

Resveratrol modulates the proliferation and migration of retinal pigment epithelial cells through TGF β 1-induced EMT signal pathway.

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Abstract

Retinal Pigment Epithelial (RPE) cells play important roles in progression of human ophthalmic diseases. Evidences have showed that Resveratrol (RV) presents benefits for the treatment of ophthalmic diseases. In this study, we investigated the potential molecular mechanism mediated by RV in RPE cells. Transforming Growth Factor beta1 (TGF β 1) and Epithelial-Mesenchymal Transition (EMT) signal pathway was analysed in RPE cells determined by Western blot analysis and RT-PCR. Results demonstrated that RV treatment (10 mg/ml) significantly inhibited proliferation and migration of RPE cells. We showed that RV treatment inhibited TGF β 1 and EMT markers Fibronectin (FIB), alpha-smooth muscle actin (α -SMA), and Vimentin (VIM) expression in RPE cells. Inhibition of endogenous TGF β 1 decreased FIB, α -SMA and VIM expression levels in RPE cells as well as inhibited proliferation and migration of RPE cells. TGF β 1 overexpression stimulated EMT markers FSP1, E-cadherin and Snail expression levels in RPE cells. Overexpression of TGF β 1 cancelled RV-down-regulated FIB, α -SMA and VIM expression in RPE cells. Overexpression of TGF β 1 also abolished RV-suppressed proliferation and migration of RPE cells. In conclusion, this study describes the RV-regulated molecular mechanism in RPE cells through TGF β 1-induced EMT signaling and suggests that RV would be a potential therapeutic agent for the prevention or treatment of Proliferative Vitreoretinopathy (PRV).

Keywords: Resveratrol, Retinal pigment epithelial, TGF β 1, Epithelial-mesenchymal transition.

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Introduction

Currently, proliferation and migration of Retinal Pigment Epithelium (RPE) cells plays important role in the progression of Proliferative Vitreoretinopathy (PVR) [1,2]. Previous reports have showed that distribution of Transforming Growth Factor- β (TGF- β) and cells derived from RPE cells can be regarded as a major source for TGF- β in Subretinal Strands (SRs), originating from patients with Proliferative Vitreoretinopathy (PVR) and proliferative diabetic vitreoretinopathy [3,4]. In addition, Study also demonstrated that EMT molecular markers play important role in the many ophthalmic diseases *via* regulation of proliferation of RPE cells [5]. Therefore, understanding the role of TGF- β and EMT in RPE cells is essential to present and treat human ophthalmic diseases.

Resveratrol (RV) is multifunctional biological polyphenol. RV plays ameliorative effects on higher inflammatory factor concentration, metabolic syndrome and neurological diseases [6,7]. Previous study has showed that RV can protect against ultraviolet A-mediated inhibition of the phagocytic function of human retinal pigment epithelial cells *via* large-conductance calcium-activated potassium channels [8]. In addition, inhibitory effects of RV on proliferation of human retinal RPE cells have been investigated *in vitro*, which may aid treatment

of proliferative PVR [9]. Furthermore, inhibitory effects of RV on PDGF-BB-induced retinal pigment epithelial cell migration have showed association with PDGFR β , PI3K/Akt and MAPK pathways [10]. However, the associations between RV and TGF β 1/EMT signal pathway in RPE cells have not been well understood yet.

In this study, we investigated the role of RV in the proliferation and migration of RPE cells. We also explored the potential mechanism mediated by RV in RPE cells. In addition to this, we reported that RV inhibited proliferation and migration of RPE cells through TGF β 1-induced EMT signal pathway.

Materials and Methods

Cell culture and reagents

The ARPE-19 human RPE cell line were obtained from PromoCell GmbH (Heidelberg, Germany) and cultured in DMEM medium (GIBCO, Invitrogen) with 1% penicillin/streptomycin sulfate (GIBCO, Invitrogen), 1% L-glutamine (GIBCO, Invitrogen) and 10% fetal bovine serum (GIBCO, Invitrogen) in humidified atmosphere containing 5% of CO₂ at 37°C. RV was purchased from Sigma-Aldrich.

Transfection of small interference RNA (Si-RNA)

All si-RNAs were synthesized by Invitrogen (Shanghai, China) including Si-RNA-TGF β 1 (Si-TGF β 1) or Si-RNA-vector. PRE cells (1×10^6) were transfected with 100 pmol of Si-TGF β 1 targeting TGF β 1 (Applied Biosystems) with Si-RNA-vector as control (Applied Biosystems) by using a Cell Line Nucleofactor kit L (Lonza).

Construction of lentivirus for TGF β overexpression

The TGF β 1 was cloned into Lentivirus plasmid using Lentivirus vector system (System Biosciences, Inc.) with vector as control and named pvector (Control) and pTGF β , respectively. All DNA sequences were synthesized by Invitrogen. All of the plasmids were confirmed by DNA sequencing. Plasmid of pvector or pTGF β was transfected into RPE cells using lipofectamine 2000. The cells transfected with pvector or pTGF β were used for further analysis.

Quantitative real-time PCR

The human RPE cells were cultured and transfected with pTGF β 1 or knockdown of TGF β 1 using Si-RNA (Si-TGF β 1). When the concentration of cells grown to 85%, the cells was randomly divided into three groups: Control group, TGF- β 1 added to RPE cells for 24 h. Total RNA was isolated from RPE cells using TRIzol reagent (Invitrogen) and transcribed into cDNA using Super Script VILO cDNA Synthesis Kit (Life Technologies). All the forward and reverse primers were synthesized by Invitrogen. Relative mRNA expression changes were calculated by $2^{-\Delta\Delta C_t}$. The results are expressed as the n-fold way compared to control.

Wound healing assay

Wound healing assay was performed by following the protocol provided in the literature [11]. Si-vector, Si-TGF β 1, pvector or pSi-TGF β 1-treated PRE cells were cultured in a 12-well plate for 24 h. After washing with culture medium to remove cell debris, the cells were allowed to migrate for 48 h, followed by observation under a microscope.

Cell proliferation assay

Non-treated, Si-TGF β , pvector or pSi-TGF β 1-treated PRE cells proliferation was detected using CCK-8 kit according to the manufacturer's instructions. Briefly, RPE cells were cultured in 48-well plates at the density of 1×10^4 cells/well and then cultured for 24 h. Finally, 10 μ l of CCK-8 solution was added to each well and incubated for 2 h. The results were measured using a microplate reader at 570 nm.

Western blot

Human PRE cells were collected and lysed in RIPA buffer (M-PER reagent for the cells and T-PER reagent for the tissues, Thermo Scientific) followed homogenized at 4°C for 10 min. A total of 20 μ g protein extracts was electrophoresed on 12.5% polyacrylamide gradient gels and then transferred to

nitrocellulose membranes. The membranes were incubated in blocking buffer (5% milk) prior to incubation with primary antibodies at 4°C overnight. The primary rabbit anti-human antibodies used in the immunoblotting assays were: TGF β 1 (1:200, ab92486, Abcam), FIB (1: 500, ab6328, Abcam), α -SMA (1: 500, ab7817, Abcam), VIM (1: 500, ab92547, Abcam) and β -actin (1: 500, ab8226, Abcam). Horseradish peroxidase-conjugated anti-rabbit IgG (Bio-Rad, Hercules, CA, USA) were used at a 1:5000 dilution and detected using a Western Blotting Luminol Reagent.

Statistical analysis

The experiments data were expressed as mean \pm standard (SD) deviation. The significant difference ($p < 0.05$) of data of different groups were calculated using Duncan's multiple range test using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

RV inhibits proliferation and migration of human RPE cells

The inhibitory effects of RV were investigated in RPE cells *in vitro*. Results demonstrated that RV treatment (10 mg/ml) significantly inhibited proliferation compared to control (Figure 1A). We also showed that RV treatment (10 mg/ml) suppressed migration of RPE cells compared to control (Figure 1B). These results suggest that RV treatment can significantly inhibit proliferation and migration of human RPE cells.

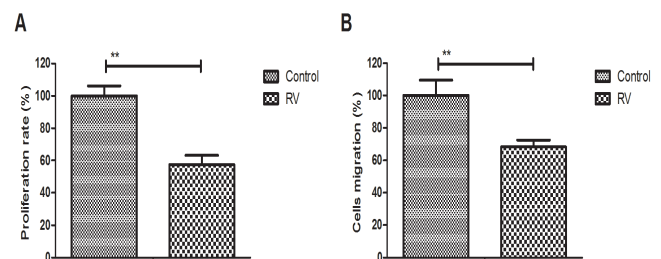


Figure 1. RV inhibits proliferation and migration of human RPE cells. (A) RV treatment (10 mg/ml) significantly inhibits proliferation compared to control. (B) RV treatment (10 mg/ml) suppresses migration of RPE cells compared to control.

RV inhibits TGF β 1 and EMT markers expression in human PRE cells

Previous study showed that TGF β 1 and EMT signal pathway is associated with proliferation and migration of human RPE cells. We showed that RV treatment inhibited TGF β 1 mRNA and protein expression levels in RPE cells (Figures 2A and 2B). Results revealed that EMT markers FIB, α -SMA and VIM expression levels were down-regulated by RV treatment in RPE cells (Figures 2C and 2D). RV treatment can inhibit TGF β 1 and EMT markers expression in human PRE cells.

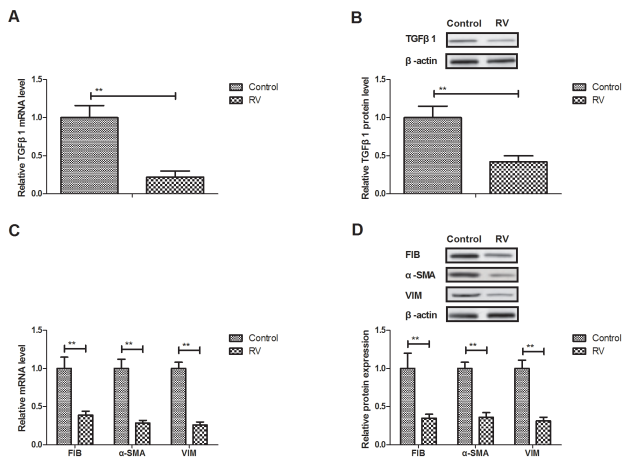


Figure 2. RV inhibits TGFβ1 and EMT markers expression in human PRE cells (A-B) RV treatment inhibits TGFβ1 mRNA (A) and protein (B) expression levels in RPE cells. (C-D) RV treatment inhibits EMT markers FIB, α-SMA and VIM mRNA (C) and protein (D) expression levels in RPE cells.

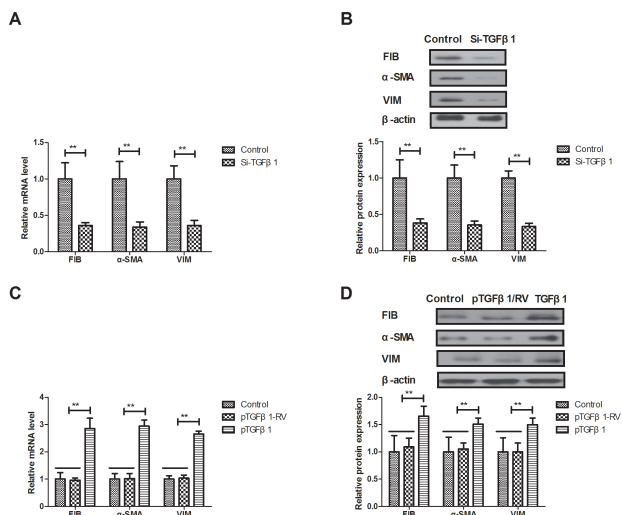


Figure 3. TGFβ1 overexpression promotes TGFβ1 and EMT markers expression in human PRE cells. (A-B) TGFβ1 down-regulation (Si-TGFβ1) inhibits EMT markers FIB, α-SMA and VIM mRNA (A) and protein (B) expression levels in RPE cells. (C-D) TGFβ1 overexpression promotes canceled RV-down-regulated FIB, α-SMA and VIM mRNA (C) and protein (D) expression in RPE cells.

TGFβ1 overexpression promotes TGFβ1 and EMT markers expression in human PRE cells

The efficacy of TGFβ1 in the EMT signal pathway was studied in human PRE cells. As shown in Figures 3A and 3B, TGFβ1 down-regulation (Si-TGFβ1) inhibited EMT markers FIB, α-SMA and VIM mRNA and protein expression levels in RPE cells. Notably, TGFβ1 overexpression promoted and canceled RV-down-regulated FIB, α-SMA and VIM expression in RPE cells (Figures 3C and 3D). These results suggest that TGFβ1 overexpression can canceled RV-inhibited EMT markers expression in human PRE cells.

TGFβ1 overexpression inhibits RV-suppressed proliferation and migration of human RPE cells

The association between RV and TGFβ1 was analysed in human PRE cells. As shown in Figure 4A, we showed that TGFβ1 overexpression abolished RV-suppressed proliferation of human PRE cells. We observed that overexpression of TGFβ1 also abolished RV-suppressed migration of human RPE cells (Figure 4B). These results suggest that TGFβ1 overexpression can lead to abolishment of RV-suppressed proliferation and migration of human RPE cells.

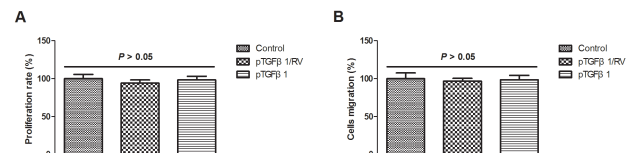


Figure 4. TGFβ1 overexpression inhibits RV-suppressed proliferation and migration of human RPE cells. (A) TGFβ1 overexpression abolishes RV-suppressed proliferation of human PRE cells. (B) Overexpression of TGFβ1 also cancels RV-suppressed migration of human RPE cells.

Discussion

RPE cells play essential role in maintaining the normal function of the retina, especially the photoreceptor in the optic nerve system [12,13]. The dysfunctions of RPE cells can lead to retina and choroid damage, which further result in the occurrence of retinal degeneration diseases and even impaired vision [14,15]. Importantly, research has showed that RV can suppress expression of VEGF in human RPE cells that indicate RV may be useful as nutraceutical in controlling pathological choroidal neovascularization processes in age-related macular degeneration [16]. In this study, we reported that RV treatment significantly suppressed proliferation and migration of human RPE cells through TGFβ1-induced EMT signal pathway. Findings have suggested that TGFβ1 overexpression can canceled RV-inhibited EMT markers expression in human RPE cells.

Previous study has RV can inhibit EMT of RPE cells and is beneficial for treatment of proliferative VRP [17]. In addition, evidences have indicated that viability of RPE cells is associated with many ophthalmic diseases through regulation of EMT molecular markers [18]. Our results showed that RV treatment present significantly inhibitory effects for proliferation and migration of human RPE cells. Sheu et al. have indicated that RV could protect human RPE cells in acrolein-induced damage [19]. In this study, we indicated that RV down-regulated TGFβ1 expression levels and decreased EMT markers FIB, α-SMA, and VIM expression in RPE cells.

Previous study has showed the regulation of EMT in RPE cells by drugs-regulated signaling may be a valuable therapeutic approach for the prevention or treatment of proliferative PVR [20]. In addition, effect of magnolol on TGFβ1 and FIB expression in human retinal pigment epithelial cells has been analysed under diabetic conditions [21]. Furthermore, TGFβ1

can induce α -SMA expression and fibronectin synthesis in cultured human retinal pigment epithelial cells [22]. We showed that TGF β 1 overexpression stimulated EMT signal pathway and abolished RV-inhibited proliferation and migration of human RPE cells.

In conclusion, the current study identified the role of RV in the proliferation of human RPE cells. Findings have indicated that RV treatment inhibits proliferation and migration of RPE cells through down-regulating TGF β 1-mediated EMT signal pathway in RPE cells. Therefore, RV is a potential agent by modulating the proliferation and migration of RPE cells through regulation of TGF β 1-mediated EMT signal pathway. However, more reports should be investigated in future.

Conflict of Interest

The authors declare that they have no conflict of interest.

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