

# Artesunate inhibits proliferation and migration of RPE cells and TGF- $\beta$ 2 mediated epithelial mesenchymal transition by suppressing PI3K/AKT pathway

Zi-Yi Wang, Yu Zhang, Ling-Dan Wu, Jie Chen, Mei-Ling Chen, Ci-Min Chen, Qi-Hua Xu

The Affiliated Eye Hospital of Nanchang University; Jiangxi Clinical Research Center for Ophthalmic Disease, Nanchang 330000, Jiangxi Province, China

**Co-first authors:** Zi-Yi Wang and Yu Zhang

**Correspondence to:** Qi-Hua Xu. The Affiliated Eye Hospital of Nanchang University, Nanchang 330000, Jiangxi Province, China. xu7ganggang@163.com

Received: 2021-04-20 Accepted: 2021-09-15

## Abstract

• **AIM:** To study the braking effectiveness of artesunate on transforming growth factor (TGF)- $\beta$ 2 mediated epithelial-mesenchymal transition (EMT) in retinal pigment epithelium (RPE) *in vitro*.

• **METHODS:** The fostered ARPE-19 cells were processed with artesunate alone or combined with the TGF- $\beta$ 2. The CCK-8 examination was utilized to test the cell propagation. Cell migration was detected by scratch as well as the Transwell examination. The EMT characters and activation of PI3K/Akt signal channel were estimated by Western blotting and immunofluorescence. The Western blotting was utilized in order to confirm the vitreous of controls as well as patients with proliferative vitreoretinopathy (PVR) were collected and the levels of PI3K, phospho-PI3K, Akt.

• **RESULTS:** Disposal of ARPE-19 cells with artesunate (50-150  $\mu$ mol/L) obviously suppressed their propagation and immigration, which dependent on the concentration and time. Artesunate suppressed the EMT which was induced by TGF- $\beta$ 2 in ARPE-19 cells through sustaining the expression of vimentin and  $\alpha$ -SMA through the suppression of PI3K, phospho-PI3K, phospho-Akt and Akt. Levels of PI3K, phospho-PI3K, AKT and phospho-Akt was increased in the vitreous in PVR ( $P < 0.05$ ).

• **CONCLUSION:** Such findings indicate that PI3K/Akt signal channel is highly activated in vitreous of PVR. Artesunate is an operative depressor of the propagation, immigration and TGF- $\beta$ 2-mediated EMT of ARPE-19 cells by reduced the expression of PI3K/Akt channel.

• **KEYWORDS:** proliferative vitreoretinopathy; artesunate; epithelial-mesenchymal transition; migration

**DOI:**10.18240/ijo.2022.02.02

**Citation:** Wang ZY, Zhang Y, Wu LD, Chen J, Chen ML, Chen CM, Xu QH. Artesunate inhibits proliferation and migration of RPE cells and TGF- $\beta$ 2 mediated epithelial mesenchymal transition by suppressing PI3K/AKT pathway. *Int J Ophthalmol* 2022;15(2):197-204

## INTRODUCTION

Proliferative vitreoretinopathy (PVR) is a common complication of ocular trauma. It can significantly reduce the visual acuity in a short time and seriously affect the quality of life of patients. The main feature of PVR is to form the anterior retinal membrane through the wound repair process. With the formation and contraction of the anterior retinal membrane, the retina will eventually wrinkle and produce tractive retinal detachment, resulting in decreased visual acuity and even blindness<sup>[1]</sup>. Surgical repair is the only effective treatment at present. The high recurrence rate makes scholars actively looking for effective drug adjuvant therapy. It is found that the anterior retinal membrane contains many cell types such as retinal pigment epithelium (RPE) cells, fibroblasts and macrophages. RPE cells are considered to be the main participants in the pathophysiological process of PVR<sup>[2-3]</sup>. RPE cells in PVR undergo a procedure which is named epithelial mesenchymal transition (EMT), which involves many changes, such as phenotypic transformation, extracellular matrix (ECM) accumulation and mesenchymal cell marker expression. Studies have shown that the EMT process of RPE cells is a key procedure in the pathogenesis of PVR<sup>[4]</sup>.

There may be many molecular mechanisms of EMT regulation in RPE cells. In PVR, RPE cells own the capability of autocrine cytokines. A large quantity of growth drivers show overexpression in vitreous or subretinal effusion in patients who are with PVR. These growth drivers and their acceptors occupy significant position in the existence and occurrence of PVR. After the blood-retina barrier is destroyed, the physiological equilibrium of growth drivers is broken, after

which the RPE cells are impulses by growth drivers to produce EMT, immigration and propagation, and then form preretinal membrane with other cells and intercellular substance<sup>[5-6]</sup>. It was found that RPE cells autocrine TGF- $\beta$ . It can make wound closure, collagen contraction and cell morphology change. The EMT process of RPE cells which are induced occupies significant position in the existence, occurrence and prognosis of PVR. It has been shown that multiple downstream trails Smads, phosphatidylinositol-3-kinase (PI3K) and MAPK can be activated to perform the EMT induction<sup>[7-8]</sup>. Recently, the stimulation of PI3K/Akt trail has also become a vital signal trail regulating EMT. Protein kinase B (Akt) is able to promote the phosphorylation of a large quantity of proteins and regulate cell survival, apoptosis and migration. But PI3K/Akt signal channel is in TGF- $\beta$ . The function of RPE cell mediated EMT remains to be confirmed.

Artesunate is a derivative of artemisinin, which is extensively applied as an anti-malaria traditional Chinese medicine. Artesunate is extensively applied in clinic because of its good water solubility and various biological activities. Artesunate has the functions of anti-proliferation, anti-fibrosis and immune regulation<sup>[9]</sup>. In the laboratory, artesunate is widely used in the treatment of glaucoma, intraocular neovascularization, ocular tumors and uveitis<sup>[10-18]</sup>. Researches have revealed that artesunate is able to restrain the EMT process of alveolar epithelial as well as renal tubular epithelial cells<sup>[19-20]</sup>, but its inhibitory effect on the EMT process of RPE cells is not clear. This experiment examined the effects of artesunate on the propagation, immigration and effect of TGF- $\beta$  induced RPE cells on EMT process.

## MATERIALS AND METHODS

**Ethical Approval** All patients involved in this research were fully aware of the research and the consent forms were offered and signed. This research followed the Declaration of Helsinki and it gained approval from the Ethics Review Committee of Ophthalmic Hospital. The utilization of human organizations confirm to the application rules in Helsinki Declaration.

**Collection of Samples** Patients who suffered from cancer, autoimmune illness and other types of severe systemic illness were kept out. PVR-a ( $n=3$ ), PVR-b ( $n=3$ ), and PVR-c ( $n=3$ ) were collected. PVR grading was carried out referring to the standard of the American retinal Nomenclature Committee in 1983. The vitreous bodies were gained from the patients who donate the cornea.

**Cell Culture and Disposal** It is from Shanghai Yubo Biotechnology Co., Ltd. that the ARPE-19 was acquired and fostered in DMEM/F12 medium which contained 10% fetal bovine serum (04-001-1acs) and 1% penicillin/streptomycin, and raised in thermo (USA) at the temperature of 37°C, and the cells attached completely to the wall after 24h. The cells were

fostered until the fusion degree become 80%-90% and starved in medium which was free of serum for one night. DMEM/F12 which contained 1% fetal bovine serum was made use of for the purpose of replacing DMEM/F12. Cells were treated with artesunate which had various concentrations (lot#418A024. Solarbio, China). Cell propagation and migration were examined at different time. The degree of EMT was detected by pretreatment with TGF- $\beta$ 2 (catalog, 100-11, lot #081701-1, peprotech) for 48h and artesunate for 48h.

**CCK-8 Detecting the Propagation Rate of ARPE-19 Cells** When the cells grew to 80%-90% fusion, the cells were digested from the culture dish, centrifuged, discarded the supernatant, added the cell culture media, after which the cell concentration was adjusted to  $1 \times 10^5$ /mL, and inoculated the cells to 96 well plate. After 24h, the control group whose drug concentration was 0 and the experimental group whose concentration was 75, 100, 150, and 200  $\mu$ mol/L were added with 100  $\mu$ L cell culture media whose drug intensity were different, and incubated in the incubator at the temperature of 37°C. The initial culture media was aspirated when the incubation lasted 0, 24, 48, and 72h, and 10% CCK-8 was incubated for one hour. The absorbance a value of 450 nm wavelength was determined by thermo, USA. The experiment was repeated three times.

**Cell Scratch Assay** Mark a line on the back of the 6-hole plate, and each hole passes through two straight lines parallel to the edge of the plate and equidistant from the center. When the cells grew to 80%-90% fusion, the cells were digested from the culture dish, centrifuged, discarded the supernatant, added DMEM/F12 media which contained 10% fetal bovine serum, adjusted the cell density to  $1 \times 10^7$ /mL, added 2 mL per well into a 6-well plate marked with lines on the back in advance, and then incubated in an incubator at the temperature of 37°C and 5% CO<sub>2</sub> saturated humidity. After 24h, the cells were basically attached to the wall and covered the bottom of the 6-well plate. The serum - free medium was used to starve for 24h, 20-200  $\mu$ L gun head was used to make a scratch perpendicular to the bottom of the plate. The wound width was 25 mm. The phosphate buffered saline (PBS) was applied to wash the cells twice, and treated with different concentration of artesunate (0, 75, 150  $\mu$ mol/L) without the existence of TGF (20 ng/mL) in a serum-free medium. Incubated in a thermostatic chamber at the temperature of 37°C and 5% CO<sub>2</sub> saturated humidity for 36h. At 0, 12, 24, and 36h, cell immigration was assessed by counting the scratch zone in three regions which are selected at random.

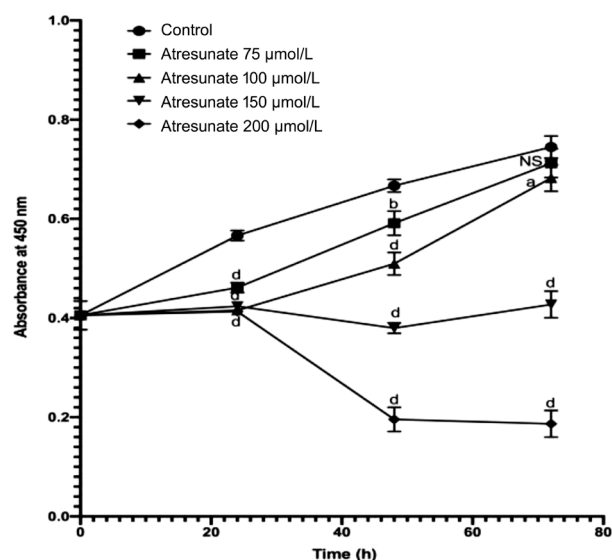
**Cell Migration Test** After raised with various densities of artesunate (0, 75, 150  $\mu$ mol/L) for 24h, the cells were produced by trypsin method and washed twice with DMEM/F12. For the purpose of detecting cell immigration, the 24 well plate

was separated into the upper and lower chambers using an 8  $\mu\text{mol/L}$  cell chamber (Transwell, Corning, USA). The inferior chamber was full of 0.6 mL DMEM/F12 which contained 10% fetal bovine serum. Totally  $5 \times 10^4$  cells were suspended in 100  $\mu\text{L}$  DMEM/F12 medium without bovine serum albumin (BSA). After 24h of culture, the cotton swab was utilized to remove the cells on the surface of Transwell membrane. There is 20 $\times$  objective select the images at random were taken with an inverted microscope (ckx41-f32f, Olympus, Japan) for the purpose of counting stained cells and quantify cell migration ability.

**Western Blot** The PBS was utilized for the purpose of washing cells and tissues twice (phosphate buffered saline, 02-024-1acs, Israel biological industries), and then centrifuged at 12000 rpm for 15min with protease depressor (PMSF, ar1178, boster). The supernatant lysate was collected for bicinchoninic acid (BCA) Protein Quantification Kit (e112-01/02, Nanjing novozan Biotechnology Co., Ltd., China). The same quantity of protein was loaded on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (SDS-PAGE gel preparation kit, boster). Totally 5% skimmed milk (skimmed milk powder, BD company, USA) was used to seal the membrane at ambient temperatures in tris buffered saline tween (TBST), rabbit anti-human against PI3K (1:1000, Cell Signaling Technology, Danvers, USA), rabbit anti-human against P-PI3K (1:1000, Cell Signaling Technology, Danvers, MA, USA), rabbit anti-human against Akt (1:1000, Cell Signaling Technology, Danvers, MA, USA), rabbit anti-human against P-Akt. After cleaning with TBST, the membrane was raised with Goat anti-rabbit IgG (ba1054, Wuhan shibode Bioengineering Co., Ltd., China) at room temperature for one hour, after which it was scanned on the exposure machine. Then, the imaging software (Image J) was utilized to confirm the full intensity of all detection bands. These tests were reiterated not less than three times.

**Immunofluorescence** ARPE-19 cells were fostered on a plate which had 6 wells covering plate for 48h, as described above. Then, PBS was washed twice and 4% paraformaldehyde was fixed at ambient temperatures which lasted 15min. The PBS was applied to wash the fix cells twice, after which they were sealed in a room temperature moist chamber with 10% normal goat serum, and raised with rabbit anti-vimentin (1:200, d21h3, XP, rabbit mAb, and CST) at 4 $^{\circ}\text{C}$  for one night. And the cells were raised with Cy3 labeled Goat anti-rabbit antibodies at ambient temperatures for 1h the next day. The cells were fixed with DAPI, added with anti-fluorescence quenching agent, and observed by laser scanning inverted microscope (ckx41-f32f, Olympus, Japan).

**Statistical Analysis** Excel and GraphPad Prism 8 software were utilized for the intention of handling and investigating



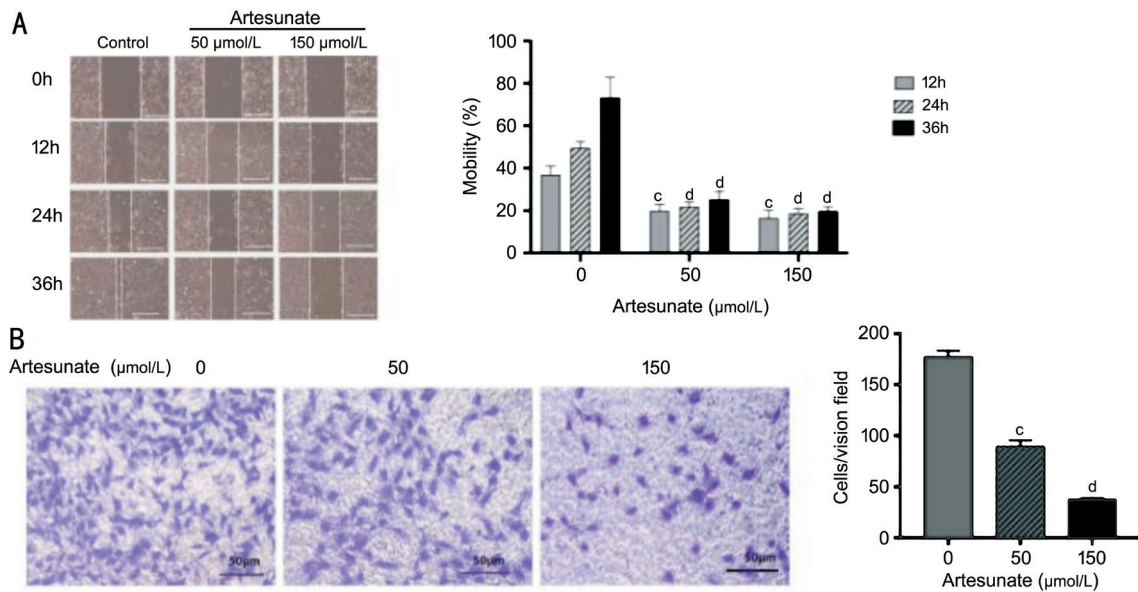
**Figure 1 Artesunate inhibits RPE cell proliferation as determined by the CCK-8 assay** ARPE-19 cells were left untreated with different concentrations of artesunate (0, 75, 100, 150, and 200  $\mu\text{mol/L}$ ) for 24, 48, and 72h. Absorbance at 450 nm showed a significant decrease in the growth of artesunate-treated cells compared with that in the control cells in a dose-and-time-dependent manner. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$ , <sup>d</sup> $P < 0.0001$ . The data are presented as the mean  $\pm$  SD ( $n = 3/\text{group}$ ).

the experimental data. Two-independent specimen *t*-test was utilized for comparison between two groups, and one-way ANOVA was applied to perform the comparison for the results of multiple groups. A value of  $P < 0.05$  showed statistical significance.

## RESULTS

**Artesunate Restrains Propagation and Immigration of RPE Cells** For the purpose of achieving the best effectiveness on ARPE-19 survival rate, CCK-8 experiment was carried out to verify the optimal intensity of artesunate, such as drug concentration and treatment time. Different concentrations of artesunate had different suppression capacity on ARPE-19 cell propagation at different time. As the concentration and time change, the inhibitory capacity was enhanced (Figure 1). In order to achieve 50% cell inhibition without increasing the time of action, the best condition was treated with 150  $\mu\text{mol/L}$  for 48h.

We conducted scratch test and Transwell test *in vitro* to determine whether artesunate can regulate ARPE-19 cell migration. As shown in the figure (Figure 2), the cells in the scratch test and no drug treatment group showed strong cell migration ability, which almost covered the scratch area after 36h, while the cell migration capability of the drug treatment group was significantly inhibited, and the results were statistically significant and time concentration dependent. The higher the drug concentration, the more obvious the inhibition of ARPE-19 cell migration. Transwell experiment also



**Figure 2 Artesunate inhibits cell proliferation, migration** ARPE-19 cells are treated with artesunate at 0, 50, and 150  $\mu\text{mol/L}$  for 0, 12, 24, and 36h. A: Scratch assay were measured cell horizontal migration. <sup>c</sup> $P < 0.001$ , <sup>d</sup> $P < 0.0001$ . Data are presented as mean  $\pm$  SD.  $n = 3/\text{group}$ . Scale bar: 250  $\mu\text{m}$ . B: In the Transwell assay, the cells that migrated into the detection zone (the lower chamber) were measured. <sup>d</sup> $P < 0.0001$ . Data are presented as mean  $\pm$  SD.  $n = 3/\text{group}$ . Scale bar: 50  $\mu\text{m}$ .

obtained results which are similar. As 24h passed, the cells in the high concentration artesunate treatment group were much less than those without drug treatment, and the results indicated statistical significance. The two migration tests showed that artesunate disposal obviously suppressed cell migration by a means which was dependent on the dose.

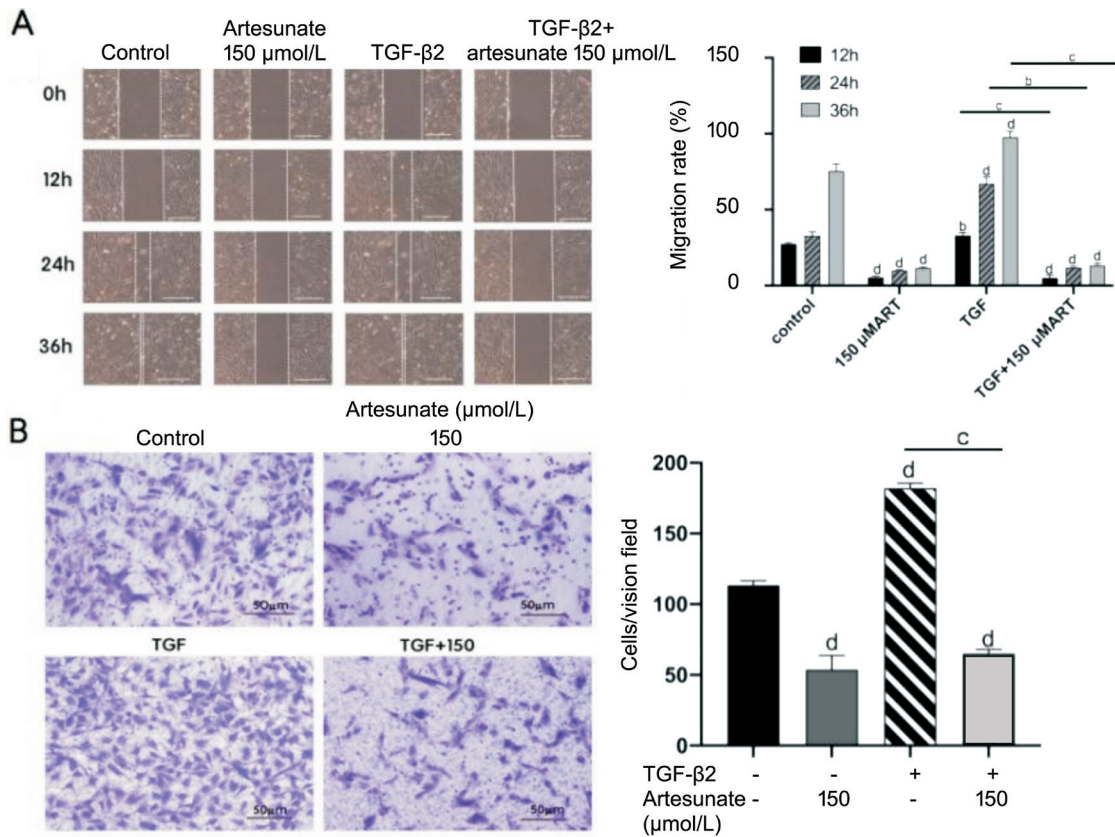
#### Effect of Artesunate on ARPE-19 Migration Induced by TGF- $\beta$ 2

**Artesunate restrains TGF- $\beta$ 2 mediated scratch in ARPE-19 cells** The EMT course of ARPE-19 cells mediated by TGF occupies a significant position in PVR, which involves the propagation, immigration and fibrosis of ARPE-19 cells. Therefore, we used cell scratch test to verify the effectiveness of artesunate on the migration of ARPE-19 treated with TGF- $\beta$ 2. Pretreatment with serum-free media for 24h inhibited cell proliferation and made cell scratch. Pictures were taken at 0, 12, 24, and 36h. Compared with the control group, artesunate treatment group could inhibit wound healing (Figure 3A); however, TGF- $\beta$ 2 treatment could promote wound healing. When artesunate was added to ARPE-19 cells which were induced by TGF- $\beta$ 2, the wound healing rate was slowed down. By referring to the discovery, our findings indicate that artesunate restrains wound healing in ARPE-19 cells treated with TGF- $\beta$ 2. Since cell propagation and immigration are crucial processes in wound closure, we pretreated ARPE-19 cells with serum-free medium, which reduced the effect of cell proliferation on wound healing. Therefore, these findings revealed that that artesunate inhibited the migration of ARPE-19 cells processed with TGF- $\beta$ 2.

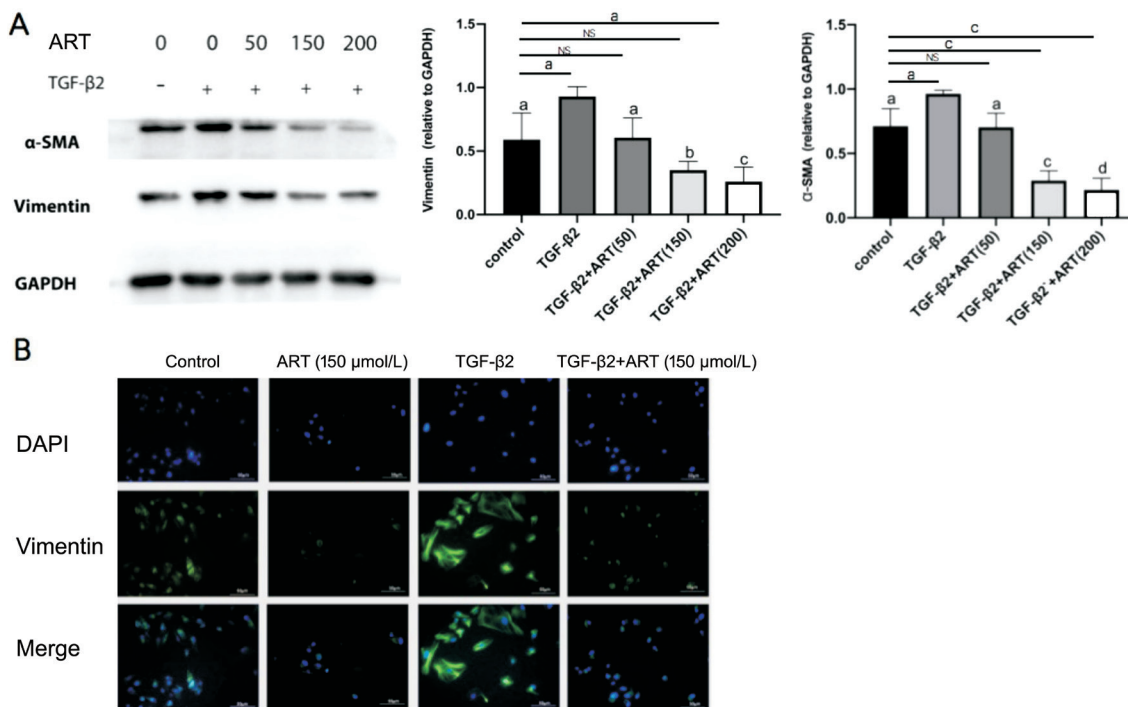
**Artesunate restrains TGF- $\beta$ 2 mediated migration of ARPE-19 cells** Transwell cell immigration examination was used to verify the effectiveness of artesunate on the vertical immigration of ARPE-19 cells processed with TGF- $\beta$ 2. In comparison with the control group, art treatment group could inhibit the vertical immigration of ARPE-19 cells (Figure 3B). In comparison with the control group, TGF  $\beta$  treatment group promoted the vertical migration of ARPE-19 cells. In comparison with the control group and TGF treated group, 100 m artesunate inhibited the vertical migration of ARPE-19 cells handled with TGF- $\beta$ 2.

**Artesunate Restrains TGF- $\beta$ 2 Mediated EMT in ARPE-19 Cells** In order to examine the inhibitive ability of artesunate on TGF- $\beta$  mediated EMT in ARPE-19 cells, we detected the alternation of cell morphology and EMT markers processed with artesunate (Figure 4). The morphology of RPE cells processed with 20 ng/mL TGF- $\beta$ 2 significantly changed from cubic to flattened structure, and increased the protein levels of  $\alpha$ -SMA and vimentin. TGF-mediated mesenchymal transition was suppressed by 150  $\mu\text{m}$  artesunate. The increase of  $\alpha$ -SMA and vimentin content in ARPE-19 cells induced by TGF- $\beta$ 2 could be inhibited by artesunate 100 and 150  $\mu\text{mol/L}$ . The results of immunofluorescence staining confirmed that the expression of vimentin protein in ARPE-19 cells induced by TGF- $\beta$ 2 increased, and decreased after 100  $\mu\text{mol/L}$  artesunate intervention. These results suggest that artesunate can inhibit the EMT induced by TGF in ARPE-19 cells.

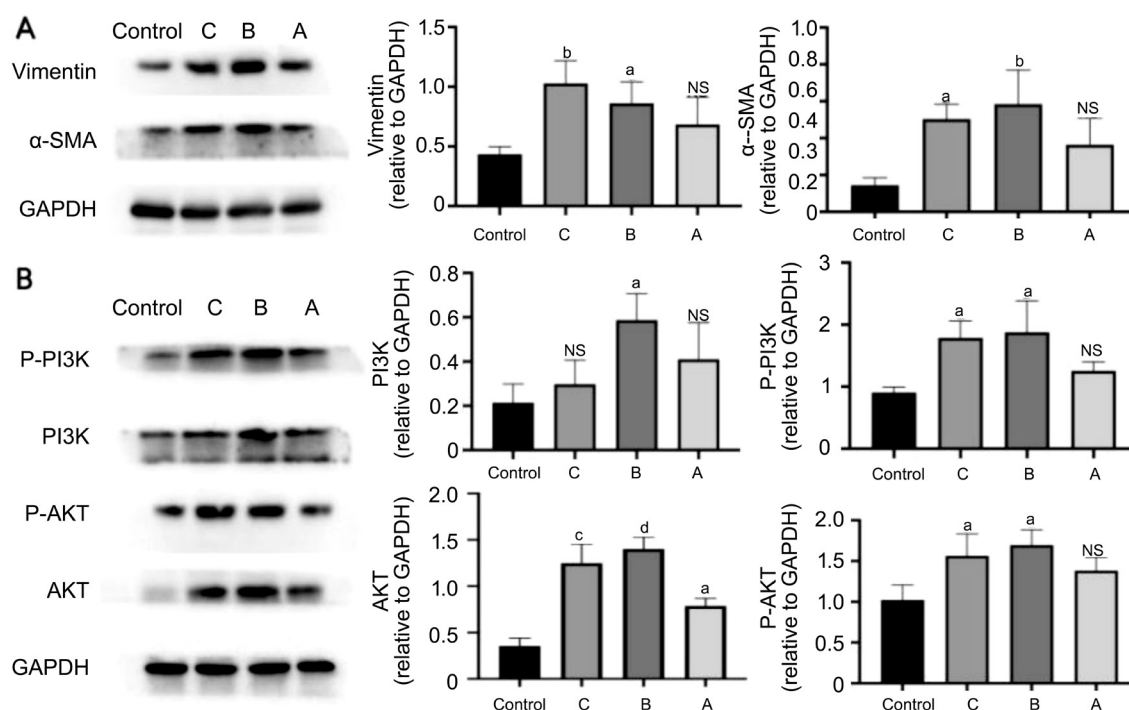
**PI3K/Akt Signal Channel Activated in Proliferative Vitreoretinopathy** The findings revealed that the expression



**Figure 3** Artesunate inhibited TGF-β2 mediated migration of ARPE-19 cells After 48h of pretreatment with TGF-β2, ARPE-19 cells were treated with or without artesunate for 12, 24, and 36h. A: Scratch assay was measured cell horizontal migration. <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$ , <sup>d</sup> $P < 0.0001$ . Data are presented as mean±SD ( $n=3$ /group). Scale bar: 250 μm. B: In the Transwell assay, the cells that migrated into the detection zone (the lower chamber) were measured. <sup>d</sup> $P < 0.0001$ . Data are presented as mean±SD ( $n=3$ /group). Scale bar: 50 μm.



**Figure 4** Artesunate inhibits TGF-β2 mediated EMT in ARPE-19 cells After 48h of pretreatment with TGF, ARPE-19 cells used for EMT assay were treated with or without ART for up to 48h. A: Western blot analysis levels of vimentin, α-SMA and GAPDH in the lysates of ARPE-19 cells after treatment for 48h. NS ( $P > 0.05$ ), <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ , <sup>c</sup> $P < 0.001$ . The data are presented as the mean±SD. ( $n=3$ /group). B: Immunofluorescence staining for vimentin (green) and nuclei (blue) in ARPE-19 cells after treatment for 48h. Scale bar: 50 μm. ART: Artesunate.



**Figure 5 PI3K/Akt signaling pathway is activated in proliferative vitreoretinopathy** Vitreous tissue were collected from clinical patients. A: Western blot analysis levels of vimentin,  $\alpha$ -SMA and the GAPDH in the lysates of clinical tissue. NS ( $P>0.05$ ), <sup>a</sup> $P<0.05$ , <sup>b</sup> $P<0.01$ . The data are presented as the mean $\pm$ SD ( $n=3$ /group). B: Western blot analysis levels of PI3K, P-PI3K, Akt, P-Akt and the housekeeping protein GAPDH in the lysates of clinical tissue. NS ( $P>0.05$ ), <sup>a</sup> $P<0.05$ , <sup>b</sup> $P<0.01$ , <sup>c</sup> $P<0.001$ , <sup>d</sup> $P<0.0001$ . The data are presented as the mean $\pm$ SD ( $n=3$ /group). ART: Artesunate.

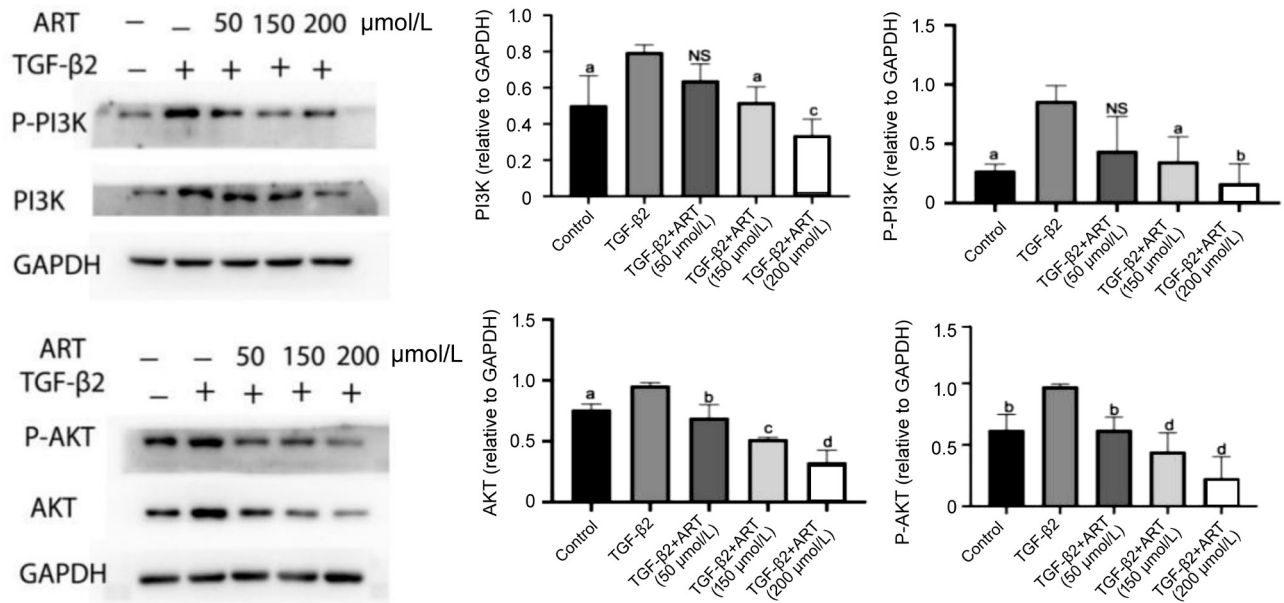
levels of vimentin and  $\alpha$ -SMA in patients with grade C and B lesions were higher than those in the control group, but there was no obvious disparity between two groups nor in the expression of vimentin and  $\alpha$ -SMA protein between the two groups. In addition, the activation of PI3K/Akt signal channel was the same as that of EMT. The expression and activation of PI3K/Akt signal channel were down adjusted in patients with grade C and grade B (Figure 5). Therefore, we speculated that the process of EMT is in connection with the expression and activation of PI3K/Akt trail.

**Effect of Artesunate on TGF- $\beta$ 2 Induced PI3K-Akt Signal Channel in ARPE-19 cells** Artesunate can induce the inhibition of PI3K/Akt channel. Akt, P-Akt, PI3K and P-PI3K levels were measured by Western blotting (Figure 6). After artesunate treatment, both PI3K and phosphorylated PI3K (P-PI3K) decreased, suggesting that the down regulation of P-PI3K may be due to the down regulation of PI3K. Akt, the downstream target of PI3K, also showed a similar trend. The decrease of PI3K and Akt induced by artesunate showed statistical significance ( $P<0.05$  or 0.01).

## DISCUSSION

This research was aimed at studying the effect of artesunate on TGF- $\beta$ 2 induced EMT and the mechanism of artesunate on RPE cells. Artesunate inhibits the propagation, immigration and EMT procedure of RPE cells through PI3K/Akt signal

channel. Based on the inhibitory effectiveness of artesunate on PVR *in vitro*, we further believe that artesunate might be a target which is potential for the disposal of PVR. In the development of PVR, EMT switch of RPE cells has become a key process, mainly manifested in the decreased expression of E-cadherin and vimentin in epithelial cells, fibronectin and  $\alpha$ -SMA increased and the morphology, migration and propagation of epithelial cells transformed into the phenotype of mesenchymal cells. TGF- $\beta$ 2 was added to RPE cells build the model of EMT process<sup>[21]</sup>, the cell morphology changes from hexagonal to spindle, and the cell ductility increased. The morphological changes were more obvious with the increase of concentration of TGF- $\beta$ 2. The content of vimentin and SMA increased. When artesunate was added in this process, the propagation and immigration of transformed cells were suppressed, and the content of EMT marker protein decreased. TGF- $\beta$  is a cytokine with broad spectrum of biological. The research confirmed that TGF- $\beta$  can regulate EMT process in epithelial cells of multiple human organs and tissues. The expression level of TGF- $\beta$ 2 was positively linked to the seriousness of PVR. In tumor cells, TGF- $\beta$  uncontrolled binding of and its receptors activates a variety of downstream trails, including Smad, RAS and MAP kinases, PI3K/Akt, which results in cell dysfunction. Among them, PI3K/Akt signal channel occupies a significant position in EMT transformation



**Figure 6 Artesunate reduce PI3K-Akt signaling pathway in transformed ARPE-19 cells** After 48h of pretreatment with TGF, ARPE-19 cells stimulated with or without artesunate for 48h. Western blot analysis levels of PI3K, P-PI3K, Akt, P-Akt and GAPDH in the lysates of ARPE-19 cells after treatment for 48h. NS ( $P>0.05$ ), <sup>a</sup> $P<0.05$ , <sup>b</sup> $P<0.01$ , <sup>c</sup> $P<0.001$ , <sup>d</sup> $P<0.0001$ . The data are presented as the mean±SD ( $n=3$ /group). ART: Artesunate.

of tumor cells<sup>[22-24]</sup>. PI3K is a family of lipid kinases that adjust a variety of signal channels, physiological functions and cellular processes. It is involved in the cell transformation and metastasis of diseases. Akt activated by PI3K is transferred to the cell compartment, where it phosphorylates a variety of protein substrates, such as mTOR<sup>[25]</sup>, to regulate cell metabolism and cell cycle. Therefore, blocking PI3K/Akt signaling channel can effectively control cell proliferation cycle, reduce cell adhesion and inhibit cell migration. Researches have revealed that after going through retinal detachment, it is normal for RPE to be away from their normal position and go into the vitreous chamber and sub-retina to autocrine TGF-β and activate PI3K/Akt signal channel to promote EMT of RPE cells<sup>[9,26]</sup>. In vitreous clinical specimens, PVR specimens were class B and C. Compared to the control group and class a PVR specimens, the expression level of SMA, the increase and elevation of EMT signaling molecules in PI3K/Akt signal channel, and the stimulation of PI3K/Akt expression also increased, which further verified the function of PI3K/Akt signal channel in the existence and occurrence of PVR. TGF-β2-induced somatic cells, PI3K/Akt signal channel and PI3K/Akt phosphorylation increased. After artesunate treatment, PI3K and P-PI3K decreased, and the downstream target Akt showed a similar trend. As a result, we concluded that artesunate inhibited the development of EMT in RPE cells by restraining the expression of PI3K/Akt trail.

#### ACKNOWLEDGEMENTS

**Conflicts of Interest:** Wang ZY, None; Zhang Y, None; Wu LD, None; Chen J, None; Chen ML, None; Chen CM, None; Xu QH, None.

#### REFERENCES

- 1 Li XH, Zhao MW, He SK. RPE epithelial-mesenchymal transition plays a critical role in the pathogenesis of proliferative vitreoretinopathy. *Ann Transl Med* 2020;8(6):263.
- 2 Pastor JC, Rojas J, Pastor-Idoate S, di Lauro S, Gonzalez-Buendia L, Delgado-Tirado S. Proliferative vitreoretinopathy: a new concept of disease pathogenesis and practical consequences. *Prog Retin Eye Res* 2016;51:125-155.
- 3 Ren YX, Ma JX, Zhao F, An JB, Geng YX, Liu LY. Effects of curcumin on epidermal growth factor in proliferative vitreoretinopathy. *Cell Physiol Biochem* 2018;47(5):2136-2146.
- 4 Tamiya S, Liu L, Kaplan HJ. Epithelial-mesenchymal transition and proliferation of retinal pigment epithelial cells initiated upon loss of cell-cell contact. *Invest Ophthalmol Vis Sci* 2010;51(5):2755-2763.
- 5 Lei HT, Rheaume MA, Kazlauskas A. Recent developments in our understanding of how platelet-derived growth factor (PDGF) and its receptors contribute to proliferative vitreoretinopathy. *Exp Eye Res* 2010;90(3):376-381.
- 6 Chen ZN, Shao Y, Li XR. The roles of signaling pathways in epithelial-to-mesenchymal transition of PVR. *Mol Vis* 2015;21:706-710.
- 7 Chen XY, Xiao W, Wang WC, Luo LX, Ye SB, Liu YZ. The complex interplay between ERK1/2, TGFβ/Smad, and Jagged/Notch signaling pathways in the regulation of epithelial-mesenchymal transition in retinal pigment epithelium cells. *PLoS One* 2014;9(5):e96365.
- 8 Yokoyama K, Kimoto K, Itoh Y, Nakatsuka K, Matsuo N, Yoshioka H, Kubota T. The PI3K/Akt pathway mediates the expression of type I collagen induced by TGF-β2 in human retinal pigment epithelial cells. *Graefes Arch Clin Exp Ophthalmol* 2012;250(1):15-23.

- 9 Wang N, Chen HX, Teng YP, Ding XH, Wu H, Jin XQ. Artesunate inhibits proliferation and invasion of mouse hemangioendothelioma cells *in vitro* and of tumor growth *in vivo*. *Oncol Lett* 2017;14(5):6170-6176.
- 10 Jiang WW, Cen YY, Song Y, Li P, Qin RX, Liu C, Zhao YB, Zheng J, Zhou H. Artesunate attenuated progression of atherosclerosis lesion formation alone or combined with rosuvastatin through inhibition of pro-inflammatory cytokines and pro-inflammatory chemokines. *Phytomedicine* 2016;23(11):1259-1266.
- 11 Cen YY, Liu C, Li XL, Yan ZF, Kuang M, Su YJ, Pan XC, Qin RX, Liu X, Zheng J, Zhou H. Artesunate ameliorates severe acute pancreatitis (SAP) in rats by inhibiting expression of pro-inflammatory cytokines and Toll-like receptor 4. *Int Immunopharmacol* 2016;38:252-260.
- 12 Zhao YY, Jiang WW, Li B, Yao Q, Dong JQ, Cen YY, Pan XC, Li J, Zheng J, Pang XL, Zhou H. Artesunate enhances radiosensitivity of human non-small cell lung cancer A549 cells via increasing NO production to induce cell cycle arrest at G2/M phase. *Int Immunopharmacol* 2011;11(12):2039-2046.
- 13 Li B, Li J, Pan XC, Ding GF, Cao HW, Jiang WW, Zheng J, Zhou H. Artesunate protects sepsis model mice challenged with *Staphylococcus aureus* by decreasing TNF- $\alpha$  release via inhibition TLR2 and Nod2 mRNA expressions and transcription factor NF- $\kappa$ B activation. *Int Immunopharmacol* 2010;10(3):344-350.
- 14 Murray J, Gannon S, Rawe S, Murphy JEJ. *In vitro* oxygen availability modulates the effect of artesunate on HeLa cells. *Anticancer Res* 2014;34(12):7055-7060.
- 15 Vandewynckel YP, Laukens D, Geerts A, Vanhove C, Descamps B, Colle I, Devisscher L, Bogaerts E, Paridaens A, Verhelst X, van Steenkiste C, Libbrecht L, Lambrecht BN, Janssens S, van Vlierberghe H. Therapeutic effects of artesunate in hepatocellular carcinoma: repurposing an ancient antimalarial agent. *Eur J Gastroenterol Hepatol* 2014;26(8):861-870.
- 16 Raffetin A, Bruneel F, Roussel C, Thellier M, Buffet P, Caumes E, Jauréguiberry S. Use of artesunate in non-malarial indications. *Med Mal Infect* 2018;48(4):238-249.
- 17 Yang Y, Wu ND, Wu YH, Chen HT, Qiu J, Qian XB, Zeng JT, Chiu K, Gao QY, Zhuang J. Artesunate induces mitochondria-mediated apoptosis of human retinoblastoma cells by upregulating Kruppel-like factor 6. *Cell Death Dis* 2019;10(11):862.
- 18 He Y, Fan J, Lin H, Yang X, Ye Y, Liang L, Zhan Z, Dong X, Sun L, Xu H. The anti-malaria agent artesunate inhibits expression of vascular endothelial growth factor and hypoxia-inducible factor-1 $\alpha$  in human rheumatoid arthritis fibroblast-like synoviocyte. *Rheumatol Int* 2011;31(1):53-60.
- 19 Li H, Gu C, Ren Y, Dai Y, Zhu X, Xu J, Li Y, Qiu Z, Zhu J, Zhu Y, Guan X, Feng Z. The efficacy of NP11-4-derived immunotoxin scFv-artesunate in reducing hepatic fibrosis induced by *Schistosoma japonicum* in mice. *J Biomed Res* 2011;25(2):148-154.
- 20 Zhang YQ, Li HH, Zhu JJ, Wei TT, Peng YX, Li R, Xu R, Li M, Xia AZ. Role of artesunate in TGF- $\beta$ 1-induced renal tubular epithelial-mesenchymal transdifferentiation in NRK-52E cells. *Mol Med Rep* 2017;16(6):8891-8899.
- 21 Ma Z, Lou SP, Jiang Z. PHLDA2 regulates EMT and autophagy in colorectal cancer via the PI3K/AKT signaling pathway. *Aging* 2020;12(9):7985-8000.
- 22 Li YL, Wang TT, Sun YJ, Huang TF, Li CP, Fu Y, Li YC, Li CZ. p53-mediated PI3K/AKT/mTOR pathway played a role in PtoxD<sup>Pl</sup>-induced EMT inhibition in liver cancer cell lines. *Oxidative Med Cell Longev* 2019;2019:2531493.
- 23 Chen HT, Wang HF, An JB, Shang QL, Ma JX. Plumbagin induces RPE cell cycle arrest and apoptosis via p38 MARK and PI3K/AKT/mTOR signaling pathways in PVR. *BMC Complement Altern Med* 2018;18(1):89.
- 24 Roybal CN, Velez G, Toral MA, Tsang SH, Bassuk AG, Mahajan VB. Personalized proteomics in proliferative vitreoretinopathy implicate hematopoietic cell recruitment and mTOR as a therapeutic target. *Am J Ophthalmol* 2018;186:152-163.
- 25 Cheng R, Li C, Li CY, Wei L, Li L, Zhang Y, Yao YC, Gu XQ, Cai WB, Yang ZH, Ma JX, Yang X, Gao GQ. The artemisinin derivative artesunate inhibits corneal neovascularization by inducing ROS-dependent apoptosis in vascular endothelial cells. *Invest Ophthalmol Vis Sci* 2013;54(5):3400-3409.
- 26 Wang XQ, Liu HL, Wang GB, Wu PF, Yan T, Xie J, Tang Y, Sun LK, Li C. Effect of artesunate on endotoxin-induced uveitis in rats. *Invest Ophthalmol Vis Sci* 2011;52(2):916-919.