·Basic Research·

Protein tyrosine phosphatase 1B regulates migration of ARPE-19 cells through EGFR/ERK signaling pathway

Zhao-Dong Du, Li-Ting Hu, Gui-Qiu Zhao, Qian Wang, Qiang Xu, Nan Jiang, Jing Lin

Department of Ophthalmology, Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong Province, China

Correspondence to: Gui-Qiu Zhao. Department of Ophthalmology, Affiliated Hospital of Qingdao University, No.16 Jiangsu Road, Qingdao 266003, Shandong Province, China. guiqiuzhao@126.com

Received: 2014-06-12 Accepted: 2015-03-03

Abstract

• AIM: To evaluate whether protein tyrosine phosphatase 1B (PTP1B) contributed to initiate human retinal pigment epithelium cells (ARPE)-19 migration and investigate the signaling pathways involved in this process.

• METHODS: ARPE-19 cells were cultured and treated with the siRNA-PTP1B. Expression of PTP1B was confirmed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). AG1478 [a selective inhibitor of epidermal growth factor receptor (EGFR)] and PD98059 (a specific inhibitor of the activation of mitogen-activated protein kinase) were used to help to determine the PTP1B signaling mechanism. Western blot analysis verified expression of EGFR and extracellular signal-regulated kinase (ERK) in ARPE-19 cells. The effect of siRNA-PTP1B on cell differentiation was confirmed by immunostaining for α-smooth muscle actin (α-SMA) and qRT-PCR. Cell migration ability was analyzed by transwell chamber assay.

• RESULTS: The mRNA levels of PTP1B were reduced by siRNA-PTP1B as determined by qRT-PCR assay. SiRNA-PTP1B activated EGFR and ERK phosphorylation. α-SMA staining and qRT-PCR assay demonstrated that siRNA-PTP1B induced retinal pigment epithelium (RPE) cells to differentiate toward better contractility and motility. Transwell chamber assay proved that PTP1B inhibition improved migration activity of RPE cells. Treatment with AG1478 and PD98059 abolished siRNA-PTP1B-induced activation of EGFR and ERK, α-SMA expression and cell migration.

• CONCLUSION: PTP1B inhibition promoted myofibroblast differentiation and migration of ARPE-19 cells, and EGFR/ERK signaling pathway played important role in migration process.

• **KEYWORDS:** protein tyrosine phosphatase 1B; retinal pigment epithelium; cell migration; epidermal growth factor receptor; extracellular signal-regulated kinase

DOI:10.3980/j.issn.2222-3959.2015.05.07

Du ZD, Hu LT, Zhao GQ, Wang Q, Xu Q, Jiang N, Lin J. Protein tyrosine phosphatase 1B regulates migration of ARPE-19 cells through EGFR/ERK signaling pathway. *Int J Ophthalmol* 2015;8(5):891-897

INTRODUCTION

Proliferative vitreoretinopathy (PVR) is a common complication of retinal detachment and open-globe injury in the posterior segment [1]. The presence of a retinal break appears necessary for the incitement of PVR allowing cells often thought to originate from ocular tissues, to migrate into the vitreous cavity [2]. Retinal pigment epithelium (RPE) cells are considered to be a key element in this process. They are activated and migrate through the provisional extracellular matrix and retinal holes to form pathologic membranes on both surfaces of the neural retina[3]. However, the cellular mechanisms involved in this migration process are not well understood[4].

In response to PVR, a wide variety of tyrosine kinase growth factor receptors such as epidermal growth factor receptor (EGFR) are activated in the retina [5]. Accumulating evidence indicates that epidermal growth factor (EGF)-EGFR signaling can induce intracellular mitogenic signal transduction pathways leading to cell proliferation, differentiation and migration [6-10]. Furthermore, EGFR is widely expressed in the fibro proliferative membranes in PVR [5], and plays a key role in the activation of RPE cell, which is the major cell involved in PVR [9]. Therefore, regulation of EGFR activity may be used as a potential method for the prevention and treatment of RPE-mediated ocular proliferative disorders, such as PVR.

Tyrosine phosphorylation of membrane receptors and intracellular signaling molecules is reversible, and this dynamic process is controlled by the protein tyrosine phosphatases (PTPs)[11]. The PTPs comprise a diverse family of transmembrane and cytoplasmic enzymes, which catalyze the dephosphorylation of tyrosyl-phosphorylated proteins[12,13]. Our previous research indicated that sodium orthovanadate

(SOV)-induced PTPs inhibition could accelerate the cell cycle of RPE cells, induce RPE cells to differentiate toward better motility and improve their migration activity [14]. This result strongly suggested that PTPs may play an important role in the regulation of RPE cells activity.

Among subfamilies of PTPs, protein tyrosine phosphatase 1B (PTP1B) is an ubiquitously expressed PTP that is localized on intracellular membranes. Previous studies have shown that internalization of the EGFR is required for their interaction with PTP1B, suggesting a potential relationship between the interaction of EGFR with PTP1B and its trafficking [15]. PTP1B can negatively regulate several receptor tyrosine kinases (RTKs) including EGFR by dephosphorylation, and subsequently attenuating activation of their downstream signaling pathways[16-18]. However, it remains unclear whether PTP1B-EGFR interaction would affect the biological functions of RPE cells, in particular, their activation and migration, and if so, what mechanisms are involved. In this study, we addressed these issues using *in vitro* cultured human retinal pigment epithelium cells (ARPE-19).

MATERIALS AND METHODS

Reagents Monoclonal rabbit-anti-human EGFR, p-EGFR, extracellular signal-regulated kinase (ERK)1/2 and p-ERK1/2 were obtained from Cell Signaling Technology (Danvers, MA, USA). Monoclonal mouse-anti-human α-smooth muscle actin (α-SMA) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Rabbit-anti-human β-actin was obtained from Biomedical Technologies (Stoughton, MA, USA). AG1478 (a selective inhibitor of EGFR), PD98059 (a specific inhibitor of the activation of mitogen-activated protein kinase kinase) were purchased from Sigma (St Louis, MO, USA). Bovine serum albumin (BSA) was purchased from Fisher Scientific (Pittsburgh, PA, USA).

Cell Culture of Human Retinal Pigment Epithelium Cells ARPE-19 (CRL-2302) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium/nutrient mixture F-12 (DMEM/F12; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals, Norcross, GA, USA). The cells were seeded into a 25 cm^2 plate at a density of 3.0×10^6 and grown in an incubator in saturating humidity and 5% CO_2 at 37℃. The cells were passaged after being grown for 3d in cultured media. Experimentation was performed using 80% to 90% confluent cells at a cell passage of 3 to 6. All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Knockdown of Protein Tyrosine Phosphatase 1B by Small Interfering RNA To determine whether PTP1B contributes to the migration of ARPE-19 cells, we used small interfering RNA (siRNA) to inhibit the expression of PTP1B in these cells. SiRNA-PTP1B and nonsilencing siRNA were purchased from Genesil Biotech (Wuhan, Hubei Province, China). The sequence of siRNA-PTP1B was positive-sense strand 5' -GAACAGGUACCGAGAUGUCUU-3' and antisense strand 3'- GACAUCUCGGUACCUGUUCUU-5'. Sequences serving as controls were as follows: sense 5'-AGACUACCGUUGUUAUAGGUG-3' and antisense 5'-GACCUAUAACAAUGGUAGUUU-3'. Lyophilized siRNA was dissolved in dH_2O into a stock concentration of 20 μM and stored at -20℃.

ARPE-19 cells were seeded in a 6-well culture plate in complete growth medium for 24h. After replacing with fresh culture medium, the cells were transfected with siRNA-PTP1B and nonsilencing siRNA using the lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Briefly, 10 μL stored siRNA was dissolved in 250 μL Opti-MEM (Invitrogen-Gibco), and 5 μL lipofectamine 2000 was dissolved in 250 μL Opti-MEM and incubated for 5min. These two solutions were mixed together and incubated for 20min. Cultures of ARPE-19 cells were incubated in an antibiotic-free, serum-free medium for 24h to ensure a cell density of 50% to 70%. The mixed solution and another 1.5 mL growth DMEM were then added to every well. The solution containing siRNA was removed 6h later, and then culture medium with 10% FBS was added for cell culture. After transfection, the ARPE-19 cells were incubated for another 24h to ensure that the cells were equilibrated in their original environment.

Quantitative Real –time Polymerase Chain Reactions Real-time PCR (RT-PCR) was conducted using Applied Biosystems (ABI Prism 7900HT) according to the manufacturer's instructions. The ARPE-19 cells were harvested and saved at -80℃. Total RNA of the isolated cells was extracted using RNAiso plus reagent (TaKaRa, Dalian, Liaoning Province, China) and rapidly quantified using spectrophotometry. Complementary DNA was generated by reverse transcription of 2 μg of total RNA and then used in the following quantitative PCR reactions with SYBR Green using specific primers: 95℃ for 30s, followed by 40 cycles of 95℃ for 5s, 60℃ for 30s, followed by a final stage of 95℃ for 15s, 60℃ for 30s, and 95℃ for 15s. The oligonucleotide primers used were as follows: PTP1B forward primer, 5'-AACAGCAACACATAGCCTGAC-3' and PTP1B reverse primer, 5'-AGCATGGTACTCTCTTTCACTCC-3"; α-SMA forward primer, 5'-GCTCACGGAGGCACCCCTGAA-3' and α-SMA reverse primer, 5'-CTGATAGGACATTGTTAGCAT-3'; β-actin forward primer, 5'-TAACACCCAGCACAATGAA-3' and β-actin reverse primer, 5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'. The gene expression levels were quantified by RT-PCR using the housekeeping gene β-actin as an internal control. Quantification was performed using the $2^{-\Delta\Delta Ct}$ method. Each experiment was repeated at least three separate times.

Immunocytochemical Localization For immunocytochemical (ICC) analysis, ARPE-19 cells were seeded onto chamber slides. After washing three times with PBS, cells were fixed in 95% ethanol at room temperature for 10min followed by a further wash with PBS. The fixed cells were then permeabilized with 1% Triton X-100 in PBS for 10min. Then cells were blocked, incubated with primary antibody overnight at 4℃, and then washed in PBS. The concentration of anti-α-SMA was 1:100. FITC-conjugated secondary antibody was applied for 1h with a 1:200 dilution at 37℃. Cells were then washed with PBS and mounted in medium containing DAPI for visualization of nuclei. Positive staining was visualized using the confocal laser scanning microscope.

Western Blot Analysis Protein preparation and Western blot analysis were performed as described previously [15]. ARPE-19 cells grown in culture plates were trypsinized, suspended in buffer containing 1% Triton X-100, 250 mmol/L sodium chloride (NaCl), 2 mmol/L ethylenediaminetetraacetic acid (EDTA), 50 mmol/L tris (hydroxymethyl) aminomethane (Tris)-HCl, 10 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride (all from Sigma-Aldrich), and homogenized. Equal amounts of extracted protein were loaded on 4%-12% polyacrylamide gels (Invitrogen) for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Corp, Bedford, MA, USA). Non-specific binding was blocked by incubation overnight at 4℃ with 5% nonfat dry milk in PBS. The membranes were then incubated at room temperature for 2h with anti- EGFR, anti- p-EGFR, anti-ERK, anti-p-ERK at the dilution of 1:500. Anti-β-actin was used as an internal control for the immunoblot. The membranes were washed three times with the blocking buffer and then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody at a final dilution of 1:1000. After final washes with 0.1% Triton X-100 in PBS, signal was detected by enhanced chemiluminescence following manufacturer's instructions (Pierce, Rockford, IL, USA) and exposed to autoradiographic film.

Transwell Chamber Assay Cell migration was determined using a transwell assay: 1×10^4 cells were placed in the upper chamber (Costar, Cambridge, MA, USA) with a volume of 200 μL serum-free medium. Next, DMEM/F12 medium with 10% FBS were placed in the bottom chamber with a volume of 600 μL per well. After 12h incubation, the cells were fixed in 95% ethanol for 10min, stained with hematoxylin for 5min, and washed in Dulbecco's Calcium and Magnesium free PBS (Gibco, Invitrogen). The remaining cells on the upper surface of the filter (pore size 8 μm) were removed by wiping with a cotton swab. Then the filters were cut off, dehydrated using graded ethanol, hyalinized by dimethylbenzene, and fixed by neutral resins. Cells migration was quantified by the number of cells that migrated across the filter toward the lower surface in five random fields per filter under microscope. All migration assays were performed in triplicates.

Statistical Analysis The statistical analysis was performed using software SPSS version 15.0 (SPSS, Chicago, IL, USA). Data are expressed as means ±standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) and the Student's t-test. $P<0.05$ was considered statistically significant.

RESULTS

Protein Tyrosine Phosphatase 1B Inhibition Promoted Activation of Epidermal Growth Factor Receptor and Extracellular Signal-regulated Kinase in Human Retinal Pigment Epithelium Cells We hypothesized that PTP1B may be the phosphatase that mediates inactivation of EGFR signaling and maintains RPE cells in a nonreplicative state. To test this hypothesis, we firstly examined whether inhibition of PTP1B would affect the activation of EGFR and ERK in ARPE-19 cells. ARPE-19 cells were pretreated with siRNA-PTP1B for 6h. As shown in Figure 1A, siRNA-PTP1B inhibited the expression of PTP1B significantly compared with control and nonsilencing siRNA. Subsequently, we found that siRNA-PTP1B significantly increased phosphorylation of EGFR and ERK compared with the control group in ARPE-19 cells (Figure 1B, 1C). The activation of EGFR and ERK by PTP1B inhibition was blocked by treatment with AG1478 and PD98059 respectively (Figure 1D, 1E). Collectively, these data indicate that PTP1B plays a critical role in dephosphorylation of EGFR/ERK and subsequent blockage of its downstream signaling pathway.

Protein Tyrosine Phosphatase 1B Regulated α-SMA Expression Through Epidermal Growth Factor Receptor/Extracellular Signal-regulated Kinase Signaling Pathway Smooth muscle actin is a highly conserved protein and a major component of microfilaments, which control cell morphology and motility [19]. The altered expression of α-SMA can thus be used as a sensitive marker of change in cell motility. It has been reported that EGFR signaling plays a critical role in the activation of RPE cells and development of PVR [5,9]. To examine whether PTP1B would also modulate α-SMA expression through EGFR/ERK signaling pathway, we treated ARPE-19 cells with siRNA-PTP1B and nonsilencing siRNA, and detected the expression of α-SMA in the presence or absence of AG1478 and/or PD98059. Figure 2 indicated α-SMA expression of deficiency in most of the ARPE cells, and siRNA-PTP1B increased basal level of α-SMA expression. However, the

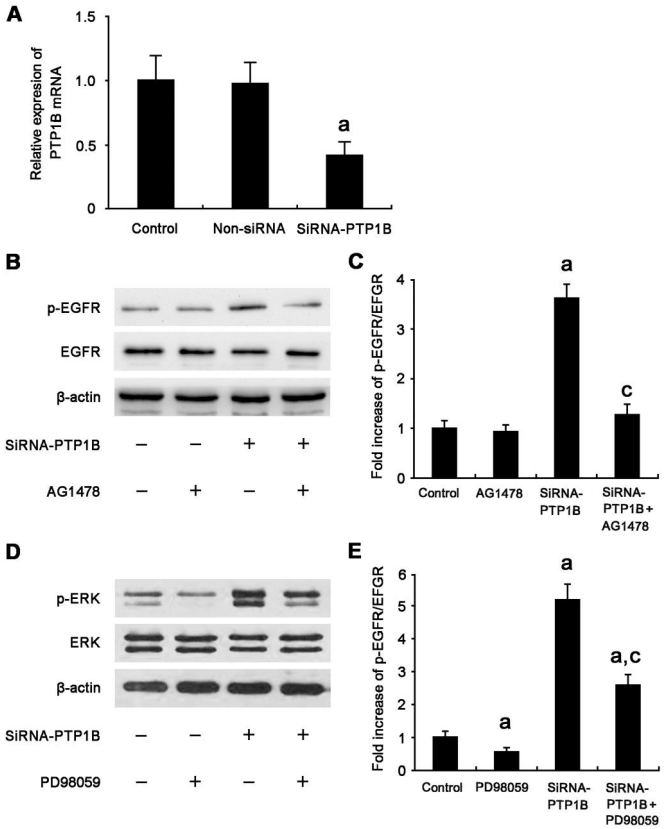

Figure 1 Phosphorylation of EGFR and ERK induced by siRNA –PTP1B A: ARPE-19 cells were incubated with siRNA-PTP1B and nonsilencing siRNA, levels of PTP1B were determined with quantitative RT-PCR. B, C: After incubated with siRNA-PTP1B for 6h, ARPE-19 cells were treated with 20 μmol/L AG1478 for 30min for assay of EGFR phosphorylation, levels of phosphorylated and total EGFR were determined with Western blot analysis. D, E: After incubated with siRNA-PTP1B for 6h, ARPE-19 cells were treated with 20 μmol/L PD98059 for 30min for assay of ERK phosphorylation, levels of phosphorylated and total ERK were determined with Western blot analysis. The data represent the mean±standard deviation (SD) of three independent experiments. $^{a}P<0.05$, compared with control group; $^{c}P<0.05$, compared with siRNA-PTP1B-treated alone.

expression could also be largely inhibited by EGFR inhibitor and/or ERK inhibitor, and the proportion of positive cells clearly decreased. This result suggests that PTP1B suppressed the expression of α-SMA through EGFR/ERK signaling pathway. It may be one of the mechanisms of the PTP1B-mediated regulation in RPE cells migration.

Protein Tyrosine Phosphatase 1B Regulated Migration of Human Retinal Pigment Epithelium Cells through Epidermal Growth Factor Receptor/Extracellular Signal–regulated Kinase Signaling Pathway Having found that siRNA-PTP1B treatment activated the EGFR/ERK signaling pathways and enhanced α-SMA expression in RPE cells, we examined whether the PTP1B- mediated activation of the EGFR/ERK signaling pathways plays a vital role in ARPE-19 cells migration. Results of an *in vitro* migration assay are shown in Figure 3. Cells were measured in a transwell chamber in which ARPE-19 cells migrated through a porous membrane. The mean number of migrated cells in the siRNA-PTP1B-treated RPE cells was significantly higher than the mean number of migrated control cells and nonsilencing siRNA-treated RPE cells. Fewer migrated cells were observed in the AG1478 or PD98059 treatment group than in the siRNA-PTP1B group. The combined blockade of EGFR and ERK could more obviously reduce the mean number of migrated cells.

DISCUSSION

RPE cells are a mosaic of polygonal postmitotic cells interposed between the choroid and the neural retina that serves as the outer blood-retinal barrier regulating retinal homeostasis and visual function, and it does not normally divide or migrate away from this layer [9,20,21]. RPE cells exposed to a new surrounding environment following a rhegmatogenous retinal detachment, resulting in RPE cell activation and separation from the monolayer. Activated RPE cells migrate through a provisional extracellular matrix within the subretinal space and through retinal holes to form pathologic membranes on both surfaces of the neural retina[3]. Contraction of these pathologic membranes (epiret and subretinal) may lead to recurrent retinal detachment and blindness[22].

We have found PTPs regulate the proliferation, differentiation and migration of RPE cells [14]. As one of classic isoforms, the endoplasmic reticulum-localized non-receptor PTP1B has been confirmed to regulate cell cycle progression and proliferation [23]. Here, we further demonstrated that PTP1B regulates the migration of RPE cells *in vitro*.

PTP1B is associated with oncogenic, metabolic, and cytokine-related signaling and functionally targets multiple RTKs for dephosphorylation, such as EGFR. EGFR ligand activation promotes RPE cell proliferation and survival, signaling through the MEK/ERK and PI3K/AKT pathways[9]. However, the signaling mechanism of PTP1B in RPE cells is unclear. To probe the relationship between PTP1B and EGFR, and gain further insight into the molecular mechanisms by which PTP1B inhibition induces migration in RPE cells, we analysed the effects of PTP1B depletion on the activation of EGFR and examined intracellular signaling pathways. We found that treatment of RPE cells with siRNA-PTP1B significantly increased phosphorylation of EGFR and ERK. Treatment with the EGFR inhibitor AG1478 and ERK inhibitor PD98059 blocked the siRNA-PTP1B-induced activation of EGFR and ERK in ARPE-19 cells, indicating that the function of PTP1B depended on EGFR/ERK signaling pathway.

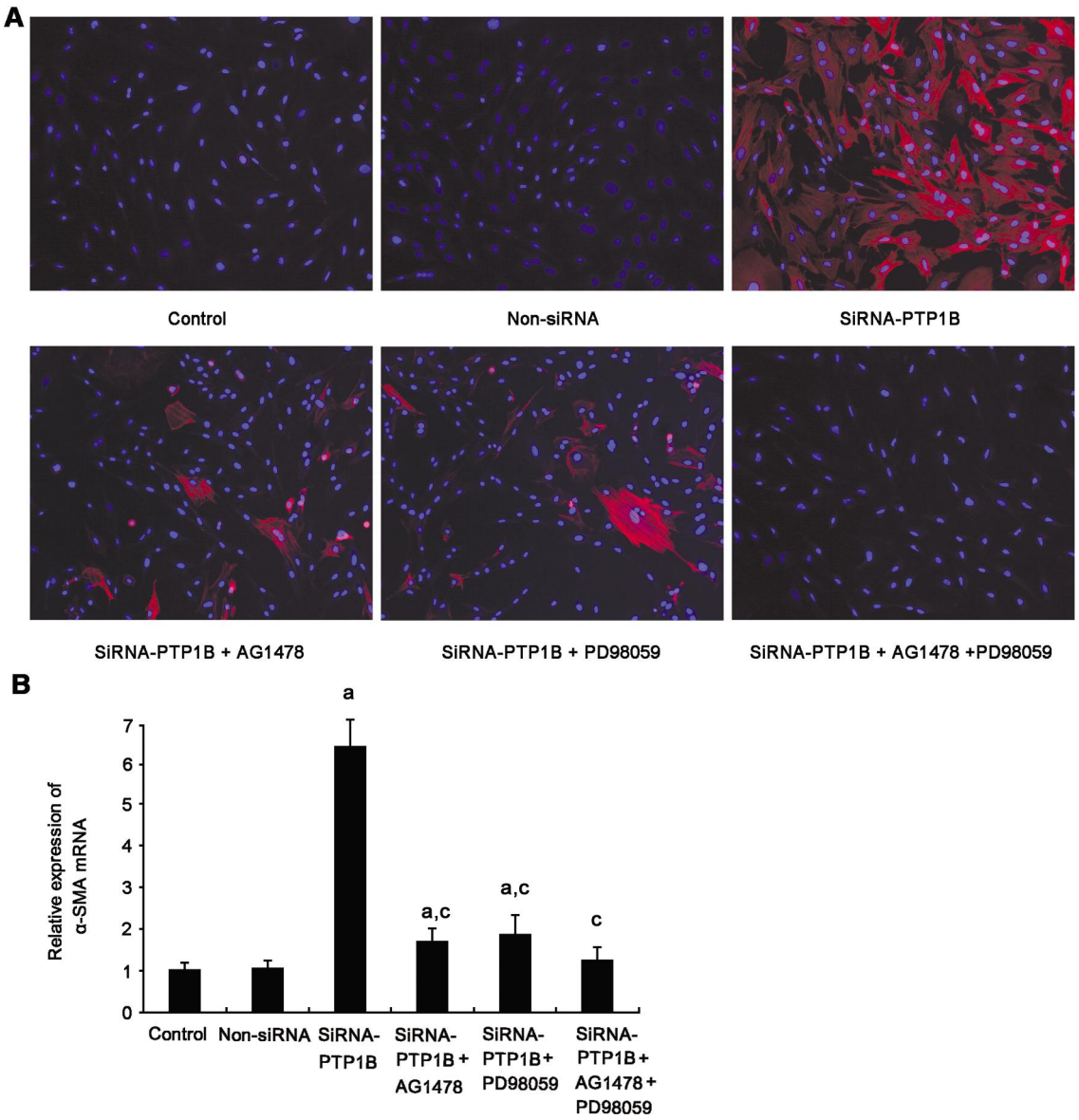

Figure 2 Immunolocalization of α-SMA in cultured ARPE-19 cells. After incubated with siRNA-PTP1B for 6h, ARPE-19 cells were treated with 20 μmol/L AG1478 and/or 20 μmol/L PD98059 for 30min A: ICC studies confirmed the expression of α-SMA (red) in the cells. 4',6-Diamidino- 2-phenylindole (DAPI) staining (blue) indicates nuclei. B: Levels of α-SMA were determined with quantitative RT-PCR. The data represent the mean±standard deviation (SD) of three independent experiments. [a]P<0.05, compared with control group; [c]P<0.05, compared with siRNA-PTP1B-treated alone.

We next examined the functional involvement of the EGFR/ERK signaling pathway in siRNA-PTP1B-induced α-SMA expression and migration in RPE cells. Our results showed that siRNA-PTP1B-induced PTP1B inhibition enhanced α-SMA expression significantly. After myoid differentiation, the RPE cells will acquire better contractility and motility. This transformation of RPE cells into contractile myofibroblast-like cells allows migration into the vitreous where they contribute to PVR membrane formation and the contractile properties of these membranes [24]. The effect of PTP1B inhibition on RPE cell migration was determined using a transwell assay. Treatment with siRNA-PTP1B significantly increased RPE cell migration. Furthermore, we also found that treatment with AG1478 and PD98059 blocked siRNA-PTP1B-induced α-SMA expression and migration in RPE cells. It suggested that PTP1B may be involved in regulation of differentiation and migration of RPE cells through EGFR/ERK signaling pathway.

As we know, the interaction of PTP1B to the EGFR induces dimerization of the receptor and the activation of its intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and the phosphorylation of tyrosine residues in various downstream signaling molecules, including ERK, AKT, STAT3, and PKC [25]. Previous studies have shown that EGFR signaling mediated by the MEK/ERK pathway is essential for RPE cell proliferation and survival[9]. Here, our current study indicated that EGFR signaling mediated by the ERK pathway is essential for RPE cell migration. One molecule downstream from EGFR can trigger the expression of target genes that are involved in cell myoid

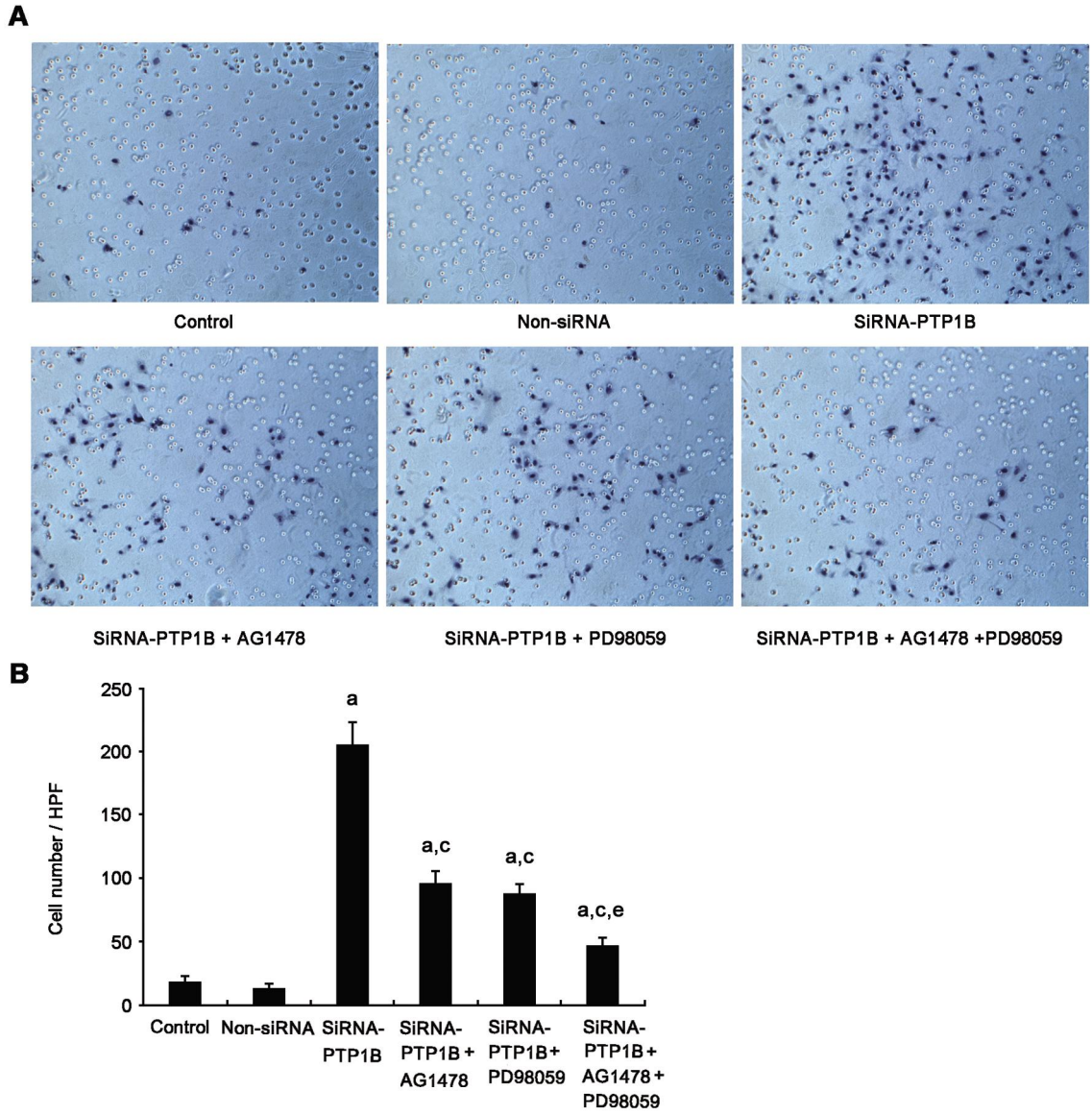

Figure 3 The inhibition effect of AG1478 and PD98059 on siRNA-PTP1B-induced migration in ARPE-19 cells A: Inhibition of siRNA-PTP1B-induced migration by pretreatment with 20 μmol/L AG1478 and/or 20 μmol/L PD98059 for 30min, as measured with a transwell assay after 12h incubation. B: The number of migrated cells per HPF was shown. The data represent the mean±standard deviation (SD) of three independent experiments. [a]$P<0.05$, compared with control group; [c]$P<0.05$, compared with siRNA-PTP1B-treated alone; [e]$P<0.05$, compared with siRNA-PTP1B + AG1478 (or) PD98059 group.

differentiation, such as α-SMA. PTP1B is responsible for the dephosphorylation of EGFR/ERK and inhibition of RPE cells migration.

The results of our study may have bearing on the pathogenesis and treatment of PVR. Both previous findings and our own strongly suggest that EGFR signal transduction pathway plays a key role in the pathogenesis of PVR. We have shown that PTP1B can suppress the migration of RPE cells, the main target cells for the treatment of PVR. Our data support the hypothesis that PTP1B effectively suppresses the migration of ARPE-19 cells through modulation of the levels of EGFR, and the inhibition of EGFR transactivation.

The results of our *in vitro* investigation suggest the possibility that targeted therapy which aims at PTP1B might play a role in the clinic as a potential method for the treatment or prevention of PVR.

ACKNOWLEDGEMENTS

Foundations: Supported by Shandong Provincial Natural Science Foundation, China (No.ZR2012HQ004); the Research Fund for Fundamental Research Project of Qingdao (No.13-1-4-180-jch); the Scientific Research Fund of Huangdao District of Qingdao City (No.2014-1-74); the Young People Scientific Research Fund of Affiliated Hospital, Qingdao University (No.QDFY134).

Conflicts of Interest: Du ZD, None; **Hu LT,** None; **Zhao GQ,** None; **Wang Q,** None; **Xu Q,** None; **Jiang N,** None; **Lin J,** None.

REFERENCES

1 Pastor JC. Proliferative vitreoretinopathy: an overview. *Surv Ophthalmol* 1998;43:3-18

2 Feist RM Jr, King JL, Morris R, Witherspoon CD, Guidry C. Myofibroblast and extracellular matrix origins in proliferative vitreoretinopathy. *Graefes Arch Clin Exp Ophthalmol* 2014;252(2):347-357

3 Hiscott P, Sheridan C, Magee RM, Grierson I. Matrix and the retinal pigment epithelium in proliferative retinal disease. *Prog Retin Eye Res* 1999;18(2):167-190

4 Qiu S, Jiang Z, Huang Z, Chen X, Qian X, Gao Q, Zheng H. Migration of retinal pigment epithelium cells is regulated by protein kinase Cα in vitro. *Invest Ophthalmol Vis Sci* 2013;54(10):7082-7090

5 Heidenkummer HP, Kampik A. Immunohistochemical localization of epidermal growth factor receptor in a human epiretinal membrane. *Graefes Arch Clin Exp Ophthalmol* 1991;229(5):492-496

6 Wouters E, Hudson CA, McArdle CA, Bernal AL. Central role for protein kinase C in oxytocin and epidermal growth factor stimulated cyclooxygenase 2 expression in human myometrial cells. *BMC Res Notes* 2014;7(1):357

7 Reiter BC, Kamanga-Sollo E, Pampusch MS, White ME, Dayton WR. Epidermal growth factor receptor is required for estradiol-stimulated bovine satellite cell proliferation. *Domest Anim Endocrinol* 2014;48:48-55

8 Spraul CW, Kaven C, Lang GK, Lang GE. Effect of growth factors on bovine retinal pigment epithelial cell migration and proliferation. *Ophthalmic Res* 2004;36(3):166-171

9 Yan F, Hui YN, Li YJ, Guo CM, Meng H. Epidermal growth factor receptor in cultured human retinal pigment epithelial cells. *Ophthalmologica* 2007;221(4):244-250

10 Steindl-Kuscher K, Krugluger W, Boulton ME, Haas P, Schrattbauer K, Feichtinger H, Adlassnig W, Binder S. Activation of the beta-catenin signaling pathway and its impact on RPE cell cycle. *Invest Ophthalmol Vis Sci* 2009;50(9):4471-4476

11 Ponnusamy M, Ma L, Zhuang S. Necrotic renal epithelial cell inhibits renal interstitial fibroblast activation: role of protein tyrosine phosphatase 1B. *Am J Physiol Renal Physiol* 2013;304(6):F698-709

12 Haj FG, Markova B, Klaman LD, Bohmer FD, Neel BG. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatase-1B. *J Biol Chem* 2003;278(2):739-744

13 Tiganis T. Protein tyrosine phosphatases: dephosphorylating the epidermal growth factor receptor. *IUBMB Life* 2002;53(1):3-14

14 Du ZD, Hu LT, Hu YT, Ma ZZ. The effects of sodium orthovanadate-induced phosphatase inhibition on rat retinal pigment epithelium cell activity. *Cutan Ocul Toxicol* 2010;29(4):261-268

15 Eden ER, White IJ, Tsapara A, Futter CE. Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. *Nat Cell Biol* 2010;12(3):267-272

16 Liu F, Chernoff J. Protein tyrosine phosphatase 1B interacts with and is tyrosine phosphorylated by the epidermal growth factor receptor. *Biochem J* 1997;327(Pt 1):139-145

17 Chang Y, Ceacareanu B, Zhuang D, Zhang C, Pu Q, Ceacareanu AC, Hassid A. Counter-regulatory function of protein tyrosine phosphatase 1B in platelet-derived growth factor- or fibroblast growth factor-induced motility and proliferation of cultured smooth muscle cells and in neointima formation. *Arterioscler Thromb Vasc Biol* 2006;26(3):501-507

18 Harris DL, Joyce NC. Protein tyrosine phosphatase, PTP1B, expression and activity in rat corneal endothelial cells. *Mol Vis* 2007;13:785-796

19 Tsukamato H, Mishima Y, Hayashibe K, Sasase A. Alpha-smooth muscle actin expression in tumor and stromal cells of benign and malignant human pigment cell tumors. *J Invest Dermatol* 1992;98(1):116-120

20 Grierson I, Hiscott P, Hogg P, Robey H, Mazure A, Larkin G. Development, repair and regeneration of the retinal pigment epithelium. *Eye(Lond)* 1994;8(Pt 2):255-262

21 Zhao S, Rizzolo LJ, Barnstable CJ. Differentiation and transdifferentiation of the retinal pigment epithelium. *Int Rev Cytol* 1997; 171:225-266

22 Campochiaro PA. Pathogenic mechanisms in proliferative vitreoretinopathy. *Arch Ophthalmol* 1997;115(2):237-241

23 Ishino Y, Zhu C, Harris DL, Joyce NC. Protein tyrosine phosphatase-1B (PTP1B) helps regulate EGF-induced stimulation of S-phase entry in human corneal endothelial cells. *Mol Vis* 2008;14:61-70

24 Bastiaans J, van Meurs JC, van Holten-Neelen C, Nagtzaam NM, van Hagen PM, Chambers RC, Hooijkaas H, Dik WA. Thrombin induces epithelial-mesenchymal transition and collagen production by retinal pigment epithelial cells via autocrine PDGF-receptor signaling. *Invest Ophthalmol Vis Sci* 2013;54(13):8306-8314

25 Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. *Mol Syst Biol* 2005;1: 2005.0010.Epub 2005 May 25