Epigallocatechin gallate enhances human lens epithelial cell survival after UVB irradiation via the mitochondrial signaling pathway

QIUXIN WU[1,2], JIKE SONG[1,2], YAN'E GAO[1,3], YINGYING ZOU[1,2], JUNGUO GUO[1,3], XIUYAN ZHANG[1,2], DONGMEI LIU[1,2], DADONG GUO[3] and HONGSHENG BI[1-3]

[1]Affiliated Eye Hospital, Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250002; [2]Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250014; [3]College of Ophthalmology and Optometry, Shandong Provincial Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Therapy of Ocular Diseases, Shandong Academy of Eye Disease Prevention and Therapy, Jinan, Shandong 250002, P.R. China

Received January 25, 2021; Accepted November 17, 2021

DOI: 10.3892/mmr.2022.12603

Abstract. The aim of the present study was to explore the mechanism underlying the ultraviolet B (UVB) irradiation-induced apoptosis of human lens epithelial cells (HLECs), and to investigate the protective effect of epigallocatechin gallate (EGCG) against the UVB-induced apoptosis of HLECs. HLECs were exposed to different concentrations of EGCG plus UVB (30 mJ/cm^2). Cell viability was determined using the MTT assay. Furthermore, mitochondrial membrane potential (Δψm) and apoptosis were assessed by flow cytometry with JC-1 and Annexin V/PI staining, respectively. Moreover, the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), as well as the levels of GSH, hydrogen peroxide (H_2O_2) and hydroxyl free radicals were determined using biochemical assay techniques. Reverse transcription-quantitative PCR and western blotting were used to detect the mRNA and protein expression levels of Bcl-2, Bax, cytochrome *c*, caspase-9 and caspase-3, respectively. The results revealed that UVB irradiation reduced the Δψm of HLECs and induced apoptosis. Notably, EGCG significantly attenuated the generation of H_2O_2 and hydroxyl free radicals caused by UVB irradiation in HLECs, and significantly increased CAT, SOD and GSH-Px activities, however, the GSH levels were not significantly increased. EGCG also reduced UVB-stimulated Bax, cytochrome *c*, caspase-9 and caspase-3 expression, and elevated Bcl-2 expression, suggesting that EGCG may possess free radical-scavenging properties, thus increasing cell viability. In conclusion, EGCG may be able to protect against UVB-induced HLECs apoptosis through the mitochondria-mediated apoptotic signaling pathway, indicating its potential application in clinical practice.

Correspondence to: Professor Dadong Guo, College of Ophthalmology and Optometry, Shandong Provincial Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Therapy of Ocular Diseases, Shandong Academy of Eye Disease Prevention and Therapy, 48 Yingxiongshan Road, Jinan, Shandong 250002, P.R. China
E-mail: dadonggene@163.com

Professor Hongsheng Bi, Affiliated Eye Hospital, Shandong University of Traditional Chinese Medicine, Jinan, Shandong 250002, P.R. China
E-mail: hongshengbi1@163.com

Key words: epigallocatechin gallate, ultraviolet B, apoptosis, human lens epithelial cells

Introduction

Cataracts are a common lens disease worldwide and are one of the main causes of blindness. Ultraviolet B (UVB) irradiation is considered an important factor leading to the formation of cataracts by inducing the apoptosis of human lens epithelial cells (HLECs) (1,2). The photobiological effects of UVB may lead to reactive oxygen species (ROS) generation (3), DNA damage (4) and apoptosis (5). Experimental evidence has indicated that oxidative stress caused by free radical accumulation may serve a crucial role in the pathogenesis of cataracts, and this process can be prevented and/or ameliorated by antioxidants (6).

In recent years, the interest in green tea has grown because of its lack of toxicity and good efficacy in a wide range of organs, such as retinal, brain and skin (7-9). Green tea contains >3,000 compounds, of which nearly one-third are polyphenols that include catechins such as (-)-epicatechin (EC), (-)-EC gallate (ECG), (-)-epigallocatechin (EGC) and (-)-EGC gallate (EGCG) (8). The EC isomers share a similar backbone but have varying locations and numbers of hydroxyl groups (Fig. 1). As the most abundant catechin derivative, EGCG is also considered to be the most effective antioxidant (10). The antioxidant activities of EGCG are caused by the presence of phenolic groups, which are sensitive to oxidation and can produce quinones. The trihydroxyl structure of the D-ring in EGCG further improves its antioxidant activity (Fig. 1) (11). Previous

studies have confirmed that EGCG exerts a mild protective effect against UVB-induced oxidative damage (12,13); however, the detailed mechanism remains unclear.

The mitochondria-mediated apoptotic signaling pathway is a crucial endogenous apoptosis pathway in which caspase-dependent and caspase-independent mechanisms have significant roles. Mitochondrial apoptosis-inducing factor (AIF) and endonuclease G (Endo G) are involved in caspase-independent apoptosis (14). In the caspase-dependent apoptosis pathway, the formation of the apoptosome contributes to mitochondrial permeabilization, which promotes the activation of caspases and in turn triggers the production of other apoptosis-related proteins, leading to cell death (15). Bax and Bcl-2 are closely related to the mitochondrial apoptosis pathway (16). During apoptosis, the activation of Bax leads to the release of numerous mitochondria-related proteins, including the transport of cytochrome *c* from the mitochondria to the cytoplasm via Bax, which overcomes the Bcl-2-mediated regulation of mitochondrial membrane protein permeability; in addition, cytochrome *c*, procaspase 9, activating factor 1 (Apaf-1) and dATP combine to form an apoptotic complex that activates caspase-9, which can in turn activate caspase-3, ultimately triggering caspase-dependent apoptosis (17,18).

UVB can lead to the apoptosis of HLECs through the caspase-dependent pathway (1), whereas EGCG may reduce oxidant damage and thus protect HLECs from apoptosis (19-21). Heo *et al* (22) first reported in 2008 that EGCG increased the cell viability and cell count after UVB irradiation of cultured HLECs, indicating that EGCG may be able to protect HLECs against UVB damage. However, whether EGCG reduces UVB-induced oxidative damage to HLECs via caspase signaling remains unclear. The present study explored the effect of EGCG on the human lens epithelial B-3 (HLE B-3) cell line, which was treated with or without UVB irradiation. The findings of the present study may provide novel insights into the EGCG-mediated protection of HLECs under UVB irradiation.

Materials and methods

Cell culture and treatment with EGCG. HLE B-3 cells were obtained from the American Type Culture Collection (ATCC); this cell line was authenticated by STR. The cells were cultured in RPMI 1640 medium (HyClone; Cytiva) containing 10% fetal bovine serum (HyClone; Cytiva) at 37˚C in a humidified environment containing 5% CO_2. After reaching 75-80% confluence, the cells were irradiated with 30 mJ/cm^2 UVB at room temperature for 2 min or pretreated with EGCG (MilliporeSigma) for 2 h prior to UVB irradiation at 37˚C. At the designated time points, the cells were collected for different measurements.

UVB exposure. In the present study, UVB exposure was provided by a UVB lamp (Nanjing Huaqiang Electronic Co., Ltd.). The UVB spectral range was 290-320 nm and the peak irradiance was 297 nm. To obtain a good irradiation effect, the distance between the UVB lamp and the bottom of the culture plate was adjusted, and the central irradiation intensity was 0.25 mW/cm^2. UVB exposure dose (30 mJ/cm^2)=irradiation intensity (0.25 mW/cm^2) x irradiation time (120 sec). Before UVB irradiation, the cells were washed three times with warm phosphate-buffered saline (PBS; pH 7.4) to remove nonattached cells and residual serum, and a small amount of PBS was left to cover the cells. The cells were then exposed to UVB. After irradiation, fresh medium was added to each well, and the cells were further cultured until the required time.

Cell viability assay. HLE B-3 cells were seeded into 96-well plates ($8x10^3$ cells/well) and exposed to UVB irradiation (30 mJ/cm^2) alone or following pretreatment with various concentrations of EGCG (0.78, 1.56, 3.125, 6.25, 12.5, 25 or 50 μM) for 2 h. Subsequently, the cells were cultured for an additional 24, 48 and 72 h. Then, 20 μl MTT solution (5 mg/ml) was added to each well for MTT detection. After 4 h of incubation at 37˚C, the supernatant was discarded and the formazan in the cells was dissolved in dimethyl sulfoxide. Finally, the absorbance was measured at a wavelength of 490 nm (Multimode Plate Reader; PerkinElmer, Inc.). The cell viability was expressed as a percentage of the control.

Detection of mitochondrial membrane potential ($\Delta\psi m$). HLE B-3 cells were seeded into 6-well plates ($7.5x10^4$ cells/well) overnight, and each well was exposed to UVB irradiation (30 mJ/cm^2) in the presence or absence of EGCG pretreatment (50 μM) for 2 h. JC-1 (5, 5', 6, 6'-tetrachloro-1,1', 3, 3'-tetra-ethylbenzimidazolylcarbocyanine iodide; Beyotime Institute of Biotechnology) was used as a probe to detect the changes in $\Delta\psi$m following an additional 12 h of treatment with EGCG at 37˚C. JC-1 monomers emit green fluorescence (488 nm) when the mitochondria are polarized, whereas JC-1 aggregates emit red fluorescence under excitation at 585 nm. The red (PI, 585 nm) and green (FITC-A, 488 nm) fluorescence was measured simultaneously using a BD FACSVerse™ flow cytometer (BD FACSVerse™ Flow Cytometer; cat. no. 651154; BD Biosciences). A total of $1x10^4$ cells were analyzed for each sample.

Detection of antioxidants and oxidants. HLE B-3 cells were seeded into 6-well plates ($5.0x10^5$ cells/well) overnight and were then exposed to UVB irradiation (30 mJ/cm^2) in the presence or absence of EGCG pretreatment (50 μM) for 2 h, followed by a 24 h culture. At the specified time point, the cells were harvested, the cell pellets were collected by centrifugation at 400 x g for 5 min at 4˚C, followed by suspension in PBS and ultrasonication on ice at moderate energy for 5 sec/time for a total of 10 min. Furthermore, the supernatants were collected after centrifugation at 5,000 x g for 10 min at 4˚C. According to the manufacturer's instructions, the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), as well as the levels of GSH, H_2O_2 and hydroxyl free radicals were detected using the corresponding kits. The following kits were used (all purchased from Nanjing Jiancheng Bioengineering Institute): CAT assay kit (cat. no. A007-1-1), SOD assay kit (cat. no. A001-3-2), GSH-Px assay kit (cat. no. A005-1-2), reduced GSH assay kit (cat. no. A006-2-1), hydrogen peroxide assay kit (cat. no. A064-1-1), hydroxyl free radical assay kit (cat. no. A018-1-1). Finally, the absorbance was measured at a suitable wavelength (Multimode Plate Reader; PerkinElmer, Inc.).

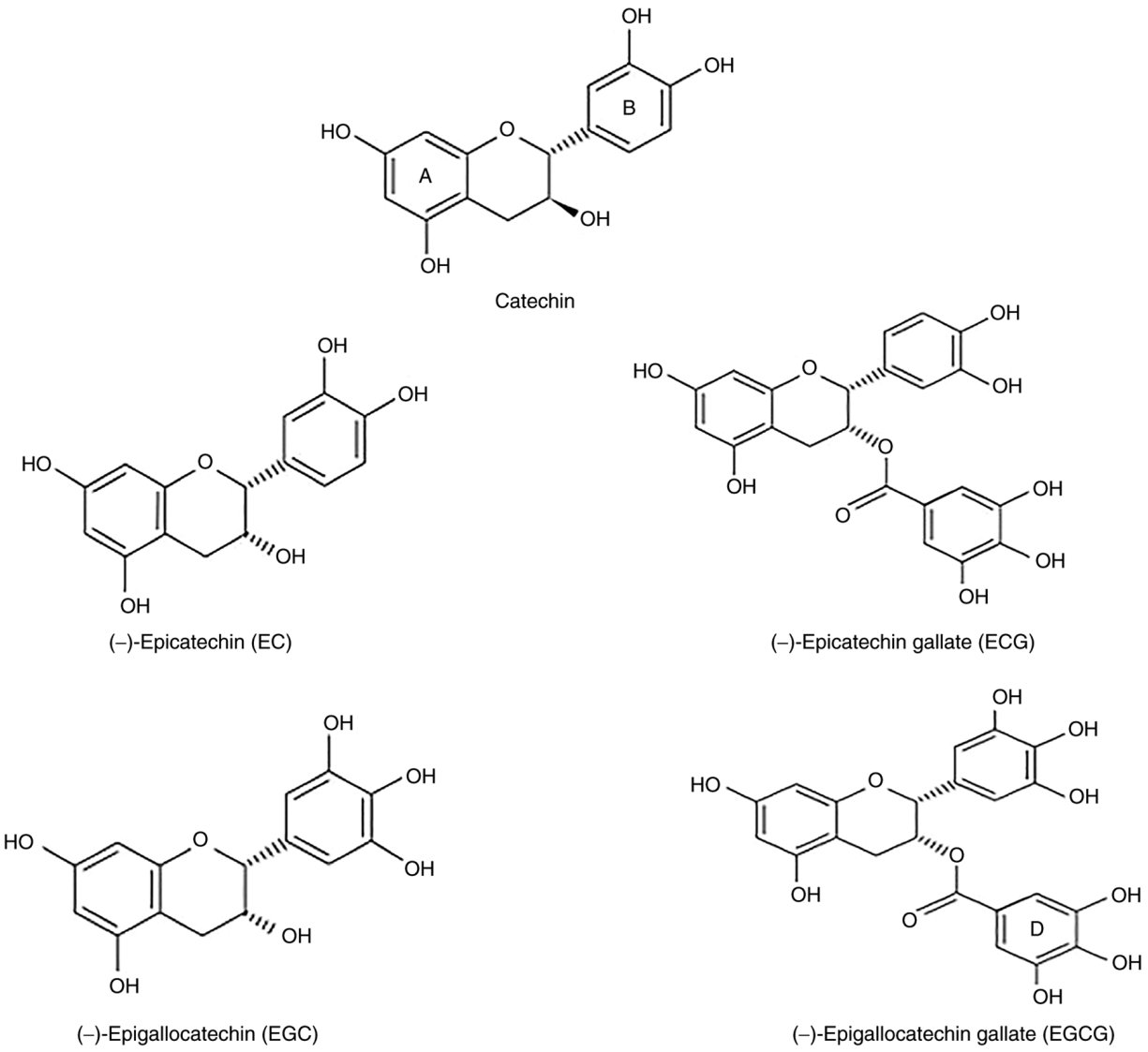

Figure 1. Schematic diagram of the molecular structure of catechins.

Apoptosis assay. A total of 16 h after the aforementioned treatments, the cells were harvested, and apoptosis was detected using an Annexin V-FITC/PI apoptosis detection kit (Bipec Biopharma Corporation). The collected cells were stained according to the manufacturer's protocol. The collected data were analyzed using BD FACSuite software and a FACSVerse flow cytometer (version 1.0) (both from BD Biosciences). A total of $1x10^4$ cells were analyzed for each experiment.

Reverse transcription-quantitative PCT (RT-qPCR). A total of 24 h after the aforementioned treatments, the cells were washed with PBS twice and collected to extract total RNA using an RNAqueous TM total RNA isolation kit (Thermo Fisher Scientific, Inc.). The extracted RNA was reverse transcribed into cDNA using a RevertAid First-Strand cDNA Synthesis Kit (cat. no. K1621; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Furthermore, qPCR was conducted in a 20 μl reaction using 2X SYBR-Green qPCR Mix (Thermo Fisher Scientific, Inc.). The PCR reaction was carried out using the Stratagene Mx3000p sequence detection system (Agilent Technologies, Inc.) under the following conditions: 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 1 min. The target genes Bcl-2, Bax, cytochrome *c*, caspase-9 and caspase-3 were amplified, and the GAPDH gene was used as the internal control. The primer sequences are listed in Table I. The $2^{-\Delta\Delta Cq}$ formula was used to calculate the relative mRNA transcription levels (23).

Western blotting. A total of 24 h after the aforementioned treatments, the cells were washed with ice-cold PBS and lysed in RIPA buffer (Beyotime Institute of Biotechnology) containing 10 mM phenylmethylsulfonyl fluoride. After incubation on ice for 30 min, the supernatant was collected following centrifugation at 8,000 x g for 10 min at 4˚C, and the target proteins (50 μg) were then separated by 5-12% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked in Tris-buffered saline solution (pH 7.6) containing 0.1% Tween-20 and 5% non-fat dried milk for 1 h at room temperature and probed with primary antibodies (all from Abcam) against Bcl-2 (rabbit; cat. no. ab32124; 1:1,000), Bax (rabbit; cat. no. ab182733; 1:2,000), cytochrome *c* (rabbit; cat. no. ab76107; 1:500), caspase-9 (rabbit; cat. no. ab185719;

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Primer sequence (5'-3')
GAPDH	F: ACTTTGGTATCGTGGAAGGACTCAT
	R: GTTTTTCTAGACGGCAGGTCAGG
Bcl-2	F: ATCGCCCTGTGGATGACTGA
	R: GAGACAGCCAGGAGAAATCAAAC
Bax	F: TTTTGCTTCAGGGTTTCATCCA
	R: TGCCACTCGGAAAAAGACCTC
Cytochrome *c*	F: CTTGGACTTAGAGAGTGGGGACG
	R: GTGGCACTGGGAACACTTCATAA
Caspase-9	F: TGGACATTGGTTCTGGAGGATT
	R: AGCACCATTTTCTTGGCAGTCA
Caspase-3	F: TGGAAGCGAATCAATGGACTCT
	R: TGAATGTTTCCCTGAGGTTTGC

F, forward; R, reverse.

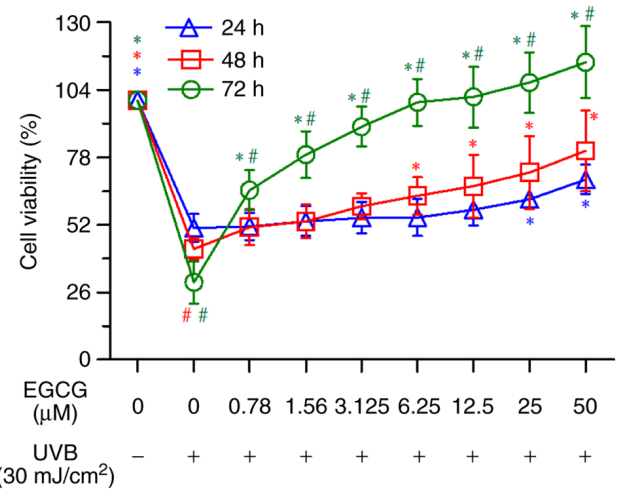

Figure 2. Protective effect of EGCG against human lens epithelial cell damage induced by UVB irradiation. The cells were cultured in different concentrations of EGCG for 2 h before UVB irradiation (30 mJ/cm^2), and the cell viability (%) was recorded at 24, 48 and 72 h. Cells that received neither irradiation nor EGCG treatment were used as the control group. Data are presented as the mean ± SD (n=3). At the same time point, *P<0.05 vs. the UVB irradiation group; at the same EGCG concentration, $^\#$P<0.05 vs. the 24 h time point. EGCG, epigallocatechin gallate; UVB, ultraviolet B.

1:1,000), caspase-3 (rabbit; cat. no. ab32042; 1:500) and β-actin (rabbit; cat. no. ab8227; 1:1,000) overnight at 4˚C. Subsequently, the membranes were washed with PBS and probed with HRP-conjugated goat anti-rabbit IgG secondary antibodies (cat. no. ab205718; 1:5,000; Abcam) for 2 h at room temperature. Finally, visualization was performed with an Immobilon Western Chemiluminescent HRP Substrate (MilliporeSigma) using the FUSION-FX7 imaging system (Vilber Lourmat) and quantified using the Fusion CAPT software (version 15.06a; Vilber Lourmat). The levels of β-actin were used to normalize the protein expression levels.

Statistical analysis. SPSS 22.0 (IBM Corp.) was used for statistical analysis. All of the experiments were repeated three times and data are presented as the mean ± SD. One-way ANOVA followed by the LSD post hoc test was used to analyze data containing ≤3 groups. One way ANOVA followed by Tukey's post hoc test was used to analyze data containing >3 groups. The effects of time and different treatments on cell viability were assessed using two-way ANOVA followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Protective effect of EGCG against UVB irradiation-induced HLEC damage. In our previous experiments, it was revealed that 30 mJ/cm^2 UVB irradiation reduced the survival rate of HLE B-3 cells to 50.79±5.34% (20); therefore, 30 mJ/cm^2 UVB irradiation was selected to perform the relevant experiment in the present study. The present study investigated the effect of EGCG on the survival rate of HLECs after UVB irradiation by MTT assay. The results indicated that following pretreatment with various concentrations of EGCG, the cell viability after exposure to UVB irradiation was increased in a time-dependent manner (Fig. 2). Moreover, EGCG pretreatment at concentrations between 0.78 and 50 μM for 2 h could efficiently improve cell viability compared with the UVB group at 72 h (Fig. 2). Notably, pretreatment with EGCG at a concentration of 50 μM exhibited the strongest protective effect on HLECs. Therefore, 50 μM EGCG was selected for subsequent studies.

Effect of EGCG on changes in Δψm caused by UVB irradiation. The JC-1 distribution results demonstrated that the UVB group (72.03±3.17%; Fig. 3C) had a lower Δψm compared with that in the control group (94.55±1.89%; Fig. 3A), and a significant improvement in Δψm was observed in the EGCG + UVB group (82.63±4.49%; Fig. 3D) compared with that in the UVB group. In the EGCG group, the Δψm was 96.15±1.67% (Fig. 3B), which was similar to the control group. The relative Δψm values of the groups compared with the control group are shown in Fig. 3E. These results indicated that EGCG could protect the Δψm of HLECs under conditions of UVB irradiation.

Effect of EGCG on the changes in enzyme activities induced by UVB irradiation. Compared with in the control group, the enzyme activities of CAT and SOD were significantly reduced by UVB irradiation (Fig. 4A and B), whereas GSH-Px activity was not significantly decreased by UVB irradiation (Fig. 4C); however, after pretreatment with EGCG, the activities of CAT, SOD and GSH-Px were significantly enhanced compared with UVB irradiation group (Fig. 4A-C). These results indicated that EGCG may enhance the activities of CAT, SOD and GSH-Px, and thus may serve a protective role against UVB irradiation-mediated oxidative damage.

Effect of EGCG on changes in GSH levels induced by UVB irradiation. A significant decrease in GSH levels was observed in the UVB group (5.04±0.83 μmol/g) compared with in the control group (8.81±1.99 μmol/g). By contrast, GSH levels were

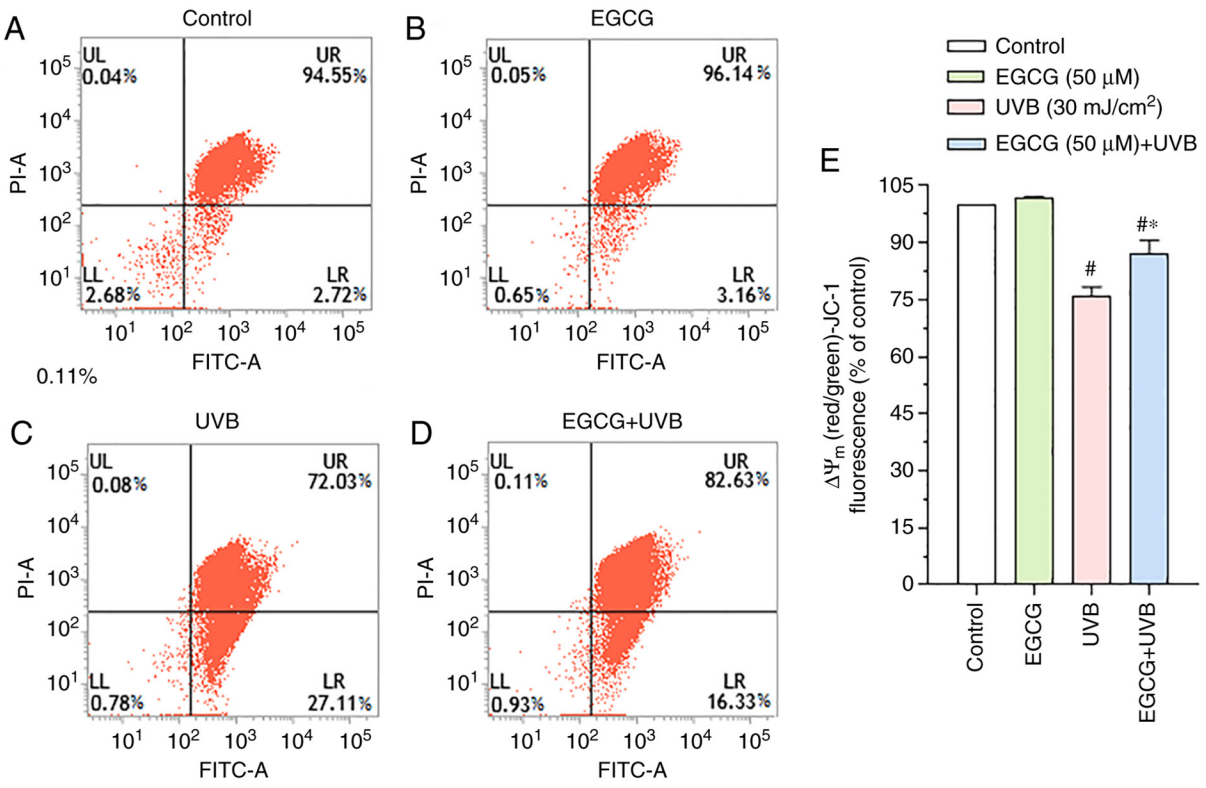

Figure 3. Protective effect of EGCG on the Δψm of HLECs. Δψm was measured by flow cytometry using JC-1 staining in the (A) control, (B) EGCG, (C) UVB and (D) EGCG + UVB groups. (E) Changes of Δψm relative to the control group. Data are presented as the mean ± SD (n=3). *P<0.05 vs. the UVB group; #P<0.05 vs. the control group. Δψm, mitochondrial membrane potential; EGCG, epigallocatechin gallate; LL, lower left; LR, lower right; UL, upper left; UR, upper right; UVB, ultraviolet B.

not significantly increased in HLECs after pretreatment with EGCG before UVB irradiation (6.77±1.87 μmol/g) compared with in the UVB group (Fig. 4D).

Effect of EGCG on the levels of H_2O_2 and hydroxyl free radicals induced by UVB irradiation. ROS levels were measured by assessing the production of intracellular H_2O_2 and hydroxyl free radicals. As shown in Fig. 5, a marked increase in H_2O_2 levels was observed in the UVB group (16.85±3.86 mmol/l) compared with in the control group (7.69±1.20 mmol/l). By contrast, a significant decrease in the H_2O_2 levels was observed in HLECs following pretreatment with EGCG before UVB irradiation (8.40±1.27 mmol/l) compared with in the UVB group (P<0.05).

In addition, the levels of hydroxyl free radicals were markedly elevated after UVB irradiation (984.53±62.07 mmol/l) compared with in the control group (879.56±61.36 mmol/l) (Fig. 5). However, a significant reduction in hydroxyl free radicals was observed in HLECs following pretreatment with EGCG before UVB irradiation (856.81±66.06 mmol/l) compared with in the UVB group (Fig. 5).

These results suggested that EGCG may have potent scavenging activity toward H_2O_2 and hydroxyl free radicals, indicating that EGCG may efficiently prevent UVB-induced intracellular ROS production within HLECs.

Effect of EGCG on apoptosis induced by UVB irradiation. A prominent increase in the number of early apoptotic cells (shown in the lower right quadrant) was observed in the UVB group (14.6±1.57%; Fig. 6C) compared with in the control group (0.39±0.25%; Fig. 6A). Conversely, a significant decrease in the number of early apoptotic cells was observed in the EGCG + UVB group (6.77±1.28%; Fig. 6D) compared with in the UVB group. Furthermore, the percentage of late apoptotic/necrotic cells (shown in the upper right quadrant) was 18.62±2.63% in the UVB group (Fig. 6C), 0.25±0.03% in the control group (Fig. 6A) and 2.79±0.15% in the EGCG group (Fig. 6B). Notably, following pretreatment with EGCG, a significant decrease in the number of late apoptotic/necrotic HLECs was detected compared with in the UVB group (10.34±1.16%; Fig. 6D). UVB irradiation significantly increased the percentage of the total apoptotic cells (including early and late apoptotic cells), whereas EGCG intervention significantly decreased the total apoptosis rate (Fig. 6E). These observations indicated that EGCG could efficiently decrease the HLEC apoptosis induced by UVB irradiation.

Effect of EGCG on the mRNA expression levels of Bcl-2, Bax, cytochrome c, caspase-9 and caspase-3 induced by UVB irradiation. RT-qPCR was used to explore whether EGCG pretreatment influenced the expression levels of Bcl-2, Bax, cytochrome *c*, caspase-9 and caspase-3 in HLECs following UVB irradiation. The results indicated that pretreatment with EGCG prior to UVB irradiation (EGCG + UVB group) significantly increased the expression levels of Bcl-2, but decreased the expression levels of Bax, cytochrome *c*, caspase-9 and caspase-3 compared with

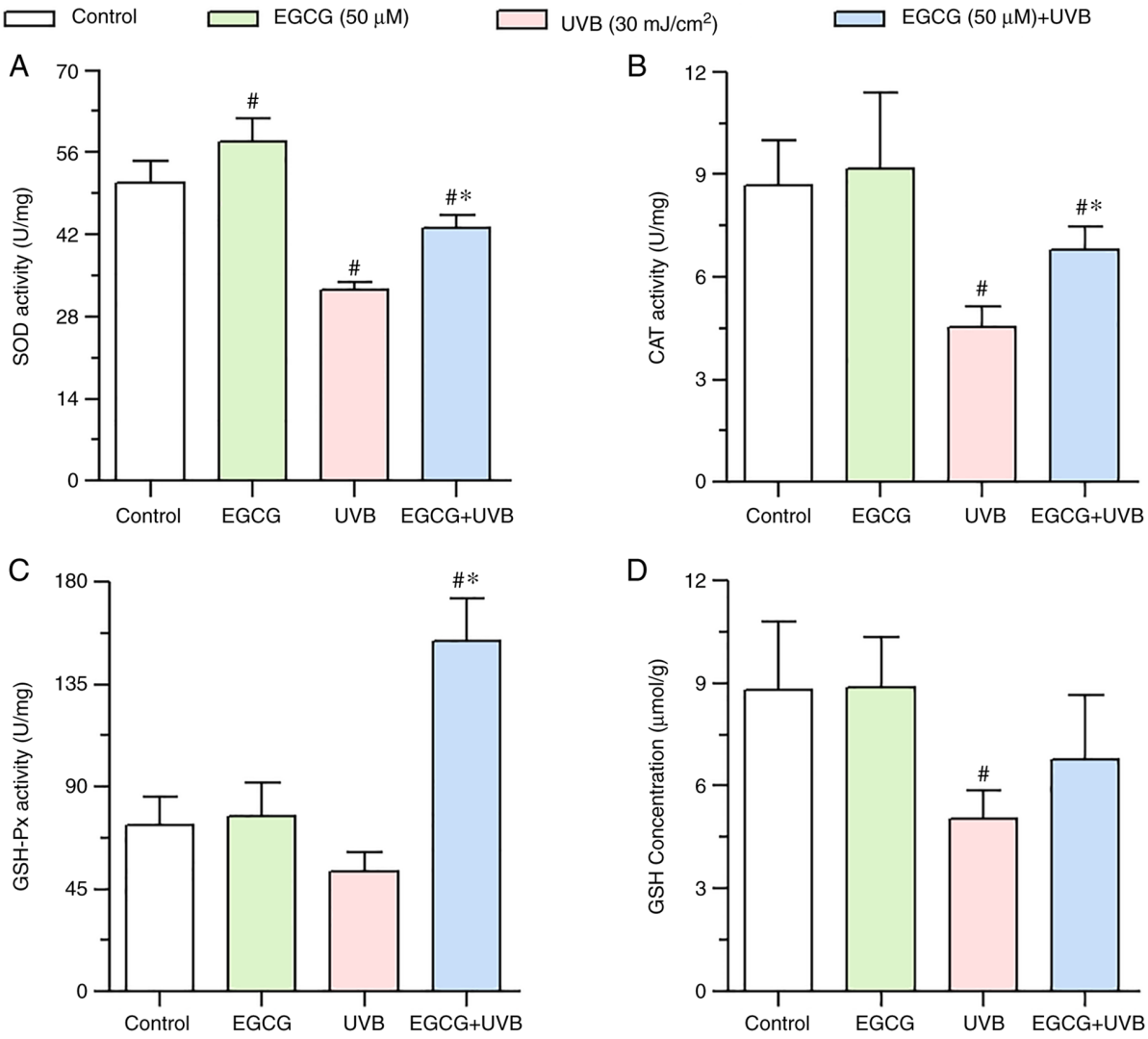

Figure 4. Effect of EGCG on antioxidant enzyme activity and GSH concentration under UVB irradiation. (A) SOD activity, (B) CAT activity, (C) GSH-Px activity and (D) GSH concentration 24 h after UVB irradiation, with or without EGCG pretreatment. The results showed that EGCG significantly enhanced the activities of CAT, SOD and GSH-Px, but did not significantly increase the levels of GSH. Data are presented as the mean ± SD (n=3). *P<0.05 vs. the UVB group; $^{\#}$P<0.05 vs. the control group. CAT, catalase; EGCG, epigallocatechin gallate; GSH, glutathione; GSH-Px, GSH peroxidase; SOD, superoxide dismutase; UVB, ultraviolet B.

in the UVB group (Fig. 7). The Bcl-2/Bax ratio was significantly decreased after UVB irradiation compared with the control group, but EGCG treatment significantly increased this ratio (Fig. 7F).

Effect of EGCG on the protein expression levels of Bcl-2, Bax, cytochrome c, caspase-9 and caspase-3 induced by UVB irradiation. The caspase-dependent pathway is an important pathway for inducing apoptosis, and includes Bcl-2, Bax, cytochrome *c*, caspase-9, caspase-3 and other proteins. As shown in Fig. 8, the western blotting results indicated that the protein expression levels of Bax, cytochrome *c*, caspase-9 and caspase-3 were downregulated in the EGCG + UVB group compared with those in the UVB group. By contrast, the expression levels of Bcl-2 were increased in the EGCG + UVB group compared with those in the UVB group (Fig. 8). Overall, these findings suggested that EGCG alleviated the UVB-induced apoptosis of HLECs by inhibiting the caspase-dependent apoptosis pathway.

Discussion

UVB causes harm to living organisms mainly through damaging DNA, proteins and cell membranes, as well as inducing oxidative stress through the generation of ROS, such as hydroxyl free radicals, hydroxyl peroxide and superoxide, leading to apoptosis (24-27). It has been well demonstrated that UVB-induced ROS production mediates cell apoptosis (25,28,29).

The intracellular ROS homeostasis depends on the dynamic balance between normal cellular aerobic metabolism and the antioxidant defense system. The antioxidant system includes enzymatic antioxidants (such as CAT, SOD, GSH-Px and GSH reductase) and nonenzymatic antioxidants (such as bilirubin, GSH, and vitamins C and E) (30-33). Oxidative stress arises when the balance between antioxidant and pro-oxidant levels is disrupted (34).

Previous studies have shown that EGCG, a crucial component in green tea, exerts protective effects against oxidative

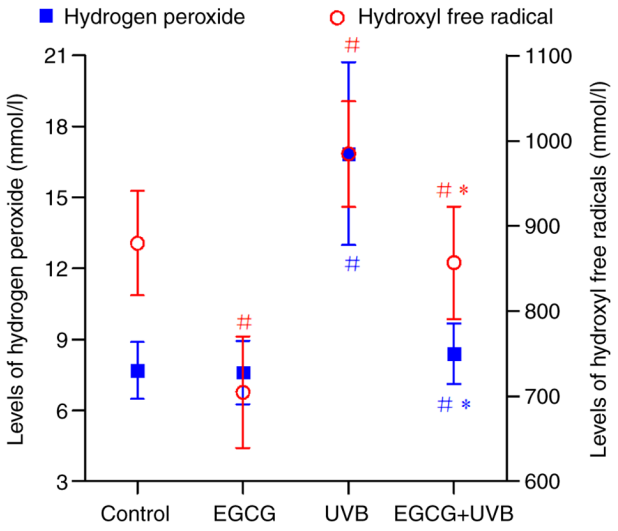

Figure 5. Protective effect of EGCG on the hydrogen peroxide and hydroxyl free radical levels induced by UVB irradiation. Data are presented as the mean ± SD (n=3). *P<0.05 vs. the UVB group; #P<0.05 vs. the control group. EGCG, epigallocatechin gallate; UVB, ultraviolet B.

stress in HLE B-3 cells (19,20). Yao *et al* (19) and our previous study (20) indicated that EGCG could increase the survival and reduce the apoptosis of HLECs by decreasing the H_2O_2- or UVB-induced generation of ROS and the loss of $\Delta\psi m$. Notably, the present study reached a similar conclusion that is consistent with those of these previous studies.

Katiyar *et al* (35) revealed that the intracellular levels of H_2O_2 in normal human epidermal keratinocytes cultured with EGCG for 24 h did not exhibit a significant decrease compared with that in the control cells. Similar results were observed in the present study. The H_2O_2 levels in the EGCG group (7.61±1.33 mmol/l) were slightly lower than those in the control group (7.69±1.20 mmol/l), but the difference was not statistically significant. Yamamoto *et al* (36) and Cao *et al* (37) reported that EGCG reduced the intracellular ROS levels in normal cells. H_2O_2 and hydroxyl free radicals are the main ROS within cells. In the present study, the hydroxyl free radical levels in the EGCG group (704.61±65.30 mmol/l) were significantly lower compared with those in the control group (879.56±61.36 mmol/l). EGCG has been shown to serve a dual role in promoting and inhibiting the production of H_2O_2 (38). On the one hand, some studies suggest EGCG is an antioxidant. For example, EGCG can inhibit ultraviolet radiation-induced oxidative stress in skin (39,40). On the other hand, other reports claim that EGCG exerts pro-oxidant actions. EGCG automatically oxidizes and produces H_2O_2 in cell culture media with and without cells (38). At an appropriate concentration, EGCG can stimulate cells to produce a low concentration of intracellular ROS, which stimulates the activation of various signaling pathways to promote cellular protective mechanisms (40). It was hypothesized that this may be the reason why EGCG cannot significantly down-regulate the level of H_2O_2 in normal cells as it can hydroxyl free radicals; however, the exact mechanism requires further study.

GSH is known to protect cells from the toxic effects of lipid peroxidation. Furthermore, GSH is essential to maintain cellular redox status and its consumption is considered to be an indicator of oxidative stress (41). GSH-Px can promote the breakdown of H_2O_2 and reduce toxic peroxides to nontoxic hydroxyl compounds, thereby protecting the structure and function of the cellular membrane from oxide damage. The activity of GSH-Px is a significant marker of the antioxidant capacity of cells. CAT defends against free radicals, and is responsible for the catalytic decomposition of H_2O_2 into water and molecular oxygen (42). SOD can efficaciously scavenge oxygen radicals, protect cells from oxidative damage and eliminate the oxidative stress caused by superoxide anion (43). In addition, SOD stops the free radical chain reaction by transforming superoxide radicals into H_2O_2. H_2O_2 and reducing GSH are decomposed into water and oxygen by CAT or GSH reductase (44).

Previous studies have suggested that UVB irradiation can increase the concentration of H_2O_2 and hydroxyl free radicals. UVB irradiation has also been reported to inhibit the activities of CAT, SOD and GSH-Px, and reduce the concentration of GSH, thus causing oxidative damage to cells (24,25,45). Previous studies have revealed that EGCG can protect against light (12,13), and that it can also reduce the oxidative damage caused by UVB irradiation by increasing CAT, SOD, GSH-Px and GSH activities (10,46). In the present study, pretreatment with 50 μM EGCG exerted a protective effect on HLECs against UVB irradiation by reducing the production of H_2O_2 and hydroxyl free radicals, significantly promoting the activities of CAT, SOD and GSH-Px, but not significantly increasing the levels of GSH. Notably, CAT and SOD activities, and GSH levels were markedly decreased following treatment with UVB irradiation (GSH-Px activity was not significantly decreased), whereas the activity of antioxidant enzymes (such as CAT, SOD and GSH-Px) was enhanced by EGCG pretreatment, which can remove free radicals in a highly efficient manner.

Apoptosis serves an important role in maintaining homeostasis. Changes in mitochondrial structure and function are associated with cell apoptosis, and these changes are mainly manifested by the release of proapoptotic factors, abundant production of ROS and an imbalance in intracytoplasmic calcium levels (47-49). UVB irradiation-induced cell apoptosis can be mediated by the mitochondria-initiated apoptotic pathway (50). In the present study, Annexin V-FITC/PI staining confirmed that UVB irradiation induced HLECs apoptosis and that EGCG reduced apoptosis. The specific mechanism underlying the ability of EGCG to reduce apoptosis through the mitochondrial pathway requires further study. It has been reported that mitophagy is critical for maintaining mitochondrial quality, energy metabolism and organ function (51). Mitochondrial quality and mitochondrial mitophagy may also be the possible mechanisms by which EGCG reduces UVB damage to HLECs. The present study did not perform the relevant investigations to explore the effect of EGCG on mitochondrial autophagy and mitochondrial quality; therefore, we aim to further study the possible mechanism underlying the effects of EGCG on apoptosis from the aspects of mitochondrial autophagy and mitochondrial quality in future studies.

The levels of cytochrome *c* in the cytoplasm are a sign of mitochondrial damage, which contributes to cell apoptosis. EGCG has been reported to inhibit the release of cytochrome *c* from the mitochondria into the cytoplasm, and to

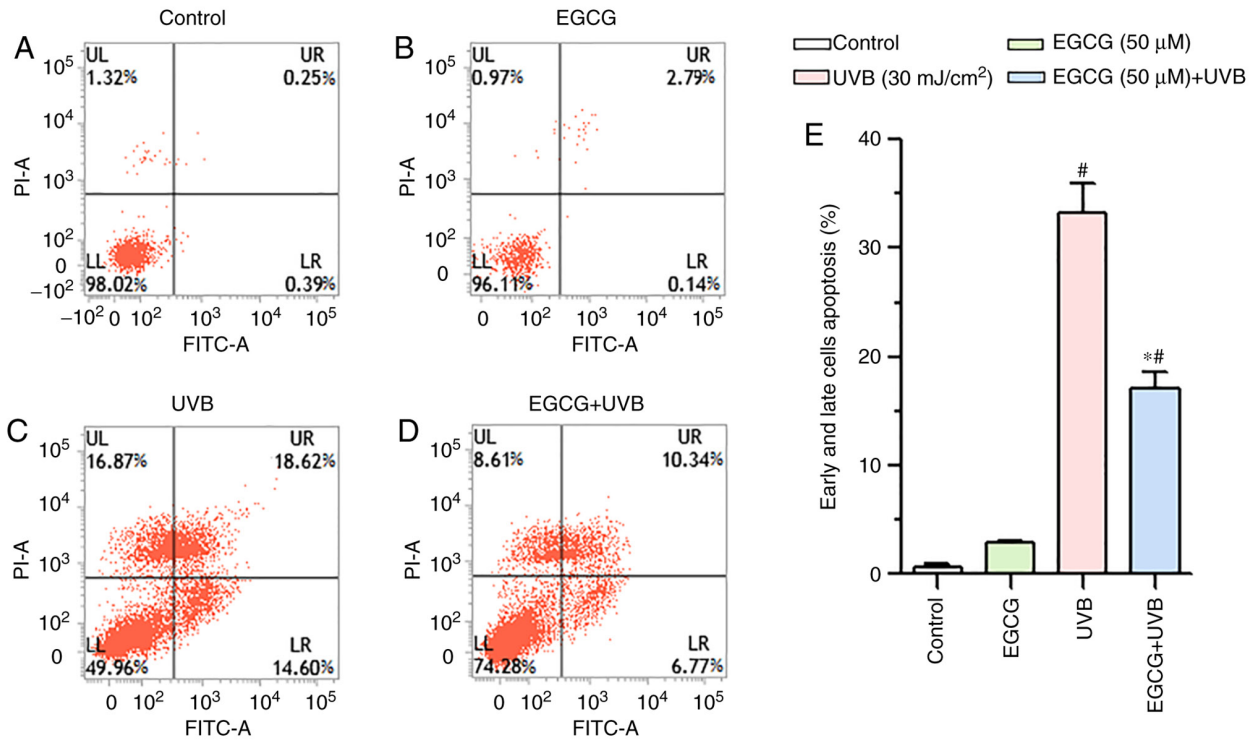

Figure 6. Protective effect of EGCG against HLECs apoptosis under UVB irradiation. HLECs were treated with 50 μM EGCG for 2 h prior to UVB irradiation (30 mJ/cm^2) and cultured for 16 h. Cell apoptosis was detected by flow cytometry using Annexin V/PI staining in the (A) control, (B) EGCG, (C) UVB and (D) EGCG + UVB groups. LL, viable non-stained cells; LR, early apoptotic Annexin V-FITC-stained cells; UR, late apoptotic/necrotic Annexin V-FITC and PI-stained cell; UL, dead PI-stained cells. (E) Proportion of early apoptotic and late apoptotic cells. Data are presented as the mean ± SD (n=3). *P<0.05 vs. the UVB group; #P<0.05 vs. the control group. EGCG, epigallocatechin gallate; LL, lower left; LR, lower right; UL, upper left; UR, upper right; UVB, ultraviolet B.

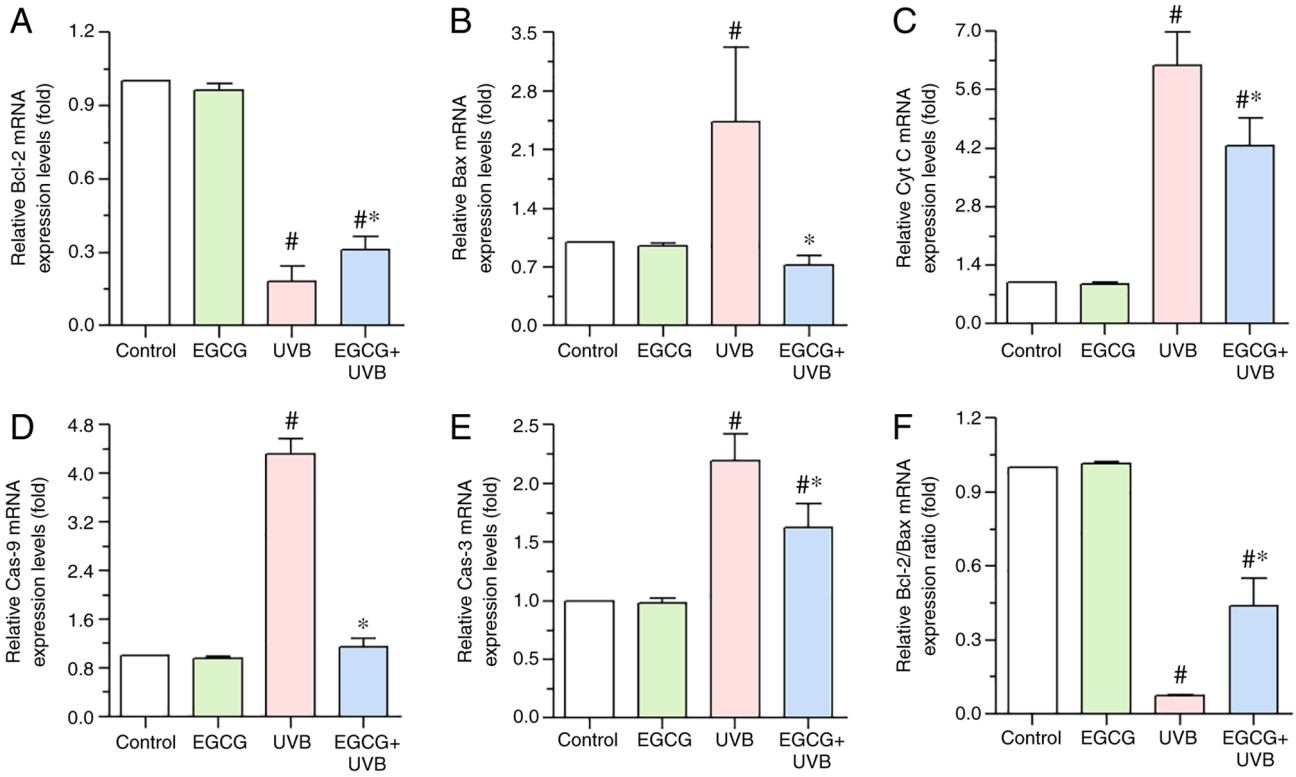

Figure 7. Alterations in the mRNA expression levels of (A) Bcl-2, (B) Bax, (C) Cyt C, (D) Cas-9 and (E) Cas-3 following pretreatment with EGCG before UVB irradiation were assessed using RT-qPCR. EGCG inhibited the UVB irradiation-induced expression of Bax, Cyt C, Cas-9 and Cas-3, and promote Bcl-2 expression in HLECs. (F) RT-qPCR analysis of the Bcl-2/Bax ratio in HLECs. GAPDH was used as the internal control. *P<0.05 vs. the UVB group; #P<0.05 vs. the control group. Cas-3, caspase-3; Cas-9, caspase-9; Cyt C, cytochrome *c*; EGCG, epigallocatechin gallate; HLECs, human lens epithelial cells; RT-qPCR, reverse transcription-quantitative PCR; UVB, ultraviolet B.

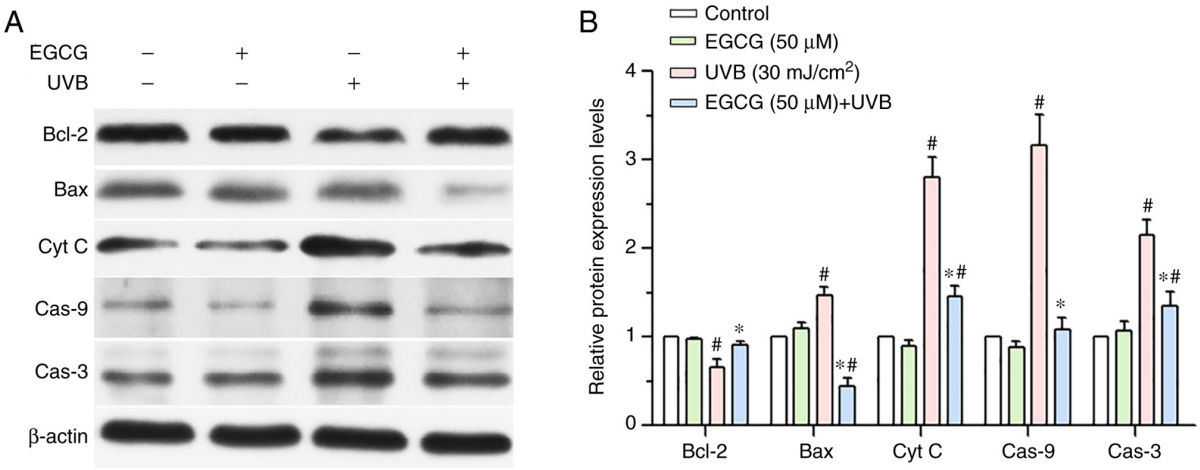

Figure 8. EGCG inhibits the UVB irradiation-induced expression of Cyt C, Cas-9 and Cas-3 and modulates the UVB irradiation-induced expression of Bcl-2 family proteins in human lens epithelial cells. (A) Protein expression levels of Bcl-2, Bax, Cyt C, Cas-9 and Cas-3 were measured by western blotting. (B) Histogram analysis of protein expression levels. Data are presented as the mean ± SD (n=3). *P<0.05 vs. the UVB group; #P<0.05 vs. the control group. Cas-3, caspase-3; Cas-9, caspase-9; Cyt C, cytochrome *c*; EGCG, epigallocatechin gallate; UVB, ultraviolet B.

reduce mitochondria-mediated apoptosis (52). Cytochrome *c* is released from the mitochondria into the cytoplasm and binds to Apaf-1 and pro-caspase-9 to form the apoptotic body, where caspase-9 is activated. Active caspase-9 is further cleaved and activates effector caspases, such as caspases-3 and -7, which execute the death program. Caspase activation is the main initiator of apoptosis. Zhang *et al* (53) reported that with the occurrence of apoptosis, the expression levels of the active form of caspase-3 were increased and caspase-3 activity simultaneously enhanced. EGCG can reduce the increased caspase-3 activity induced by oxidative damage and inhibit cell apoptosis (54). The present study demonstrated that EGCG pretreatment efficiently protected HLECs against UVB-induced cell apoptosis by inhibiting the mRNA and protein expression levels of cytochrome *c*, caspase-9 and caspase-3, suggesting that EGCG may block the mitochondria-dependent cell death pathway. However, the present study only analyzed the expression of caspase-3, which significantly increased following UVB irradiation. By contrast, following EGCG intervention, a decrease in caspase-3 expression observed. It is hypothesized that the decrease in caspase-3 may be related to the change in the expression of active caspase-3. Active caspase-3 (cleaved caspase-3) could stimulate the apoptotic cascade, and active caspase-3 may reflect the apoptotic status of target cells (55). In the present study, only the caspase-3 protein were measured; therefore, the lack of detection of active caspase-3 expression in the present study is a limitation and the effect of EGCG on cleaved caspase-3 (active caspase-3) requires further investigation.

Bcl-2 family members, such as Bax and Bcl-2, are crucial regulators of various apoptotic pathways. Bax serves a central role in the execution of mitochondrial apoptosis and an increase in Bax expression affects mitochondrial membrane permeability; this change leads to the release of cytochrome *c*, which further activates caspases. By contrast, anti-apoptotic proteins, such as Bcl-2, are known to downregulate Bax expression, suppressing cytochrome *c* release and inhibiting cell apoptosis through the mitochondrial pathway. Changes in the ratio of anti-apoptotic/pro-apoptotic Bcl-2 family proteins are essential for determining whether apoptosis is executed (56). In the present study, HLECs treated with UVB irradiation exhibited downregulated expression of Bcl-2, upregulated expression of Bax, and a decreased Bcl-2/Bax ratio, which are closely associated with HLECs apoptosis. However, pretreatment with EGCG effectively reversed the expression levels of Bax and Bcl-2, and increased the Bcl-2/Bax ratio. This result is identical to the results of previous investigations, which revealed that UV irradiation could induce apoptosis of lens epithelial cells by regulating Bax and Bcl-2 expression (57,58) and that EGCG could suppress downregulation of the Bcl-2/Bax ratio induced by oxidative stress (59). These observations indicated that Bcl-2 family proteins may have an important role in regulating HLECs apoptosis under UVB irradiation and that EGCG may be able to prevent UVB irradiation-induced HLECs apoptosis by regulating the expression levels of Bax and Bcl-2.

EGCG has been suggested to protect HLECs from H_2O_2-induced apoptosis by regulating the caspase, MAPK and Akt pathways (19). EGCG may protect against high glucose-induced HLECs apoptosis by regulating the gene expression levels of the Bcl-2 family, c-fos, c-myc and p53 (60). EGCG may also block the adverse effects of UVB radiation by modulating the JNK1/c-Jun pathway in ARPE19 cells (37). In addition, it has been reported that EGCG can reduce UVB-induced photodamage and apoptosis by inhibiting the expression levels of p53, p21 and c-fos genes in HaCaT cells (61). In our previous study (20), it was demonstrated that EGCG reduced UVB-induced apoptosis through AIF/Endo G signaling pathways. The present study further supplemented the findings of our previous study. Based on these results, it was suggested that EGCG could notably attenuate UVB-induced apoptosis through the caspase-dependent and caspase-independent mitochondrial apoptosis pathways. As shown in Fig. 9, it was inferred that EGCG may scavenge free radicals by enhancing the activities of CAT, SOD and GSH-Px, and increasing GSH levels, thus increasing the survival rate of HLECs. EGCG also efficiently prevented UVB irradiation-induced HLECs apoptosis by modulating the expression of Bcl-2/Bax, cytochrome *c*, caspases and AIF/Endo G (20),

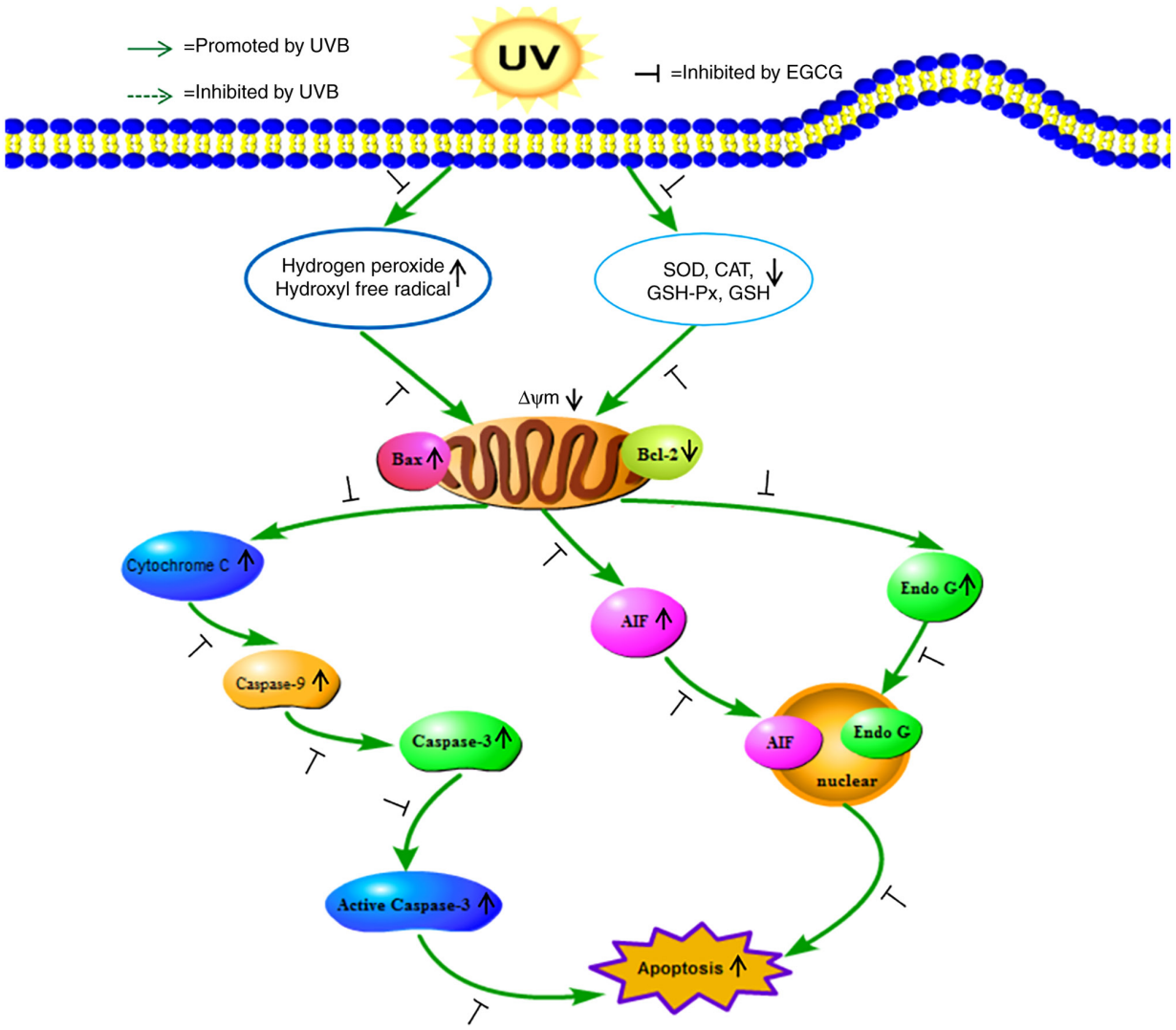

Figure 9. EGCG inhibits UVB-induced human lens epithelial cell apoptosis through the caspase-dependent pathway and caspase-independent pathway. Δψm, mitochondrial membrane potential; AIF, apoptosis-inducing factor; CAT, catalase; EGCG, epigallocatechin gallate; Endo G, endonuclease G; GSH, glutathione; GSH-Px, GSH peroxidase; SOD, superoxide dismutase; UVB, ultraviolet B.

thereby playing a protective role in HLECs survival under conditions of UVB irradiation.

In conclusion, the present study investigated the protective effect of EGCG on HLECs under conditions of UVB irradiation *in vitro*. The results indicated that UVB irradiation could induce HLECs apoptosis, which is driven by oxidative stress via the mitochondrial signaling pathway. In addition, EGCG scavenged free radicals, protected HLECs viability, improved Δψm, and enhanced CAT, SOD and GSH-Px activities, but did not significantly increase GSH levels. EGCG may also efficiently prevent UVB irradiation-induced HLECs apoptosis by modulating Bcl-2, Bax, cytochrome *c*, caspase-9 and caspase-3 expression, indicating its protective role and potential application in preventing cataract in clinical practice.

Acknowledgements

The authors would like to thank Dr Wenjun Jiang (Shandong Provincial Key Laboratory of Integrated Traditional Chinese, Shandong, China) for kindly providing guidance in the experiment and statistical analysis.

Funding

This work was supported by the Natural Science Foundation of Shandong province (grant nos. ZR2017PH002 and ZR2014HL048), the National Key Research and Development Project (grant nos. 2019YFC1710200 and 2019YFC1710203) and the National Natural Science Foundation of China (grant no. 82104937).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QW wrote the main manuscript. QW, DG, JS, YG, YZ, JG and XZ carried out the experiments and statistical analysis, and generated all figures. DG, DL and HB jointly participated in the research design. DG and HB are the corresponding

authors. HB, DG and QW confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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