Supplementation of procyanidins B2 attenuates photooxidation-induced apoptosis in ARPE-19 cells

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ABSTRACT
During the aging process, dimers of dietary vitamin A accumulated in retinal pigment epithelium (RPE) cells. Vitamin A dimer-mediated photooxidation resulted in RPE apoptosis, which is associated with age-related degenerative disease of retina, leading to blindness. It has been reported that proanthocyanidin-rich grape seed extract reduces oxidative stress in the eye. In this study, we investigated the underlying mechanism of photooxidation-induced apoptosis inhibition by procyanidins B2 (PB2), one of the main components of grape seed proanthocyanidin. To mimic vitamin A dimer-mediated photooxidation, ARPE-19 cells that accumulated vitamin A dimer, A2E, were used as a model system. Exposure of A2E loaded ARPE-19 cells to blue light induced ER stress and resulted in significant apoptosis. Pretreatment of blue light-exposed A2E containing ARPE-19 cells with PB2 inhibited apoptosis, increased the ratio of Bcl-2/Bax in the mitochondria, attenuated ROS and cytochrome c release, and decreased caspase cleavage. Additionally, PB2 inhibited the phosphorylation of ER stress markers eIF2α and IRE1α and reduced CHOP expression. Moreover, PB2 inhibition of apoptosis is dependent on the UPR chaperone GRP78, indicating PB2 inhibits vitamin A dimer-mediated apoptosis in RPE cells by activating the UPR.

ARTICLE HISTORY
Received 6 March 2016
Revised 4 May 2016
Accepted 11 May 2016
Published online 2 June 2016

KEYWORDS
Apoptosis; oxidative stress; photooxidation; procyanidins B2; retinal pigment epithelium

Introduction
Retinal pigment epithelium (RPE) senescence is associated with age-related degenerative disease of retina, leading to blindness (Suter et al. 2000). During the aging process, dimers of dietary vitamin A, such as N-retinyleydin-N-retinylethanolamin (A2E), accumulated in RPE cells. Once vitamin A dimerized, the special orientation of polyene chains make them susceptible to blue light, the most phototoxic visible light to which retina is routinely exposed (Sparrow et al. 2000; Mihai & Washington 2014). The blue light induces oxidative degradation of vitamin A dimers, which leads to the formation of oxidation products, such as reactive aldehyde and epoxide toxicant. The photooxidation can induce generation of reactive oxygen species by mitochondrial cytochromes, inhibit cytochrome oxidase, and lead to apoptosis in RPE, which is a major reason responsible for retina degeneration (Wielgus et al. 2010).

The endoplasmic reticulum (ER) is engaged in protein folding and trafficking. Oxidative stress disrupts ER function, which results in ER stress and subsequent activation of unfolded protein response (UPR). The self-protective UPR upregulates UPR chaperones, such as GRP78, which assists in the folding of newly synthesized proteins, and promotes clearance of misfolded proteins to combat ER stress. However, if the duration and intensity of ER stress overwhelms the capacity of the UPR to restore ER homeostasis, apoptotic pathways are activated (Rao et al. 2004). Severe and prolonged ER stress is implicated in many human diseases including degenerative disease of retina (Zhang et al. 2014). Activation of the UPR pathways plays a pivotal role in maintaining ER function and homeostasis and may prevent the onset and progression of retina degeneration.

Investigators have studied multiple approaches to mitigating photooxidative damage to eyes. RPE cell apoptosis induced by photooxidative stress can be alleviated by dietary antioxidants. Among these antioxidants, proanthocyanidin-rich grape seed extract, a dietary antioxidant consumed worldwide, provides excellent protection against free radicals (Bagchi et al. 2002; Josepa Salvado et al. 2015; Xi et al. 2015). Accumulating evidence demonstrates the eye protective effect of grape seed proanthocyanidin. Yamakoshi et al.
showed that grape seed extract significantly prevented and postponed development of cataract formation in rats (Yamakoshi et al. 2002; Ritch 2007). Also, grape seed extract reduced oxidative stress-induced apoptosis in the retinal ganglion cell line sssRGC-5 (Yang et al. 2012). In addition, grape seed extract showed neuroprotective effects against oxidative stress in the rat central nervous system (Feng et al. 2005). Dimeric procyanidins B2 (PB2) is one of the main components of grape seed proanthocyanidin, composed of two molecules of the flavan-3-ol (2)-epicatechin linked by a 4b→8 bond (Rodriguez-Ramiro et al. 2012). It has been reported that PB2 provides potent biological activities and health benefits (Stoupi et al. 2010), including anti-inflammatory activity (Lei et al. 2014), reducing oxidative stress, etc. (Fernández-Iglesias et al. 2014). Moreover, grape seed proanthocyanidin extracts were reported to alleviate ER stress (Ding et al. 2013). Based on these reports, we hypothesized that PB2 may protect RPE cells against apoptosis induced by photooxidative damage through inhibition of ER stress. To investigate this hypothesis, ARPE-19 cells that accumulate vitamin A dimer, A2E, were used as a model system to mimic vitamin A dimer-mediated photooxidation. Briefly, A2E was supplemented in culture media to facilitate uptake into cultured ARPE-19 cells at comparable levels to RPE cells in healthy culture media to facilitate uptake into cultured ARPE-19 cells. A2E was delivered at concentrations of 25 μM in culture media. Confluent cultures were incubated with A2E for 2 h, washed with PBS to remove any A2E that had not been internalized, and cultured in DMEM/F12 for 24 h. The medium was then changed to DMEM/F12 with 10% fetal bovine serum (FBS). For uptake into cultured ARPE-19 cells, A2E was delivered at concentrations of 25 μM in culture media. Confluent cultures were incubated with A2E for 2 h, washed with PBS to remove any A2E that had not been internalized, and cultured in DMEM/F12 for 24 h. The medium was then changed to DMEM/F12 with 10% FBS. Cells were incubated for additional 12 h after exposure to blue light (2000 Lux) for 30 min in the presence or absence of PB2. Cells that had neither accumulated A2E nor exposed to blue light were used as controls.

**Cell apoptosis assays**

ARPE-19 cells were grown to reach confluence in 6-cm dishes and treated with A2E as described above. Apoptotic cells were evaluated by flow cytometry. As A2E exhibited autofluorescence with the emission maximum of 565–570 nm, APC (excitation at 633 nm, emission at 660 nm) and DAPI (excitation at 340 nm, emission at 488 nm) were used to detect apoptotic cells, which were unaffected by A2E fluorescence (Sparrow et al. 2000). Briefly, cells were detached from the dish and incubated with APC Annexin V (5%) and DAPI (0.05 μg/ml) for 5 min. Cells were analyzed using a BD LSRFortessa™ cell analyzer (BD, Franklin Lakes, NJ), and the collected data were analyzed with FACSDiva Version 6.2.

**RNA interference-based gene silencing experiment**

shRNA sequences targeting HSPA5 (GRP78) were synthesized and inserted into pGLV3/H1/GFP + Puro vector (GenePharma, Shanghai, China). The shRNA sequences used for HSPA5 silencing were as follows: 5′-GATCCGGAACCTTGAGAAGAAAATTTGCTA TTCAG AGATAGAACAATTTTCTCCAGTTCCT TTTTGTG-3′ and 5′-AATTCAGA AGGAACTTGAAGAAATTTGTTCATCTTTGAAATGACACATTCTTCTCCAGTTCG-3′. The shRNA sequences used for negative control (NC) were as follows: 5′-GATC CGTTCGAGAAGAAAATTTGCTA TTCAG AGATAGAACAATTTTCTCCAGTTCCT TTTTGTG-3′ and 5′-AATTCAGA AGGAACTTGAAGAAATTTGTTCATCTTTGAAATGACACATTCTTCTCCAGTTCG-3′.

**Materials and methods**

**Materials**

Grape seed PB2 (>90%) was obtained from JF-Natural Corporation (Tianjin, China). A stock solution of PB2 was prepared in dimethyl sulfoxide (DMSO) and diluted with Dulbecco’s modified Eagle medium (DMEM). The highest DMSO concentration in the final solution was 0.1%. Antibodies against GRP78, CHOP, Bcl-2, Bax, caspase-9 and caspase-3 were from Cell Signaling Technology (Beverly, MA). IRE1α antibody was from Novus Biologicals (Littleton, CO). elf2α antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). APC Annexin V was purchased from BD Biosciences (Franklin Lakes, NJ) and DAPI was from Sigma-Aldrich (St. Louis, MO).

A2E was prepared as described by Parish et al. (1998) and HPLC chromatograms of synthesized A2E was given in our previous report (Feng et al. 2014). A2E was stored as a stock solution in DMSO and kept at −80°C in the dark. ARPE-19 cells (American Type Culture Collection, Manassas, VA) were grown in DMEM/F12 (volumetric ratio of 1:1; Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (FBS). For uptake into cultured ARPE-19 cells, A2E was delivered at concentrations of 25 μM in culture media. Confluent cultures were incubated with A2E for 2 h, washed with PBS to remove any A2E that had not been internalized, and cultured in DMEM/F12 for 24 h. The medium was then changed to DMEM/F12 with 10% FBS. Cells were incubated for additional 12 h after exposure to blue light (2000 Lux) for 30 min in the presence or absence of PB2. Cells that had neither accumulated A2E nor exposed to blue light were used as controls.

**A2E synthesis and treatment**

The shRNA sequences used for negative control (NC) were as follows: 5′-GATCCGGAACCTTGAGAAGAAAATTTGCTA TTCAG AGATAGAACAATTTTCTCCAGTTCCT TTTTGTG-3′ and 5′-AATTCAGA AGGAACTTGAAGAAATTTGTTCATCTTTGAAATGACACATTCTTCTCCAGTTCG-3′. The shRNA sequences used for negative control (NC) were as follows: 5′-GATC CGTTCGAGAAGAAAATTTGCTA TTCAG AGATAGAACAATTTTCTCCAGTTCCT TTTTGTG-3′ and 5′-AATTCAGA AGGAACTTGAAGAAATTTGTTCATCTTTGAAATGACACATTCTTCTCCAGTTCG-3′. The shRNA sequences used for negative control (NC) were as follows: 5′-GATC CGTTCGAGAAGAAAATTTGCTA TTCAG AGATAGAACAATTTTCTCCAGTTCCT TTTTGTG-3′ and 5′-AATTCAGA AGGAACTTGAAGAAATTTGTTCATCTTTGAAATGACACATTCTTCTCCAGTTCG-3′.
GAACG-3′. The nucleotides specific to GRP78 and NC are shown in italics. Virus packaging was performed in 293T cells after cotransfection of target plasmids using Lipofectamine 2000 (Invitrogen). Viruses were harvested at 72 h after transfection. The ARPE-19 cells (10 × 105) were infected with the filtered lentivirus and 0.5 µg/ml polybrene (Sigma-Aldrich). The transfected cells were screened with 5 µg/ml puromycin (Sangon, Shanghai, China).

**Western blotting**

For preparation of whole cell extracts, cells were harvested and then lysed in RIPA buffer (Beyotime Inst. Biotech, Shanghai, China). After centrifugation, supernatants containing total cellular proteins were collected. The protein concentrations were determined using a BCA protein assay kit (Beyotime). Cytoplasmic and mitochondrial extracts were isolated separately using Beyotime Nuclear and Cytoplasmic, and Mitochondria Extraction Reagents (Beyotime Inc., Nantong, Jiangsu, China) according to the manufacturer’s instructions. For protein immunoblot analysis, an appropriate amount of lysate was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes. Membranes were incubated with target protein specific antibodies overnight at 4°C, followed by incubation with HRP-conjugated secondary antibodies for 1 h. Peroxidase activity was visualized using an enhanced chemiluminescence (ECL) kit (Bio-Rad, Hercules, CA) with a Chemi™ Doc XRS (Bio-Rad). For equal loading and normalization purpose, β-actin was used as an internal control.

**RNA extraction and real-time PCR**

Total RNA was isolated from cells using TRIzol reagent and reverse-transcribed using High-Capacity cDNA Reverse Transcription Kits (Invitrogen, Carlsbad, CA). Real-time PCR was performed using ABI 7900 HT Real Time PCR System (Applied Biosystems, Carlsbad, CA). PCR amplification was performed using initial 2 min step at 50°C, 10 min denaturing step at 95°C, followed by two-step PCR comprising 15 s at 95°C and 1 min at 60°C, with 40 cycles for Bax, CHOP and β-actin. For Bax, the forward primer was 5′-CTCAGGATGGTCCACCAAGA-3 and the reverse primer was 5′-CGGCGGTCGCAAAATGAGA-3′. For CHOP, the forward primer was 5′-CCACAGAGGTCACCAGACGC-3′ and the reverse primer was 5′-CGCACGTGACCATCTCTGTTC-3′. Results were calculated based on the 2−ΔΔCT method. Relative mRNA levels were normalized to those of β-actin and described as the fold change from the normal control group.

**Flow cytometric analysis of intracellular ROS**

ARPE-19 cells were treated as described in “A2E synthesis and treatment”, then stained with 2.5 µM dihydroethidium (DHE, Invitrogen) at 37°C for 30 min, and harvested. The pellets were suspended in PBS. The fluorescence intensity in cells was determined by flow cytometry (BD, Franklin Lakes, NJ). Excitation wavelength was at 480–535 nm and emission wavelength was at 590–610 nm.

**Statistical analysis**

One-way analysis of variance (ANOVA) followed by Tukey’s test was used to statistically evaluate differences in apoptosis, protein expression of apoptosis or ER markers, and ROS level. Statistical analysis was performed using SPSS Statistics 17.0. p Values <0.05 were considered to be statistically significant.

**Results**

**Procyanidins B2 inhibits vitamin a dimer-mediated RPE cell apoptosis**

To determine whether PB2 inhibits apoptosis, we assessed apoptosis in a model cell system to mimic vitamin A dimer-mediated photooxidation. A2E was delivered in culture media at concentrations of 25 µM and ARPE-19 cells containing accumulated A2E were subsequently exposed to blue light. Apoptosis was assessed by flow cytometer, and double staining of APC and DAPI were used. Although apoptotic cells did not significantly segregate as another cell population due to the effect of A2E fluorescence, apoptosis could be quantified by flow cytometer. Significant apoptosis occurred in A2E loaded cells exposed to blue light, and blue light alone did not induce apoptosis. PB2 inhibited apoptosis of RPE cells induced by photooxidation in a dose dependent manner up to 0.1 µM (30%, p < 0.05). However, apoptosis inhibition gradually decreased when the concentration of PB2 was higher than 0.1 µM (Figure 1(A,B)).

**Procyanidins B2 suppressed photooxidation-induced activation of the mitochondrial apoptosis pathways**

To investigate the effect of PB2 on mitochondrial apoptosis proteins, A2E-loaded ARPE-19 cells were supplemented with PB2 before exposure to blue light and expression levels of mitochondrial apoptosis pathways.
proteins were detected. As shown in Figure 2, supplementation with PB2 attenuated the increase of mRNA Bax expression induced by photooxidation, although this was statistically insignificant (Figure 2(A)), and increased the ratio of Bcl-2/Bax protein expression in mitochondria (Figure 2(B,C)). Moreover, PB2 reduced cytochrome c release (Figure 2(D,E)). Consistent with the increase in cytosolic cytochrome c, exposure to blue light led to increased caspase-9 and caspase-3 cleavage in ARPE-19 cells loaded with A2E compared to control cells. Importantly, caspase-3 and -9 cleavage induced by photooxidation were reversed by PB2 (Figure 2(F,G)). In addition, A2E-loaded cells showed an apparent increase in intracellular ROS levels after exposure to blue light, which demonstrated that blue light may induce generation of reactive oxygen species by mitochondrial (Chen 1993). ROS elevation was attenuated by PB2 treatment (Figure 3(A,B)). These data suggest that photooxidation-induced ER stress in RPE cells was attenuated by PB2.

**Procyanidins B2 alleviates photooxidation-induced ER stress**

In response to oxidative stress, accumulation of unfolded or misfolded proteins triggers a cellular adaptive response known as ER stress. However, if ER stress is severe or prolonged, a proapoptotic pathway is triggered. In the present study, phosphorylation and expression of the ER stress markers elF2α, IRE1α and CHOP/GADD153 in ARPE-19 were investigated by Western blot. As shown in Figure 4, phosphorylation of elF2α and IRE1α, and CHOP protein expression increased in cells treated with A2E and subsequently exposed to blue light compared to control, untreated cells. Importantly, PB2 decreased both CHOP mRNA and protein expression, and attenuated IRE1α and elF2α phosphorylation induced by photooxidation. These data suggest that photooxidation-induced ER stress in RPE cells was attenuated by PB2.

**Procyanidins B2 inhibits photooxidation-induced apoptosis via activating the UPR**

To restore ER homeostasis, the ER triggers the UPR, which selectively promotes transcription of the machinery required for protein folding and degradation (Sano & Reed 2013), including the UPR chaperone, GRP78/BiP. To determine whether PB2 promotes induction of the UPR, GRP78/BiP was monitored. PB2 supplementation resulted in increased GRP78 expression in A2E loading cells exposed to blue light (Figure 5(A,B)). To determine whether PB2 inhibited apoptosis by promoting GRP78 expression, we silenced GRP78
Figure 2. PB2 suppressed photooxidation-induced activation of mitochondrial apoptosis pathways. ARPE-19 cells were treated with A2E and incubated for 12 h after exposure to blue light in the presence and absence of PB2, and changes in mRNA were detected by real-time PCR (A) and protein expression was detected by Western blot. The cells were lysed, and cytoplasmic and mitochondrial extracts were isolated separately. Mitochondrial protein fractions were used to detect Bax and Bcl-2 expression levels (B and C). Cytoplasmic protein fractions were used to detect cytochrome c release (D and E). Total cell lysate was used to detect caspase-9, cleaved caspase-9, caspase-3 and cleaved caspase-3 (F and G). Values are mean ± SD of three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 relative to control cells that were treated with neither A2E nor blue light; #p < 0.05; ##p < 0.01 relative to cells that had been loaded with A2E and exposed to blue light.
with shRNA (Figure S1). Silencing GRP78 abrogated PB2 apoptosis inhibition (Figure 5(C,D)). In addition, to clarify whether PB2 treatment alone induced GRP78 protein expression, we investigated the dose-dependent expression level of GRP78 in ARPE-19 cells treated with PB2. As shown in Figure 5(E,F), GRP78 expression was not upregulated after PB2 treatment. These results suggest that PB2 inhibits photooxidation-induced apoptosis in a GRP78-dependent manner.

**Discussion**

Dimerization of vitamin A and its accumulation in RPE are common chemical events that occur at the early stage of some age-related-neurodegenerative retinal disorders (Mihai & Washington 2014). The dimerized vitamin A, such as A2E, mainly accumulates in periphery of retina with age (Ablonczy et al. 2013; Pallitto et al. 2015) and can induce protracted cell death. Also, the dimerized vitamin A can act as a photooxidizing agent in the presence of short wavelength (400–430 nm) blue light, which induces injury of RPE cells (Schutt et al. 2000). The photodamage observed in RPE cells is oxygen-dependent and is diminished by antioxidants, which suggests that the blue light effect is associated with oxidative stress (Wielgus et al. 2010). Although dimerized vitamin A may photogenerate a little amount of reactive oxygen species (Cantrell et al. 2001), the formation of oxidation products is highly toxic to RPE cells (Wielgus et al. 2010).

Oxidative stress and ER stress are interrelated biological events and both are implicated in retina degeneration. Oxidative stress triggers ER stress in retinal cells (Cano et al. 2014) and ER stress also enhance the production of reactive oxygen species. During ER stress, the UPR chaperone, GR78/BiP, dissociates from and activates PERK/eIF2α, IRE1 and ATF6 to initiate a survival signaling pathway. If ER stress is irreversible, CHOP is activated resulting in apoptosis (Sato et al. 2000). We found that vitamin A dimer-mediated photooxidation-induced severe ER stress in RPE cells, which was supported by increased expression of p-IRE1α, p-eIF2α and CHOP in A2E-loaded ARPE-19 cells after exposure to blue light. ER stress can regulate apoptosis-associated proteins that localize on the mitochondrial membrane, including the Bcl-2 family members. We also found that exposure of vitamin A dimer-loaded ARPE-19 cells to blue light decreased the ratio of Bcl-2/Bax expression in mitochondria, induced cytosolic cytochrome c and caspase cleavage, and increased ROS production, resulting

**Figure 3.** PB2 attenuated photooxidation-induced ROS elevation. ARPE-19 cells were treated with A2E and incubated for 12h after exposure to blue light in the presence and absence of PB2. Intracellular ROS levels were measured using DHE staining and fluorescence intensity in cells was determined by flow cytometry (excitation at 480–535 nm, emission at 590–610 nm). Values are mean ± SD of three independent experiments carried out in triplicated. *p < 0.05 relative to control cells that were treated with neither A2E nor blue light; #p < 0.05 relative to cells that had been loaded with A2E and exposed to blue light.
in significant apoptosis. These results suggest that vitamin A dimer-mediated photooxidation in RPE cells induces ER stress which activates the mitochondrial apoptosis pathway.

Furthermore, results from this study showed that PB2 treatment attenuated photooxidation induced apoptosis in RPE cells, consistent with studies investigating PB2 in other cell types. Li et al. (2011) demonstrated that grape seed procyanidin B2 significantly inhibited human umbilical endothelial cell apoptosis and ROS production. Rodriguez-Ramiro similarly demonstrated that PB2 reduced oxidative stress in human colonic cells (Rodriguez-Ramiro et al. 2011). It is also known that there is a direct link between oxidative stress, mitochondria malfunction and cell death (Ott et al. 2007). Bcl-2 is an antiapoptotic protein located in mitochondrial membrane, and inhibits ROS production induced by mitochondria malfunction. Thus, Bcl-2 blocks the effect of ROS on mitochondrial membrane permeability and cytochrome c release (Hochman et al. 1998). We found that PB2 increased Bcl-2 expression in the mitochondria and reduced ROS and cytochrome c release, indicating PB2 inhibited mitochondrial apoptosis pathway activated by photooxidation. Additionally, PB2 attenuated IRE1α and eIF2α phosphorylation induced by photooxidation. Phosphorylated IRE1α stimulates activation of JNK and p38 MAPK, inducing CHOP activation (Ron &
Phosphorylation of eIF2α promotes translation of UPR-dependent proteins including ATF4, which also increases CHOP translation (Sano & Reed 2013). As a downstream target of eIF2α and IRE1α, CHOP expression was also decreased by PB2. The present study indicates that PB2 inhibition of photioxidation-induced apoptosis in RPE cells is related to attenuating ER stress. PB2 also significantly increased GRP78/Bip expression, an UPR chaperone with antiapoptotic properties. GRP78 may interact with apoptosis pathway by blocking caspase activation and reducing CHOP expression (Gorbatyuk et al. 2010). A role for the UPR in oxidative stress control and cell survival has been demonstrated in RPE (Chen et al. 2014; Zhang et al. 2014). PB2 inhibited apoptosis in a GRP78-dependent manner, suggesting UPR response is required. These results were also supported by the reduced caspase cleavage and CHOP expression in vitamin A dimer-loaded ARPE-19 cells treated with PB2 before exposure to blue light. However, our results are inconsistent with some reports showing that antioxidants attenuate oxidative stress-induced upregulation of GRP78. For example, N-Acetylcysteine increases H₂O₂ exposed mouse corneal endothelial cell survival when GRP78 was downregulated (Kim et al. 2014). Ginkgolide B, an active component of Ginkgo biloba, attenuated ROS-mediated upregulation of GRP78 in SH-SYSY cells and protected against cell apoptosis (Li et al. 2013). The different effects of antioxidants on GRP78 might be due to cell-type-specific differences in apoptosis responses, stimuli, or the contexts of experimental conditions, and should be investigated further. Moreover, PB2 did not increase GRP78 expression in ARPE-19 cells that were treated with neither A2E nor blue light, indicating that PB2 may not induce ER stress and UPR under normal physiological conditions.

It has been reported that PB2 and its metabolites are present in serum after ingestion (Serra et al. 2011; Margalef et al. 2014). Baba et al. showed that after PB2 administration, PB2 is absorbed and excreted in urine, and a portion of the PB2 is degraded to epicatechin and to the metabolized conjugated and/or methylated epicatechin internally in the rat (Baba et al. 2002). Moreover, Spencer et al. showed that epicatechin and a small amount of non-methylated and methylated procyanidin dimer were detected on the serosal side by perfusion of the
small intestine with PB2 (Spencer et al. 2001). A major portion of ingested procyanidins is degraded by human microbiota in the colon into various phenolic compounds. Ou et al. identified 5-(3',4'-dihydroxyphenyl)-γ-valerolactones and 5-(3'-hydroxyphenyl)-γ-valerolactones as the microbial metabolites of procyanidin B2 after anaerobic fermentation with human microbiota (Ou et al. 2014). Recently, Wiese et al. showed that 5-(3',4'-dihydroxyphenyl)-α-valerolactones as the microbial metabolites of procyanidin B2 after anaerobic fermentation with human microbiota (Ou et al. 2014). Recently, Wiese et al. showed that 5-(3',4'-dihydroxyphenyl)-γ-valerolactone represents an important in vivo metabolite of procyanidin produced by the gut microbiota (Wiese et al. 2015). These results suggest that procyanidin oligomers are utilized in the body. However, little is known about retinal concentrations of PB2 and its metabolites after dietary intake of procyanidins, and need to be determined in future studies.

Conclusion
Supplementation of PB2 protected RPE cells from vitamin A dimer-mediated apoptosis by activating the UPR response, indicating PB2 and grape seed extracts rich in PB2 may be utilized as part of diet to reduce the risk of age-related degenerative disease of retina.

Disclosure statement
The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

Funding information
This study was supported by the National Natural Science Foundation of China (No. 31271843).

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