The International Journal of Biochemistry & Cell Biology 44 (2012) 808–814

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Oxidized low density lipoprotein-induced senescence of retinal pigment epithelial cells is followed by outer blood–retinal barrier dysfunction

Jin Hyoung Kim[a], Sung-Joon Lee[b], Kyu-Won Kim[c], Young Suk Yu[a,d], Jeong Hun Kim[a,d,*]

[a] Fight against Angiogenesis-Related Blindness (FARB) Laboratory, Clinical Research Institute, Seoul National University Hospital, Seoul 110-744, Republic of Korea
[b] Division of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Institute of Biomedical Science and Safety, Korea University, Seoul 136-713, Republic of Korea
[c] NeuroVascular Coordination Research Center, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Republic of Korea
[d] Department of Ophthalmology, Seoul National University College of Medicine, Seoul 110-744, Republic of Korea

ARTICLE INFO

Article history:
Received 12 November 2011
Received in revised form 30 January 2012
Accepted 5 February 2012
Available online 13 February 2012

Keywords:
Outer blood–retinal barrier
Oxidized low density lipoprotein
Retinal pigment epithelial cell
Senescence

ABSTRACT

Age-related macular degeneration is the most common cause of vision loss in the elderly, which starts from aging processes of retinal pigment epithelial cells. Among variable risk factors in occurrence and progression of age-related macular degeneration, oxidized low density lipoprotein could be causally involved in pathobiological changes of RPE cells. Herein we showed that oxidized low density lipoprotein-induced senescence of retinal pigment epithelial cells is followed by outer blood–retinal barrier dysfunction. Under sub-lethal concentration, oxidized low density lipoprotein could promote advanced senescence of retinal pigment epithelial cells. Interestingly expression of CRALBP and RPE 65, indicators of retinal pigment epithelial cell differentiation, was decreased by oxidized low density lipoprotein. In addition, oxidized low density lipoprotein induced reactive oxygen species production and up-regulated inflammatory factors such as tumor necrosis factor-α and vascular endothelial growth factor, when β-catenin, a critical mediator of the canonical Wnt pathway, was also elevated. Oxidized low density lipoprotein increased paracellular permeability of retinal pigment epithelial cells, when zonula occludens-1 at intercellular junctions markedly decreased as well. Furthermore, in retinal pigment epithelial cells and choriocapillaris of human apolipoprotein E2 transgenic mouse eye, increased vascular endothelial growth factor and decreased zonula occludens-1 expression was observed. Therefore, our results suggest that oxidized low density lipoprotein could promote senescence of retinal pigment epithelial cells which leads to induce outer blood–retinal barrier dysfunction as an early pathogenesis of age-related macular degeneration.

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of visual impairment in elderly people (Klein et al., 2004). Depending on clinico-pathological features AMD may be grouped as two types of dry form and wet form. The dry form is demonstrated by the progressive degeneration of retinal pigment epithelial (RPE) cells and the subsequent loss of photoreceptors whereas the wet form is characterized by choroidal neovascularization which may lead to the sudden vision loss from subretinal hemorrhage or edema (Jager et al., 2008). Regardless of different clinico-pathological features, AMD is assumed to start from common age-related pathological processes of RPE cells including drusen and pigmentary changes limited in RPE cells and adjacent structures (Roth et al., 2004; Feher et al., 2006). Accordingly, an approach to intervene the senescence-associated changes of RPE cells would be helpful for preventing development or progression of AMD. Although the cause of AMD remains to be elucidated, it has been proposed that variable risk factors including nutritional, medical, genetic factors as well as lifestyles may play a role in occurrence and progression of AMD (Van Leeuwen et al., 2003; Clemons et al., 2005; DeWan et al., 2007; Chakravarthy et al., 2010). In particular, cardiovascular risk factors are closely linked to AMD development and progression. In addition to previous history of cardiovascular diseases, increased low-density lipoprotein (LDL) in serum was related to the increased risk of AMD whereas increased high-density lipoprotein (HDL) was related to decreased risk (Tan et al., 2007; Chakravarthy et al., 2010). We also reported that the eyes of human apolipoprotein E2 transgenic mouse (apoE2) with high serum cholesterol develop lipid accumulation in RPE cells, a typical characteristic of AMD (Lee et al., 2007). Furthermore, native LDL up-regulated expression of

* Corresponding author at: Fight against Angiogenesis-Related Blindness (FARB) Laboratory, Clinical Research Institute, Seoul National University Hospital and Department of Ophthalmology, Seoul National University College of Medicine, Seoul 110-744, Republic of Korea. Tel.: +82 2 2072 2438; fax: +82 2 741 3187.
E-mail address: steph25@snu.ac.kr (J.H. Kim).

1357-2725/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2012.02.005

vascular endothelial growth factor (VEGF), a major angiogenic and inflammatory factor in RPE cells (Lee et al., 2007).

RPE cells in the retina form a monolayer between photoreceptors and choroidal vessels which is a barrier to maintain the normal structural and functional integrity of the retina. The blood–retinal barrier (BRB) is composed of inner BRB of retinal microvascular endothelial cells and outer BRB of RPE cells (Cunha-Vaz, 1976). As the BRB is essential to serve functions in the eye, the BRB breakdown could lead to serious visual impairment. For example, AMD is closely related to outer BRB breakdown whereas diabetic retinopathy is a common cause of inner BRB breakdown (Kim et al., 2006; Jo et al., 2010). Physiologically outer BRB could be maintained by intercellular tight junction between RPE cells, which is however disrupted with the redistribution of tight junction proteins and increased paracellular permeability under pathological conditions (Kim et al., 2006, 2010a).

Oxidized LDL has been known to be relevant to pathobiological changes of variable human diseases including artherosclerosis, which is mediated by oxidative stress (Steinberg, 1997). Interestingly, the oxidative stress has been thought to play a critical role in the pathogenesis of AMD, for RPE cells are basically prone to oxidative stress from high oxygen tension of high metabolic activity, physiological phagocytosis as well as life-long light illumination (Roth et al., 2004; Feher et al., 2006; Jo et al., 2010). Although transient fluctuations of reactive oxygen species from normal oxidative condition could play some regulatory roles in cellular physiology, abnormally increased and sustained oxidative stress could lead to pathobiological changes including outer BRB breakdown and senescence of RPE cells (Martindale and Holbrook, 2002; Bailey et al., 2004). Therefore, based on our and other researchers' reports that high cholesterol could link to AMD development and progression (Tan et al., 2007; Chakravarthy et al., 2010), oxidized LDL could be causally involved in pathobiological changes of RPE cells (Lee et al., 2007; Kamei et al., 2007; Yu et al., 2009).

In the present study, we for the first time demonstrated that oxidized LDL-induced senescence of RPE cells is followed by outer BRB dysfunction. Interestingly, oxidized LDL promoted advanced senescence of RPE cells and inhibited differentiation of RPE cells. With treatment of oxidized LDL, ROS production as well as expression of inflammatory factors such as tumor necrosis factor (TNF)-α and VEGF was significantly increased, when β-catenin was also up-regulated. Surprisingly, oxidized LDL increased paracellular permeability of RPE cells, when ZO-1 at intercellular junctions markedly decreased. Furthermore, VEGF expression was increased in RPE cells and choriocapillaris of apoE2 eyes compared to control whereas ZO-1 expression was significantly decreased in apoE2 eyes. Taken together, oxidized LDL may promote senescence of RPE cells which would lead to outer BRB dysfunction via oxidative stress and inflammation.

2. Materials and methods

2.1. Cell culture

ARPE-19 cells were used for human RPE cells. ARPE-19 cell was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). According to the standard procedure, the cells were kept in Dulbecco's modified Eagle's medium (Invitrogen, Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), 100 U/ml of penicillin (Sigma–Aldrich, St. Louis, MO, USA), 100 μg/ml of streptomycin (Invitrogen, Gibco, Carlsbad, CA, USA), and 1 mM of sodium pyruvate (Sigma–Aldrich, St. Louis, MO, USA). ARPE-19 cells used in this study were taken from passages 4 to 6.

2.2. Mouse

Human apolipoprotein E2 transgenic mouse (B6.129P2-$^{Apoetm1(APOE2)Mae}$ N8) and C57BL/6J mice were purchased from Taconic (Germantown, NY, USA) and Samtako (Seoul, Korea), respectively. The apoE2 specifically express human apoE2 but not mouse apoE (mapoe$^{-/-}$/hapoe2$^{+/+}$) (Sullivan et al., 1998). Care, use, and treatment of all animals in this study were in strict agreement with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Mice were kept in standard 12-h dark–light cycles and approximately 23 °C room temperature. Ten mice per each group, aged 30–36 weeks, were fed normal chow or chow containing high fat (high-fat diet: 15% cacao butter, 0.5% cholate, 1% cholesterol, 40.5% sucrose, 10% corn starch, 1% corn oil, and 4.7% cellulose) for four weeks. At the end of this period, the enucleated eyes for imunohistochemistry were fixed in 4% paraformaldehyde and subsequently embedded in paraffin.

2.3. Preparation of oxidized LDL

With modification from a previous report (Chen et al., 2003), native LDL (Sigma, St. Louis, MO, USA) was oxidized by exposure to 10 mM $CuSO_4$ in phosphate-buffered saline (PBS) at 37 °C for 24 h. After the oxidation was terminated by adding 0.3 mM EDTA, the preparation was dialyzed and preserved in nitrogen-filled tubes. Protein concentration of the prepared LDL was quantified by a modification of Lowry's method.

2.4. Cell viability assay

Cell viability was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. ARPE-19 cells (1×10^5 cells) were incubated with variable concentrations of oxidized LDL (10–100 μg/ml) for 24 h. The medium was then replaced with fresh medium containing 0.5 mg/ml MTT for 4 h. After incubation, the medium was carefully removed from the plate and dimethyl sulfoxide was added to solubilize formazan produced from MTT by the viable cells. Absorbance was measured at 540 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.5. Senescence-associated β-galactosidase (SA-β-gal) staining

According to a previous report (Dimri et al., 1995), ARPE-19 cells (1×10^5 cells) were incubated with variable concentrations of oxidized LDL (10–50 μg/ml) and fixed with 2% formaldehyde/0.2% glutaraldehyde and incubated under light protection at 37 °C for 8 h with fresh SA-β-gal stain solution containing 1 mg/ml 5-bromo-4-chloro-3-indoyl-b-D-galactopyranoside, 40 mM citric acid/sodium phosphate, pH 6.0/5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, 2 mM $MgCl_2$. The RPE cells with SA-β-gal staining were evaluated by two masked and independent observers (Kim JH and Lee SJ) on randomly selected 10 fields at a ×400 magnification under light microscopy (Carl Zeiss, Chester, VA, USA).

2.6. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

For mRNA measurement of *CRALBP* and *RPE 65*, ARPE-19 cells (1×10^5 cells) were maintained in culture for 1 week or 5 weeks after seeding, and then incubated with 50 μg/ml oxidized LDL. For mRNA measurement of *TNF-α* and *VEGF*, ARPE-19 cells (1×10^5 cells) were incubated with 50 μg/ml oxidized LDL. Total RNA from cells was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First-stranded cDNA was synthesized with 3 μg each of DNA-free total