Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;47:2136-2146

DOI: 10.1159/000491525

Published online: July 04, 2018

Accepted: May 25, 2018

Original Paper

Effects of Curcumin on Epidermal Growth Factor in Proliferative Vitreoretinopathy

Yan-xin Ren[a] Jing-xue Ma[a] Feng Zhao[b] Jian-bin An[a] Yu-xin Geng[a] Li-ya Liu[a]

[a]Department of Ophthalmology, The Second Hospital of Hebei Medical University, [b]Department of Ophthalmology, Nanjing First Hospital, Nanjing Medical University, China

Key Words

Curcumin • EGF • RPE cell • PVR

Abstract

Background/Aims: Proliferative vitreoretinopathy (PVR) is a common refractory eye disease that causes blindness and occurs after retinal detachment or retinal reattachment. Epidermal growth factor (EGF) has been shown to play an important role in the migration and proliferation of retinal pigment epithelium (RPE) cells, which promote PVR. Curcumin inhibits RPE cell proliferation, but it is not known whether it participates in the formation of PVR. Curcumin regulates the biological functions of EGF, which plays important roles in the development of PVR. This study aimed to evaluate the effect of curcumin on the regulation of EGF in PVR. ***Methods:*** Rabbit RPE cells were cultured, and EGF expression was detected by immunocytochemistry. MTT assay was conducted to determine cell proliferation induced by different concentrations of EGF. Immunocytochemical staining was used to detect EGF expression after treatment with curcumin at varying concentrations. Real-time PCR (RT-PCR) and western blot analysis were used to detect the concentrations of EGF mRNA and protein after treatment with curcumin. After RPE cells and curcumin were injected into experimental rabbit eyes, the cornea, aqueous humor, lens, and vitreous opacity were observed and recorded simultaneously by indirect ophthalmoscopy, fundus color photography, and B-ultrasonography. The vitreous body was extracted, and the EGF content in the vitreous humor was measured by enzyme-linked immunosorbent assay (ELISA). ***Results:*** At each time point (24, 48, and 72 h), cell proliferation gradually increased with increasing EGF concentrations (0, 3, 6, and 9 ng/mL) in a dose-dependent manner. Cell proliferation between EGF concentrations of 9 and 12 ng/mL were no different, which suggested that 9 ng/mL EGF was the best concentration to use to stimulate RPE cell proliferation *in vitro*. Under all EGF concentrations (0, 3, 6, 9, and 12 ng/mL), RPE cell proliferation increased with time (from 24 to 72 h), suggesting a time–effect relationship. Curcumin downregulated EGF expression in RPE cells, which also indicated time–effect and dose–effect relationships. The best curcumin concentration for the inhibition of EGF expression was 15 μg/mL. RT-PCR and western blot analyses indicated that the EGF mRNA and expression of the protein in RPE cells treated with curcumin significantly decreased with time. Ocular examinations revealed that the vitreous opacity was lower and the proliferative membrane was thinner in the curcumin group compared with the control

Prof. Jing-xue Ma

Department of Ophthalmology, The Second Hospital of Hebei Medical University
215 Heping Road, Shijiazhuang 050000, Hebei, (PR China)
E- Mail hbeyyk@163.com

group. The PVR grade and the incidence of retinal detachment were significantly lower in the experimental group than in the control group. ELISA results showed that the EGF content in vitreous humor was higher in the control group than in the curcumin group. The curcumin and control groups were significantly different at each time point. ***Conclusion:*** Curcumin inhibited RPE cell proliferation by downregulating EGF and thus effectively inhibited the initiation and development of PVR.

Introduction

Proliferative vitreoretinopathy (PVR) is a retinal detachment disease caused by the proliferation, migration, and contraction of retinal pigment epithelium (RPE) cells and glial cells, among others. PVR is considered a common refractory eye disease that causes blindness. Numerous studies have shown that PVR occurs and develops via a combination of factors. By these factors, the release and migration of RPE cells into the vitreous plays an important role. Cytokines are key factors that promote the uncontrolled proliferation of RPE cells [1-5]. As such, their roles in the mechanisms leading to PVR have become the current areas of focus. Previous studies have emphasized the important role of epidermal growth factor (EGF) in the migration and proliferation of RPE cells, which promotes the occurrence of PVR [6].

PVR is mainly treated by surgical procedures. Surgery can remove the hyperplastic tissue but can neither prevent nor inhibit cell proliferation, resulting in a high rate of recurrence after treatment. Drug-assisted treatments are therefore applied to both animal models of different pathogenic factors and patients at different stages of disease to improve the efficacy of surgery. The drugs generally used include low-molecular-weight heparin, 5-FU, corticosteroids, antioxidants, extracellular matrix synthesis inhibition agents, and cell signal transduction inhibitors [4, 7]. Most western drugs used for clinical treatment have one or more limitations, such as unique pharmacological effects and high cost. Curcumin is a natural chemical component present in the rhizomes of Zingiberaceae. It exhibits many pharmacological effects, such as anti-inflammatory, anti-proliferative, and antimicrobial effects [8, 9]. The pharmacological effects of curcumin satisfy the requirements for a PVR treatment drug. In addition, curcumin has many benefits, such as a high level of safety, low toxicity, a wide range of treatment uses, and low cost. Curcumin also inhibits RPE cell proliferation [10-12], although it is unknown whether it plays an important role in the prevention of PVR formation. However, curcumin has been reported to regulate the biological functions of EGF, which plays important roles in the development of PVR. In this study, *in vitro* cell experiments and *in vivo* animal experiments demonstrated the role of curcumin in the regulation of EGF in PVR treatment and provided a theoretical basis for the prevention and treatment of PVR via curcumin in the clinical setting.

Materials and Methods

Culture and identification of rabbit RPE cells

Purple-blue rabbits (healthy with no eye disease) were provided by the Experimental Animal Center of Hebei Medical University, Hebei Province, China. Rabbit eyes were collected under sterile conditions. The eyeballs were cut 2 mm behind the edge of the sawtooth, and the front part of the eye and the vitreous were removed. The retinal nerve layer was removed from the disc to expose the RPE of the eye cup and washed with D-Hank's buffer twice. The extracted eye cup tissue was digested with 0.25% trypsin for 30 min. The supernatant of the digested tissue was transferred into a centrifuge tube. Dulbecco's modified Eagle's medium (DMEM, Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Bude Biological Engineering Co., Ltd., Wuhan, China) was used to stop the digestion reaction. The supernatant was centrifuged at 1500 g/min for 10 min. It was then transferred into a culture flask and incubated in a 5% CO_2 incubator. The medium was changed every 2–3 days, and cell passage was processed when cells

were about 90% confluent. Third-generation cells were used for the experiment [13]. The streptavidin-biotinylated peroxidase complex method was used to detect cell purity with the anti-human cytokeratin monoclonal antibody (Sigma-Aldrich, St. Louis, MO) [12].

Immunocytochemistry

Immunocytochemistry was performed in accordance with the instructions provided by the manufacturer. The primary antibodies included primary IGF1, IGF2, IGF1R, and IGF2R anti-rabbit IgG polyclonal antibodies (Bioworld, Dublin, USA), as well as mouse anti-human keratin monoclonal antibodies MNFll6 and anti-EGF (Sigma-Aldrich). An EGF-positive signal was located in the cytoplasm. High-magnification images (n = 10) from each slice were collected using a BX53 biological microscope (Olympus, Tokyo, Japan). Image analysis was performed using the Motic Med 6.0 image analysis system (Motic, Carlsbad, CA). Antigen expression was measured using the mean optical density of the positive cells. Four levels were classified according to signal strength: weak positive (+), light yellow; positive (++), yellow-brown; strong positive (+++), brown; and negative (−), no color.

MTT assay for detecting cell proliferation

The 3-(4, 5-Dimethylthiazole-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) assay was conducted to evaluate cell viability and proliferation. The third-generation RPE cells (1×10^4/ mL) were seeded and cultured in three 96-well plates in the cell culture incubator for 6 h. The experimental groups were supplemented with 3, 6, 9, and 12 ng/mL EGF diluted in 200 μL DMEM medium with 10% fetal bovine serum. The control group was treated in the same way but without the drug. For each drug group and control group, 6 wells were set up. MTT assay was used to determine cell viability for 24, 48, and 72 h. Up to 20 μL MTT (5 mg/mL) was added to each well and incubated in an incubator at 37 °C for 4 h. The supernatant was removed from each well, and 100 μL dimethyl sulfoxide (DMSO; Sigma-Aldrich) was added into the wells. After shaking, the absorption (A) of each well was read at 490 nm via an enzyme-linked immunosorbent assay (ELISA) microplate reader. The rate of cell proliferation inhibition (R) was calculated using the equation R = [(A0 − At)/A0] × 100%, where A0 denotes the average absorbance of the control group and At is the average absorbance of the experimental group.

Real-Time PCR experiment

The experiments showed that 9 ng/mL EGF was the best concentration to stimulate cell proliferation *in vitro*, and curcumin (purity ≥ 98.5%, Alexis, Lausen, CH) inhibited EGF expression at an optimal concentration of 15 μg /mL in RPE cells. The cell groups in the real-time PCR (RT-PCR) experiment were as follows: blank control group, 15 μg/mL curcumin group, 9 ng/mL EGF group, and 15 μg/mL curcumin + 9 ng/mL EGF group. The cells were collected at 24, 48, and 72 h after treatment, and total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. A_{260}/A_{280} was determined by UV spectrophotometry to determine the RNA content and purity. Syntheses of complementary DNA and RT-PCR were performed using the PrimeScript® RT reagent kit and SYBR® Premix Ex Taq™ II (Takara Bio Inc., Kusatsu, Japan), respectively, according to the manufacturer's protocol. The forward and reverse primers were as follows: EGF, forward primer 5′- ATCA- CTGTGGTGGCTGTCTG-3′, reverse primer 5′-TGGTCGATG-AAAGCTTCGCT-3′, 402 bp amplified fragment; GAPDH (control), forward primer 5′-AACGGGAAACTCA-CTGGCAT-3′, reverse primer 5′-TGCTGTCGAG-ACTTTATTGATGG-3′, 539 bp amplified fragment. Following electrophoresis, the electrophoretic bands were presented using Quantity One software (Tianmei, China). The optical density ratio of the target gene to the internal reference was calculated to compare the difference.

Western blot analysis

Cells were divided into groups as described earlier and collected at 24, 48, and 72 h. Total protein was extracted with a RIPA lysis buffer and quantified. Proteins were separated by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Waltham, USA). The membrane was incubated with the primary anti-EGF antibody (1:250) (PeproTech, Rocky Hill, NJ) and secondary antibody (1: 6500). GAPDH on the same membrane was used as a loading control. Signal intensity was determined using Quantity One software and normalized to the amount of PDGFR in each sample.

Replication, administration, and ocular examination of the PVR animal model

Healthy, purple-blue rabbits (n = 30) weighing 1.5–2.0 kg were treated in accordance with the ARVO statement for the use of animals in ophthalmic and vision research. All rabbits were anesthetized by intraperitoneal injection of chloral hydrate (25 mg/kg), and their pupils were dilated with compound tropicamide eye drops. Subsequently, 10% sulfoacetate sodium was used to rinse the conjunctival sac. Ocular surface anesthesia was achieved with 0.5% ciprofloxacin hydrochloride.

PVR was induced via injections of rabbit RPE cells [14]. By indirect ophthalmoscopy, the vitreous cavity was accessed via the superotemporal sclera 4–5 mm posterior to the limbus. Up to 0.2 mL vitreous humor was extracted from each eye of each rabbit with a l-mL syringe. Each eye was treated with 2×10^6 RPE cells from the third passage in 0.1 mL normal saline injected into the vitreous cavity (a process that is used in the culture and identification of rabbit RPE cells mentioned above). PVR was induced in both eyes of each animal. One eye of each rabbit was immediately selected for intravitreal injection of 0.1 mL curcumin (0.1 mg/0.1 mL) as the curcumin group (30 eyes); each of the remaining eyes was injected with 0.1 mL 0.5‰ DMSO saline, which served as the control group (30 eyes). After the treatment, tobramycin and dexamethasone eye drops were applied [15].

The cornea, aqueous humor, lens, and vitreous opacity were observed and recorded by slit lamp biomicroscopy at 3, 7, 14, 21, and 28 days after treatment. The retinal proliferative membrane, retinal detachment, vitreous opacity, and the PVR grade of the eyes were measured by indirect ophthalmoscopy, fundus color photography, and B-ultrasonography [5]. PVR severity was graded on a scale of I to V, as follows: grade I, proliferating band of the vitreous cavity; grade II, focal traction, localized vascular engorgement, dilatation, and elevation; grade III, localized retinal detachment at the medullary ray; grade IV, extensive retinal detachment, total medullary ray detachment, and peripapillary retinal detachment; and grade V, total retinal detachment.

Detection of EGF content in the vitreous

After the eye examinations, 6 rabbits were sedated via intraperitoneal injection of chloral hydrate. The vitreous was extracted with a 5-mL syringe, following the aforementioned steps at different time points (3, 7, 14, 21, and 28 days). The test was processed according to instructions provided in the ELISA kit (LifeSpan Biosciences, Seattle, WA). The EGF content of the vitreous body was evaluated using a streptavidin-coated assay plate of the ELISA kit. The plates were then read at 450 nm via an ELISA microplate reader. The results of the experiment were calculated by subtracting the blank value from all optical density values. The concentration is the abscissa, and the optical density value is the ordinate. Commercial EGF was used to generate a standard curve. The EGF content in each experimental sample was calculated based on the standard curve.

Statistical analysis

We used SPSS ver. 16.0 (SPSS Inc., Chicago, IL) for all statistical analyses. All data are presented as the mean ± standard deviation (SD) from at least 3 replicates. Single-factor analysis of variance was used to conduct multiple comparisons. Multiple comparisons between samples were analyzed using the least significant difference method. The incidence of retinal detachment in the curcumin and control groups was analyzed using the Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

Rabbit RPE cell growth and identification

Primary RPE cells were spherical and translucent. Many melanin particles were present in the cytoplasm, and the nuclei were indistinct. After incubation for 48 h, the cells adhered to the bottom of the dish and became flat and irregular polygons. The nuclei were transparent and round, and binucleated cells were seen. Numerous melanin particles were found in the cytoplasm. After passage, the cells grew rapidly and became confluent after 5–6 days, exhibiting spindly and irregular shapes. Cytoplasmic pigment particles disappeared, whereas the fat particles multiplied. No pigment particles were observed in the third generation (Figs. 1A–1C). Cell anti-keratin was stained brown (Figs. 1D and 1E).

Effects of EGF on RPE cell proliferation

Concentration-dependence: At all time-points (24, 48, and 72 h), the optical density of RPE cells gradually increased with increasing EGF concentration (0–9 ng/mL; $P < 0.05$), whereas no difference was observed in the 9 and 12 ng/mL EGF groups ($P > 0.05$). This result suggested that 9 ng/mL EGF was the optimal concentration for stimulating RPE cell proliferation *in vitro* (Fig. 2). Time-dependence: Under the influence of all concentrations of EGF (0, 3, 6, 9, and 12 ng/mL), the optical density of RPE cells increased with time (24–72 h). Significant differences in optical density between each pair were observed ($P < 0.05$; Fig. 2). These results indicated that EGF could promote RPE cell proliferation in relation to both dose and incubation time.

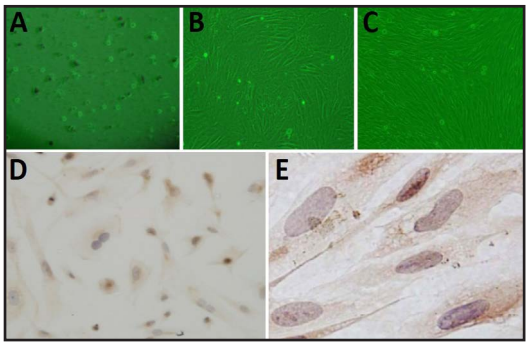

Fig. 1. Morphology of RPE cells under a light microscope. A. RPE cells isolated from rabbit eyes are round and full of melanin (×200). B. Rabbit RPE cells of passage 1 close to confluence showed many binuclear cells. C. Rabbit RPE cells of passage 3 close to confluence showed a network-like distribution and lost their pigment (×200). D. Rabbit RPE cells of passage 3 were identified by immunohistochemistry for keratin (StreptAvidin-Biotin Complex (SABC) ×200). E. Rabbit RPE cells of passage 3 identified by immunohistochemistry showed strong EGF expression (SABC×400). All experiments were repeated 3 times.

Inhibition of EGF expression in RPE cells by curcumin

The results of immunocytochemistry indicated that EGF was expressed in the cytoplasm (brown, Fig. 1E). At different time points, EGF expression was significantly lower in the curcumin-treated group than in the control group ($P < 0.05$), but no difference between the 15 and 20 μg/mL curcumin groups was found ($P > 0.05$). EGF expression decreased with an increase in curcumin dose ($P < 0.05$). At the same concentration of curcumin, the expression of intracellular EGF significantly decreased ($P < 0.05$) with longer incubation times (Fig. 3). These results showed that the downregulation of EGF expression by curcumin is related to both the incubation time and drug dose. The optimal concentration for the inhibition of EGF expression was 15 μg/mL of curcumin.

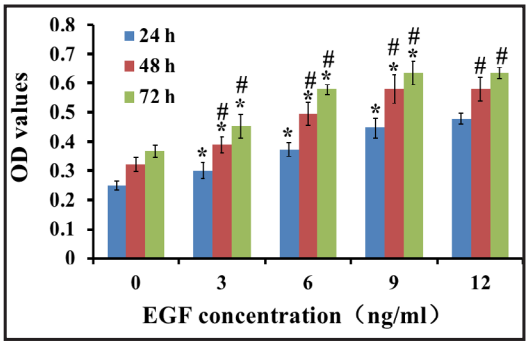

Fig. 2. Effects of EGF on RPE cell proliferation at different concentrations and times. MTT assays were used to evaluate the probability of RPE cell proliferation. All experiments were repeated 3 times. All data are presented as the mean ± SD. # P<0.05 versus the previous time (at the same concentration). * P<0.05 versus the previous group (at the same time point). OD, optical density.

Effects of curcumin on EGF mRNA content in RPE cells

RT-PCR results showed that the content of EGF mRNA in RPE cells was decreased significantly by 15 μg/mL curcumin by time ($P < 0.05$); at each time point, the EGF mRNA content was lower than that in the control group ($P < 0.05$; Fig. 4). The EGF mRNA content in the 15 μg/mL curcumin + 9 ng/mL EGF group was reduced by time ($P < 0.05$); the EGF mRNA content at each time point was significantly lower than that of the 9 ng/mL EGF group ($P < 0.05$; Fig. 5).

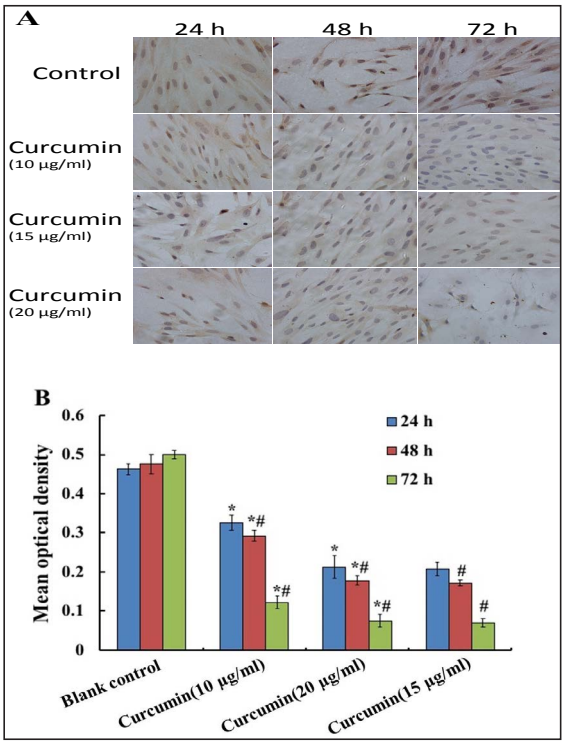

Fig. 3. Inhibition of the expression of EGF in RPE cells by curcumin. A. The relative expression of EGF in RPE cells in all groups by immunocytochemistry staining (SABC ×200). B. Comparison of EGF expression in all groups, using mean optical density. Expression was increased in the blank control group, but decreased in the different curcumin groups. All experiments were repeated 3 times. All data are presented as the mean ± SD. * P<0.05 versus the former group (at the same time point). # P<0.05 versus the previous time point (in the same group).

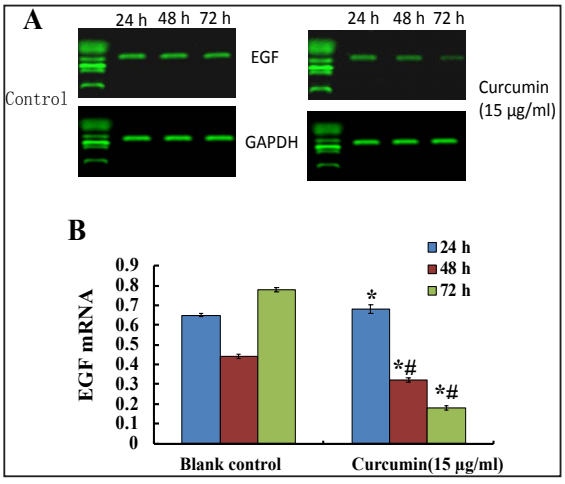

Fig. 4. Expression of EGF mRNA in RPE cells at different time points by RT-PCR. A. Representative images. B. Expression of EGF mRNA in the 15 μg/mL curcumin and control groups. All experiments were repeated 3 times. All data are presented as the mean ± SD. * P<0.05 versus the previous group (at the same time point). # P<0.05 versus the previous time point (in the same group).

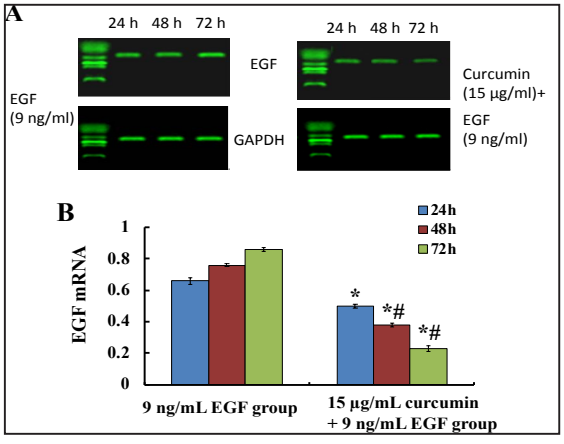

Fig. 5. Expression of EGF mRNA in RPE cells at different time points by RT-PCR. A. Representative images. B. Expression of EGF mRNA in the 5 μg/mL curcumin + 9 ng/mL and the 9 μg/mL EGF groups. All experiments were repeated 3 times. All data are presented as the mean ± SD. * P<0.05 versus the previous group (at the same time point). # P<0.05 versus the previous time point (in the same group).

Effects of curcumin on EGF protein expression in RPE cells

The results of western blot analysis indicated that EGF protein expression in the curcumin group (15 μg/mL) decreased with time, and significant differences were observed between the 3 time points ($P < 0.05$). At each time point, EGF protein expression was lower in the curcumin group than in the control group ($P < 0.05$; Fig. 6). EGF protein expression in RPU cells was decreased at 24, 48, and 72 h in the 15 μg/mL curcumin + 9 ng/mL EGF group. The EGF protein expression levels were significantly different at the 3 time points ($P < 0.05$), and lower than those in the 9 ng/mL EGF group ($P < 0.05$; Fig. 7).

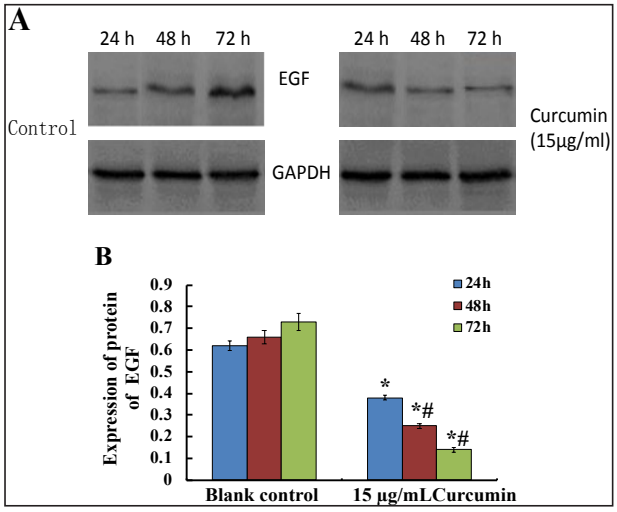

Fig. 6. Expression of EGF protein in RPE cells at different time points by western blot analysis. A. Representative images. B. Expression of EGF protein in the 15 μg/mL curcumin and control groups. All experiments were repeated 3 times. All data are shown as the mean ± SD. * P<0.05 versus the previous group (at the same time point), # P<0.05 versus the previous time point (in the same group).

Eye examination

In both groups, only a small amount of floating matter was found in the anterior chambers on day 3 post-injection of RPE cells by slit lamp biomicroscopy and indirect ophthalmoscopy, but it disappeared on day 7. The vitreous was cloudy on day 3 post-injection in both groups, while the vitreous opacities were low in the curcumin group. The proliferation membrane was thinner in the curcumin group than in the control group on day 7 post-treatment recorded by B-ultrasonography. The PVR grade is judged based on comprehensive results from indirect ophthalmoscopy, fundus color photography, and B-ultrasonography. PVR II was assigned to 17 eyes in the control group and 2 in the experimental group. On day 14 post-treatment, PVR was observed in all 18 eyes in the control group; 7 were PVR II and 11 were PVR III. The incidence of retinal detachment was 61%. Meanwhile, the curcumin group had only 6 PVR eyes (4 PVR I and 2 PVR III), and the incidence of retinal detachment was 11%, which was significantly lower than that in the control group (P <0.05). On day 21 post-treatment, PVR was observed in all 12 eyes in the control group; among them, 3 were classified as PVR I, 4 as PVR III, and 5 as PVR IV. The incidence of retinal detachment in the control group was 75%. However, PVR was present in only 4 of 12 eyes in the curcumin group (2 PVR I and 2

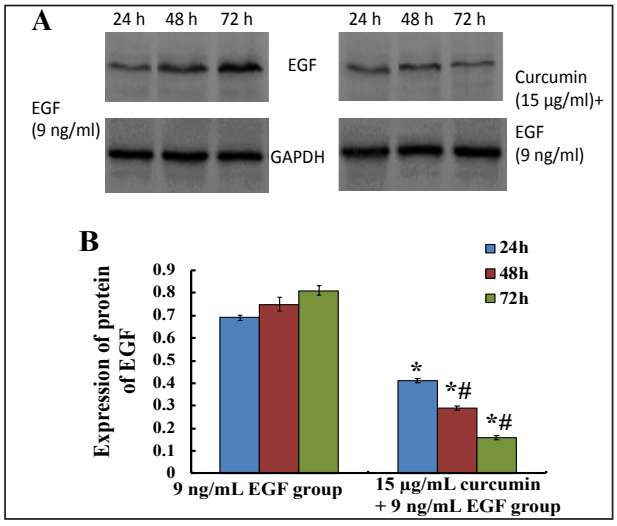

Fig. 7. Expression of EGF protein in RPE cells at different time points by western blot analysis. A. Representative images. B. Expression of EGF protein in the 5 μg/mL curcumin + 9 ng/mL EGF and the 9 μg/mL EGF groups. All experiments were repeated 3 times. All data are shown as the mean ± SD. * P<0.05 versus the previous group (at the same time point). # P<0.05 versus the previous time point (in the same group).

PVR III eyes). The incidence of retinal detachment was 16%, which was significantly lower than that in the control group ($P < 0.05$). On day 28 post-treatment, PVR was observed in all 6 eyes in the control group, including 1 PVR I, 1 PVR III, and 4 PVR IV; the incidence of retinal detachment was 83%. PVR was observed in only 2 of 6 eyes in the curcumin group (1 PVR I and 1 PVR III) and the incidence of retinal detachment was 16%, which was significantly lower than that in the control group ($P < 0.05$; Figs. 8 and 9 and Table 1). These results indicate that curcumin significantly inhibited the initiation and development of PVR.

EGF content in the vitreous

After intravitreal injection of RPE cells and curcumin, the vitreous fluid was extracted at each time point to measure the EGF content in the vitreous. The results indicated that the EGF content was significantly lower in the curcumin group than in the control group ($P < 0.05$; Figure 10).

Discussion

The pathogenesis of PVR is complex, and the causative processes must be clarified. However, this study demonstrated that the incidence is closely related

Table 1. Incidence of retinal detachment after injection of RPE cells. All experiments were repeated at least 3 times. * P < 0.05 versus the control groups

Time (d)	Control group	Curcumin group
14	61% (11/18)	11/% (2/18)*
21	75% (9/12)	16% (2/12) *
28	83% (5/6)	16% (1/6) *

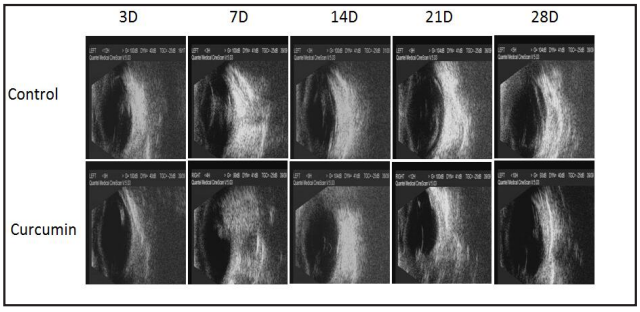

Fig. 8. Ultrasonic photograph of rabbit eyes on different days. Day 3: The vitreous opacity ranged from low to moderate in the control group (6 eyes), and slight vitreous opacities were observed in the curcumin group (6 eyes). Day 7: 17 of 24 eyes in the control group and 2 of 24 eyes in the curcumin group exhibited proliferation of the cords. Day 14: 7 of 18 eyes in the control group had proliferation bands in the vitreous; the remaining 11 exhibited limited retinal detachment; 4 of 18 eyes had proliferative bands in the vitreous, and 2 exhibited localized detachment in the curcumin group. Day 21: 3 of 12 eyes in the control group presented with proliferative membranes in the vitreous, 4 showed limited retinal detachment, and the remaining 5 s exhibited wide retinal detachment; 2 of 12 eyes in the curcumin group showed proliferating bands in the vitreous, and 2 exhibited limited retinal detachment. Day 28: 1 of 6 eyes in the control group had proliferating cords in the vitreous, 1 had limited retinal detachment, while the other 4 had a wide range of retinal detachment stages. 1 of 6 eyes in the curcumin group had proliferative cords in the vitreous, and 1 eye had localized retinal detachment.

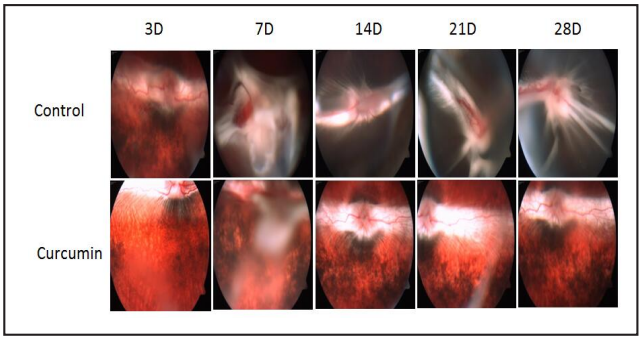

Fig. 9. Fundus photograph of a rabbit eye in the curcumin group on different days. The results are consistent with the results obtained by B-scan ultrasonography.

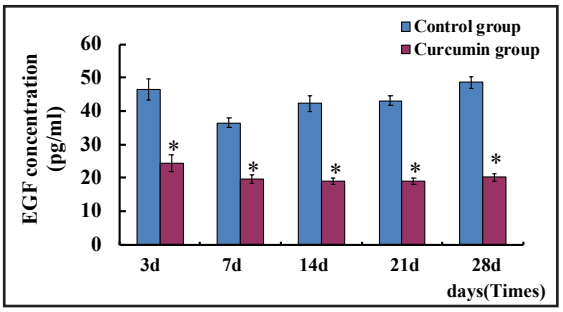

Fig. 10. EGF content in the vitreous. ELISA assays were employed to determine the EGF content in the vitreous in the control and curcumin groups at different times. All experiments were repeated more than 6 times. All data are shown as the mean ± SD. * P<0.05 versus the control group (at the same time point).

to RPE cells. After the RPE cell enters the vitreous, its morphology and function change significantly. It can be transformed into macrophage-like and fibroblast-like cells, among others, which possess multiple functions (including the synthesis of inflammatory factors, collagen, and several other proteins) and promote the formation of the proliferation film. Previous studies show that PVR formation is closely related to various cytokines that not only promote cell chemotaxis, migration, and aggregation, but also help regulate RPE cell differentiation and construction of the extracellular matrix [16-18]. EGF is a small molecule peptide growth factor secreted by platelets that can promote multiple cell division *in vivo* [19]. Numerous studies have reported that different concentrations of EGF exert different stimulatory effects on the proliferation of human RPE cells *in vitro* [20]. The results of our study show that EGF is expressed in RPE cells, suggesting that EGF can be synthesized in these cells. The effect of EGF on RPE cell proliferation showed a time–effect and a dose–response relationship.

At present, PVR treatment is conducted mainly by surgery, which can remove the pathological tissues but not inhibit cell proliferation, leading to a high rate of PVR recurrence. In addition, the vision and recovery outcomes of the patients are not ideal. Therefore, it has become both the focus and a challenge to find an effective drug to prevent and treat PVR in the ophthalmology field. Thus, depending on the different pathogenic factors and stages of disease, drug-assisted treatment (such as low-molecular-weight heparin and 5-FU, corticosteroids, antioxidants, extracellular matrix synthesis inhibition agents, and cell signal transduction inhibitors) can improve the condition of the patients and the efficacy of surgical treatments [4, 7]. However, most western medicines are expensive and exert a single pharmacological effect. No efficient medicine for clinical application has been found to date. Therefore, a Chinese herbal medicine with a wide range of pharmacological effects on the treatment of PVR must be identified. Curcumin is a monomer component from traditional Chinese medicine that exhibits anti-proliferation, anti-tumor, and anti-inflammatory effects, among others [8, 9]. Previous studies show that curcumin can inhibit RPE cell proliferation *in vitro* [10, 11]; however, there are no reports on whether this inhibition is achieved by downregulating EGF expression. In the current study, different concentrations of curcumin were used to treat RPE cells cultured *in vitro*. The results indicated that curcumin exerted a significant effect on the inhibition of EGF expression in RPE cells and that this effect was related to treatment time and dose. The ideal inhibitory concentration is 15 μg/mL curcumin. To further study the mechanism of the action of curcumin on the inhibition of EGF, we used the ideal curcumin concentration to treat rabbit RPE cells *in vitro*. Curcumin was found to decrease EGF mRNA and inhibit the expression of EGF protein in RPE cells; protein expression gradually decreased with increasing treatment time. We used EGF to stimulate RPE cell proliferation *in vitro*, which simulates the natural formation of PVR, and used curcumin as a treatment. The results showed that the inhibitory effect of curcumin gradually increased with increasing treatment times, suggesting that curcumin decreased EGF mRNA and inhibited the expression of EGF protein in RPE cells in a time-dependent manner.

To further explore the pathogenesis of PVR and provide effective prevention and control methods, third-generation rabbit RPE cells, with or without curcumin, were injected into the vitreous cavity of rabbits. Animal models for the PVR study were successfully

produced. The initiating factors for PVR formation are RPE cells. RPE cells leave the retinal pigment epithelium, reach the surface of the retina and cavity of the vitreous body under cytokinetic action and begin to deform and proliferate. We chose rabbit RPE cells cultured *in vitro* to establish the PVR model by intravitreal injection, which is in line with the natural development of PVR and conducive to PVR research. The results showed that curcumin could effectively reduce the occurrence and development of PVR. Simultaneously, ELISA showed that curcumin significantly reduced EGF expression in RPE cells, which confirmed that curcumin could inhibit the initiation and development of PVR by decreasing the amount of EGF in the vitreous cavity during PVR formation.

In summary, both *in vivo* and *in vivo* studies demonstrated that curcumin can inhibit RPE cell proliferation and the development of PVR by downregulating the expression of EGF. This finding provides a theoretical basis for the use of curcumin in the prevention and treatment of PVR.

Acknowledgements

This work was supported by a grant from the National Natural Science Foundation of China (No. 30973252).

Disclosure Statement

The authors have declared that no competing interests exist.

References

1 Garweg JG, Tappeiner C, Halberstadt M. Pathophysiology of proliferative vitreoretinopathy in retinal detachment. Surv Ophthalmol 2013;58:321-329.
2 Por ED, Greene WA, Burke TA, Wang HC. Trichostatin A Inhibits Retinal Pigmented Epithelium Activation in an *In vitro* Model of Proliferative Vitreoretinopathy. J Ocul Pharmacol Ther 2016;32:415-424.
3 Ducournau Y, Boscher C, Adelman RA, Guillaubey C, Schmidt-Morand D, Mosnier JF, Ducournau D. Proliferation of the ciliary epithelium with retinal neuronal and photoreceptor cell differentiation in human eyes with retinal detachment and proliferative vitreoretinopathy. Graefes Arch Clin Exp Ophthalmol 2012;250:409-423.
4 Ishikawa K, He S, Terasaki H, Nazari H, Zhang H, Spee C, Kannan R, Hinton DR. Resveratrol inhibits epithelial-mesenchymal transition of retinal pigment epithelium and development of proliferative vitreoretinopathy. Sci Rep 2015;5:16386.
5 Fastenberg DM, Diddie KR, Dorey K, Ryan SJ. The role of cellular proliferation in an experimental model of massive periretinal proliferation. Am J Ophthalmol 1982;93:565-572.
6 Dithmer M, Fuchs S, Shi Y, Schmidt H, Richert E, Roider J, Klettner A. Fucoidan reduces secretion and expression of vascular endothelial growth factor in the retinal pigment epithelium and reduces angiogenesis *in vitro*. PLoS One 2014;9:e89150.
7 Sundaram V, Barsam A, Virgili G. Intravitreal low molecular weight heparin and 5-Fluorouracil for the prevention of proliferative vitreoretinopathy following retinal reattachment surgery. Cochrane Database Syst Rev 2013;CD006421.
8 Al-Rubaei ZM, Mohammad TU, Ali LK. Effects of local curcumin on oxidative stress and total antioxidant capacity *in vivo* study. Pak J Biol Sci 2014;17:1237-1241.
9 Rashmi R, Kumar S, Karunagaran D. Human colon cancer cells lacking Bax resist curcumin-induced apoptosis and Bax requirement is dispensable with ectopic expression of Smac or downregulation of Bcl-XL. Carcinogenesis 2005;26:713-723.

10 Sun Y, You ZP. Curcumin inhibits human retinal pigment epithelial cell proliferation. Int J Mol Med 2014;34:1013-1019.
11 Zhu W, Wu Y, Meng YF, Wang JY, Xu M, Tao JJ, Lu J. Effect of curcumin on aging retinal pigment epithelial cells. Drug Des Devel Ther 2015;9:5337-5344.
12 Zhou X, Kuang X, Long C, Liu W, Tang Y, Liu L, Liu H, He J, Huang Z, Fan Y, Zhang Q, Shen H. Curcumin Inhibits Proliferation and Epithelial-Mesenchymal Transition of Retinal Pigment Epithelial Cells Via Multiple Pathways. Curr Mol Med 2017;17:312-319.
13 Uebersax ED, Grindstaff RD, Defoe DM. Survival of the retinal pigment epithelium *in vitro*: comparison of freshly isolated and subcultured cells. Exp Eye Res 2000;70:381-390.
14 Radtke ND, Tano Y, Chandler D, Machemer R. Simulation of massive periretinal proliferation by autotransplantation of retinal pigment epithelial cells in rabbits. Am J Ophthalmol 1981;91:76-87.
15 Bai Y, Yu W, Han N, Yang F, Sun Y, Zhang L, Zhao M, Huang L, Zhou A, Wang F, Li X. Effects of semaphorin 3A on retinal pigment epithelial cell activity. Invest Ophthalmol Vis Sci 2013;54:6628-6638.
16 Chen X, Xiao W, Liu X, Zeng M, Luo L, Wu M, Ye S, Liu Y. Blockade of Jagged/Notch pathway abrogates transforming growth factor beta2-induced epithelial-mesenchymal transition in human retinal pigment epithelium cells. Curr Mol Med 2014;14:523-534.
17 Dvashi Z, Goldberg M, Adir O, Shapira M, Pollack A. TGF-beta1 induced transdifferentiation of rpe cells is mediated by TAK1. PLoS One 2015;10:e0122229.
18 Zhang L, Wang F, Jiang Y, Xu S, Lu F, Wang W, Sun X, Sun X. Migration of retinal pigment epithelial cells is EGFR/PI3K/AKT dependent. Front Biosci (Schol Ed) 2013;5:661-671.
19 Yan F, Hui Y. Epidermal growth factor receptor exists in the early stage of proliferative vitreoretinopathy. Can J Ophthalmol 2012;47:e24-25.
20 Yan F, Hui YN, Li YJ, Guo CM, Meng H. Epidermal growth factor receptor in cultured human retinal pigment epithelial cells. Ophthalmologica 2007;221:244-250.