

# EGCG protects against UVB-induced apoptosis via oxidative stress and the JNK1/c-Jun pathway in ARPE19 cells

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Received April 7, 2011; Accepted August 31, 2011

DOI: 10.3892/mmr.2011.582

**Abstract.** Ultraviolet B (UVB) radiation is part of the spectrum of light produced by the sun. This form of radiation has been implicated as one of the potential etiological factors causing age-related macular degeneration (AMD). Oxidative injury to the retinal pigment epithelium (RPE) has also been thought to play a key role in AMD. The aim of the present study was to determine the mechanism by which UVB causes damage to the RPE cells, whether it occurs through oxidative stress and the mitogen-activated protein kinase (MAPK) pathway and whether the green tea extract, (-)-epigallocatechin gallate (EGCG), has a protective role. Cell viability assays were used to determine the viability of the cells under different conditions. Cell death caused by apoptosis was determined using fluorescein isothiocyanate conjugated-annexin V/PI labeling, followed by flow cytometry. Intracellular reactive oxygen species (ROS) levels were measured by flow cytometry. Western blot analysis was used to detect UVB-induced MAPK signaling pathways. The findings showed that UVB induced apoptosis, which increased intracellular ROS in ARPE19 cells. Inhibition of c-Jun NH2-terminal kinase (JNK) with a specific inhibitor augmented this apoptosis, and anisomycin (an activator of JNK) attenuated this apoptosis. In addition, UVB decreased the phosphorylation of JNK1 and c-Jun. Finally, EGCG

reduced the ROS generation and apoptosis, and also partially blocked the decreased phosphorylation of JNK1 and c-Jun by UVB irradiation. The findings show that UVB irradiation is able to induce apoptosis in ARPE19 cells through oxidative stress, but EGCG treatment attenuates this damage. In this situation, the JNK pathway plays an anti-apoptotic role. The use of selective activators or antioxidants may be useful in reducing the oxidative damage occurring in AMD.

## Introduction

Ultraviolet (UV) radiation is part of the sunlight spectrum and is divided into three bands (UVA, UVB and UVC). The damaging role of UVB has been extensively studied in skin pathologies due to the high risk of exposure of the skin to UVB. The eye is another organ exposed to UVB, as the cornea and lens largely filter and dilute UVB radiation that enters the eye. It is commonly, and erroneously, assumed that only a very small proportion reaches the retina (1). For this reason, little is known about the role of UVB radiation to the retina. However, as the depletion of the ozone layer increases, there is a considerable increase in the accumulated lifetime exposure of the retina to UVB, especially after cataract removal (2). Thus, we believe that UVB can greatly affect the retina.

Retinal pigment epithelial (RPE) cells, which are located in the outermost layer of the retina, are critical for the normal functioning and health of photoreceptors (3). It has been suggested that RPE degeneration or death is one of the leading early causes of age-related macular degeneration (AMD), which is the leading cause of blindness in individuals over 50 years of age. Light-induced pathological changes to the retina mainly occur in the RPE cells and photoreceptors. In this setting, the RPE cells serve to protect the retina, where they selectively absorb the lower wavelength light particles (4). Therefore, we believe that RPE cells may be the main target of UVB light reaching the retina.

The mitogen-activated protein kinases (MAPKs) are a part of a phosphorylation cascade, which includes the extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK) and p38 MAPK. The ERK pathway plays a major role in regulating cell growth, survival and differentiation (5), whereas

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**Key words:** epigallocatechin gallate, ultraviolet B, mitogen-activated protein kinase, retinal pigment epithelial cells, cell apoptosis

JNK and p38 are activated by stress signals, and the activation of these kinases is strongly associated with cell apoptosis (6,7). These UV-activated signal transduction pathways are mediated primarily through signaling cascades involving MAPKs (8), and MAPKs are activated in the UVC-induced apoptosis of ARPE19 cells (9).

Oxidative stress, which refers to cellular damage caused by reactive oxygen species (ROS), has been implicated in many diseases, especially age-related disorders, including AMD. Studies have shown that photochemical retinal injury can be ascribed to oxidative stress, and that the antioxidant vitamins C, E and A protect against photochemical retinal injury (10). The green tea extract, (-)-epigallocatechin gallate (EGCG), is a type of antioxidant which protects several cell types from oxidative stress. Our previous study also showed that EGCG may protect human lens epithelial cells (HLECs) against UVB irradiation-induced oxidative stress damage (11).

In this study, we investigated the effect of UVB radiation and MAPK signaling pathways, as well as whether oxidative stress and EGCG play a role in UVB-induced reaction in RPE cells.

## Materials and methods

*UV light apparatus.* Consistent with previous studies (12), the UV irradiation apparatus used in this study consisted of four F36T12 UV tubes. A Kodacel TA401/407 filter was mounted 4 cm in front of the tubes to remove wavelengths of <290 nm (UVC). Irradiation intensity was monitored using an IL443 phototherapy radiometer and a SED240/UV/W photodetector. Before UV irradiation, the cells were washed twice with pre-warmed PBS and a thin layer of PBS was left. The cells were irradiated at the desired intensity without a plastic dish lid. After UV irradiation, the cells continued to be incubated in basal Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM/F12; Gibco Life Technologies) containing the treatments for the indicated time periods.

*Cell culture.* Human RPE cells (ARPE19 cell line) were obtained from Dr Fu Shang at Tufts University, Boston, MA, USA. The spontaneously arising human RPE cell line, ARPE19, was used as the model to study the relationship between RPE and UVB. The ARPE19 cells were maintained in DMEM/F12 supplemented with 10% fetal bovine serum (Hyclone), penicillin/streptomycin (1:100; Sigma), 4 mM L-glutamine and 0.19% HEPES, in a humidified incubator at 37°C and 5% CO<sub>2</sub>. The cells were reseeded and, upon reaching 70-80% confluence, they were starved in serum-free DMEM/F12 overnight before further treatment.

*Flow cytometry analysis of apoptosis.* With or without pre-treatment, the cells were exposed to UVB and incubated in serum-free DMEM/F12 for an additional 24 h. The cells were trypsinized, centrifuged, washed and resuspended twice in warm PBS for the quantitative analysis of apoptosis. The harvested cells were incubated for 10 min with annexin V and PI in calcium-containing HEPES buffer (Apoptosis Detection kit; Becton-Dickinson, Franklin Lanes, NJ, USA), then immediately analyzed with a FACS machine (Becton-Dickinson).

*Measurement of ROS.* The cultured ARPE19 cells were deprived of serum overnight and pre-treated with EGCG (5 µg/ml) for 2 h, then exposed to UVB irradiation. The control cells did not receive EGCG and/or UVB irradiation. After the cells were incubated in the serum-free DMEM/F12 medium for 30 min, they were treated with 10 µM of the fluorescent dye, 2,7-dichlorofluorescein diacetate (DCFH-DA; Reactive Oxygen Species Assay kit; Beyotime, China), that reacted with the ROS in the cells and resulted in fluorescence. The generation of ROS was measured by FACS analysis. Induction of ROS generation was expressed in arbitrary units.

*Cell viability assays.* The viability of the cells was determined by a 3, 4, 5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) reduction assay. Briefly, following pre-treatment, the cells were exposed to UVB and incubated for an additional 24 h (the control cells were not treated). After a brief wash with the medium, 0.5 mg/ml MTT in DMEM/F12 were used for the quantification of the living metabolically active cells. Mitochondrial dehydrogenase metabolizes MTT to form a purple formazan dye, which can be analyzed photometrically at 490 nm.

*Western blot analysis.* The treated (or untreated) cultured ARPE19 cells were washed with cold PBS and lysed in RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% NP40, 1 mM EDTA, 0.25% sodium deoxycholate, phosphatase inhibitors and complete protease inhibitors. After incubation on ice for 30 min, the cells were scraped off and the lysate was centrifuged (13,000 x g for 10 min at 4°C), and 60 µg of protein were separated by 10% SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked for 60 min in blocking buffer (Tris-buffered saline, pH 7.6, 0.1% Tween and 5% nonfat dry milk) and incubated with anti-phospho-JNK (1:500), anti-JNK (1:500; both from Cell Signaling Technology, Inc., Beverly, MA, USA), anti-phospho-c-Jun (1:200), anti-c-Jun (1:500; both Santa Cruz Biotechnology, CA, USA). After incubation with the appropriate secondary anti-IgG-horseradish peroxidase conjugate, antibody binding was developed with SuperSignal chemiluminescent substrate (Pierce, Rockford, IL, USA).

*Statistical analysis.* Values were expressed as the means ± standard deviation (SD). Statistical analysis of the data between the control and treated groups was performed by a Student's t-test. Values of P<0.05 were considered to be significant.

## Results

*UVB induces apoptosis of cultured ARPE19 cells.* To study the impact of UVB radiation on ARPE19 cells, the cultured ARPE19 cells were exposed to a number of doses of UVB radiation (0, 5, 50, 100 and 200 mJ/cm<sup>2</sup>). Apoptosis was determined 24 h after UVB irradiation using fluorescein isothiocyanate (FITC) conjugated-annexin V/PI labeling followed by flow cytometry. The percentage of apoptosis for the different doses of radiation was 5.08, 5.5, 12.21, 24.63 and 30.76%, respectively. It was observed that UVB induced apoptosis in the ARPE19 cells in a dose-dependent manner.

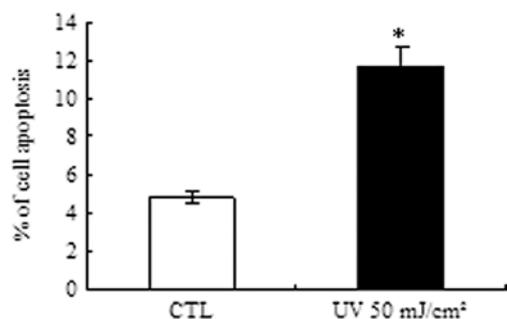


Figure 1. UVB induced apoptosis of cultured ARPE19 cells. The ARPE19 cells were cultured as described in Materials and methods. The cells were exposed to UVB radiation (50 mJ/cm<sup>2</sup>). The treated cells were then incubated in serum-free medium for 24 h. Cell apoptosis was determined by flow cytometry. The results are represented as the means  $\pm$  SEM determined from three independent experiments. The P-value is for comparison between the control and UVB-irradiated cells. \*P<0.001 vs. control.

Fig. 1 shows that there was a significant increase in apoptosis in the cells that received a UVB dose of 50 mJ/cm<sup>2</sup>. Thus, this dosage was selected for use in subsequent studies.

*Inhibition of JNK augments UVB-induced ARPE19 cell apoptosis and activation of JNK reduces this apoptosis.* Several roles have been ascribed to MAPKs, including modulating apoptosis. To determine whether the inhibition of the MAPKs would alter the survival of cells, the cells were pre-treated with specific JNK, p38 and ERK inhibitors, known as SP600125, SB203580 and PD98059, respectively (Enzo Life Science, Switzerland) at different concentrations (1, 5, 10 and 20  $\mu$ M) for 1.5 h before irradiation. Cell viability was measured by the MTT assay and cell apoptosis was determined using flow cytometry. Fig. 2A-C shows that SP600125, an inhibitor of JNK, augments UVB-induced ARPE19 cell death at a low concentration (5  $\mu$ M). The inhibition of p38 and ERK had no effect on cell survival (Fig. 2E and F). Furthermore, the ARPE19 cells were pre-treated with the specific JNK activator, anisomycin (2  $\mu$ g/ml), for 1.5 h before irradiation. Fig. 2D shows that the JNK activation reduced UVB-induced apoptosis in the ARPE19 cells. These results suggest that JNK may play an anti-apoptotic role in UVB irradiation.

*UVB increases ROS production and EGCG decreases UVB-induced ROS production and cell apoptosis in ARPE19 cells.* Numerous studies have shown that oxidative stress is involved in the cell damage that occurs during exposure to UV irradiation. We have previously investigated whether UVB increases the ROS production in HLECs and whether the antioxidant, quercetin, and EGCG have any protective effect against UVB-induced cataract formation (12). We therefore tested whether UVB radiation can increase intracellular ROS production and whether EGCG is capable of inhibiting it. The amount of intracellular ROS in the ARPE19 cells was measured using DCFH-DA, a dye that has been shown to react with ROS and is extensively used for the detection of intracellular ROS. Fluorescence microscopy showed that intracellular fluorescence was significantly enhanced after UVB exposure, but was reduced as a result of EGCG treatment (Fig. 3A). Flow cytometry revealed that mean fluorescence (i.e., intracellular

ROS production) was increased in the UVB-exposed cells, whereas the increase in intracellular ROS was inhibited through treatment with EGCG (Fig. 3B and C). In addition, treatment of the cells with EGCG inhibited UVB-induced cell apoptosis in the ARPE19 cells (Fig. 3D). These results suggest that UVB irradiation activates oxidative stress and that oxidative stress pathways may play a role in UVB-induced apoptosis in RPE cells.

*EGCG inhibits the UVB-induced JNK1 decrease in ARPE19 cells.* Activation of the JNK pathway is strongly associated with cell apoptosis induced by stressful stimuli (7). However, in our experiments, the inhibition of JNK augmented UVB-induced apoptosis in ARPE19 cells. Therefore JNK may play an anti-apoptotic role in UVB irradiation. Consequently, we wanted to investigate the state of JNK after UVB irradiation. The UVB-exposed cells were harvested after incubation for 24 h and analyzed by Western blot analysis. As shown in Fig. 4A, JNK1 phosphorylation was decreased in UVB-irradiated ARPE19 cells, but there was no significant change in JNK2 phosphorylation. Incubating the cells with EGCG only partially inhibited the UVB-induced decrease in JNK1. This result is consistent with our previous findings and also demonstrates that JNK, especially JNK1, may play an anti-apoptotic role in UVB irradiation. Furthermore, the inhibitory effect of EGCG indicates that oxidative stress plays a role in this process.

*EGCG inhibits the UVB-induced c-Jun decrease in ARPE19 cells.* It has been shown that c-Jun is the principal transcription factor mediating gene regulation by JNK, whereby JNK induces c-Jun transactivation by phosphorylating the c-Jun N-terminal domain, mainly at serines 63/73 (13). It has been widely reported that the JNK-c-Jun pathway contributes to stress-induced apoptosis in a number of different cell types (14). Thus, we also detected the state of c-Jun after UV irradiation. Treated cells were harvested after 24 h and analyzed by Western blot analysis. Fig. 4B shows that c-Jun phosphorylation decreased following UVB irradiation and that the EGCG treatment partially attenuated this decrease.

## Discussion

It is well known that light triggers the visual transduction cascade, but also induces cell damage and death through phototoxic mechanisms. However, despite more than 40 years of research, there is no comprehensive understanding of this. Because of similar end-point morphologies, retinal light damage has served as a model for human retinal degenerative diseases. For example, there are notable similarities between late-stage retinal cell remodeling in light-damaged rodent retinas and the anatomical changes found in advanced atrophic AMD (15). Sunlight irradiation causes damage which accumulates during a lifetime. There is an increasing body of evidence that places the damage caused by sun radiation as one of the prime etiological factors causing AMD. The UVB band is part of the sunlight spectrum and is a constant feature of our living environment. Experiments have shown that the retina is damaged as a result of exposure to sunlight, even though less than 1% of the solar UVB light penetrates it. In our experiments, we used a low dose of UVB irradiation

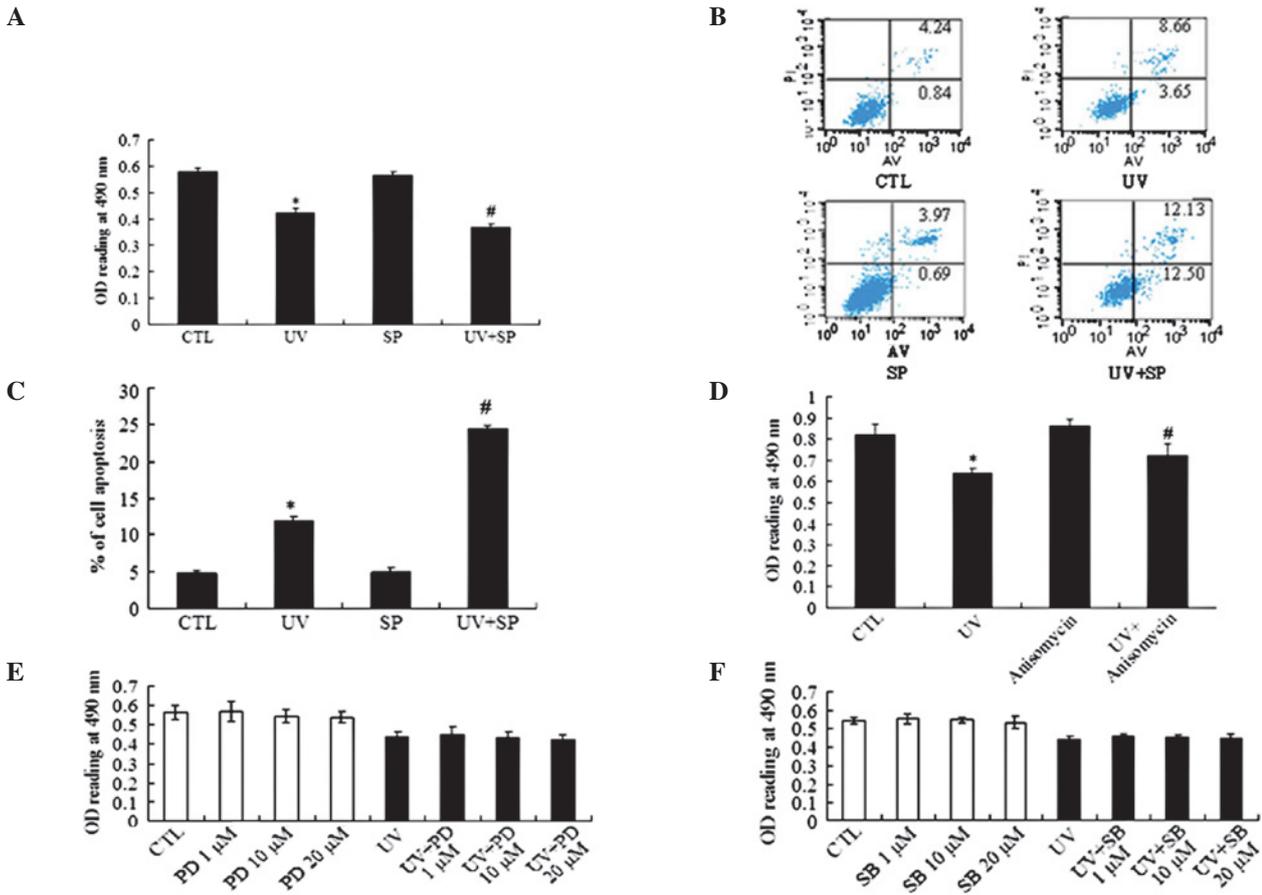


Figure 2. Inhibition of JNK augmented UVB-induced apoptosis in the ARPE19 cells and the activation of JNK reduced apoptosis. The ARPE19 cells were pre-treated with the various drugs for 1.5 h, then exposed to UVB radiation and incubated in serum-free medium for 24 h. (A) Cells in pre-treated with the specific JNK inhibitor SP600125 (5  $\mu$ M). Cell viability was assayed by MTT assay (n=6). (B) Cells pre-treated with SP600125, where apoptosis was determined by flow cytometry, in which the numbers in the image represent early- and late-stage cell apoptotic percentages. (C) statistical analysis from triplicate experiments. (D) Pre-treatment with the specific JNK activator, anisomycin (2  $\mu$ g/ml). Cell viability was assayed by MTT assay (n=6). (E and F) Cells pre-treated with the ERK- and p38-specific inhibitors, PD98059 and SB203580, respectively. Cell viability was assayed using an MTT assay (n=6). The results are represented as the means  $\pm$  SEM. \*P<0.05 control (CTL) vs. UV; #P<0.05 UV vs. UV + SP or UV + Anisomycin.

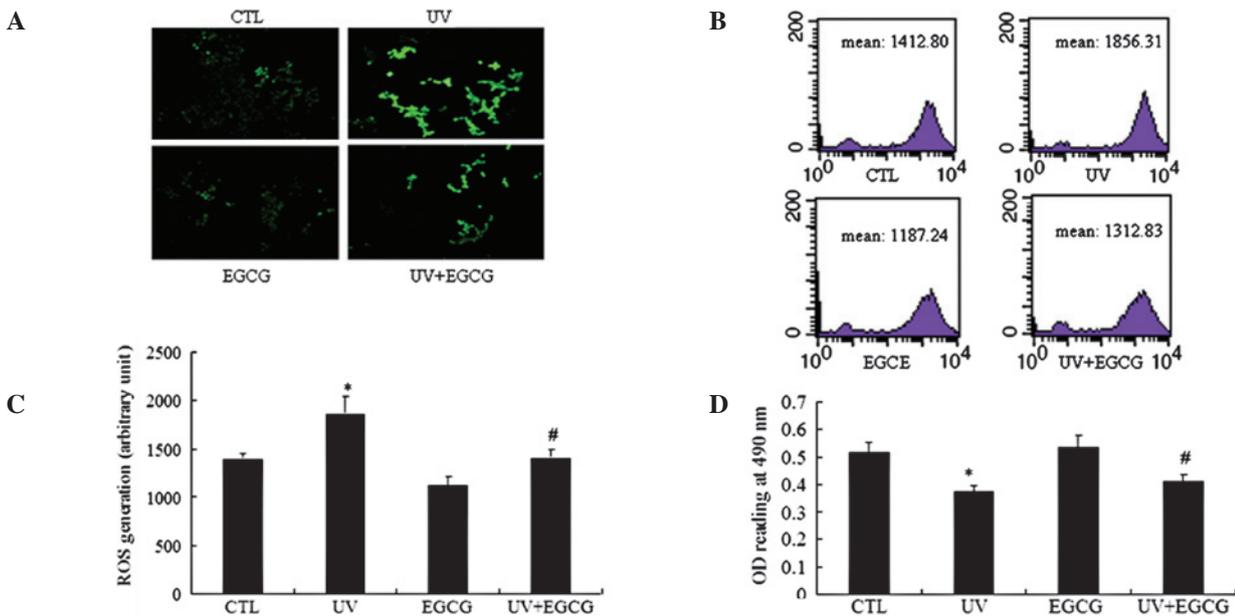


Figure 3. UVB increased ROS production, but EGCG decreased UVB-induced ROS production and cell apoptosis in the ARPE19 cells. (A) Fluorescence microscopy: DCFH-DA dye enters the cell, reacts with the intracellular ROS cleaved to DCF, which emits green fluorescence to represent intracellular ROS production. (B) After UVB exposure and incubation of the fluorescent dye for 30 min, the cells were harvested for flow cytometry. (C) Statistical analysis from triplicate experiments. (D) Pre-treated with (or without) EGCG, the cells were exposed to UVB and the viability of the cells was determined by MTT assay (n=6). The results are expressed as the means  $\pm$  SEM. \*P<0.05 control (CTL) vs. UV; #P<0.05 UV vs. UV + EGCG.

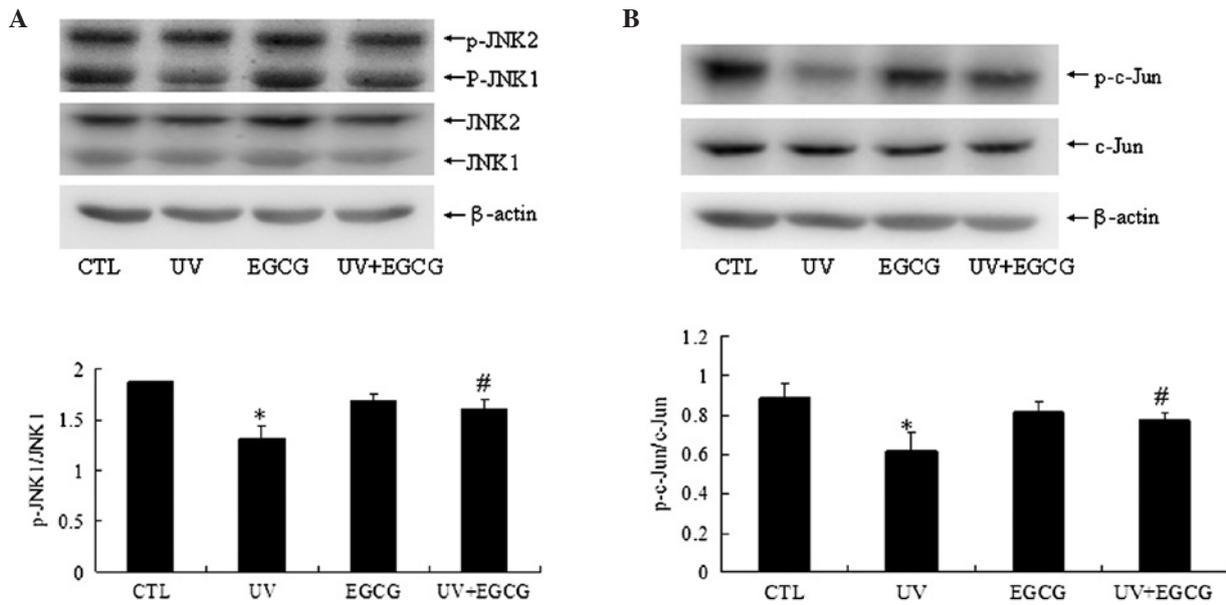


Figure 4. EGCG inhibited UVB-induced JNK1 and c-Jun phosphorylation decreased in the ARPE19 cells. (A) The irradiated and EGCG-treated cells were harvested after 24 h and analyzed by Western blot analysis with antibodies specific for phospho-JNK, JNK and  $\beta$ -actin. The ratio of phospho-JNK1 to JNK1 was determined by densitometry. (B) The irradiated and EGCG-treated cells were harvested after 24 h and analyzed by Western blot analysis with antibodies specific for phospho-c-Jun, c-Jun and  $\beta$ -actin. The ratio of phospho-c-Jun to c-Jun was determined by densitometry. The results are expressed as the means  $\pm$  SEM for four experiments. \* $P < 0.05$  control (CTL) vs. UV; # $P < 0.05$  UV vs. UV + EGCG.

(50 mJ/cm<sup>2</sup>) and found that even at this low dose, a significant degree of apoptosis occurred in the ARPE19 cells.

Three light damage hypotheses, originally presented in the study by Noell *et al* (16), have guided much of the study in this area. They describe damage as being caused by: i) A toxic photoproduct arising from vitamin A during exposure to intense light, ii) a metabolic abnormality resulting from light exposure, and iii) light-induced oxidative reactions (17). The retina is particularly susceptible to oxidative stress, which includes high oxygen consumption, high levels of cumulative irradiation, high photosensitizers and high polyunsaturated fatty acids in the photoreceptor outer segment membranes which are phagocytized by the RPE. The role of oxidative stress has been implicated in many age-related diseases (18) and a large number of studies support the key role of ROS in AMD (10). Our experiments indicate that UVB irradiation significantly increases ROS generation in ARPE19 cells, which is consistent with our presumption that oxidative stress is involved in UVB-induced light damage in RPE cells.

It has been reported that the activation of MAPK is essential for H<sub>2</sub>O<sub>2</sub>-induced apoptosis in RPE cells (19), and the UV-activated signal transduction pathways are mediated primarily through signaling cascades involving MAPKs, which includes ERK, JNK and p38 kinases (8). It is generally accepted that ERK activation is essential for cell survival, whereas the activation of JNK and p38 is thought to play a role in apoptotic signaling. Herein, we used three specific inhibitors for ERK, p38 and JNK, which were used to pre-treat the ARPE19 cells before UVB irradiation. Only SP600125 (the inhibitor of JNK) augmented UVB-induced apoptosis in the ARPE19 cells. In contrast, the inhibitors of p38 and ERK had no effect on cell survival. Furthermore, the ARPE19 cells were pre-treated with the specific JNK activator, anisomycin, prior

to irradiation, where it was found that the activation of JNK reduces UVB-induced apoptosis in the ARPE19 cells. Western blot analysis was then used to demonstrate that UVB irradiation decreases JNK1 phosphorylation. Our results confirm the anti-apoptotic role of JNK, which is contrary to the classic role of JNK. The exact role of the JNK pathway in apoptosis is highly controversial and there has been much speculation as to whether the JNK pathway is pro- or anti-apoptotic, or whether it plays any role at all (20). Supporters of the anti-apoptotic role, for example, have pointed to the activation of JNKs that have been shown to protect neurons against oxidative stress in diabetes (21). The sensitivity of cardiac myocytes to hydrogen peroxide was increased in MEKK1-negative cells (upstream of JNK), further suggesting the anti-apoptotic role of the JNK pathway (22). It has also been shown that the protective effect of cyclopentenone 15-deoxy-D12,14-prostaglandin J2 (dPGJ2), an endogenous PPAR $\gamma$  agonist that protects RPE cells from oxidative injury, is dependent on the activation of JNK and p38 (23). Thus, the JNK pathway is not simply a pro- or an anti-apoptotic pathway, which depends on cell and stimulus type, but requires co-activation of additional pathways and is also dependent on the duration of activation.

We also detected changes in c-Jun after UVB irradiation. Downstream targets of JNK include c-Jun, ATF-2, Elk2 and Nrf2. The most classic JNK substrate is the transcription factor c-Jun, from which JNK derived its name. It has been shown that mouse embryonic fibroblasts isolated from the c-JunAA63/73/c-JunAA63/73 mouse, a c-Jun mutant that cannot be phosphorylated, were insensitive to UV-induced cell death, which confirms that the phosphorylation of c-Jun by JNK is required for UV effect (24). We chose c-Jun to verify the role of JNK, wherein it was shown by Western blot analysis that there was a significant decrease in c-Jun phosphorylation

after UVB irradiation. This coincided with the change in the phosphorylation of JNK and further confirmed the anti-apoptotic role of the JNK pathway.

In order to investigate the relationship between an increase in ROS and a decrease in UVB-induced JNK, EGCG, a type of antioxidant, was used. It has been suggested that EGCG has the most protective effect among all the catechins in green tea. It is also known as a kind of oxygen-free radical scavenger (25). Our results show that EGCG nearly reverses ROS generation and partially decreases apoptosis induced by UVB irradiation. Notably, EGCG also blocked, in part, the phosphorylation of JNK and c-Jun caused by UVB irradiation. Thus, we have compelling evidence to suggest that oxidative stress is definitely involved in UVB-induced apoptosis and that it occurs partially through influencing c-Jun NH<sub>2</sub>-terminal kinase (JNK) signal transduction pathways.

Deciphering the mechanism of apoptosis in RPE cells will provide important insight into understanding the processes involved in AMD. From our investigations, we know that UVB irradiation is able to damage ARPE19 cells and induce apoptosis in these cells through oxidative stress pathways. In this setting, the JNK pathway plays an anti-apoptotic role. These results should be supported by *in vivo* experiments or based on findings from RPE cells taken from patients with AMD in order to confirm our hypothesis further. Modulating RPE cells through selective activators or antioxidants may be a useful strategy for reducing the oxidative damage that occurs in AMD.

### Acknowledgements

This study was supported in part by a grant from the Nanjing Foundation for Development of Science and Technology, China (200801093, to Q.J.), a grant from the Province Postdoctoral Science Foundation (to Q.J.), and a grant from the Natural Science Foundation for Colleges and Universities in Jiangsu Province of China (to Q.J.). The authors wish to thank Qi Chen for the guidance and help.

### References

- Norren DV and Vos JJ: Spectral transmission of the human ocular media. *Vision Res* 14: 1237-1244, 1974.
- Mainster MA: The spectra, classification, and rationale of ultraviolet-protective intraocular lenses. *Am J Ophthalmol* 102: 727-732, 1986.
- Reme C, Reinboth J, Clausen M and Hafezi F: Light damage revisited: converging evidence, diverging views? *Graefes Arch Clin Exp Ophthalmol* 234: 2-11, 1996.
- Cruickshanks KJ, Klein R, Klein BE and Nondahl DM: Sunlight and the 5-year incidence of early age-related maculopathy: the beaver dam eye study. *Arch Ophthalmol* 119: 246-250, 2001.
- Dent P, Yacoub A, Fisher PB, *et al*: MAPK pathways in radiation responses. *Oncogene* 22: 5885-5896, 2003.
- Zwang Y and Yarden Y: p38 MAP kinase mediates stress-induced internalization of EGFR: implications for cancer chemotherapy. *EMBO J* 25: 4195-4206, 2006.
- Li T, Dai W and Lu L: Ultraviolet-induced junD activation and apoptosis in myeloblastic leukemia ML-1 cells. *J Biol Chem* 277: 32668-32676, 2002.
- Bode AM and Dong Z: Mitogen-activated protein kinase activation in UV-induced signal transduction. *Sci STKE* 28: RE2, 2003.
- Roduit R and Schorderet DF: MAP kinase pathways in UV-induced apoptosis of retinal pigment epithelium ARPE19 cells. *Apoptosis* 13: 343-353, 2008.
- Beatty S, Koh H, Phil M, Henson D and Boulton M: The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 45: 115-134, 2000.
- Yao J, Liu Y, Wang X, Shen Y, Yuan S, Wan Y and Jiang Q: UVB radiation induces human lens epithelial cell migration via NADPH oxidase-mediated generation of reactive oxygen species and up-regulation of matrix metalloproteinases. *Int J Mol Med* 24: 153-159, 2009.
- Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S and Voorhees JJ: Mechanisms of photoaging and chronological skin aging. *Arch Dermatol* 138: 1462-1470, 2002.
- Davis RJ: Signal transduction by the JNK group of MAP kinases. *Cell* 103: 239-252, 2000.
- Raivich G: c-Jun expression, activation and function in neural cell death, inflammation and repair. *J Neurochem* 107: 898-906, 2008.
- Marc RE, Jones BW, Watt CB, Vazquez-Chona F, Vaughan DK and Organisciak DT: Extreme retinal remodeling triggered by light damage: implications for age related macular degeneration. *Mol Vis* 14: 782-806, 2008.
- Noell WK, Walker VS, Kang BS and Berman S: Retinal damage by light in rats. *Invest Ophthalmol* 5: 450-473, 1966.
- Organisciak DT and Vaughan DK: Retinal light damage: mechanisms and protection. *Prog Retin Eye Res* 29: 113-134, 2010.
- Finkel T and Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. *Nature* 408: 239-247, 2000.
- Ho TC, Yang YC, Cheng HC, Wu AC, Chen SL, Chen HK and Tsao YP: Activation of mitogen-activated protein kinases is essential for hydrogen peroxide-induced apoptosis in retinal pigment epithelial cells. *Apoptosis* 11: 1899-1908, 2006.
- Lin A and Dibling B: The true face of JNK activation in apoptosis. *Aging Cell* 1: 112-116, 2002.
- Price SA, Hounsom L, Purves-Tyson TD, Fernyhough P and Tomlinson DR: Activation of JNK in sensory neurons protects against sensory neuron cell death in diabetes and on exposure to glucose/oxidative stress *in vitro*. *Ann NY Acad Sci* 1010: 95-99, 2003.
- Minamino T, Yujiri T, Papst PJ, Chan ED, Johnson GL and Terada N: MEKK1 suppresses oxidative stress-induced apoptosis of embryonic stem cell-derived cardiac myocytes. *Proc Natl Acad Sci USA* 96: 15127-15132, 1999.
- Qin S, McLaughlin AP and de Vries GW: Protection of RPE cells from oxidative injury by 15-deoxy-delta12, 14-prostaglandin J2 by augmenting GSH and activating MAPK. *Invest Ophthalmol Vis Sci* 47: 5098-5105, 2006.
- Behrens A, Sibilina M and Wagner EF: Amino-terminal phosphorylation of c-Jun regulates stress-induced apoptosis and cellular proliferation. *Nat Genet* 21: 326-329, 1999.
- Katiyar SK, Afaq F, Perez A and Mukhtar H: Green tea polyphenol (-)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. *Carcinogenesis* 22: 287-294, 2001.