Original Article
EGFL6 regulates hypoxia-induced angiogenic factors in retinal pigment epithelial cells

Yixin Qu[1*], Binbin Li[1*], Qing Zhou[1], Ling Jin[2], Xiaoyong Liu[1], Xiaoxi Yang[3], Wei Xu[1], Longyan Ye[1], Jian Chen[1]

[1]Department of Ophthalmology, The First Affiliated Hospital of Jinan University, Guangzhou 510630, Guangdong, China; [2]Department of Ophthalmology, The People's Hospital of Baoan Shenzhen, Shenzhen 518101, Guangdong, China; [3]Department of Ophthalmology, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, Guangdong, China. *Equal contributors.

Abstract: Epidermal growth factor like domain 6 (EGFL6), which is a member of the epidermal growth factor repeat superfamily of proteins, has been demonstrated to be important in angiogenesis. However, the expression and role of EGFL6 in the retinal pigment epithelial (RPE) cells remains to be elucidated. The aim of the present study was to investigate the role of EGFL6 in cultured human RPE cells. ARPE-19 cells were pretreated by 200 µM $CoCl_2$ for 24 h, then transfected with siRNA-EGFL6 or control siRNA. Cell proliferation was measured using the MTT assay. The expression protein levels of EGFL6, VEGF, pigment epithelium-derived factor (PEDF), stromal cell-derived factor (SDF)-1 and IL-1β were detected using western blotting. The expression of EGFL6 was significantly elevated under hypoxic conditions. EGFL6 knockdown inhibited hypoxia-induced RPE cell proliferation. In addition, knockdown of EGFL6 inhibited hypoxia-induced pro-angiogenic factor expression and the expression of inflammatory factors in RPE cells. These results suggested that the knockdown of EGFL6 suppressed pro-angiogenic factors induced by hypoxia in RPE cells. Therefore, EGFL6 may be a potential therapeutic target for the treatment of age-related macular degeneration.

Keywords: Epidermal growth factor like domain 6, retinal pigment epithelial, hypoxia, angiogenic factors

Introduction

Age-related macular degeneration (AMD) is one of the major causes of visual impairment in the elderly population worldwide [1]. A severe form of AMD is choroidal neovascularization (CNV), in which new blood vessels grow from the choroids, through the Bruch's membrane into the subretinal space [2]. The retinal pigment epithelium (RPE), which forms a blood-retinal barrier between the neural retina and choriocapillaris, is important in maintaining the ocular angiogenic homeostasis [3]. RPE cells are the primary cells involved in CNV, and vascular endothelial growth factor (VEGF) secretion by RPE cells leads to neovascularization in the posterior segment of the eye [4]. In addition, hypoxia could contribute to the development of retinal and CNV formation [5-7]. Under hypoxic conditions, increased production of angiogenic factors, result in hypoxia-induced retinal angiogenesis [8].

The epidermal growth factor (EGF) repeat motif defines a superfamily of diverse proteins involved in mediating a variety of cellular processes, including cell adhesion, proliferation, growth, survival and differentiation [9]. EGF-like domain 6 (EGFL6), a member of the EGF repeat protein superfamily expressed in various tissues [10]. Furthermore, it was reported that EGFL6 promotes the endothelial cell tube-like structure formation and angiogenesis in a chick embryo chorioallantoic membrane [11]. However, the role of EGFL6 in RPE remains to be elucidated. The present studies, aimed to examine the effects of EGFL6 during hypoxia-induced angiogenesis in RPE cells.

Materials and methods

Cell culture and hypoxic treatment

Human RPE cells (ARPE-19) were obtained from the American Type Culture Collection (Manass-

as, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium/F-12 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, SUA), 100 U/ml penicillin, 100 µg/ml streptomycin and 4 mmol/l glutamine at 37°C in an incubator with an atmosphere containing 5% CO_2. For the hypoxia treatment, 200 µM cobalt chloride ($CoCl_2$; Sigma Chemical Co., St. Louis, MO) was added to the culture medium. The controls were cultured at 37°C in a humidified atmosphere containing 5% CO_2 for the same time period.

Small interfering RNA (siRNA) transfection

Human-specific EGFL6 siRNA (siRNA-EGFL6, 5'-GCTTCTCTCTACAACATTT-3') and non-targeting negative control siRNA (mock) were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). For siRNA transfection, ARPE-19 cells were seeded onto 6-well plates at a density of $3×10^3$ cells/well and cultured to 60-80% confluence, then siRNAs were transfected into ARPE-19 cells at a final concentration of 30 nM with siRNA Transfection Reagent (Santa Cruz Biotechnology, Inc.) according to the manufacturer's protocol.

Cell proliferation assay

Cell proliferation was measured using the MTT assay. Briefly, ARPE-19 cells transfected with siRNA-EGFL6 or mock were seeded in a 96-well plate at a density of $1×10^4$ cells/well and then cultured for 24 and 48 h. Then, the initial culture medium was replaced with fresh medium containing MTT (5 mg/ml; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and incubated for an additional 4 h. The formazan was dissolved in dimethylsulfoxide (150 µl/well; Sigma-Aldrich, Merck KGaA) for 10 min. The absorbance was subsequently measured on a Bio-Rad Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using a test wavelength of 490 nm. Each experiment was performed in triplicate.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

Total cellular RNA was isolated from cultured cells using RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) in accordance with the man-ufacturer's protocol. For RT, single-stranded cDNA was reverse transcribed from 2 µg total RNA using reverse transcriptase (Invitrogen, Carlsbad, CA, USA) with an oligo (dT) primer. The qRT-PCR was performed in a final volume of 20 µl, containing 50 ng of total RNA, 10 µl 2×SYBR Green I reagent, 6.25 U Multi-Scribe reverse transcriptase, 10 U RNase inhibitor and 0.1 mM primers. The primer sequences used were as follows: Forward 5'-TCT GTG CCT GCT GCT CAT AG and reverse 5'-TCTCCTTGGCCAC-AATG-3', for EGFL6; and forward 5'-GGTGGCTT-TTAGGATGGCAAG-3' and reverse, 5'-ACTGGA-ACGGTGAAGGTGACAG-3' for β-actin. Relative mRNA levels were evaluated by qPCR (LightCycler; Roche Applied Science, Penzburg, Germany) using Absolute Blue qPCR kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The PCR conditions comprised 40 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec and polymerization at 72°C for 30 sec. The PCR products obtained were resolved on a 1.5% agarose gel and visualized with ethidium bromide under a transilluminator. Relative levels of gene expression were expressed relative to β-actin and calculated using the $2^{-\Delta\Delta Cq}$ method [12].

Western blotting

Total protein was extracted from ARPE-19 cells using RIPA Cell Lysis Buffer (Bio-Rad, Hercules, CA, USA), containing a phosphatase inhibitor and the protease inhibitor cocktail (Sigma-Aldrich), by incubation on ice for 30 min. The concentration of the protein was measured using the BCA Protein Assay Kit (Pierce; Thermo Fisher Scientific, Inc.). Equal amounts of protein (30 µg per lane) were boiled for 10 min in sample buffer, separated by 10% SDS-PAGE and transferred to a polyvinylidene membrane. The blots were blocked in 5% nonfat milk in TBS-Tween (5 mM Tris-HCl (pH 7.4) 136 mM NaCl, 0.1% Tween 20) for 1 h at room temperature and then probed with mouse anti-human EGF-L6 polyclonal IgG (1:2500; ab-167281; Abcam, Cambridge, UK), mouse anti-human VEGF monoclonal IgG (1:2500; sc-57496), mouse anti-human pigment epithelium-derived factor (PEDF) monoclonal IgG (1:3000; sc-390172), mouse anti-human stromal cell-derived factor (SDF)-1 monoclonal IgG (1:3000; sc-74271), mouse anti-human interleukin (IL)-1β monoclonal IgG (1:3000; sc-52012) and mouse anti-human β-actin monoclonal IgG (1:2000; sc-
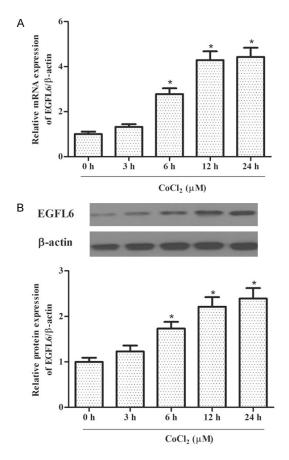
Figure 1. EGFL6 was highly expressed in retinal pigment epithelial cells under hypoxic conditions. ARPE-19 cells were exposed to 200 μM CoCl$_2$ for 0, 3, 6, 12 and 24 h. (A) Reverse transcription-quantitative polymerase chain reaction and (B) western blotting analysis were used to measure the mRNA and protein expression levels of EGFL6, respectively. Bars represent the mean and standard deviation of three independent experiments. *P<0.05 vs. 0 h. EGFL, epidermal growth factor like domain.

Figure 2. Effect of EGFL6 on RPE cell proliferation under conditions of hypoxia. ARPE-19 cells were pretreated by 200 μM CoCl$_2$ for 24 h, then transfected with siRNA-EGFL6 or control siRNA. (A) Reverse transcription-quantitative polymerase chain reaction and (B) western blotting analysis were used to measure the mRNA and protein expression of EGFL6, respectively. *P<0.05. (C) Knockdown of EGFL6 inhibited RPE cell proliferation under the hypoxic culture conditions. Bars indicate the mean and standard deviation of three independent experiments. *P<0.05 vs. normoxia group. &P<0.05 vs. hypoxia group. EGFL, epidermal growth factor like domain; RPE, retinal pigment epithelial; siRNA, small interfering RNA.

8432) (Santa Cruz Biotechnology Inc.) overnight at 4°C. Subsequently, the membranes were incubated with goat anti-mouse horseradish peroxidase-conjugated secondary antibody (1:3000; sc-2031) for 1 h at room temperature. Proteins were visualized via enhanced chemiluminescence detection (Pierce; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Statistical analysis

Statistical analyses were performed using the SPSS 17.0 (SPSS, Inc., Chicago, USA). Data were expressed as the mean ± standard deviation. Statistical analysis of data was performed
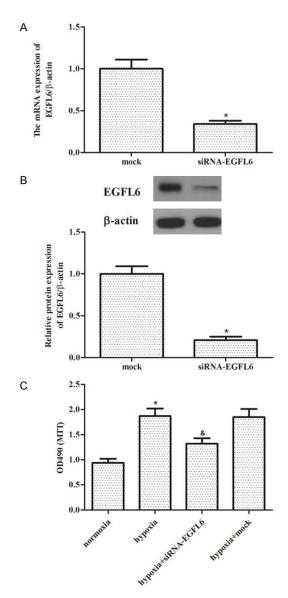
by one-way analysis of variance followed by a Student-Newman-Keuls test, and P<0.05 was considered to indicate a statistically significant difference.
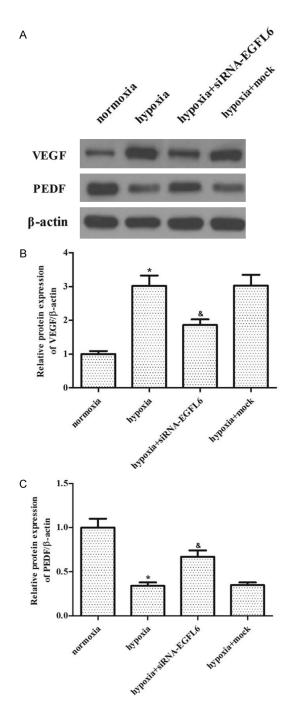
Results

EGFL6 was highly expressed in RPE cells under conditions of hypoxia

To explore the alteration of EGFL6 expression in RPE cells in response to a hypoxic stimulus, the present study examined EGFL6 mRNA and protein expression levels. As presented in **Figure 1A**, EGFL6 mRNA expression levels were significantly upregulated following 6 h incubation under hypoxic conditions compared with the 0 hgroup (P<0.05), and reached a maximum at 24 h. Western blotting results additionally demonstrated that hypoxia induced the expression of EGFL6 protein compared with 0 h group (**Figure 1B**).

Knockdown of EGFL6 inhibits RPE cell proliferation under hypoxic conditions

To investigate the effect of EGFL6 on RPE cell proliferation under hypoxic conditions, siRNA-EGFL6 was transfected into ARPE-19 cells. Following transfection, the expression of EGFL6 was inhibited at the RNA (**Figure 2A**) and protein (**Figure 2B**) levels under hypoxic conditions, as compared with the mock group. Subsequently, the MTT assay was used to determine the RPE cell proliferation *in vitro*. The results demonstrated that hypoxia significantly induced RPE cell proliferation, as compared with the control groups. However, knockdown of EGFL6 suppressed the hypoxia-induced RPE proliferation, compared with the hypoxia control groups (P<0.05; **Figure 2C**).

Effect of EGFL6 knockdown the expression of angiogenic factors in RPE cells under hypoxia

Angiogenic factors are important in vascular formation and progression. Therefore, the present study evaluated the effects of EGFL6 on the expression levels of pro-angiogenic factor, VEGF, and anti-angiogenic factor, PEDF, in ARPE-19 cells under hypoxic conditions. Western blotting results demonstrated that hypoxia significantly upregulated VEGF expression whereas it downregulated PEDF expression compared with cells in normoxia (P<0.05). However, knockdown of EGFL6 partially rescued the imbalance of pro- and anti-angiogenesis factors caused by hypoxia in ARPE-19 cells (**Figure 3**).

Figure 3. Effect of EGFL6 on the expression of angiogenic factors in retinal pigment epithelial cells under hypoxia. ARPE-19 cells were pretreated by 200 μM CoCl$_2$ for 24 h, then transfected with siRNA-EGFL6 or control siRNA. (A) Western blot analysis of protein levels of VEGF and PEDF. Quantification of the results of western blots was performed by calculating the band density ratio of (B) VEGF/β-actin and (C) PEDF/β-actin. Bars represent the mean and standard deviation of three independent experiments. *P<0.05, vs. normoxia group. &P<0.05 vs. hypoxia group. siRNA, small interfering RNA; EGFL, epidermal growth factor like domain; VEGF, vascular endothelial growth factor; PEDF, pigment epithelium-derived factor.
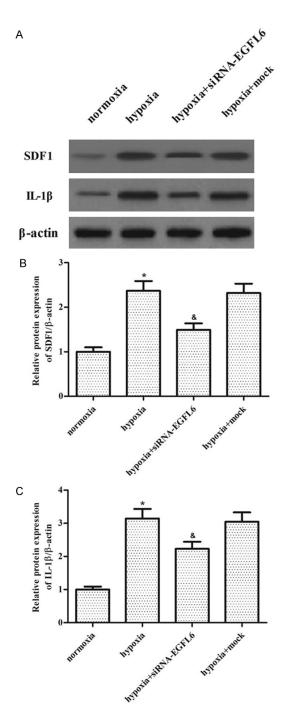
A

B

C

Figure 4. Effects of EGFL6 on the expression of inflammatory cytokines in retinal pigment epithelial cells under hypoxia. ARPE-19 cells were pretreated by 200 μM CoCl$_2$ for 24 h, then transfected with siRNA-EGFL6 or control siRNA. (A) Western blot analysis of protein levels of SDF1 and IL-1β. Quantification of the results of western blotting were performed by calculating the band density ratio of (B) SDF1/β-actin or (C) IL-1β/β-actin. Bars represent the mean and standard deviation of three independent experiments. *P<0.05 vs. normoxia group, &P<0.05 vs. hypoxia group. siRNA, small interfering RNA; EGFL, epidermal growth factor like domain; SDF, stromal cell-derived factor, IL, interleukin.

Knockdown of EGFL6 decreases the expression of inflammatory cytokines in RPE cells under hypoxia

Inflammatory cytokines, including SDF-1 and IL-1β, have been demonstrated to be involved in angiogenesis. Therefore, the present study evaluated the effects of EGFL6 on the expression levels of SDF-1 and IL-1β in ARPE-19 cells under hypoxic conditions. As presented in **Figure 4**, hypoxia treatment markedly induced the expression of SDF-1 and IL-1β, compared with the control group (P<0.05), whereas knockdown of EGFL6 inhibited the expression of SDF-1 and IL-1β induced by hypoxia in ARPE-19 cells (P<0.05).

Discussion

The present study, to the best of our knowledge, is the first to describe the expression and role of EGFL6 in human RPE cells. The expression of EGFL6 was increased by hypoxia, and EGFL6 knockdown suppressed hypoxia-induced RPE cell proliferation. In addition, knockdown of EGFL6 restored the imbalance of pro- and anti-angiogenic factors, and inhibited the expression of SDF-1 and IL-1β in hypoxia-stimulated ARPE-19 cells.

The role of EGFL6 in angiogenesis has previously been reported. Chim *et al.* [11] demonstrated that EGFL6 is highly expressed in osteoblastic-like cells, and these express EGFL6 that is capable of promoting endothelial cell migration and angiogenesis via extracellular signal-related kinase activation. Another study showed that recombinant EGFL6 enhanced proliferation of human adipose tissue-derived stromal vascular cells [13]. Similarly, herein, we observed that expression of EGFL6 was increased following hypoxic insult in a time-dependent manner and knockdown of EGFL6 suppressed hypoxia-induced RPE cell proliferation. These results suggested that EGFL6 is involved in the hypoxic response of RPE cells.

The increased production of VEGF has been identified as essential in the development and progression of CNV. It is well established that hypoxia is important in the development and progression of retinal vascular diseases. It has previously been suggested that RPE cells secrete VEGF and VEGF-associated polypeptides in response to hypoxic insult [14], and may therefore be important in the progression

of CNV. PEDF, a glycoprotein (50 kDa), is an endogenous inhibitor of angiogenesis and is present in the vitreous at high levels [15]. It has previously been demonstrated that the decrease of PEDF in the eye is correlated with CNV formation [16-18]. In addition, hypoxia may downregulate PEDF via proteolytic degradation [19]. Consistent with these previous studies, the present study observed that hypoxia significantly upregulated VEGF expression and downregulated PEDF expression. However, knockdown of EGFL6 restored the imbalance of pro- and anti-angiogenic factors in hypoxia-stimulated ARPE-19 cells. This demonstrated the angiogenic effect of EGFL6 in RPE cells under hypoxic conditions, which may be important in the development of CNV.

Inflammation is associated with the development of CNV [20-22]. A study demonstrated that the administration of an anti-inflammatory drug, bromfenac, decreased the formation of laser-induced CNV [23]. SDF-1 is important in ocular neovascularization diseases [24-26], and hypoxic insult effectively augmented the expression of SDF-1α in RPE cells [27]. Furthermore, IL-1β is the primary inflammatory cytokine produced by a variety of cells, and is fundamental in inflammatory responses [28]. Various studies have previously revealed the protein secretion and mRNA expression of VEGF in ARPE-19 cells was increased by IL-1β [29, 30]. In accordance with the previous data, the present study observed that knockdown of EGFL6 reduced the expression of IL-1β in RPE under hypoxic culture conditions. These results suggested that EGFL6 may regulate the expression of hypoxia-induced angiogenic factors in RPE cells via regulation of inflammatory factors.

In conclusion, the results of the present study demonstrated that EGFL6 exhibited an angiogenic role in CNV, via regulation of angiogenic factor expression in RPE cells under hypoxic conditions. However the exact underlying molecular mechanism remains to be elucidated in further investigations.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Jian Chen, Department of Ophthalmology, The First Affiliated Hospital of Jinan University, Guangzhou 510630, Guangdong, China. E-mail: chenjian_oph@sina.com

References

[1] Fine SL. Age-related macular degeneration 1969-2004: a 35-year personal perspective. Am J Ophthalmol 2005; 139: 405-420.

[2] Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. New Engl J Med 2008; 358: 2606-2617.

[3] Martin G, Schlunck G, Hansen LL, Agostini HT. Differential expression of angioregulatory factors in normal and CNV-derived human retinal pigment epithelium. Graef Arch Clin Exp 2004; 242: 321-326.

[4] Ferrara N, Gerber HP. Vascular endothelial growth factor molecular and biological aspects. Adv Organ Biol 1999; 7: 25-57.

[5] Zhang P, Wang Y, Hui Y, Hu D, Wang H, Zhou J, Du H. Inhibition of VEGF expression by targeting HIF-1α with small interference RNA in human RPE cells. Ophthalmologica 2007; 221: 411-417.

[6] Lin M, Hu Y, Chen Y, Zhou KK, Jin J, Zhu M, Le YZ, Ge J, Ma JX. Impacts of hypoxia-inducible factor-1 knockout in the retinal pigment epithelium on choroidal neovascularization. Invest Oph Vis Sci 2012; 53: 6197-6206.

[7] Zhu J, Wang YS, Zhang J, Zhao W, Yang XM, Li X, Jiang TS, Yao LB. Focal adhesion kinase signaling pathway participates in the formation of choroidal neovascularization and regulates the proliferation and migration of choroidal microvascular endothelial cells by acting through HIF-1 and VEGF expression in RPE cells. Exp Eye Res 2009; 88: 910-918.

[8] Stone J, Itin A, Alon T, Pe'Er J, Gnessin H, Chan-Ling T, Keshet E. Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. J Neurosci 1995; 15: 4738-4747.

[9] Davis CG. The many faces of epidermal growth factor repeats. New Biologist 1990; 2: 410-419.

[10] Buchner G, Broccoli V, Bulfone A, Orfanelli U, Gattuso C, Ballabio A, Franco B. MAEG, an EGF-repeat containing gene, is a new marker associated with dermatome specification and morphogenesis of its derivatives. Mech Develop 2000; 98: 179-182.

[11] Chim SM, Qin A, Tickner J, Pavlos N, Davey T, Wang H, Guo Y, Zheng MH, Xu J. EGFL6 promotes endothelial cell migration and angiogenesis through the activation of extracellular signal-regulated kinase. J Biol Chem 2011; 286: 22035-22046.

[12] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 2001; 25: 402-408.

[13] Oberauer R, Rist W, Lenter MC, Hamilton BS, Neubauer H. EGFL6 is increasingly expressed in human obesity and promotes proliferation of adipose tissue-derived stromal vascular cells. Mol Cell Biochem 2010; 343: 257-269.

[14] Shima D, Adamis A, Ferrara N, Yeo K, Yeo T, Allende R, Folkman J, D'Amore PA. Hypoxic induction of endothelial cell growth factors in retinal cells: identification and characterization of vascular endothelial growth factor (VEGF) as the mitogen. Mol Med 1995; 1: 182-193.

[15] Karakousis PC, John SK, Behling KC, Surace EM, Smith JE, Hendrickson A, Tang WX, Bennett J, Milam AH. Localization of pigment epithelium derived factor (PEDF) in developing and adult human ocular tissues. Mol Vis 2001; 7: 154-163.

[16] Tong JP, Chan WM, Liu DT, Lai TY, Choy KW, Pang CP, Lam DS. Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. Am J Ophthalmol 2006; 141: 456-462.

[17] Renno RZ, Youssri AI, Michaud N, Gragoudas ES, Miller JW. Expression of pigment epithelium-derived factor in experimental choroidal neovascularization. Invest Ophth Vis Sci 2002; 43: 1574-1580.

[18] Holekamp NM, Bouck N, Volpert O. Pigment epithelium-derived factor is deficient in the vitreous of patients with choroidal neovascularization due to age-related macular degeneration. Am J Ophthalmol 2002; 134: 220-227.

[19] Notari L, Miller A, Martínez A, Amaral J, Ju M, Robinson G, Smith LE, Becerra SP. Pigment epithelium-derived factor is a substrate for matrix metalloproteinase type 2 and type 9: implications for downregulation in hypoxia. Invest Ophth Vis Sci 2005; 46: 2736-2747.

[20] Nagai N, Oike Y, Izumi-Nagai K, Urano T, Kubota Y, Noda K, Ozawa Y, Inoue M, Tsubota K, Suda T, Ishida S. Angiotensin II type 1 receptor-mediated inflammation is required for choroidal neovascularization. Arterioscl Throm Vas 2006; 26: 2252-2259.

[21] Izumi-Nagai K, Nagai N, Ohgami K, Satofuka S, Ozawa Y, Tsubota K, Ohno S, Oike Y, Ishida S. Inhibition of choroidal neovascularization with an anti-inflammatory carotenoid astaxanthin. Invest Ophth Vis Sci 2008; 49: 1679-1685.

[22] Koto T, Nagai N, Mochimaru H, Kurihara T, Izumi-Nagai K, Satofuka S, Shinoda H, Noda K, Ozawa Y, Inoue M, Tsubota K, Oike Y, Ishida S. Eicosapentaenoic acid is anti-inflammatory in preventing choroidal neovascularization in mice. Invest Ophth Vis Sci 2007; 48: 4328-4334.

[23] Yoshinaga N, Arimura N, Otsuka H, Kawahara K-i, Hashiguchi T, Maruyama I, Sakamoto T. NSAIDs inhibit neovascularization of choroid through HO-1-dependent pathway. Lab Invest 2011; 91: 1277-1290.

[24] Sengupta N, Caballero S, Mames RN, Timmers AM, Saban D, Grant MB. Preventing stem cell incorporation into choroidal neovascularization by targeting homing and attachment factors. Invest Ophth Vis Sci 2005; 46: 343-348.

[25] Unoki N, Murakami T, Nishijima K, Ogino K, van Rooijen N, Yoshimura N. SDF-1/CXCR4 contributes to the activation of tip cells and microglia in retinal angiogenesis. Invest Ophthalmol Vis Sci 2010; 51: 3362-3371.

[26] Lima e Silva R, Shen J, Hackett SF, Kachi S, Akiyama H, Kiuchi K, Yokoi K, Hatara MC, Lauer T, Aslam S, Gong YY, Xiao WH, Khu NH, Thut C, Campochiaro PA. The SDF-1/CXCR4 ligand/receptor pair is an important contributor to several types of ocular neovascularization. FASEB J 2007; 21: 3219-3230.

[27] Wang YQ, Zhang XM, Wang XD, Wang BJ, Wang W. 17-AAG, a Hsp90 inhibitor, attenuates the hypoxia-induced expression of SDF-1α and ILK in mouse RPE cells. Mol Biol Rep 2010; 37: 1203-1209.

[28] Dinarello CA. The interleukin-1 family: 10 years of discovery. FASEB J 1994; 8: 1314-1325.

[29] Watanabe K, Zhang XY, Kitagawa K, Yunoki T, Hayashi A. The effect of clonidine on VEGF expression in human retinal pigment epithelial cells (ARPE-19). Graef Arch Clin Exp 2009; 247: 207-213.

[30] Dugas B, Charbonnier S, Baarine M, Ragot K, Delmas D, Ménétrier F, Lherminier J, Malvitte L, Khalfaoui T, Bron A, Creuzot-Garcher C, Latruffe N, Lizard G. Effects of oxysterols on cell viability, inflammatory cytokines, VEGF, and reactive oxygen species production on human retinal cells: cytoprotective effects and prevention of VEGF secretion by resveratrol. Eur J Nutr 2010; 49: 435-446.