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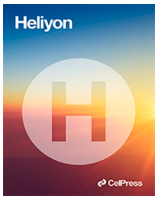

Research article

ABCA4 mediated traumatic proliferative vitreoretinopathy associated with PI3K/Akt signaling pathway

Wang Menghua [*,1], Zhirou Hu

Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, China

A R T I C L E I N F O

Keywords:
PVR
ABCA4
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A B S T R A C T

Background: Proliferative vitreoretinopathy (PVR) is the main cause of retinal detachment. However, the underlying mechanism of PVR is complex and has not yet been fully elucidated. The PI3K/Akt/mTOR signaling pathway is involved in angiogenesis and plays an important role in cell proliferation and tumor formation. Therefore, our study was designed to investigate the potential biological mechanisms of alleviating ARPE-19 cell and traumatic PVR model involving PI3K/Akt signaling pathway by targeting ABCA4.

Materials and methods: ARPE-19 cell model was induced by ABCA4 overexpression vector and si-ABCA4, then the ABCA4 overexpression vector and si-ABCA4 were constructed, the plasmids were expanded for cell transfection and verification. In addition, OE-ABCA4, shRNA NC and si-ABCA4 were transfected into ARPE-19 cells. Cell viability was detected by CCK-8 assay, cell cycle was determined by flow cytometry. The expression level and location of ABCA4 were detected by immunofluorescence. Finally, rabbit traumatic PVR model was induced by surgery, the adenovirus was injected into the vitreous body respectively, and the fundus observation was performed by direct ophthalmoscope observation combined with fundus photography, and the retinal routine histopathology HE staining was performed. Analysis of P21, CDK4, Cyclin D1, BAX, BAD, and ABCA4 was used by quantitative RT-PCR and Western blot. Besides, the expression level of ABCA4, AKT, *p*-AKT, PI3K, p-PI3K, P38, p-P38, JNK, *p*-JNK, ERK, and *p*-ERK was detected by Western blot.

Results: All results indicated that the viability of cells with high expression of ABC4A increased, while the viability of cells with inhibition of ABC4A decreased, the number of cells with high ABC4A expression was significantly higher, and the migration level of cells was significantly reduced after ABC4A inhibition ($P < 0.05$). ABC4A could affect cell apoptosis by affecting G1/G2 phase. The cell proliferation level was significantly increased with high expression of ABC4A. High expression of ABC4A increased phosphorylation levels, including *p*-AKT, *p*-PIK3, and p-P38, while inhibition of ABC4A decreased the expression levels of these proteins ($P < 0.05$). Inhibition of ABC4A could significantly improve retinopathy, indicating that the proliferation ability of cells was restored after inhibition of ABC4A.

Conclusions: Our finding suggested that inhibition of ABC4A ameliorated the injury degree of traumatic PVR and performed the potential anti-PVR effect via inhibiting PI3K/Akt signaling pathway, while promoting cell proliferation in both rabbit and ARPE-19 cells PVR model. The study has a certain innovation by building a traumatic PVR model to explore whether the ABCA4 participates in the regulation of the PI3K/AKT signaling pathway and the pathological

* Corresponding author.
E-mail address: wangmenghua0615@163.com (W. Menghua).
[1] The first author.

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mechanism of PVR regulation. At the same time, ABCA4's participation in the regulation of PI3K/Akt signaling pathway can prevent and delay the occurrence and development of PVR, which has positive significance for improving the survival rate and quality of life of patients, and also provides an important basis for its therapeutic mechanism. Therefore, our study demonstrated a significant strategy for inhibiting traumatic PVR via targeting PI3K/Akt/ABCA4 pathway.

Abbreviations

PVR	Proliferative vitreoretinopathy
RPE	Retinal pigment epithelial

1. Introduction

Proliferative vitreoretinopathy (PVR) is a common proliferative vitreoretinopathy (PVR) that is refractory and blinding, which forms a cellular membrane that could contract in front of the retina, behind the retina, and in the vitreous body, causing retinal tension, detachment, and fixation [1,2]. Traumatic, vascular and inflammatory retinopathy could lead the occurrence of PVR [3]. Retinal pigment epithelial (RPE) cells are one of the key cells for the occurrence and development of PVR [4]. Currently, the main clinical treatment for PVR is vitrectomy, but the surgical treatment effect is not ideal, which still could not inhibit the PVR recurrence. Therefore, the prevention and delay of the occurrence and development of PVR are of positive significance for improving the survival rate and quality of life of patients, and a better understanding of the potential mechanism is necessary, the quest for an effective strategy to treat PVR has attracted the attention of global researchers.

Recently, the mechanisms underlying inflammation in the PVR have not been fully investigated. Previous studies demonstrated the main cause for the onset of PVR is that there are extensive fibrous proliferative membranes in the vitreous body that are closely connected with the retina, and the contraction and pulling of the membrane result in retinal detachment [5,6]. However, regardless of the inducement, during the formation and contraction of PVR membrane, the main role in proliferation is in the retinal pigment epithelium (RPE) cells [7]. Under the influence of various factors, RPE without proliferation under normal conditions will have abnormal proliferation [8]. PI3K/Akt/mTOR signaling pathway plays an important role in cell growth and proliferation [9]. The PI3K/Akt/mTOR signaling pathway is involved in angiogenesis and plays an important role in cell proliferation and tumor formation by inhibiting cell apoptosis, accelerating cell cycle progression and increasing cell growth and proliferation [10]. PI3K can promote G1 phase progression and accelerate cell cycle through Akt mTOR and p70S6K [11]. In vitro experiments with tumor cells, inhibition of the mTOR pathway in tumor cells eliminated by PI3K/Akt pathway-mediated proliferation signaling stagnates the cell cycle and inhibits tumor growth, so mTOR has become a target for the development of new anti-proliferation drugs [12]. The mTOR specifically inhibits RAPA, which is a macrolide antibiotic. Its basic principle is to reduce the expression of mTOR, and inhibit the translation process of mRNA, and reduce the synthesis of essential proteins entering the S phase, eventually cause cell apoptosis [13]. ABCA4 is the only retina-specific protein in the ABC family [14]. Studies have found that ABCA4 is only expressed in the light-sensing nerve tissue at the back of the eyeball in the retina [15]. In ABCA4, the invertase can promote the transfer of N-retinylidene-PE and the charged substance present in the outer optic disc of the cytoplasmic surface, the all-trans conformation retinal covalent admixture with phosphatidylethanolamine [16]. However, whether ABCA4 is involved in the regulation of PI3K/Akt signaling pathway and the pathological mechanism of PVR regulation remains unclear.

Given the meaningful effects of ABCA4 and the crucial effect of PI3K/Akt, we hypothesized that inhibiting PI3K/Akt performs its effects via targeting ABCA4 to inhibit the development of PVR. Thus, our study was carried out to investigate the potential biological effects of ABCA4 on PVR by regulating PI3K/Akt through the in cell and rabbit traumatic PVR model, which could provide important role on the therapeutic mechanism.

2. Materials and methods

2.1. Materials

DMEM (Trueline, Kaukauna, WI, USA); TRIzol Reagent (Invitrogen, Waltham, MA, USA); ARPE-19 cells (American Type Culture Collection, ATCC, Manassas, VA, US); siRNA siNC (Ambion, USA, e98611); Primary antibodies against ABCA4, AKT, *p*-AKT, PI3K, p-PI3K, P38, p-P38, JNK, *p*-JNK, ERK, and *p*-ERK were provided Abcam; BCA Protein Assay Kit (Sigma, USA, GK10009); RNA later solution (Invitrogen, AM7020); Fetal bovine serum (Gibco, USA, 10,099–141).

2.2. Cell culture and treatment

The ARPE-19 cells were gently rinsed with PBS, then 1 mL of 0.25% trypsin-0.02%EDTA was added and incubated in an incubator

at 37 °C and 5%CO_2 saturated humidity. 2 mL of cell culture medium was added to terminate digestion, and cell suspension was absorbed into the centrifuge tube. After centrifugation at 1000 r/min for 5 min, the supernatant was discarded and DMEM/F12 cell culture medium containing 10% FBS.

ABCA4 overexpression vector was constructed, and cell transfection was performed according to the instructions of Lipofectamine RNAiMAX transfection reagent (Invitrogen, USA, 13,778,150) and adjusted appropriately. The ABCA4-specific small interfering RNA siABCA4 and the non-specific control siRNA siNC (Ambion, USA, e98611) dry powder were dissolved in RNase free water to prepare the working solution with the final concentration of 20 μM 200 μL transfer solution was added into each well of the 6-well plate and gently shaken. The medium can be replaced with the complete medium after 6 h culture in an incubator at 37 °C and 5% CO_2. Cells were divided into control group, NC group, OE-ABCA4 group, shRNA NC group, and si-ABCA4 group.

Cells were collected 48 h after transfection for real time PCR and Western blot analysis of transfection efficiency.

2.3. CCK-8 assay used for assessing cell viability

The viability of ARPE-19 cells was analyzed by the CCK-8 method. Briefly, the cells were grown in the logarithmic phase and harvested to inoculate into 96-well plates at a cell density of 3×10^3 cells per well. At 6, 12, 24 and 48 h after treatment, each well was added with a volume of 10 μL CCK-8 solution and maintained the reaction time for 1 h. Absorbance values were measure at 450 nm on a microplate reader and used for the calculation of cell viability.

2.4. Analysis of cells migration

ARPE-19 cells grew to 100% fusion, and the cells were scratched with a 200 μL gun head perpendicular to the ruler, causing cell scratches. The cells were rinsed twice with PBS, and the cells shed during the scratch process were washed away. At the same time, the serum-free medium was replaced, the cells were observed and photographed with an inverted microscope (0 h), and the cells were cultured in an incubator at 37 °C and 5% CO_2. The scratch was observed and photographed at 24 h after the scratch, and the difference of scratch healing among cells was calculated and compared.

2.5. Analysis of cell cycle and apoptosis

The cell cycle and apoptosis were detected by flow cytometry, the above cells were transfected with siABCA4 and siNC. Cultivate cells, wait for the cells to grow 60%–70%, deal with it according to experimental needs. After continued culture for 48 h, the samples were collected into the centrifuge tube. The cells were cleaned with PBS buffer, centrifuged with PBS buffer, counted under a microscope, and 1×10^5 cells were filtered and collected in a centrifuge tube, and 400 μL PI staining solution was added for detection by flow cytometry. The cell proliferation cycle data of each group were obtained. The cell apoptosis was detected by flow cytometry according to manufacturer's instruction.

2.6. PVR grading and H&E dyeing

The fundus was observed continuously for 28 days, and the PVR formed 28 days after surgery was graded. The PVR score was divided into 3 grades according to Francine criteria: grade 0 without hyperplastic reaction; Grade 1 vitreous hyperplasia; The formation of preretinal membrane and preretinal folds in grade 2; Grade 3 has dense white membrane formation in front of the retina, retinal folds, localized retinal detachment, with or without localized posterior capsular cataract.

Twenty-four rabbits were randomly divided into 3 groups ($n = 8$): control group, PVR model group and sh-ABCA4-AAV adenovirus group. On the 28th day after the establishment of the animal model, all rabbits were killed by excessive anesthesia, and the eyeballs, anterior knots and vitreous bodies were removed. Eyeball tissue was fixed with 10% formaldehyde by volume fraction, dehydrated with gradient ethanol, and embedded with xylene transparent paraffin. The sections were 5 μM thick and stained with hematoxylin-eosin and immunohistochemical methods, respectively.

2.7. Immunofluorescence detection

The cells were removed and washed with PBS for three times, then fixed with 4% paraformaldehyde, washed with PBS for three times, 0.2%Triton X 100 permeated for 10 min, washed with PBS, closed with the serum of the same host as the second antibody for 30 min, washed with PBS for three times, incubated with the second antibody at room temperature for 2 h, washed with PBS for three times, nucleated with DAPI, and fluorescent radiography was performed.

2.8. Quantitative RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, USA, 15,596,026), then 1 μg RNA was transcribed into cDNA transcriptase by M-MLV reverse (Promega, Madison, WI, USA). qRT-PCR was performed with SYBR premixed Ex Taq (Takara, Kusatsu, Japan) real-time PCR system on ABI 7500 fast (Applied Biosystems, Foster City, CA, USA). ACTIN mRNA was used as an endogenous control for mRNA.

2.9. Rabbit and treatments

Rabbits have large eyeballs, which are easy to operate and observe, and are the most common animals in ophthalmic studies [17]. Rabbit (body weight: 2.5 kg; male, all animal experiments have complyde with the ARRIVE guidelines) purchased from Shanghai Slack laboratory animal Co., Ltd, traumatic PVR model was induced by surgery as follows: The left eye of rabbits was dropped with chloramphenicol before traumatic PVR model establishment. Topiramide drops were used to dilate the pupil and make the pupil dilate to about 8 mm. Promecaine hydrochloride eye drops were used for surface anesthesia, and 10% chloral hydrate solution 2.5 mL/kg was used for general anesthesia through intravenous injection at the rabbit ear edge, then, the eyelid was opened fully to expose the eyeballs and flush the conjunctival. The bulbar conjunctiva and fascia at points 1–3 were separated, and the sclera was exposed. The scleral bayonet was inserted 4 mm behind the temporal limbal to make a penetrating wound parallel to the limbal of the cornea. The tip of the bayonet should be inserted into the central part of the vitreous body, and the wound should be expanded to 8 mm with scissors and parallel to the limbal of the cornea. The 0.1 mL of PRP was injected into the vitreous body 4 mm away from the corneal limbal, and the needle was directed towards the center of the vitreous body. The needle was stopped when the needle reached the center of the vitreous body. For the blank control group, 0.1 mL sterile normal saline was injected into the vitreous cavity. The specific operation method was the same as above. Chloramphenicol was used to drop the left eye within one week after surgery. During the experiment, the sex, age, weight and experimental conditions of rabbits were basically the same. 24 rabbits were randomly divided into 3 groups (n = 8): control group, PVR model group and sh-ABCA4-AAV adenovirus group. One eye of each rabbit was randomly selected as the experimental eye, and adenovirus was injected into vitreous body respectively. The contralateral eye was the control eye, and 0.1 mL normal saline was injected into the vitreous body. All animal experiments have been approved by the Ethics Committee and follow all applicable institutional and governmental regulations regarding the ethical use of animals (Research and Clinical Experiment Ethics Committee, Aproved number：2023-KY-1264-002).

2.10. Western blot analysis

Whole protein lysates were extracted from different samples using RIPA lysis buffer with EDTA-free protease inhibitor cocktail. The concentration was estimated by an enhanced BCA protein assay kit. Equal amounts of total protein were fractionated on 10% SDS-PAGE and transferred to a nitrocellulose membrane overnight. Then after being blocked with 5% nonfat dry milk for 1 h at room temperature, the membranes were probed at 4 °C overnight with the primary antibodies (ABCA4, AKT, *p*-AKT, PI3K, p-PI3K, P38, p-P38, JNK, *p*-JNK, ERK, *p*-ERK, and ACTIN) followed by secondary antibody anti-mouse IgG (1:1000; Beyotime, Shanghai, China) for 1

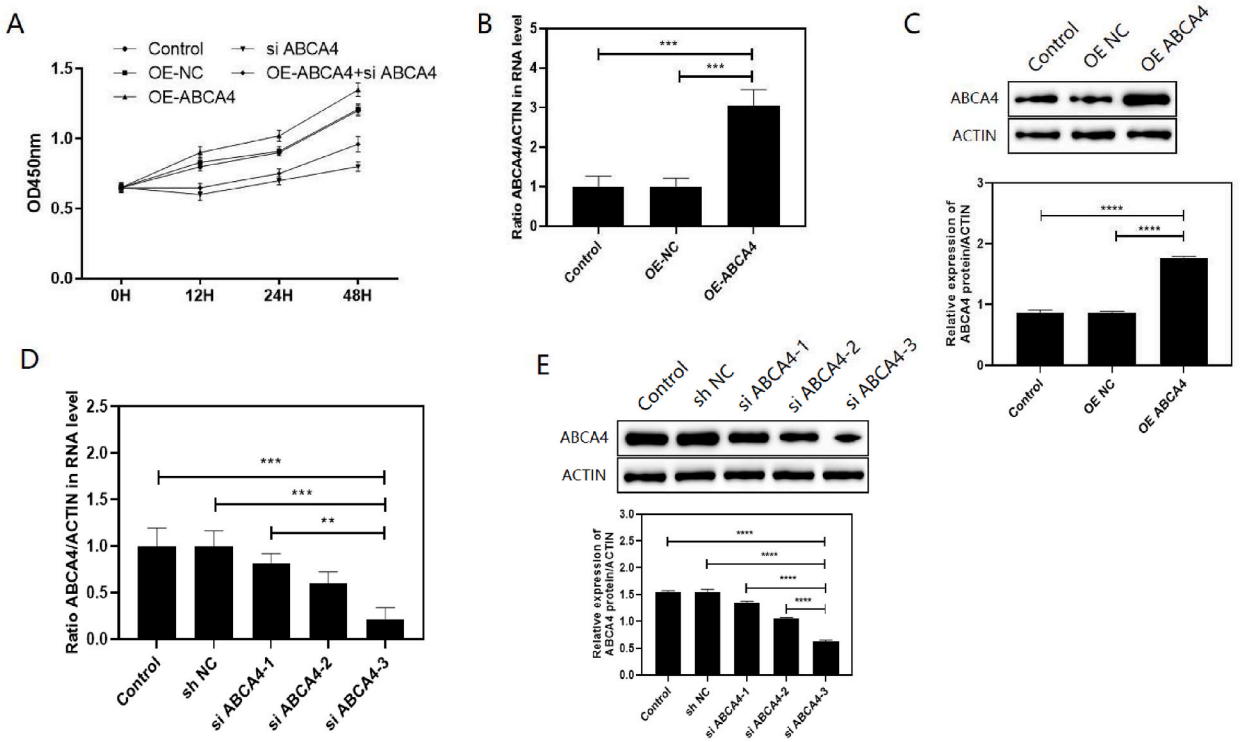

Fig. 1. Effects of overexpression ABC4A on ARPE-19 cells. (A) Cell viability; (B) Construction of overexpressed ABCA4 cell lines was verified by PCR; (C) I Construction of overexpressed ABCA4 cell lines was verified by Western blot; (D) si ABC4A with the best activity was screened by the transcriptional level. (E) si ABC4A with the best activity was screened by expression level. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

h at 37 °C. An enhanced chemiluminescence system was used for detection protein expression value.

2.11. Statistical analysis

The experiment was divided into control group and experimental group, all experiments were conducted in triplicates and data were expressed as mean + SD. All statistical analyses were done on GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA). Group comparisons were performed with ANOVA or Student's *t*-test. A P value < 0.05 indicated statistical significance.

3. Results

3.1. Effects of ABC4A on cell viability

In order to investigate the effects of ABC4A on the viability of ARPE-19 cells, the ABCA4 overexpression vector and si-ABCA4 were constructed, the plasmids were expanded for cell transfection, then treated the ARPE-19 cells. Our results showed that compared with siNC transfection, the viability of ARPE-19 cells transfected with siABCA4 was significantly inhibited over time. There was no change in cell viability between the control group and the blank group. The cell viability increased with the high expression of ABC4A, and decreased with the inhibition of ABC4A (siABC4A) (Fig. 1A), suggesting that transfection with siABCA4 may lead to inhibited proliferation and apoptosis of ARPE-19 cells.

3.2. The verification of ABCA4 overexpression vector and si-ABCA4 on ARPE-19 cells

In order to assess whether ABCA4 was overexpressed and interfered successfully, RT-PCR and Western blot detection was adopted. The construction of overexpressed ABCA4 cell was verified by PCR, and the mRNA level of ABC4A in the highly expressed cells was

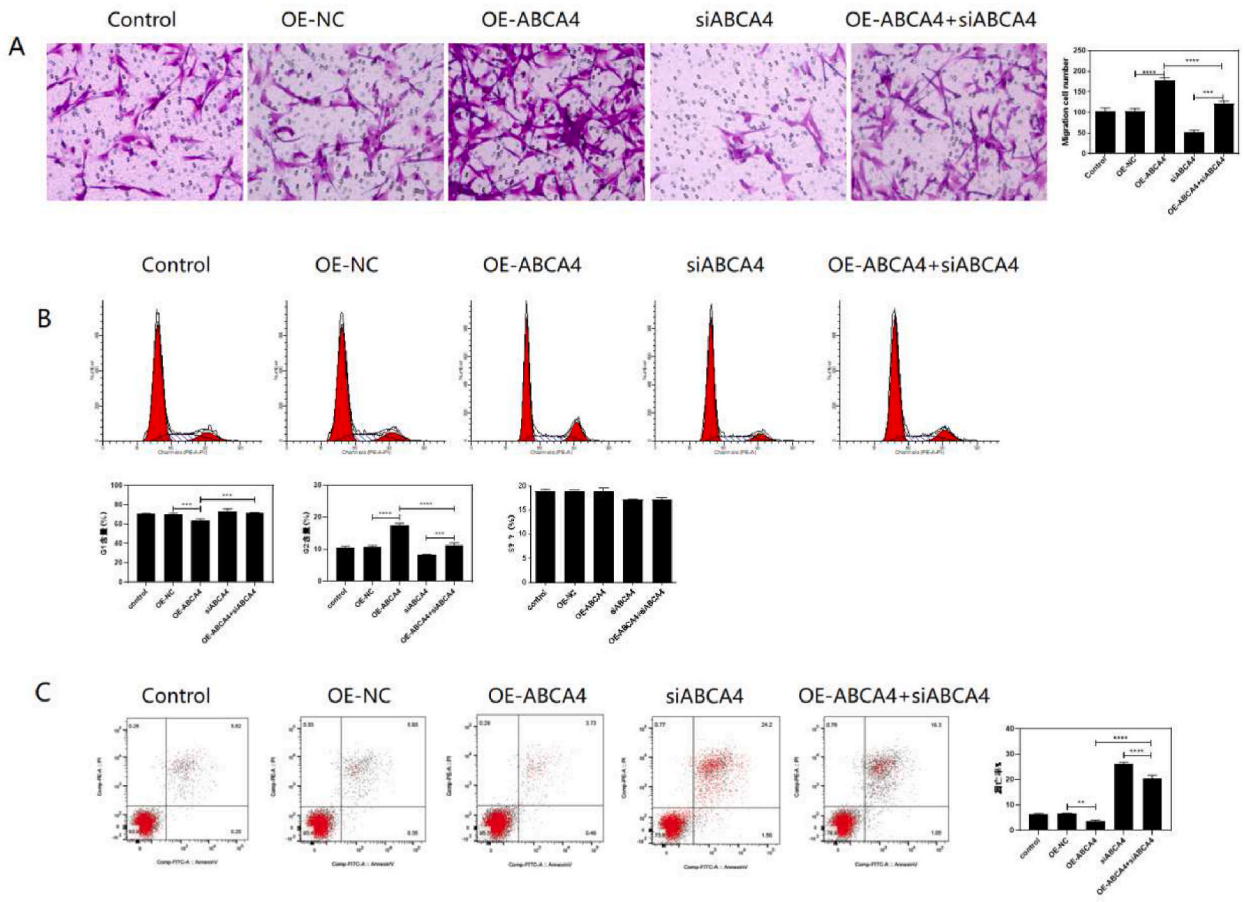

Fig. 2. Effects of ABC4A on ARPE-19 cell migration, cycle and proliferation. (A) The effects of ABC4A on cell migration; (B) The effects of ABC4A on cell cycle; (C) The effects of ABC4A on cell apoptosis; (D) ABC4A immunofluorescence staining. (E) PCNA immunofluorescence staining. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

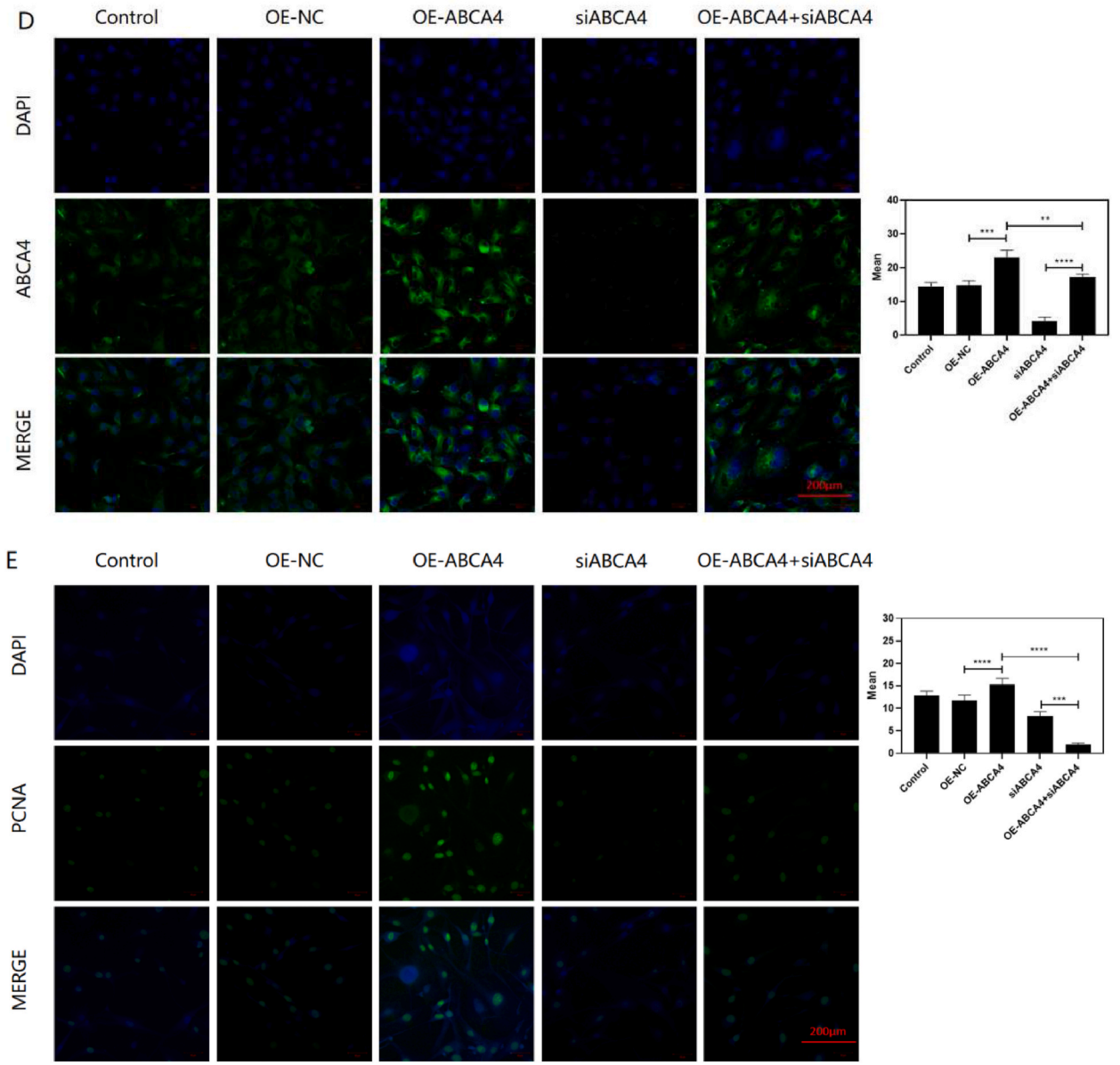

Fig. 2. (*continued*).

significantly improved compared with the blank and the control group, indicating the successful construction of the highly expressed ABC4A cells (Fig. 1B). The construction of overexpressed ABCA4 cells was verified by Western blot, and the expression level of ABC4A protein in the highly expressed cells was significantly increased compared with the blank and the control group, indicating the successful construction of the highly expressed ABC4A cells (Fig. 1C–E).

3.3. Effects of ABC4A on ARPE-19 cell migration, cycle and proliferation

To further investigate the effects of ABC4A on cell migration, cycle and proliferation, ABCA4 overexpression vector and si-ABCA4 treated cells will be constructed. The number of cells with high ABC4A expression was significantly higher than that in the blank group, and the migration level of cells was significantly reduced after ABC4A inhibition (Fig. 2A).

The G1 phase was significantly lower than that in blank group, the G2 phase was significantly higher than that in blank group, and the S phase content did not change significantly. After inhibition of ABC4A, G1 phase was significantly higher than that of blank group, G2 phase was significantly lower than that of blank group and high expression group, and the content of S phase did not change significantly, indicating that ABC4A affected cell apoptosis by affecting G1/G2 phase (Fig. 2B).

The apoptosis rate of cells with high expression of ABC4A was significantly decreased, and the apoptosis rate of cells with inhibition

of ABC4A was significantly increased compared with the blank group, and the apoptosis rate of cells in OE-ABC4A + si ABC4A group was significantly higher than that in the high expression of ABC4A group, suggesting that inhibition of ABC4A could significantly improve the level of cell apoptosis (Fig. 2C).

The fluorescence intensity of the high-expression ABC4A group was significantly higher than that of the blank group, and the fluorescence intensity was significantly reduced after inhibition of ABC4A, indicating that the expression level of ABC4A was higher in the high-expression ABC4A group, and significantly decreased after inhibition of ABC4A. The fluorescence intensity of the high-expression ABC4A group was higher, and the fluorescence intensity was significantly reduced after inhibition of ABC4A, indicating that the cell proliferation level of the high-expression ABC4A group was significantly higher than that of the blank group, and the cell proliferation level was significantly reduced after inhibition of ABC4A (Fig. 2D–E).

3.4. Effects of ABC4A on P13K/Akt signaling pathway in ARPE-19 cell model

In order to study the influence of ABC4A on the P13K/Akt signaling pathway, ABCA4 overexpression vector and si-ABCA4 treated cells will be constructed, then PCR and Western blot were used to detect the level of related proteins. Our results showed that The transcription levels of P21, BAX and BAD were significantly decreased in the group with high expression of ABC4A, while the transcription levels of CDK4, CyclinD1, ABC4A, AKT, PIK3 and P38 were significantly increased. After inhibition of ABC4A, the transcription levels of P21, BAX and BAD were significantly increased, while the transcription levels of CDK4, CyclinD1, ABC4A, AKT, PIK3 and P38 were significantly decreased (Fig. 3A).

The translation levels of AKT, PIK3 and P38 in the high-expression ABC4A group and the inhibitory ABC4A group were not significantly changed. However, the phosphorylation levels of the high-expression ABC4A group were increased, and the levels of *p*-AKT, *p*-PIK3 and p-P38 were significantly increased, while the phosphorylation levels of the inhibitory ABC4A group were significantly decreased. The levels of *p*-AKT, p-PI3K and p-P38 were significantly lower than those of the high-expression ABC4A group (Fig. 3B).

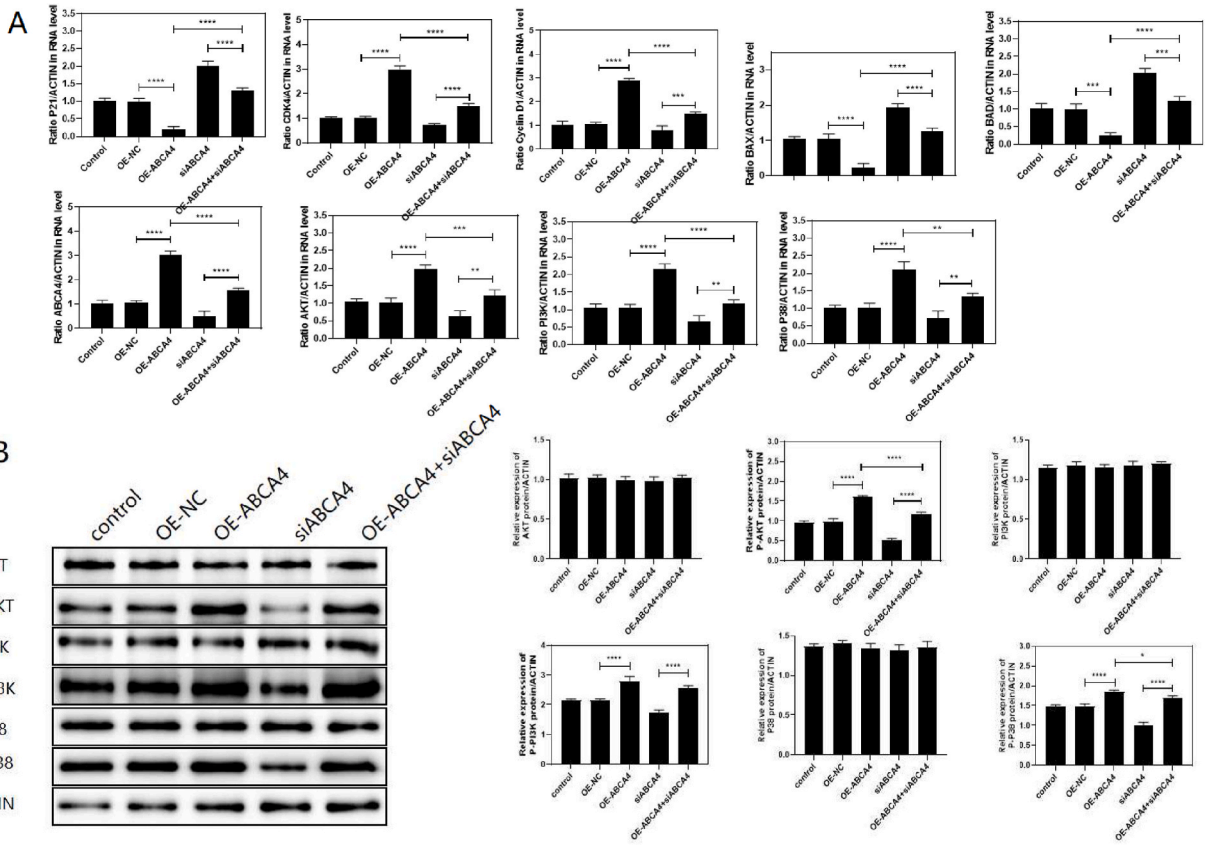

Fig. 3. Effects of ABC4A on P13K/Akt signaling pathway in ARPE-19 cell model. (A) PCR was used to detect the transcription levels of P13K/Akt signaling pathway related proteins; (B) Western blot was used to detect the expression levels of P13K/Akt signaling pathway related proteins. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

3.5. PVR scores and H&E histopathological analysis

Fundus photos were taken for rabbits in each group and PVR score was performed, A higher score indicated a higher degree of visual impairment. Our results showed that the score of the model group was significantly higher than that of the blank group, and the score decreased significantly after ABC4A inhibition, indicating that the inhibition of ABC4A can significantly improve the retinopathy (Fig. 4A). H&E histopathological analysis demonstrated that the structure of each layer of retina was clear and complete, and no cell edema or absence was observed in each layer. The pigmented cortex has neat structure, tight cell arrangement and complete morphology in the control group, however, the vitreous cavity contains a large number of reticular, cord-like red dyed collagen fibers. The retinal layers are disordered and disordered, with local distortion. Cell degeneration and disordered arrangement occurred in the outer and inner layers in the PVR model group (Fig. 4B). Therefore, the results indicated that Sh-ABC4A could ameliorate these pathological features.

3.6. Immunofluorescence analysis

The expression level of ABC4A protein in eyeball tissue have been investigated, the results showed that the expression level of ABC4A in the model group was significantly higher than that in the blank group, and the expression level of ABC4A was significantly decreased after inhibition of ABC4A. The expression level of PCNA in the model group was significantly higher than that in the blank group, and the expression level of ABC4A was significantly decreased after inhibition of ABC4A. In addition, the fluorescence intensities of Ki67 and Cyclin D1 in the model group were significantly lower than those in the blank group, while the fluorescence intensities of Ki67 and Cyclin D1 in the ABC4A inhibitory group were recovered (Fig. 4C–D), indicating that the cell proliferation ability was restored after the inhibition of ABC4A.

3.7. Effects of ABC4A on P13K/Akt signaling pathway in rabbit traumatic PVR model

In order to further the influence of inflammation ABC4A on the P13K/Akt signaling pathway, the related protein expression of the PI3K/Akt signaling pathway was measured by PCR and Western blot. Our results showed that in the model group, the transcription levels of P21, BAX, BAD, ABC4A and P38 were significantly decreased, while the transcription levels of CDK4, CyclinD1, AKT and PI3K were significantly increased, while the transcription levels of JNK and EPK were not significantly changed. In the treatment group, the transcription levels of P21, BAX, BAD, ABC4A and P38 were significantly increased, while the transcription levels of CDK4, CyclinD1, AKT and PI3K were significantly decreased, all of which were recovered to varying degrees, while the transcription levels of JNK and EPK were not significantly changed (Fig. 5A). The expression levels of AKT, PIK3, P38, JNK and ERK in model group and treatment

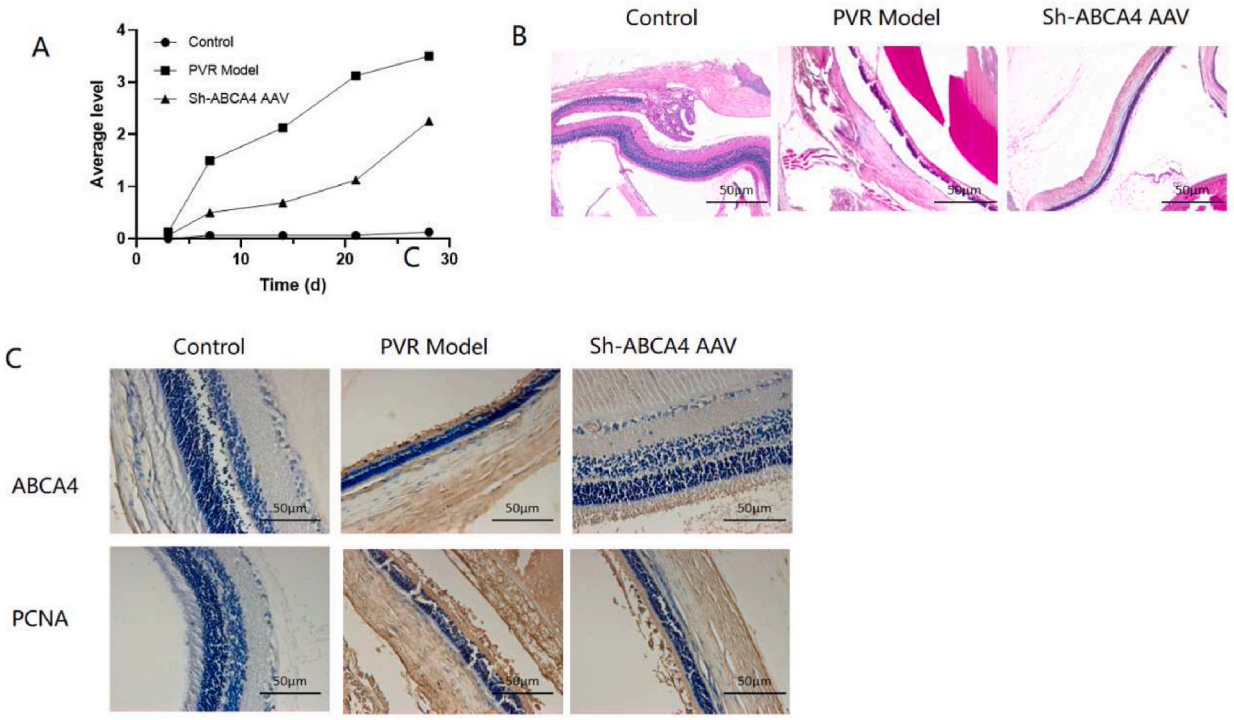

Fig. 4. Effects of ABC4A on rabbit traumatic PVR model. (A) PVR scores; (B) H&E histopathological analysis; (C) Immunohistochemistry of eyeball tissue; (D) Immunofluorescence of eyeball tissue. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

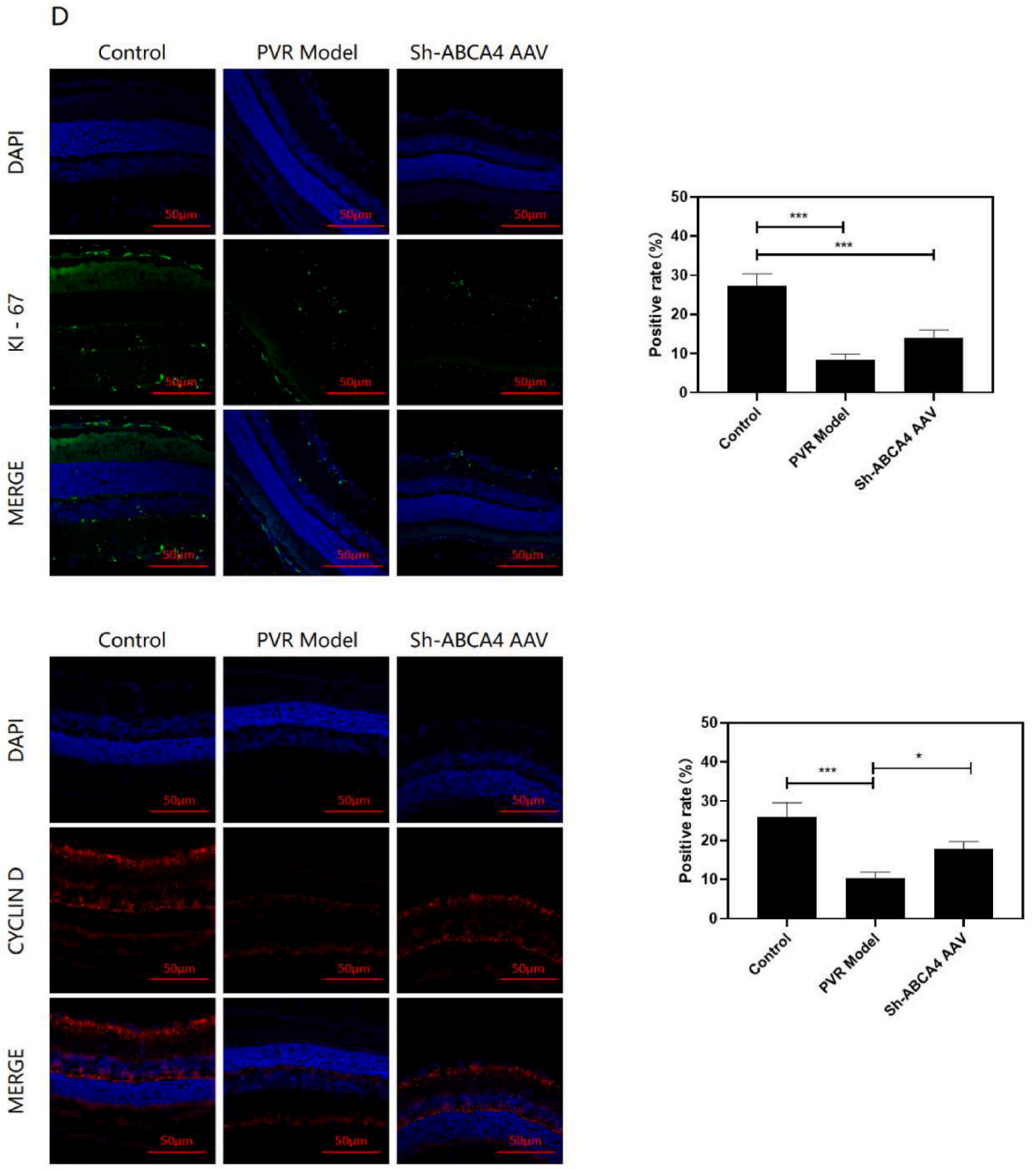

Fig. 4. (*continued*).

group were not significantly changed. The phosphorylation level of the model group was significantly decreased, and the expression levels of *p*-AKT, p-PI3K and p-P38 were significantly decreased, while the phosphorylation levels of the treatment group were significantly recovered, and the expression levels of *p*-AKT, p-PI3K and p-P38 were significantly increased compared with the model group (Fig. 5B). All these findings indicated that ABC4A involved in the process of PVR by targeting P13K/Akt signaling pathway.

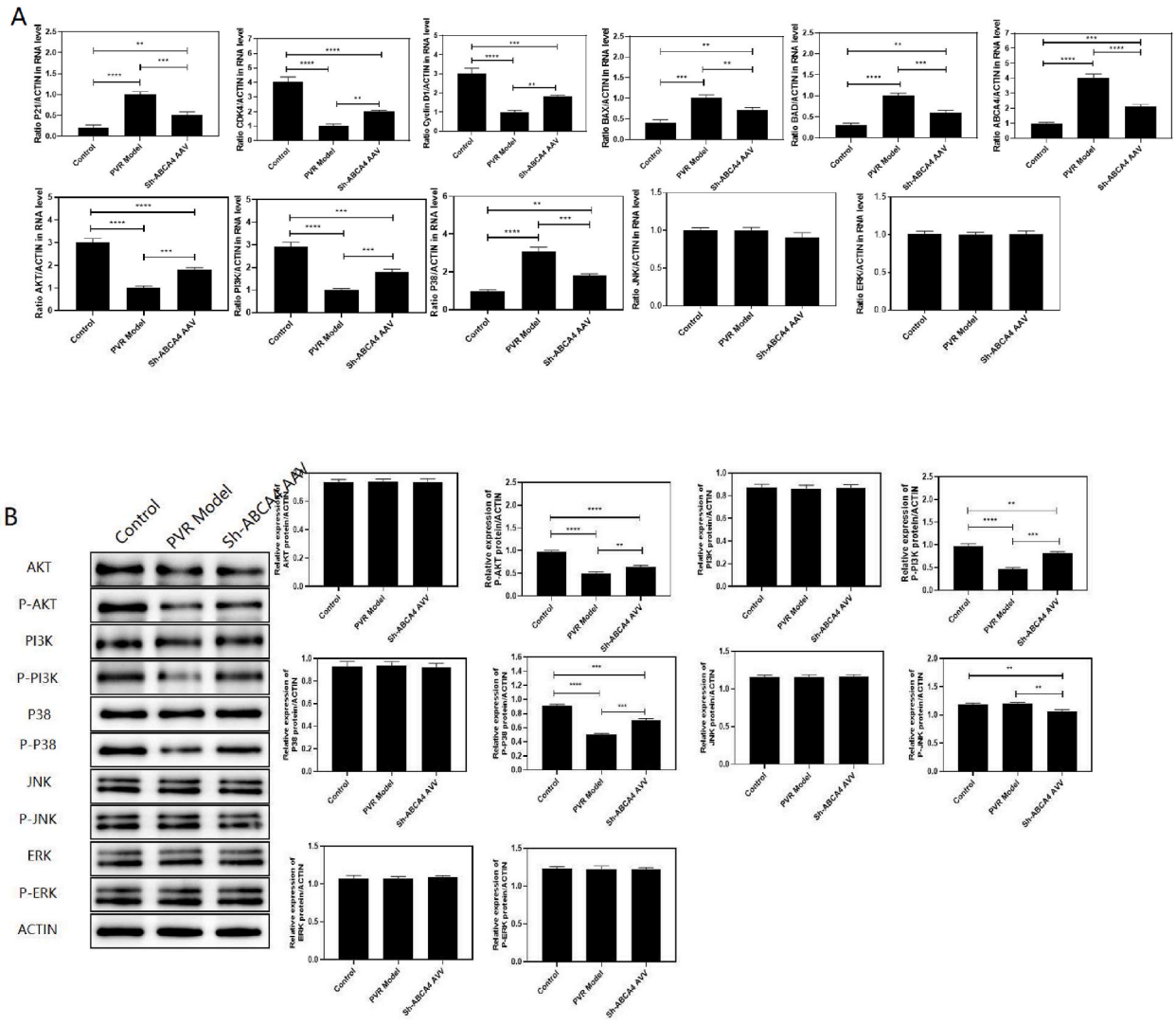

Fig. 5. Effects of ABC4A on P13K/Akt signaling pathway in rabbit traumatic PVR model. (A) PCR was used to detect the transcription levels of P13K/Akt signaling pathway related proteins; (B) Western blot was used to detect the expression levels of P13K/Akt signaling pathway related proteins. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

4. Discussion

Traumatic PVR is caused by physical, chemical, biological and other external factors that cause severe proliferation of vitreous and retinal tissue, which is also a repair process to the damage [18]. PVR is one of the leading causes of severe ocular diseases and one of the most common causes of surgical failure in rheogenic retinal detachment [19]. The pathology of PVR is the continuous migration and proliferation of photoreceptor cells, thus generating a covering cell proliferative membrane on the surface of the retina and in the vitreous cavity [20]. The proliferative membrane contains a variety of cells, such as fibroblasts, glial cells, PRE cells, inflammatory cells (including lymphocyte hull type cells) and so on. Contraction of the proliferative membrane can induce traction retinal detachment, which can lead to decreased visual function and even blindness. At present, most studies focus on the pathogenesis of PVR [21,17]. However, few studies have specifically explored the pathogenesis of traumatic PVR. Therefore, in this study, we hypothesized that ABC4A reduced hyperproliferation and inflammation alleviating inflammation response by targeting PI3K/Akt pathway. The effects of ABC4A on ARPE-19 cell model and rabbit traumatic PVR model and focused on cell proliferation. Our findings showed that inhibiting ABC4A could attenuate the injury degree of PVR, which inhibited cell proliferation by suppressing PI3K/Akt pathway, and inhibit the level of cell apoptosis. These findings may provide a novel comprehensive understanding of ABC4A inhibitor against PVR.

The ABCA4 gene encodes A photoreceptor specific transmembrane protein that transports vitamin A (retinoid) from the photoreceptor to RPE cells [22]. ABCA4 dysfunction leads to the deposition of lipofuscin, an aging pigment, in RPE cells, which usually occurs only in older eyes. The active chemical composition of lipofuscin can impair RPE function and subsequently lead to the death of

photoreceptor cells. Mutations in the gene ABCA4 are responsible for over 95% of stargardt lesions [23]. It was found that the retina-specific transmembrane enzyme ABCA4 is expressed in epidermal keratinocytes and is essential for cell passage, proliferation and activation in the later stage [24]. In ABCA4-deficient cells, light-induced atRAL clearance is slower due to ABCA4 deficiency, resulting in cellular oxidative stress, DNA damage, and p53 phosphorylation, leading to activation of bax, and thus apoptosis [25]. The expression of ABCA4 was decreased by transfection of small interfering RNA specifically targeting ABCA4 into human retinal pigment epithelial (RPE) cell line ARPE-19 cells. ARPE-19 cells with low expression of ABCA4 were constructed. Using siABCA4 to reduce the expression of ABCA4 in RPE cells inhibited cell viability and increased with culture time, inhibited cell proliferation and migration, and induced apoptosis of RPE cells. The effect of siABCA4 on RPE cell viability, proliferation and apoptosis may be related to the abnormal expression of cycle-regulating proteins p21, CDK4 and Cyclin D1, and the increased expression of pro-apoptotic proteins Bax and Bad. Therefore, ABCA4 has potential as a target for the prevention and treatment of traumatic PVR. In addition, PI3K is an important intracellular protein kinase. When cells are stimulated by growth factors and other stimulants, they become thinner intracellular PI3K is activated and converted into 3, 4, 5-triphosphate phosphatidyl alcohol (PIP3), and binds to the downstream molecule Akt [26]. The activated PI3K/Akt and TSC1/2 complex activates its downstream molecule mTOR. Activated mTOR promotes protein synthesis by regulating the main genes of protein synthesis 4E-BP1 and p70S6k in its downstream pathway, thus accelerating cell proliferation.

In recent years, it has been found that the PI3K/Akt/mTOR signaling pathway is closely related to tumorigenesis and development [27]. Interestingly, our results demonstrated that Western blot and PT-PCR results showed that the expression levels of phosphorylated PI3K and Akt in ABCA4 overexpression group were significantly higher than those in the control group. This further proves that the PI3K/Akt/mTOR signaling pathway also plays a major role in the normal growth and proliferation of cells. During the proliferation period of PVR, the PI3K/Akt/mTOR signaling pathway is activated because a large number of growth factors regulate the migration and proliferation of RPE cells, resulting in the imbalance of intraocular cell proliferation and apoptosis, resulting in increased expression of RPE cells. At the same time, activated mTOR activates downstream to increase the synthesis of ribosomal proteins and translational regulatory proteins, Further, pS6K (ribosomal S6-kinase (RSK) target proteins promoting protein synthesis and E-BP1 (eukaryotic initiation factor 4E-binding protein) were carried out the protein translation [28–31]. In addition, siABCA4 could inhibit the level of *p*-AKT, p-PI3K and p-P38, which indicated that ABCA4 involved in the activation state of PI3K/Akt signaling pathway, then promoted cell proliferation and ameliorated the injury degree of traumatic PVR. In this paper, the relationship between ABCA4-mediated traumatic hyperplastic vitreoretinopathy and PI3K/Akt signaling pathway was expounded from both micro and macro perspectives by combining cell experiments and animal experiments, which is innovative and referable to a certain extent. Based on the potential biological effects of ABCA4 on PVR through regulation of PI3K/Akt, this study provides an important basis for its therapeutic mechanism. The results indicate that ABCA4 can prevent and delay the occurrence and development of PVR by regulating PI3K/Akt, which has potential positive significance for improving the survival rate and quality of life of patients.

5. Conclusion

In a sum, our study indicated that the viability of cells with high ABC4A expression was increased, while the viability of cells with inhibition of ABC4A was decreased ($P < 0.05$). The number of cells with high expression of ABC4A was significantly increased, the level of cell proliferation and phosphorylation was significantly increased ($P < 0.05$), and the level of cell migration and protein expression was significantly decreased after inhibition of ABC4A ($P < 0.05$). Inhibition of ABC4A can significantly improve retinopathy, indicating that the proliferation ability of cells can be restored after inhibition of ABC4A. In conclusion, inhibition of ABCA4 may improve the severity of traumatic PVR by inhibiting the ABCA4-mediated PI3K/Akt signaling pathway in vitro and in vivo. In conclusion, inhibition of ABCA4 can improve the damage degree of traumatic PVR in vitro and in vivo by inhibiting the ABCA4-mediated PI3K/Akt signaling pathway, providing new insights into the inhibition of PI3K/Akt/ABCA4. Therefore, the findings demonstrate an important strategy for inhibiting traumatic PVR by targeting the PI3K/Akt/ABCA4 pathway.

Data availability statement

The data will be available on request.

CRediT authorship contribution statement

Wang Menghua: Writing – review & editing. **Zhirou Hu:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27024.

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