compared to other tissues around the eye, it is highly expressed in corneal tissues [8]. The PAX6 gene is a major regulator of ocular vision and plays an important regulatory role in vertebrate eye development, especially in the formation and development of the lens and corneal epithelium [9,10]. PAX6 consists of 16 exons and three promoter regions (P0, P1, and Pα), resulting in three isoforms of the PAX6 protein: classical PAX6, PAX6 (5a), and PAX6 (ΔPD) [11]. In addition, in vertebrates, the PAX6-encoded product affects the expression of transforming growth factor β2 (TGF-β2) and follistatin, which also regulate the function of PAX6 through positive and negative feedback [12].

Three highly homodimeric forms of TGF-β (TGF-β1, 2, and 3) mediate cellular effects through the same receptor complex and affect important physiologic processes, such as cell growth, differentiation, and immunity [13]. Among them, TGF-β1, which has the highest level of expression in human tissues, plays an important role in scleral remodeling, myofibroblast transdifferentiation, and the formation of high myopia by regulating the expression of collagen in the extracellular matrix (ECM), especially collagen type I alpha 1 chain (COL1A1) [13,14]. Scleral remodeling is a dynamic process involving continuous synthesis and degradation of the ECM, and matrix metalloproteinase type 2 (MMP-2), a member of the endopeptidase family, can degrade ECM components and participate in signal transduction [15,16]. It has been shown that MMP-2 overexpression can affect scleral tissue remodeling, which in turn leads to the onset

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and development of pathological myopia [17]. It was experimentally confirmed that knocking down MMP-2 in a mouse model of deprivation myopia significantly increased COL1A1 accumulation in the sclera during myopia [18].

Retinal pigment epithelium (RPE) cells, which are pigment cells that develop and differentiate from "optic nerve vesicles," play an important role in maintaining retinal homeostasis and integrity [19]. Oxidative stress accelerates the senescence of RPE cells, resulting in the loss of their hexagonal structure and a corresponding weakening of their barrier function, leading to altered retinal function [20,21].

Cassia seeds, originally documented in the ancient Chinese medical text *Shennong Ben Cao Jing*, are known for their rich bioactive constituents, including anthraquinones, fatty acids, naphthalene, pyrrolidone, and polysaccharides. These components confer a range of physiologic benefits, such as antioxidant and antiaging effects, blood pressure and lipid regulation, antibacterial and anti-inflammatory effects, and obesity inhibition. Specifically, cassia polysaccharides, a primary active component, exhibit antioxidant, anti-inflammatory, and immunomodulatory properties. These properties are crucial in preventing and mitigating hereditary ocular diseases such as retinitis pigmentosa [22,23]. Historically, as noted in the *Divine Husbandman's Classic of the Materia Medica*, cassia seeds have been primarily used to treat various eye-related ailments, including glaucoma, redness, swelling, pain, cataracts, and tearing. Cassia polysaccharides effectively neutralize reactive oxygen species, such as O^{2-}, -OH, and HO_2, and reduce malondialdehyde (MDA) production, thus enhancing the body's antioxidant capability. This action helps prevent eye diseases caused by oxidative damage and slows their progression [24].

Given this background, our research aims to develop a cellular model of myopia to explore the protective effects of cassia polysaccharides on human retinal pigment epithelial cells. This study will focus on the modulation of the Wnt/β-catenin signaling pathway and assess the expression of related factors, providing insights for the prevention and management of myopia.

METHODS

Materials and reagents: ARPE-19 (human retinal pigment epithelial cells) cells were purchased from the National Collection of Authenticated Cell Cultures, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM)/F12K medium was purchased from Gibco (Waltham, MA). Cassia polymorpha was purchased from Sichuan Weikeqi Biologic Technology (Sichuan, China). PAX6 antibody and sheep antirabbit antibody were purchased from Proteintech Group (Wuhan, China). Wnt2 antibody, β-catenin antibody, COL1A1 antibody, MMP-2 antibody, TGF-β antibody, cyclin D1 antibody, and proliferating cell nuclear antigen (PCNA) antibody were purchased from WanleiBio (Liaoning, China). β-Actin antibody and goat antimouse antibody were purchased from Beyotime Biotechnology (Shanghai, China).

All primer sequences were synthesized and acquired by Sangon Biotech (Shanghai, China; Table 1). Lentivirus was packaged and acquired by OBiO (Shanghai, China). XAV-939 was purchased from Aladdin Biochemical Technology (Shanghai, China).

Cell culture: ARPE-19 cells were cultured in a 37 °C, 5% CO_2 incubator, using DMEM/F12K medium containing 10% fetal bovine serum and 1% penicillin-streptomycin. A cell density of 90% or more was subcultured by digesting the cells with 0.05% trypsin for 3 min and then observing them under a microscope until they are detached and rounded. Then, the cells were centrifuged at 1000 ×g for 5 min and cultured at 1:3. Cell densities of 80% to 90% were achieved in around 5 days. The cryopreservation solution for freezing was 70% fetal bovine serum, 20% complete medium, and 10% DMSO, and the program was cooled to −80 °C and transferred to liquid nitrogen the following day.

Constructing a cellular model of myopia: ARPE-19 cells were inoculated in 6-well plates at a density of 1×10^6 cells/ml, and ARPE-19 cells were infected with a multiplicity of infection (MOI; number of viruses infected per live cell) of 0, 2.5, 5, 10, 20, and 40. An equal volume of protransfection reagent was added, and the cell infection was observed under a fluorescence microscope after incubation for 24 h with serum-free and antibiotic-free medium. After infecting the cells with an appropriate MOI, ARPE-19 cells were cultured for 2 weeks using medium containing 2 μg/l puromycin to remove the lentivirus-uninfected cells. A stable low-expression ARPE-19 cell line of the PAX6 gene was constructed, and the expression level of PAX6 was detected by reverse transcription PCR (RT-PCR) and western blot assay.

Western blot assay: Cells were washed with precooled phosphate-buffered saline (PBS), collected with a cell scraper, added with lysate containing 1/100 of trypsin inhibitor and 1/50 of phosphoproteinase inhibitor, and lysed for 30 min on ice and then centrifuged at 4 °C for 15 min at 12,000 rpm. The supernatant was collected and protein concentration was determined by the BCA method. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels were prepared and electrophoresed at 80 V to the point of separation and then changed to 120 V until the end of electrophoresis. After electrophoretic transfer with a 200-mA current for 1 to 1.5 h,

the PVDF membrane was placed in 5% sealing solution and blocked for 2 h. Subsequently, diluted antibody was added and incubated at 4 °C overnight; the secondary antibody was incubated the next day, and protein bands were detected by ECL chemiluminescence.

RT-PCR assay: Cells were washed with precooled PBS, total RNA was extracted by TRIzol, and reverse transcription was performed with the cDNA First Strand Synthesis Kit (RNase H-) Beyotime Biotechnology (Shanghai, China), with the reaction performed at 42 °C for 60 min and at 80 °C for 10 min to inactivate the reverse transcriptase and terminate the reverse transcription reaction.

The reaction system for RT-PCR was 10 µl SYBR Green, 0.4 µl each of forward primer and reverse primers, 1 µl cDNA, and double-distilled H_2O to make up to 20 µl. Reaction conditions were predenaturation at 95 °C for 30 s, 40 cycles of reaction 95 °C for 10 s and 60 °C for 30 s, and a dissolution curve at 60 °C for 60 s.

Scratch assay: ARPE-19 cells were seeded in six-well plates at a density of 1×10^6 cells/ml. When the cells were evenly confluent in the 6-well plates, the medium was replaced with serum-free and antibiotic-free medium, and a scratch was made. Thereafter, the cell migration status was observed every 24 h for a continuous 72 h, the assay was repeated three times independently.

Cell proliferation assay: ARPE-19 cells in the logarithmic growth phase were seeded into 96-well plates at a density of 5,000 cells per well. This was followed by the addition of 10 ml CCK-8 reagent with 100 ml serum-free DMEM at 24 h, 48 h, and 72 h; continued incubation for 2 h; and then measurement at OD 450 nm in a microplate (SpectraMax M3; Molecular Devices, San Jose, CA). Three independent repeated experiments were performed.

Detection of oxidative stress products: After washing cells with precooled PBS, cells in each group were collected. The contents of MDA and glutathione (GSH) in cells from each group were detected using MDA and GSH kits (Beyotime

Table 1. All primer sequences used in this experiment.

Primer	Sequence (3'-5')
PAX6	F: AGCACCAGTGTCTACCAACCAATTC
	R: TGTTTGTGAGGGCTGTGTCTGTTC
Wnt2	F: CCTGAAGGAGAAGTACGACAG
	R: GATGTAGACCAGGTCTTGTGTG
â-catenin	F: TGGATTGATTCGAAATCTTGCC
	R: GAACAAGCAACTGAACTAGTCG
COL1A1	F: AAAGATGGACTCAACGGTCTC
	R: CATCGTGAGCCTTCTCTTGAG
MMP-2	F: ATTGTATTTGATGGCATCGCTC
	R: ATTCATTCCCTGCAAAGAACAC
TGF-â	F: CTGTACATTGACTTCCGCAAG
	R: TGTCCAGGCTCCAAATGTAG
cyclin D1	F: CACAAAACTACAGGTCAAGTGG
	R: GAGAAATTTCCCGAATTGCAGT
PCNA	F: AAGGTGTTGGAGGCACTCAAGG
	R: GTCGCAGCGGTAGGTGTCG
PI3K	F: CCTGATCTTCCTCGTGCTGCTC
	R: CATGCCAATGGACAGTGTTCCTC
AKT	F: TGACCATGAACGAGTTTGAGTA
	R: GAGGATCTTCATGGCGTAGTAG
P27	F: TGAGATTGTGGAGTTCGGCTCTG
	R: TGCGGATCACTGTCACATTCAGG
â-actin	F: CCTGGACTTCGAGCAAGAGATGG
	R: CAGGAAGGAAGGCTGGAAGAGTG

Biotechnology), respectively. The experiment was independently repeated three times.

Statistical analysis: Data for each group are expressed as mean ± SD, and the significance of differences in means between groups was determined using the Student *t* test (GraphPad Prism; GraphPad Software, La Jolla, CA), with $p < 0.05$ being considered statistically significant. This was repeated at least three times for each group.

RESULTS

Establishing a myopic cell line model with stable low expression of PAX6: After infecting ARPE-19 cells with a MOI of 0, 2.5, 5, 10, 20, and 40, respectively, for 48 h, the results showed that the best infection efficiency was achieved when the MOI was 20 (Figure 1). Downregulated PAX6 gene expression was detected (Figure 2), and downregulated PAX6 protein expression was detected in ARPE-19 cells even at 72 h (Figure 3). A stable low expression of PAX6 in the ARPE-19 cell line means a myopic cell line model was successfully established.

Cassia polysaccharides can modulate the effect of low PAX6 expression on the Wnt/β-catenin pathway: The results of western blot and RT-PCR experiments showed that the expression of Wnt2 genes and proteins in both the PAX6-shRNA group and the XAV-939 group was increased compared with the blank group, but the expression of β-catenin in the XAV-939 group was downregulated compared with the blank group (Figure 4). This suggests that the reduction of PAX6 gene expression controls the activation of the Wnt/β-catenin signaling pathway by affecting the expression of upstream Wnt, rather than directly regulating downstream factors such as β-catenin. However, the differences in Wnt2 and β-catenin expression in the cassia polysaccharide group were not statistically significant compared with the blank group (Figure 4), suggesting that cassia polysaccharides can negatively regulate the overexpression of Wnt2 induced by the low expression of the PAX6 gene.

Cassia polysaccharides modulated the effect of PAX6-shRNA on scleral remodeling: Scleral remodeling also plays an important role in the development of myopia, and detecting low PAX6 gene expression is particularly important for the expression of scleral remodeling-related proteins and RNAs. We found that the expression of COL1A1 was decreased and MMP-2 and TGF-β were elevated in the PAX6-shRNA group compared with the negative control group. In contrast, the expression of COL1A1, MMP-2, and TGF-β in the cassia polysaccharide group was in the opposite direction (Figure 5), which suggests that cassia polysaccharides have a protective effect on ARPE-19 cells, caused by low expression of the PAX6 gene, which had a certain protective effect on scleral remodeling.

Regulation of PAX6-shRAN influenced the cell cycle by cassia polysaccharides: Low PAX6 expression affects the cell cycle, resulting in slower growth of ARPE-19 cells with low PAX6 expression under the same culture conditions, whereas the cell growth status of the cassia polysaccharide group did not differ much from that of the negative control group. Subsequently, we detected the expression of cell cycle–related genes and proteins using western blot and RT-PCR and found that the expression of cyclin-dependent kinase 1 (CDK1) and PCNA in the PAX6-shRNA group was reduced compared with that of the negative control group, whereas the expression of CDK1 and PCNA was normal in the cassia polysaccharide group (Figure 6). This indicates that cassia polysaccharides can regulate the cell cycle hysteresis caused by low PAX6 expression and protect the normal function of ARPE-19 cells.

Knockdown of PAX6 inhibited cell migration and proliferation: Abnormal expression of cell cycle–related genes and proteins also suggested changes in cell migration function, so we performed scratch experiments on ARPE-19 cells from different treatment groups and observed them continuously for 72 h (Figure 7). From the results, it can be seen that the low expression of PAX6 led to a decrease in cell migration, while cassia polysaccharides increased the migration of ARPE-19 cells after the low expression of the PAX6 gene to a certain extent, and the scratch wound healing was faster than in the PAX6-shRNA group. The difference in cell migration among the cassia polysaccharide group, blank group, and negative control group was not significant, indicating that cassia polysaccharide could protect the migration function of ARPE-19 cells after low expression of PAX6. The CCK-8 assay (Figure 8) was performed to confirm whether PAX6 and cassia polysaccharides could impact cell proliferation. The results showed that the absorbance (OD) at 450 nm of ARPE-19 cells in the PAX6-shRNA group was lower at 48 h and 72 h, while the proliferation of ARPE-19 cells was significantly improved after the addition of cassia polysaccharides. These data suggest that knocking down PAX6 can inhibit cell migration and proliferation, while cassia polysaccharides have a protective effect on cell functions.

Knockdown of PAX6 reduced cellular antioxidant activities: MDA is one of the end products of lipid peroxidation and a biomarker of oxidative stress. GSH, an important component of intracellular antioxidant systems, serves as a key reducing agent and participates in various redox reactions within cells. The results (Figure 9) showed that the content of MDA in ARPE-19 cells of the PAX6-shRNA group was significantly

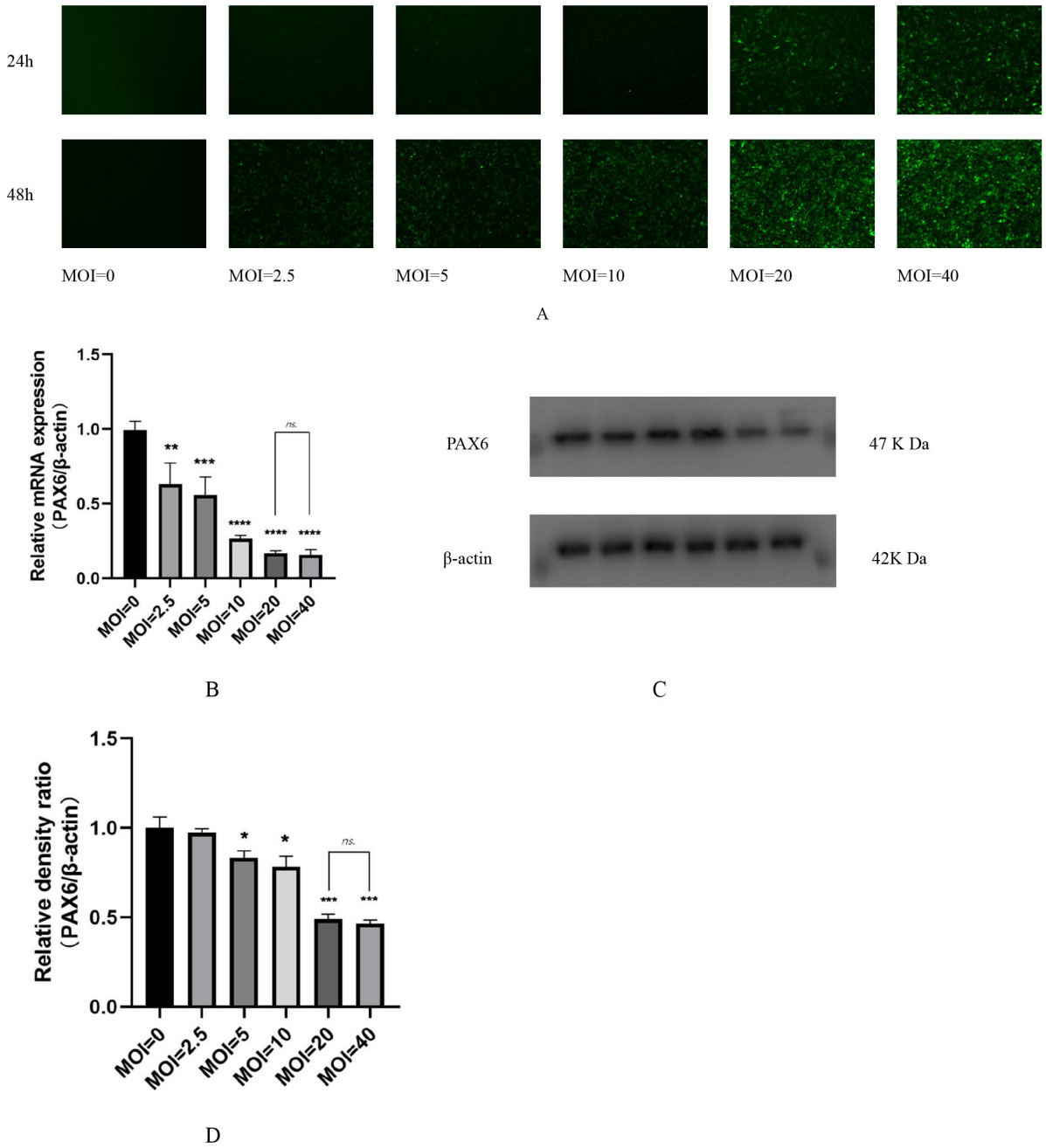


Figure 1. Experimental results of lentiviral infection of human retinal pigment epithelial cells (ARPE-19) at different multiplicities of infection (MOI = 0, 2.5, 5, 10, 20, 40; n=3). **A**: Observed under a 40× fluorescence microscope at 24 h and 48 h post-infection with lentiviruses of different MOIs, the MOI was positively correlated with fluorescence intensity. **B**: At 24h after lentiviral infection of ARPE-19 cells at different MOIs, the mRNA expression level of paired box gene 6 (PAX6) was inversely correlated with MOI, and no statistically significant difference in mRNA expression was observed when MOI>20. **C**, **D**: At 48h after lentiviral infection of ARPE-19 cells at different MOIs, the PAX6 protein expression level showed a decreasing trend, and no statistically significant difference in protein expression was detected when MOI > 20 (the MOI = 0 group exhibited statistically significant differences compared with all other groups; no statistically significant difference was observed between the MOI = 20 group and the MOI = 40 group; *p<0.05, **p<0.01, ***p<0.001).

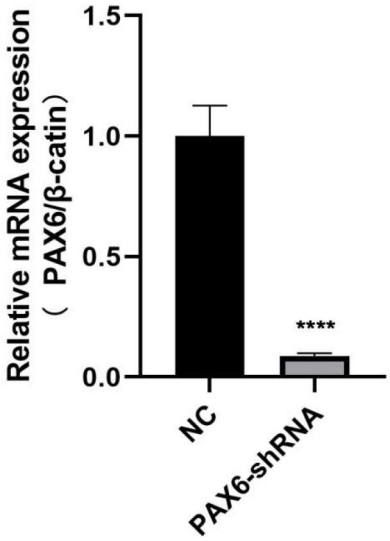


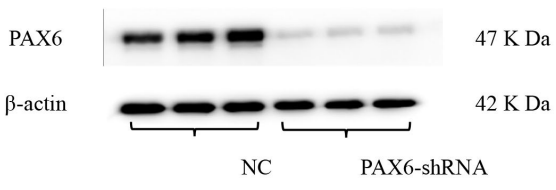
Figure 2. At 48 h after infection of human retinal pigment epithelial cells (ARPE-19) with lentivirus at a multiplicity of infection (MOI) of 20, the mRNA expression of paired box gene 6 (PAX6) showed a significant downward trend (n=3; PAX6-shRNA group versus NC-shRNA group; *p<0.05, **p<0.01, ***p<0.001).

increased, while the content of GSH was significantly decreased. After the addition of cassia polysaccharides, the intracellular MDA content decreased, and the GSH content increased. These results indicate that knocking down the PAX6 gene causes oxidative damage to ARPE-19 cells, while cassia polysaccharides may protect PAX6-shRNA ARPE-19 cells by exerting antioxidant effects.

Cassia polysaccharides can modulate the effect of low PAX6 expression on the PI3K/AKT-P27 pathway: After testing the effect of PAX6 low expression regulated by cassia seed polysaccharides on the Wnt/β-catenin pathway and cell cycle, we also assessed the expression of factors involved in the PI3K/AKT-P27 pathway. We found that PAX6 low expression inhibited the PI3K/AKT-P27 pathway, and the expression of genes and proteins of PI3K and AKT in the PAX6-shRNA group was lower than that in the control group and the cassia seed polysaccharide group, whereas the expression of P27 in the PAX6-shRNA group was elevated compared with that in the control group and the cassia seed polysaccharide group (Figure 10).

DISCUSSION

The PAX6 gene is critical for ocular development and has been linked to several ocular diseases [25,26]. To date, the association of PAX6 gene polymorphisms with myopia and refractive error remains controversial. PAX6 was not associated with myopia in Caucasians [27], whereas two single-nucleotide polymorphisms in PAX6-rs3026390 and rs3026393 were significantly associated with high myopia in Chinese [28,29]. PAX6 polymorphisms have also been



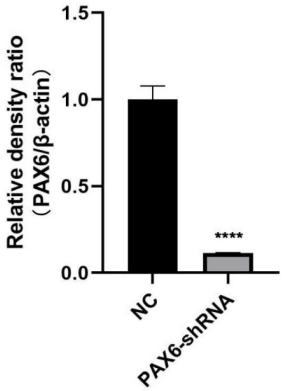


Figure 3. At 72 h after infection of human retinal pigment epithelial cells (ARPE-19) with lentivirus at a multiplicity of infection (MOI) of 20, the protein expression of paired box gene 6 (PAX6) showed a significant downward trend. (n=3; PAX6-shRAN group vs. NC-shRNA group, *p<0.05, **p<0.01, ***p<0.001).

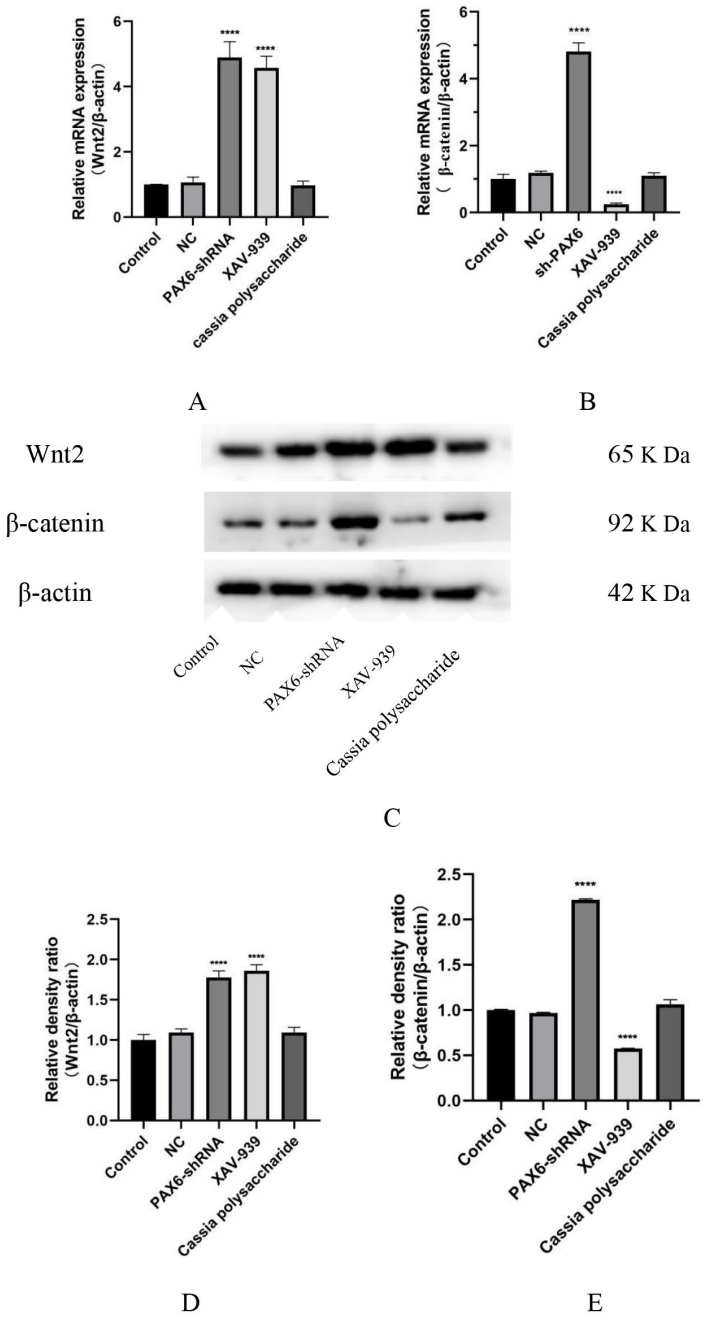


Figure 4. Effect of PAX6-shRNA mediated by cassia polysaccharide on the Wnt/β-catenin pathway. **A**: After PAX6 gene knockdown, the mRNA expression of Wnt2 in the PAX6-shRNA group and XAV-939 group was significantly up-regulated compared with the normal group and cassia polysaccharide group. **B**: After treatment with the Wnt/β-catenin pathway inhibitor, the mRNA expression of β-catenin in the XAV-939 group was significantly decreased, while the mRNA expression trend of β-catenin in the cassia polysaccharide group showed no obvious change. **C**–**E**: After PAX6 gene knockdown, the protein expression trends of Wnt2 and β-catenin in the PAX6-shRNA group, XAV-939 group and cassia polysaccharide group were consistent with their respective mRNA expression trends, using the protein expression levels of the blank group and negative control group as references (n=3; *p<0.05, **p<0.01, ***p<0.001, respectively).

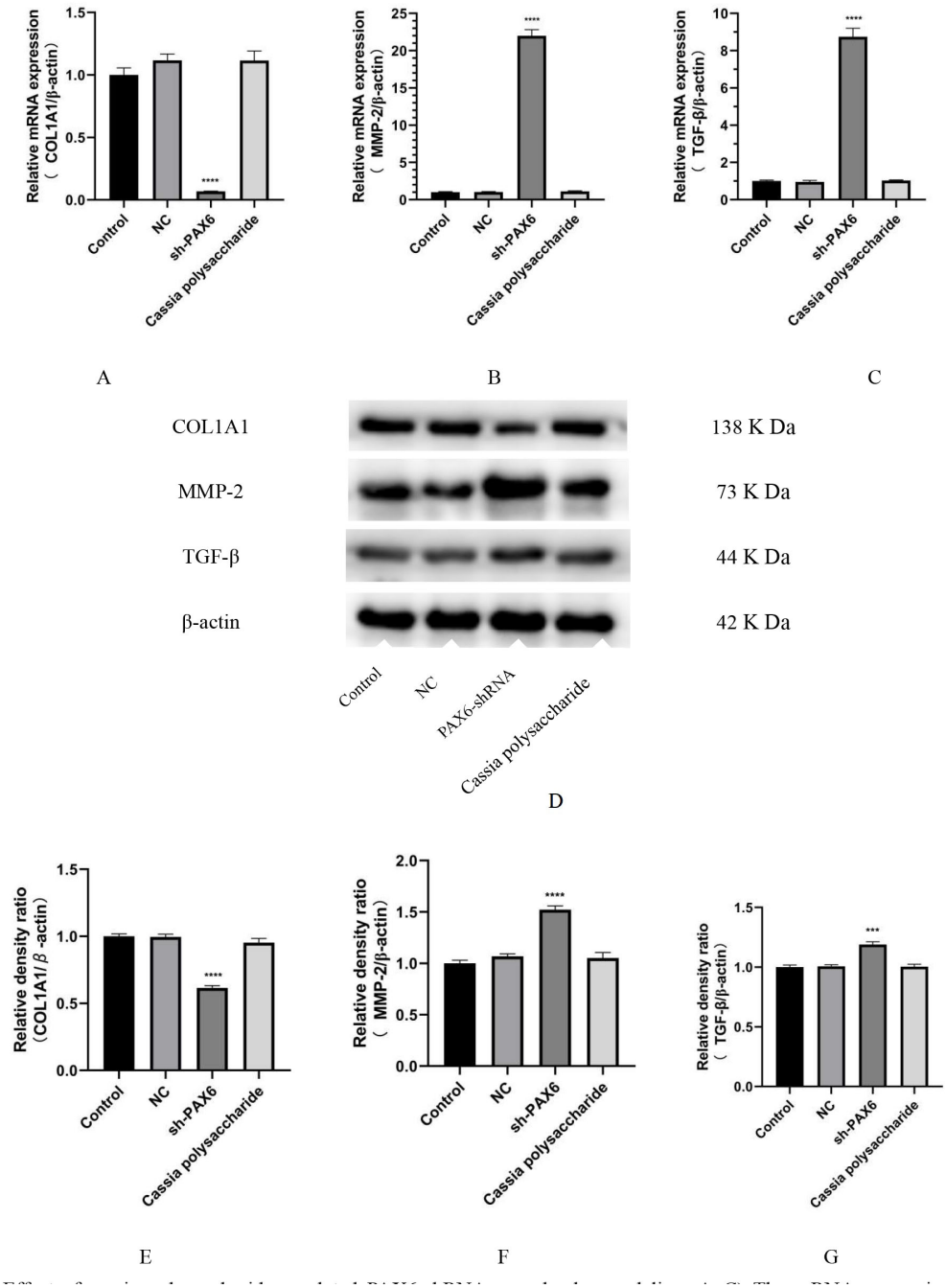


Figure 5. Effect of cassia polysaccharide-regulated PAX6-shRNA on scleral remodeling. **A**–**C**: The mRNA expression levels of COL1A1, MMP-2, and TGF-β in the cassia polysaccharide group were significantly increased compared with those in the PAX6 knockdown group, with no significant difference relative to the normal group. **D**–**G**: The protein expression levels of COL1A1, MMP-2, and TGF-β in the cassia polysaccharide group were also significantly increased compared with those in the PAX6 knockdown group, which was consistent with the mRNA expression trend (n=3; *p<0.05, **p<0.01, ***p<0.001, respectively).

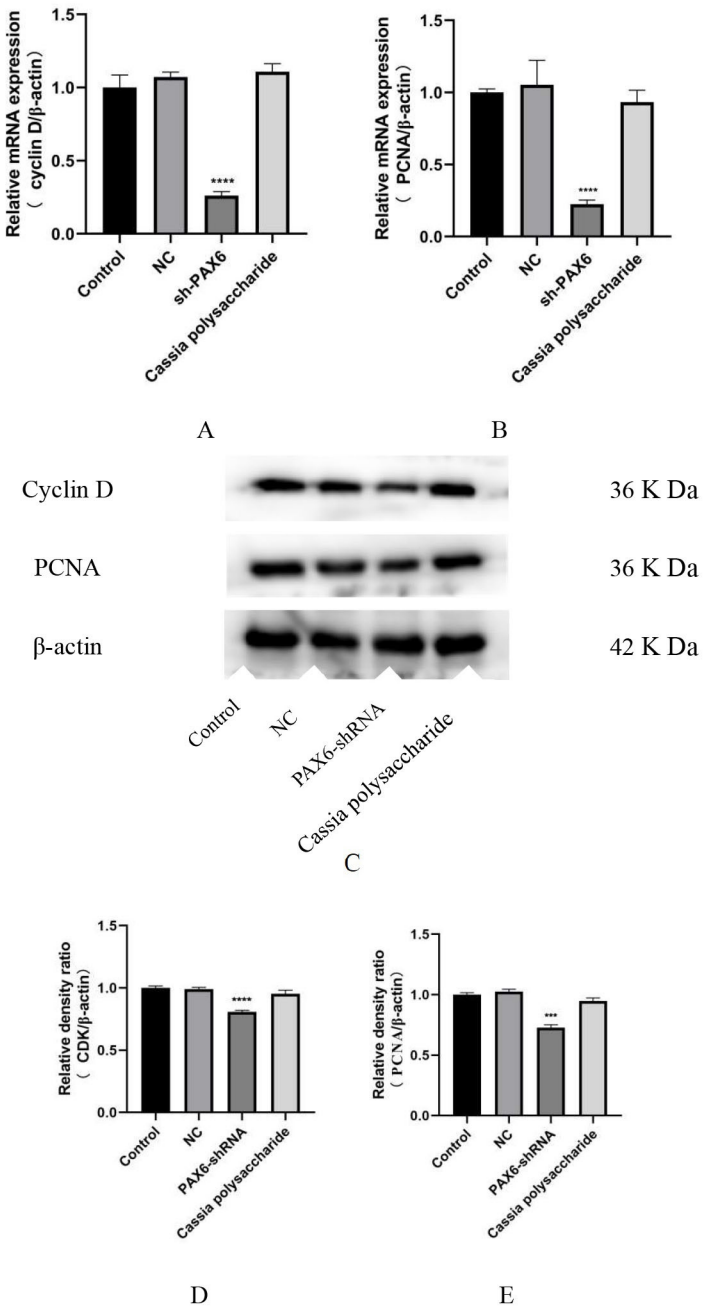


Figure 6. Regulatory effect of cassia polysaccharide on PAX6-shRNA-mediated cell cycle. **A**, **B**: The mRNA expression levels of cell cycle-related genes CDK and PCNA in the cassia polysaccharide group were increased compared with those in the PAX6-shRNA group, showing no significant difference relative to the normal group. **C**–**E**: The cassia polysaccharide group significantly up-regulated the expression of cell cycle-related proteins CDK and PCNA compared with the PAX6-shRNA group (n=3; *p<0.05, **p<0.01, ***p<0.001, respectively).

associated with high myopia in Chinese adults [30], but the association of PAX6 with high myopia has not been reported in Chinese children [31].

In this study, we used lentiviral infection of ARPE-19 cells to successfully establish a myopic cell model with stable low expression of PAX6. We found that the expression of Wnt2 and β-catenin proteins in ARPE-19 cells was elevated following low PAX6 expression, whereas it was restored to normal after the addition of cassia polysaccharides. To verify the relationship between PAX6 and cassia polysaccharides and the Wnt/β-catenin signaling pathway, we subsequently added XAV-939, a blocker of the Wnt/β-catenin signaling pathway, and found that Wnt2 expression was upregulated, whereas downstream β-catenin expression was reduced. This demonstrated that PAX6 and cassia polysaccharides affect the Wnt/β-catenin signaling pathway by regulating Wnt protein expression.

A major cause of myopia is refractive error due to growth of the eye axis, which is closely related to remodeling the scleral extracellular matrix. Scleral remodeling leads to changes in the axial length of the eye and refractive status, which are important features of myopia. TGF-β, which is expressed in the sclera, retina, and choroid, is involved in the formation of form deprivation myopia [32–34], which also affects scleral remodeling and the formation of high myopia [35]. Loss of COL1A1 function leads to systemic diseases such as osteogenesis imperfecta, scleral thinning, and myopia. Another study showed that miR-328 can reduce PAX6 expression by binding to its 3′-UTR, leading to increased MMP-2 expression, which enhances extracellular matrix degradation, causing scleral thinning and lengthening

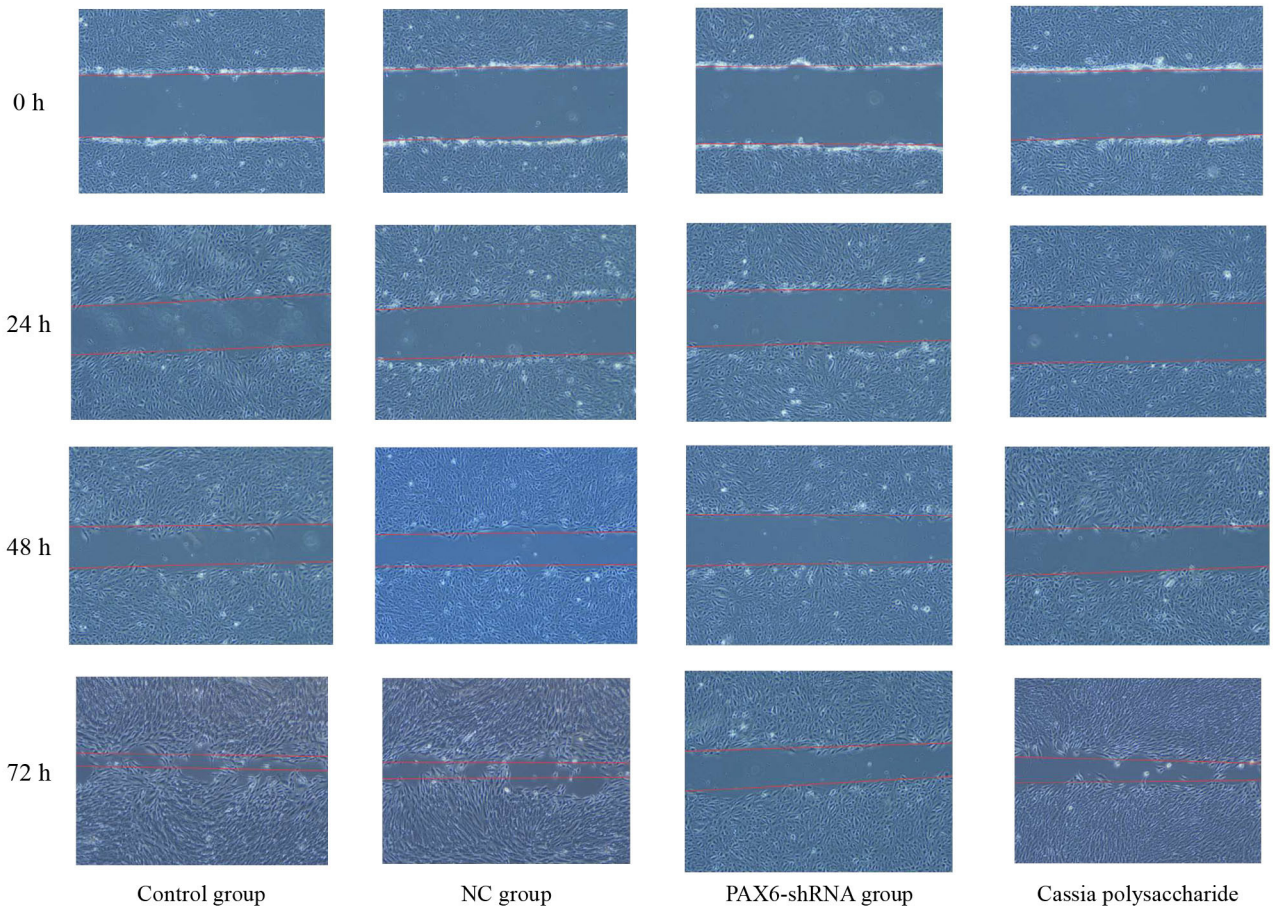


Figure 7. Wound healing rate of cell scratch assay in different treatment groups observed under a 40× microscope. Among them, the wound healing rate of the cell scratch was the fastest in the Control group and NC group, followed by the cassia polysaccharide group, which showed no significant difference from the Control group and NC group in terms of cell migration rate; while the cell migration rate was the slowest in the PAX6-shRNA group (n=3).

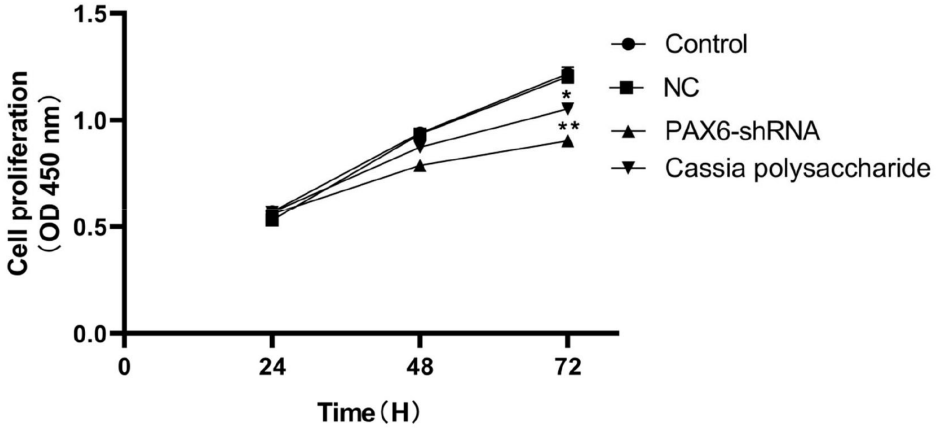


Figure 8. Protective effect of cassia polysaccharide on the proliferation ability of ARPE-19 cells after inhibition by PAX6-shRNA lentiviral vector. The results of CCK-8 assay showed that cassia polysaccharide exerted a certain proliferation-promoting effect on cells after PAX6 knockdown. Data were expressed as mean ± standard deviation (SD); (n=3; *p<0.05, **p<0.01 versus the control group).

of the ocular axis, thereby affecting the progression of myopia [36]. Upregulation of MMP-2 leads to thinning of the scleral collagen framework and changes in extracellular matrix composition, inducing the development of myopia [37]. These are consistent with the results of the present study.

In addition, we found that the ARPE-19 cells in the PAX6-shRNA group grew slower than the other groups under the same conditions during the culture of ARPE-19 cells. To investigate this, we also examined the expression of cell cycle–related genes and proteins in different groups. We found that the expression of cell cycle–related genes and proteins cyclin D1 and PCNA were reduced in ARPE-19 cells in the PAX6-shRNA group. Then, we tested the PI3K/AKT-P27 signaling pathway related to energy metabolism and found that the energy metabolism of ARPE-19 cells in the PAX6-shRNA group was slower than that of other groups (Figure 10). The results also suggest that after the occurrence of myopia, the metabolic level of ARPE-19 cells decreases, and abnormal cell metabolism may be caused by other reasons. If this process cannot be curbed, it may lead to cell apoptosis, thereby aggravating the development of myopia and bringing adverse outcomes, such as high myopia, retinal detachment, and even blindness.

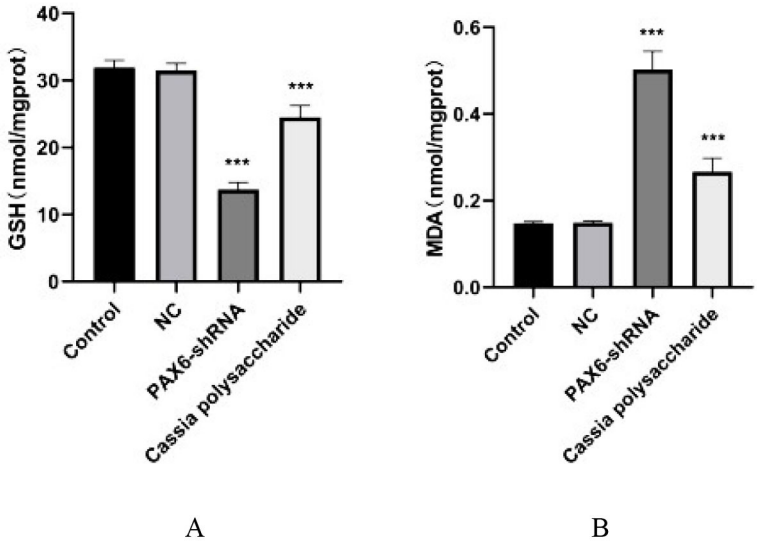


Figure 9. The contents of malondialdehyde (MDA) and glutathione (GSH) in human retinal pigment epithelial cells (ARPE-19) were determined using the corresponding detection kits. The results showed that the intracellular MDA content increased while the GSH content decreased in the PAX6 knockdown group, indicating that ARPE-19 cells suffered a certain degree of oxidative damage. Whereas cassia polysaccharide could protect the cells from oxidative stimulation to a certain extent. The detection results of MDA and GSH were expressed as mean ± standard deviation (SD; n=3; ***p<0.001 versus the control group).

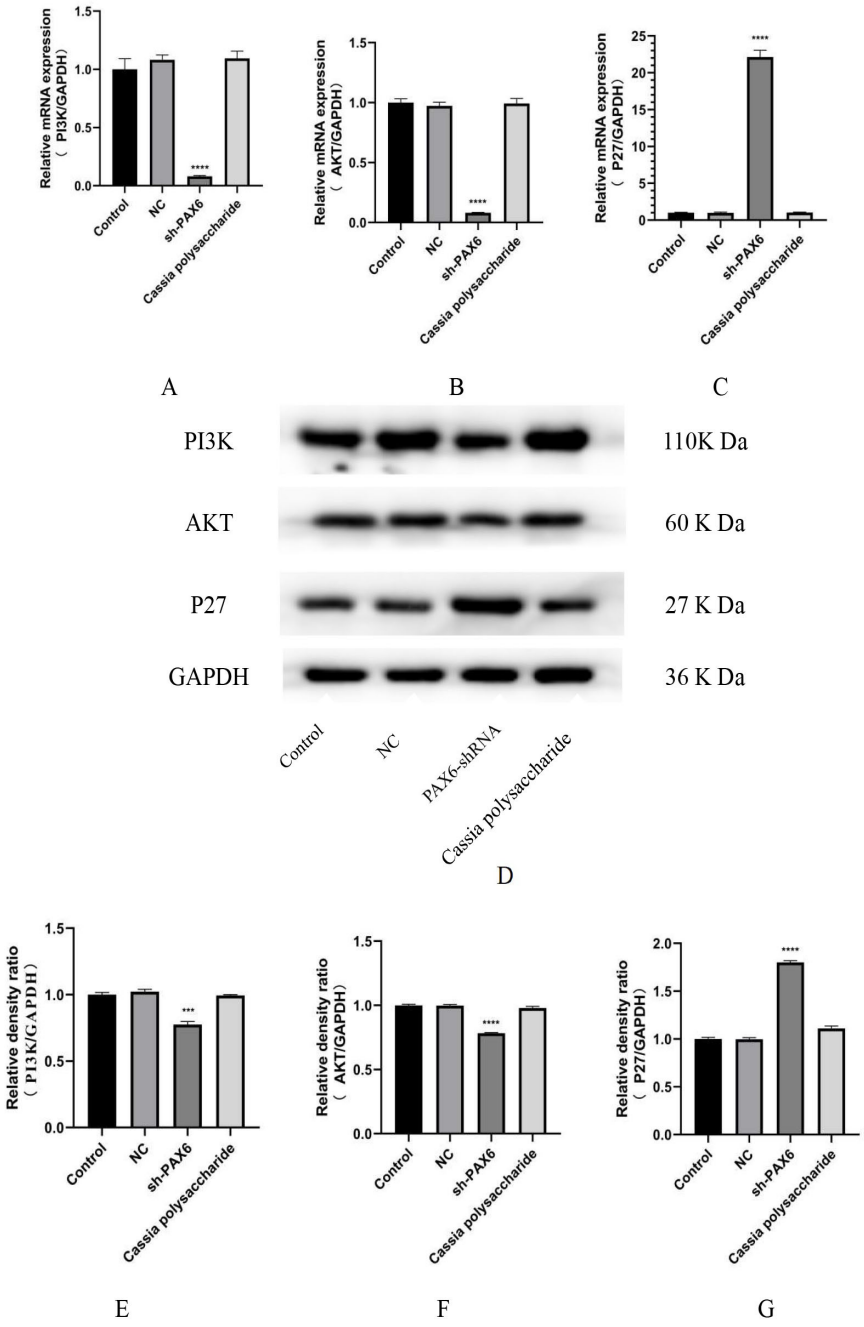


Figure 10. Effect of cassia polysaccharide regulating PAX6-shRNA on the PI3K/AKT-P27 pathway. A–**C**: After PAX6 knockdown in ARPE-19 cells, the mRNA expression of PI3K and AKT decreased while the mRNA expression of P27 increased, whereas cassia polysaccharide could counteract this phenomenon to a certain extent. **D**–G: The expression of PI3K, AKT and P27 proteins in each group, and the results indicated that cassia polysaccharide could counteract the inhibitory effect of PAX6 knockdown on the PI3K/AKT-P27 pathway to a certain degree (n=3; *p<0.05, **p<0.01, ***p<0.001).

Lipid peroxidation is a harmful peroxidation reaction of free or bound unsaturated fatty acids under the influence of free radicals. During this process, a series of oxygen free radicals can be produced, causing damage to tissues [38–40]. MDA is a product of lipid peroxidation and can reflect the degree of lipid peroxidation; GSH is an important reducing agent that participates in various redox reactions in the body. Its mechanism involves directly scavenging or neutralizing intracellular reactive oxygen species, thereby effectively reducing damage induced by reactive oxygen species to cell structure and function, and may further consolidate this protective effect by inhibiting acute inflammatory reactions [41]. Some studies have shown that cassia polysaccharides can participate in the PI3K/Akt pathway, affect the expression of Bcl-2 by playing an antiapoptotic role, inhibit the activation of TLR4, reduce the expression of NF-κB, inhibit the TLR4/NF-κB signaling pathway, and thus downregulate the expression of inflammatory factors, such as TNF-α, IL-1β, IL-6, and IL-18, which can play a preventive and protective role in hereditary ophthalmological diseases, such as retinitis pigmentosa [24].

Cassia seeds were first recorded in China's ancient medical book *Shennong Ben Cao Jing*. The main bioactive substances include anthraquinones, fatty acids, naphthalene, pyrrolidone, and polysaccharides. These compounds exhibit various physiological effects, including antioxidant, anti-aging, and anti-inflammatory properties; regulation of blood pressure and lipid levels; and inhibition of obesity. According to the *Divine Husbandman's Classic of the Materia Medica*, "Cassia seed is the main treatment for glaucoma, eye redness, swelling and pain, cataract, and tearing." Cassia polysaccharides can scavenge O^{2-}, -OH, and HO_2 produced during metabolism and reduce the production of peroxidation products, such as MDA, to enhance the body's antioxidant capacity, thus preventing ocular diseases caused by oxidative damage and slowing down their pathogenesis This study investigated only the inhibitory effect of cassia polysaccharides on myopia development; further studies are needed to examine the protective effects against other ophthalmic diseases and the effects of other components of cassia seed on ocular vision.

Conclusion: In summary, our study shows that the Wnt/β-catenin signaling pathway is overactivated in myopia, leading to a failure to improve myopia and prolonged myopia, which can enter a vicious cycle of vision loss. Cassia polysaccharides can alleviate the progression of myopia to some extent and also stop further scleral remodeling, but physical therapy or other more effective methods may be needed to completely improve the state of myopia. The present study supports the view that the Wnt/β-catenin signaling pathway is overactivated in long-term myopia, leading to a lack of improvement, suggesting that regulating its normal expression may be a potential method to control the further development of myopia. Cassia seed polysaccharides can regulate the expression of Wnt/β-catenin signaling pathway to a certain extent, which provides a new perspective to control the development of myopia.

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The authors declare that there are no potential interests or financial relationships that could be considered in the conduct of this study. Data availability statement All data are provided within this manuscript or supplementary information files. Additional data relevant to this study can be obtained by contacting the corresponding author at any time. Author contributions Luo Jincheng and Qiu Hongbin conceived and designed the research. Luo Jincheng, Li Doudou, Sun Xuewei, and Wang Xue engaged in experimental operations. Luo Jincheng and Li Doudou analyzed the data of the entire work. Luo Jincheng wrote the manuscript. Luo Jincheng and Li Jie revised the draft. All authors have checked the final manuscript, contributed to the article, and approved the submitted version. Funding: This work was financially supported by Heilongjiang Provincial Colleges and Universities Basic Scientific Research Projects, China (2020-KYYWF-0300). We sincerely thank each of the authors for their contribution to this study also thank the Northern Medicine and Functional Food Characteristic Discipline Program of Heilongjiang Province for their help.

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