Cell Biology International

Cell Biol. Int. (2011) 35, 381–389 (Printed in Great Britain)

Research Article

Inhibitory effects of Vitamin E on UVB-induced apoptosis of chicken embryonic fibroblasts

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Abstract

Apoptosis research has been focused on several model species in the past decades, whereas studies concerned with non-mammalian vertebrate, particularly birds, have rarely been involved. In accord with requirements to expand the biodiversity of apoptotic research, a chicken embryonic fibroblasts model involving UVB (ultraviolet B) as the death stimulus was established through primary explantation and serial passage. Myriads of antioxidants can inhibit UVB-induced apoptosis by virtue of scavenging reactive oxygen species. To improve our understanding of the possible anti-apoptotic effects and mechanisms of Vitamin E against UVB-induced apoptosis in chicken embryonic fibroblasts, cells treated with Vitamin E after UVB irradiation were stained with AO/EB and Fluo-3/AM to visualize chromatin distribution and calcium homoeostasis, respectively. They were also analysed by flow cytometry to detect mitochondrial transmembrane potential, and cell cycle progression and apoptotic rates were recorded. RT-PCR was used to analyse the expression of some apoptosis-related genes. Typical apoptotic events, including cell shrinkage, blebbing and nuclear condensation, occurred after radiation. In the presence of Vitamin E following irradiation, apoptotic cells were reduced. Ca^{2+} release was temporarily prevented, and cell cycle arrest at S/G2 checkpoint had almost completely reverted to normal. fas decreased, while procaspase-3 remained nearly unchanged with and without Vitamin E, and bcl2/bax ratio was up-regulated, indicating possible anti-apoptotic mechanisms through the mitochondrial pathway. This new investigation of an apoptosis model involving chicken embryonic fibroblasts expands the database of knowledge across a wider spectrum of vertebrate species.

Keywords: apoptotic model; Beijing fatty chicken; Ca^{2+}; embryonic fibroblast line; ultraviolet light; Vitamin E

1. Introduction

Apoptosis, a prevalent phenomenon in multicellular organisms, constitutes a basis for tissue homoeostasis and individual development and is responsible for the clearance of senescent cells, inhibition of canceration and immune response, the disturbance of which would almost inevitably lead to tumourigenesis, immune disorders or other major physiological disturbances in the body. Generally, apoptotic signals are transduced by death receptor and mitochondrial pathways, characterized by the formation of a DISC (death-inducing signal complex) and the release of cytochrome *c*, respectively. Programmed cell death can be induced by innumerable stimuli, including radiation, reactive oxygen species, heavy metals, aliphatic acids, virus infection, and antitumour drugs (Igney and Krammer, 2002; Leonard et al., 2004; Batista et al., 2007; Liu et al., 2009; Hori et al., 2010).

Current research concerning apoptotic mechanisms focuses on *Caenorhabditis elegans*, *Drosophila melanogaster*, rodents, human and a few species of other mammals (Mendes et al., 2006; Geng et al., 2009; Chopra et al., 2010; Chen et al., 2010; Kinjo et al., 2010), including some work on fish cells (Takle and Andersen, 2007; Krumschnabel and Podrabsky, 2009), while avian species seem to be largely neglected. However, biodiversity – the foundation of ecosystem – by definition implies that species with different genetic origins will possess distinctive biological characteristics. Therefore, research on birds is significantly important in any further development of a general theory about apoptosis.

Owing to its high efficiency and expedient application, UV light has been widely adopted as an apoptotic stimulus (Mnich et al., 2009; D'Emilio et al., 2010; Wang et al., 2010). UVB leads to the production of DNA photoproducts, mainly CPDs (cyclobutane pyrimidine dimers) and (6-4)PPs [(6-4) pyrimidine-pyrimidone photoproducts]. As DNA damage accumulates, a well-orchestrated series of self-destructive events are triggered (Batista et al., 2009). UVB-induced apoptosis and cellular damage is largely mediated by NER (nucleotide excision repair) pathway, MAPKs, ERK1/2, JNK1/2 and p38α/β, ATM/ATR, p53 signalling pathway and a number of receptor tyrosine kinases (Auclair et al., 2009). Vitamin E (tocopherol) protects against reactive oxygen species, thereby maintaining the integrity of long-chain polyunsaturated fatty acids in the membranes of cells and preserving their bioactivity (Traber and Atkinson, 2007). Evidence is accumulating that links Vitamin E with pro-apoptotic effects in many types of malignant cells and anti-apoptotic effects against oxidative stress (Gu et al., 2008; Brigelius-Flohé, 2009; Shirpoor et al., 2009; Xu et al., 2009; Huang et al., 2010). The relationship of Vitamin E and UVB-induced apoptosis

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Abbreviations: $[Ca^{2+}](i)$, free Ca^{2+}; DISC, death-inducing signal complex; PDT population doubling time; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labelling; UVB, ultraviolet B.

remains to be elucidated, and there is only a report of Portugal-Cohen et al. (2009) that suggests cream with Vitamin E has some effects against UVB-induced stress in human skin. Beijing fatty chicken (*Gallus gallus*), a local breed distinctive to the People's Republic of China, highly adaptive and genetically stable, might serve an ideal alternative in the avian world as a research model that should expand the species spectrum of apoptotic research.

The present study investigates the anti-apoptotic mechanisms of Vitamin E against UVB radiation, in an isolated and purified chicken embryonic fibroblast line. Comprehensive techniques, including flow cytometry, confocal microscopy, TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labelling) and RT-PCR, were adopted to get a better understanding of the mechanisms as well as to build up a bridge for apoptosis research between well-established models and avian species, thereby providing a valuable reference for researchers interested in apoptotic mechanisms.

2. Materials and methods

2.1. Primary cell culture and serial passage

Animal experiments were carried out in accordance with international standards on animal welfare and conformed to local and national regulations. Nine-day embryos isolated from Beijing fatty chicken eggs (Institute of Animal Sciences, Chinese Academy of Agricultural Sciences) were rinsed three times with PBS (pH 7.4), chopped into 1-mm^3 pieces and placed in tissue cultivation flasks containing MEM (minimal essential medium) (Gibco)+10% (v/v) fetal bovine serum (Hyclone) in an incubator at 37°C with 5% CO_2 in air (Freshney, 2000; Zhou et al., 2004). Primary cells were subcultured further into flasks when 80–90% confluent in the ratio of 1:2 or 1:3 (Freshney, 2000). All experimental cells were treated when they were ~80% confluent.

2.2. Growth kinetics

Cells were harvested and seeded into 24-well microplates at 1.5×10^4 per well and cultured up to 7 days. Samples were counted daily. Mean values of cell number were recorded to plot a growth curve and calculate the population doubling time (Sun et al., 2006).

2.3. Apoptotic induction

Fibroblasts in exponential phase were subject to UV radiation for 0, 0.5, 1, 2 and 4 J/cm^2, refreshed with complete MEM (Gibco) media containing different concentrations of Vitamin E and returned to the incubator before periodic sampling.

2.4. AO/EB staining

Ten microlitres of AO and EB both at 2 mg/ml in ethanol (Sigma) were added to 3 ml cell suspension harvested from a well on a six-well microplate, and the samples from different treatments were observed by confocal microscopy (Nikon TE-2000-E) with the excitation wavelengths of 488 and 543 nm.

2.5. TUNEL staining

Air-dried cell samples were fixed with 4% (m/v) paraformaldehyde (in PBS, pH 7.4, freshly prepared) and subjected to TUNEL assay using TUNEL apoptosis detection kit (KeyGEN).

2.6. Intracellular Ca^{2+} homoeostasis

Two hundred microlitres of Fluo-3/AM (Invitrogen) solution (15 μmol/l in 30 mmol/l Hepes solution) was pipetted into the microwells containing treated samples on a 24-well plate. After incubation at room temperature for 0.5–1 h, the cells were washed three times with PBS before immersion of each sample in 0.5 ml PBS at pH 7.4 for immediate examination by confocal microscopy (Nikon TE-2000-E).

2.7. FACS analysis

Annexin V–EGFP/PI (enhanced green fluorescent protein/propidium iodide) staining: cells were harvested and stained using Annexin V–EGFP/PI apoptosis detection kit (Biovision) for 0.5 h, and the cell suspension was analysed within 1 h with a flow cytometer (BD FACSCalibur).

Rhodamine-123 staining: cell suspension was harvested and centrifuged at 1000 rev./min for 5 min, washed three times with PBS, pH 7.4, stained with Rhodamine-123 (5 μg/ml in PBS, pH 7.4) and incubated at 37°C for 1 h. Subsequently, the samples were washed three times, resuspended with 0.5 ml PBS, pH 7.4, and analysed immediately by flow cytometry.

Cell cycle analysis: cells were harvested and centrifuged at 1000 rev./min at room temperature for 5 min to form a pellet. After discarding the supernatant, the pellet was washed three times with PBS at pH 7.4 and then resuspended in precooled 70% (v/v) ethanol for overnight storage at 4°C. The samples were centrifuged to remove the ethanol, washed once with PBS at pH 7.4, resuspended in PI solution (PI 0.05 mg/ml, RNase 0.02 mg/ml, NaCl 0.585 g/ml, sodium citrate 1 mg/ml, pH 7.2–7.6) and incubated at 4°C for 30 min in the dark. Samples were analysed by flow cytometry.

2.8. RT-PCR

RNA was extracted from cell samples according to the protocol described by Chomczynski and Sacchi (1987). Primers (SBS Genetech) are listed corresponding to the standards of O'Driscoll et al. (1993) (Table 1). PCR was conducted according to the protocol described by Saiki et al. (1988). Amplified cDNA products were recovered with the TIANgel Midi Purification Kit (TIANGEN), ligated to pGEM-T vector (Promega) and used to transform *Escherichia coli* DH5α according to the protocol of Hanahan (1983). Sixty microlitres of positive bacterium suspension as template was used to perform PCR for each assay. Amplification products were detected by agarose gel electrophoresis, sequenced (SBS Genetech), analysed with ContigExpress software and subjected to online BLAST using the NCBI website.

Table 1 **Primer sequences for internal control and apoptosis-related genes**
F, forward primer; R, reverse primer.

cDNA	Primer sequence	Tm (°C)	Length (bp)
Gapdh	F: 5′-ACCACAGTCCATGCCATCAC-3′ R: 5′-TCCACCACCCTGTTGCTGTA-3′	55	500
Bcl-2	F: 5′-CGCCGCTACCAGAGGGACTT-3′ R: 5′-GCATCCCATCCTCCGTTGT-3′	53	275
Bax	F: 5′-TTTTGCTTCAGGGTTTCATC-3′ R: 5′-AGAGGAGGCCGTCCCAACCA-3′	55	765
Fas	F: 5′-ATGATTTGGACGAGTGTA-3′ R: 5′-CGTAGTGTCATCTCCCTCA-3′	52	454
Procaspase-3	F: 5′-AAAAGATGGACCACGCTCAG-3′ R: 5′-GACTGAATAAACCAAGAGCC-3′	55	661

3. Results

3.1. Growth kinetics

A precise understanding on the growth kinetics of the fibroblasts provides a basis for setting appropriate detection intervals of subsequent experiments. The growth curve of chicken embryonic fibroblasts (Figure 1) was typically sigmoidal, with a population doubling time (PDT) of ~24 h. The lag time or latency phase corresponding to the adaptation and recovery period of the cells following trypsinization was ~24 h, after which they proliferated rapidly and entered exponential phase. As the cell density continually increased, proliferation slowed as a result of contact inhibition. Cell density was maximal by day 4, and a plateau phase was seen from day 5.

3.2. AO/EB staining

Fibroblasts exposed to UVB for 1 J/cm^2 were cultured for 24 h. AO/EB staining was used to visualize chromatin distribution and to evaluate the integrity of membrane via their binding with DNA and RNA in relation to permeability differences (AO permeable, whereas EB impermeable). Cells exposed to UVB showed chromatin aggregation and nuclear condensation, typical of apoptosis (Figure 2).

3.3. TUNEL staining

Fibroblasts treated with UVB for 1 J/cm^2 were cultured for 48 h. DNA fragmentation was labelled *in situ* via TUNEL assay. There was an obvious increase in the number of positive cells in the radiated populations, and their proportion was substantially greater in cells not treated with Vitamin E after UVB exposure (Figure 3D). The anti-apoptotic effect of Vitamin E was dose-dependent.

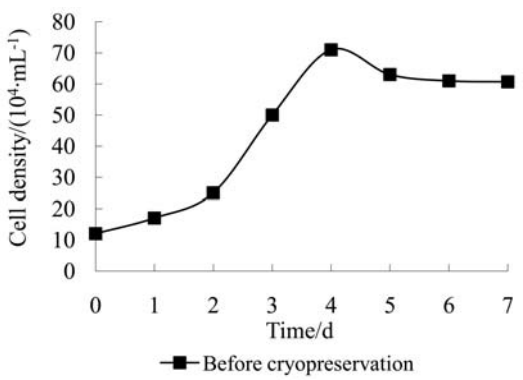

Figure 1 **Growth curve of chicken embryonic fibroblasts**

3.4. Ca^{2+} dynamics analysis

Fibroblasts exposed to UVB at 0.5 J/cm^2 were cultured for 24 and 48 h. They were subsequently labelled with the molecular probe Fluo-3/AM to visualize [Ca^{2+}](i) (free Ca^{2+}). The results show that controls released negligible amounts of Ca^{2+} (Figure 4), unlike those exposed to UVB. Cells treated with Vitamin E alone showed some relief of the alteration after 24 h; however, all the samples treated with UVB and Vitamin E showed greater Ca^{2+} release than the controls not given Vitamin E at 48 h, except in those receiving 10 μmol/l. Positive cells were lowest in samples treated with Vitamin E of 20 μmol/l at 24 h (Figure 4G) and 10 μmol/l at 48 h (Figure 4F).

3.5. FACS analysis

Cells treated with 20 μmol/l Vitamin E and those not at 24, 48 and 72 h after exposure to UVB were harvested and analysed by FACS to investigate the effects of Vitamin E on mitochondrial transmembrane potential, cell cycle progression and apoptotic rates upon UVB induction.

The results showed that apoptosis rates decreased in the presence of Vitamin E (Figure 5A). Groups treated with 20 μmol/l Vitamin E had lower transmembrane voltages, which were especially obvious at 72 h (Figure 5B). Groups at 48 h upon radiation for 1 J/cm^2 (Table 2) were arrested at S/G_2 checkpoint, leading to a decrease in the G_2/M population, which was mitigated by treatment with 20 μmol/l Vitamin E.

3.6. RT-PCR

Since the expression of several apoptosis-related genes could be influenced by UVB induction and treatment with Vitamin E, RT-PCR was used to compare the differences at the mRNA level. Sequencing software analysis and online BLAST verified the high accuracy of the amplified products. RT-PCR results of the genes of interest from treated samples at 48 h (Figure 6) showed that, upon UVB exposure, expression of bcl-2, fas and procaspase-3 was significantly increased, whereas in the case of bax, the situation was more complicated. The expression of bax was clearly elevated

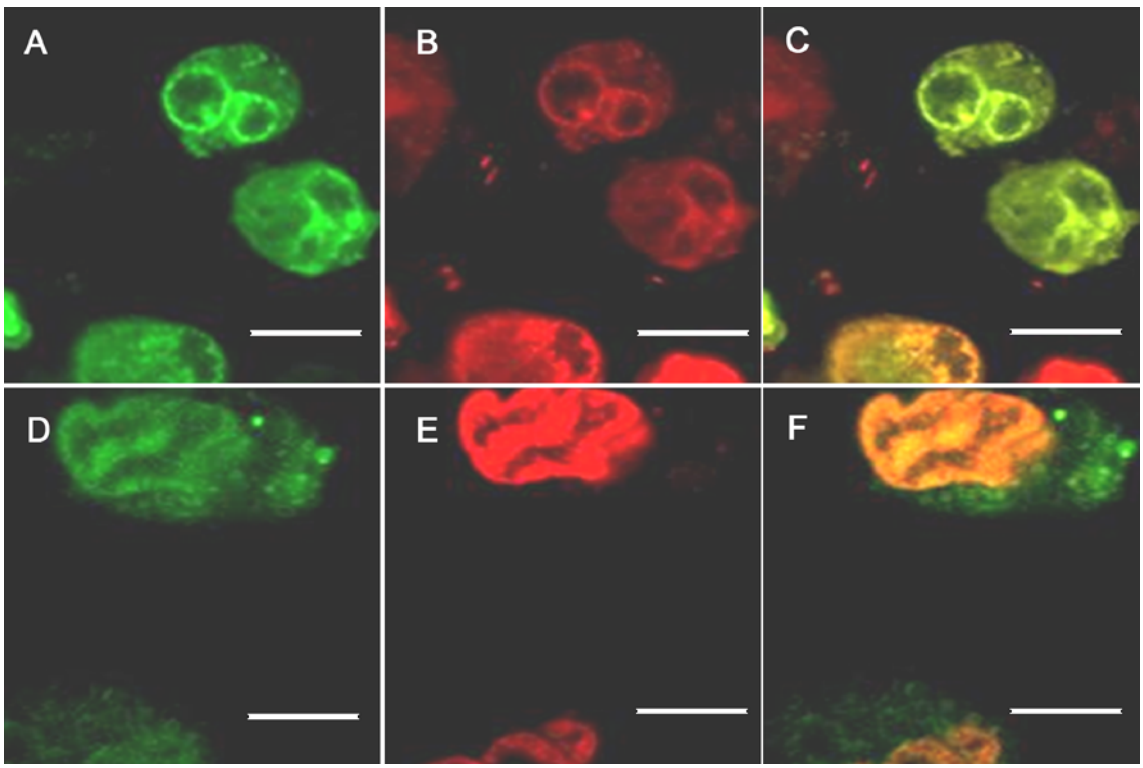

Figure 2 **AO/EB staining for chicken embryonic fibroblasts upon UVB exposure of 1 J/cm^2 at 24 h**
(**A**, **D**) AO+/EB−; (**B**, **E**) AO−/EB+; (**C**, **F**) AO+/EB+. Scale bars=10 μm.

after exposure to UVB, but at 4 J/cm^2, it became down-regulated. Upon treatment with 20 μmol/l Vitamin E, bcl-2 was elevated, fas decreased, procaspase-3 remained highly expressed and bax was down-regulated, except after exposure to 4 J/cm^2 UVB.

4. Discussion

A chicken embryonic fibroblast line has been isolated, purified through adherent culture and serial passaged for further research. A minimal number of passages, 2–6, is recommended to reduce cellular damage and to obtain relatively pure populations of cells.

There are many characteristic changes associated with apoptosis, including nuclear fragmentation, blebbing, chromatin aggregation, cytoplasmic condensation, cell partitioning and the emergence of apoptotic bodies containing intact cytoplasmic organelles (Gerschenson and Rotello, 1992; Cohen, 1993). Cells damaged with UVB suffered similar changes, such as shrinkage, blebbing, nuclear condensation, etc.

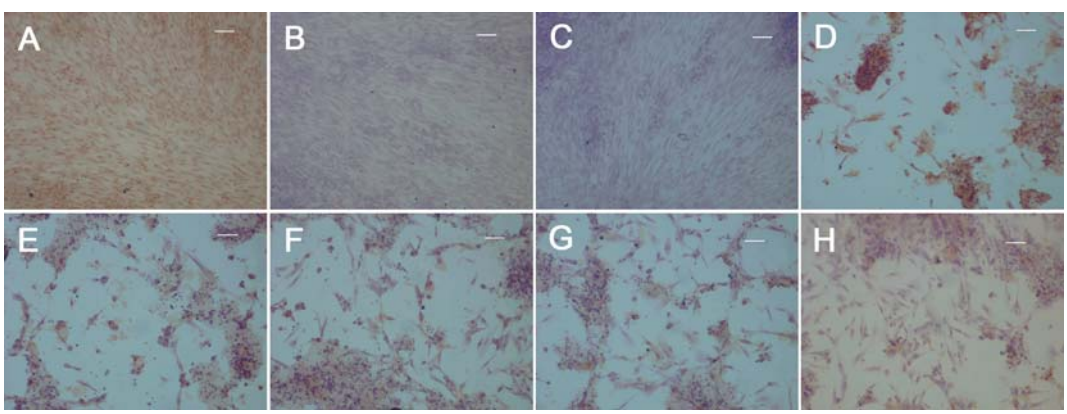

Figure 3 **TUNEL microslides for chicken embryonic fibroblasts upon UVB for 1 J/cm2 at 48 h**
(**A**) Positive control; (**B**) negative control; (**C**) control; (**D**, **E**, **F**, **G**, **H**) corresponding to groups treated with 0, 10, 20, 50 and 100 μmol/l Vitamin E, respectively. Positive cells were stained brown, indicating that DNA fragmentation had taken place. This characteristic apoptotic event due to UVB exposure was greatly reduced after Vitamin E treatment. A representative experiment of at least three similar replicates is shown. Scale bars=100 μm.

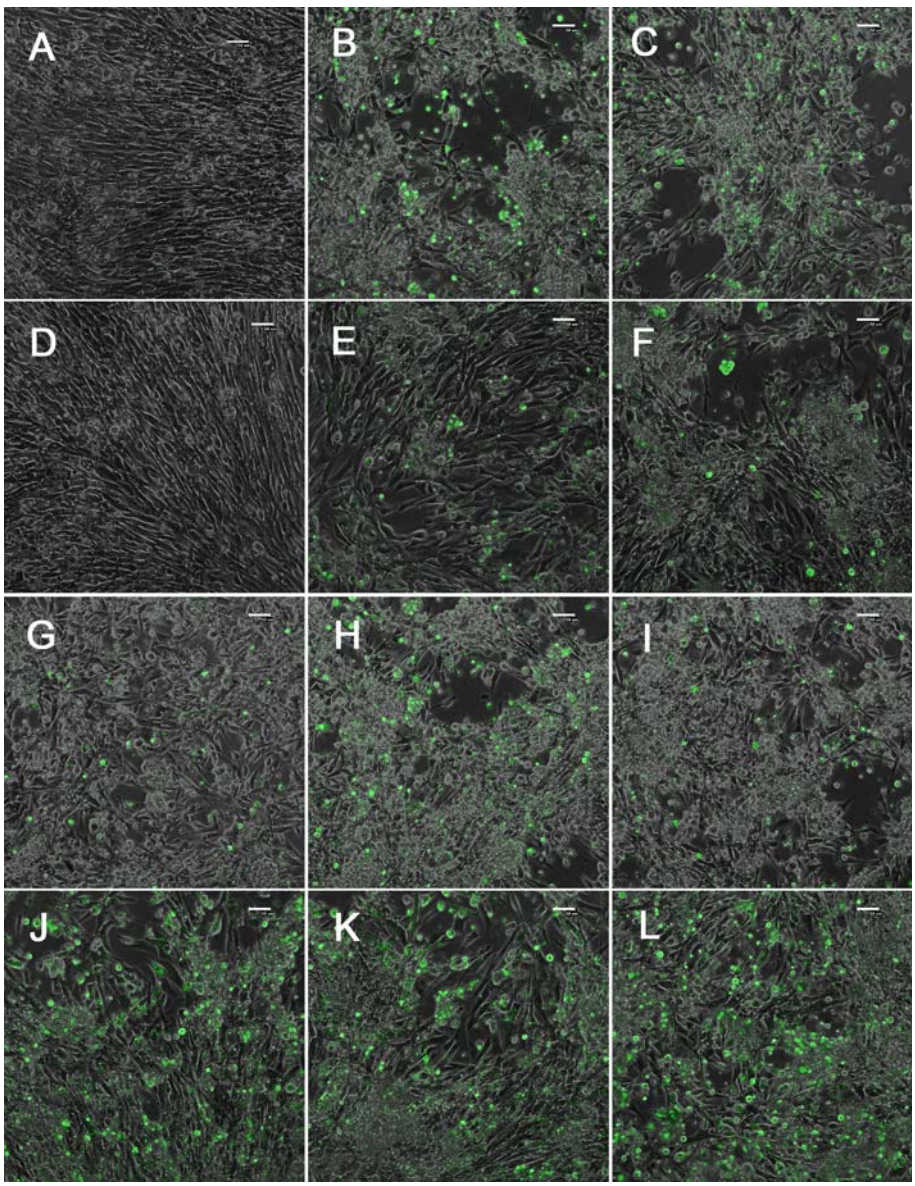

Figure 4 **Effects of UVB and Vitamin E on intracellular Ca^{2+} homoeostasis: (A) control at 24 h; (D) control at 48 h; (B, C, G, H, I) corresponding to control and samples treated with 0, 10, 20, 50 and 100 μmol/l Vitamin E after UVB exposure to 0.5 J/cm^2 at 24 h, respectively; (E, F, J, K, L) corresponding data at 48 h, respectively**

Perturbations of intracellular calcium homoeostasis were characterized with the green fluorescence emitted by the specific binding of released free Ca^{2+} with Fluo-3/AM. Positive cells were lowest in the sample treated with 20 μmol/l Vitamin E at 24 h and with 10 μmol/l Vitamin E at 48 h. A representative experiment of at least three similar replicates is shown. Scale bars=50 μm.

Prunella vulgaris extract and its main phenolic acid component, RA (rosmarinic acid), *Lonicera caerulea* and *Vaccinium myrtillus* fruit polyphenols are known to suppress UVB-induced DNA fragmentation in human keratinocytes HaCaT (Svobodová et al., 2009; Vostálová et al., 2010). Our TUNEL assay data suggest that massive DNA fragmentation occurs after UVB exposure and that Vitamin E effectively mitigated some of the damage. TUNEL-positive cells decreased as the concentration of Vitamin E rose. Although the mechanisms are unclear, Vitamin E may well have inhibited the activation of CAD (caspase-activated deoxyribonuclease), endonuclease G and other autocatalytic enzymes.

Intracellular accumulation of $[Ca^{2+}](i)$ is sufficient to induce apoptosis (Jiang et al., 1994), the mechanisms targeting mitochondria and ER (endoplasmic reticulum) and activating caspases and calpains, thereby triggering the signal cascade (Kass and Orrenius, 1999; Verbert et al., 2007). Previous research has suggested that basal $[Ca^{2+}](i)$ decreased immediately after 250 J/m^2 UVB irradiation in human keratinocyte HaCaT cells (Li et al., 2004). We observed that Vitamin E was effective in preventing Ca^{2+} from being released, but the effect was only transitory. Considering that apoptosis is a multifunctional mechanism, it was likely that other signal transduction pathway finally leads to the disturbance of Ca^{2+} homoeostasis.

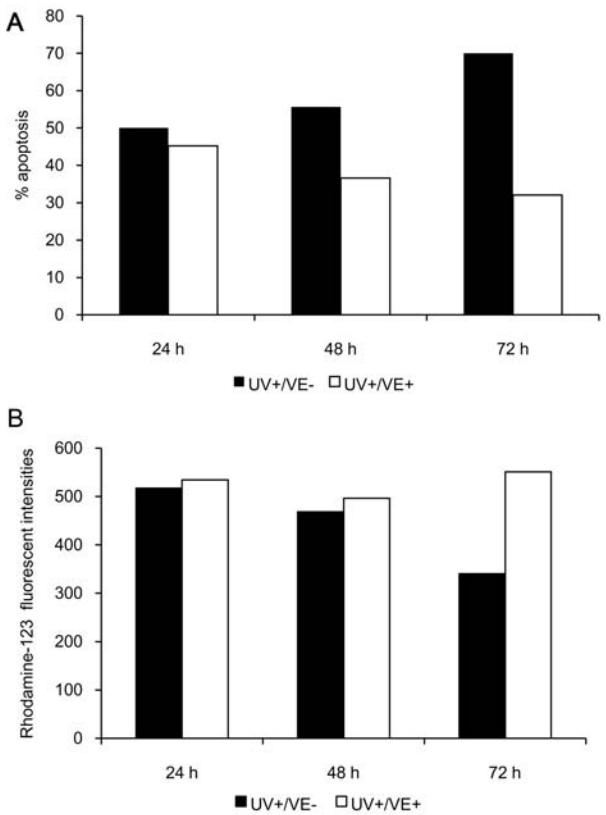

Figure 5 FACS analysis
(**A**) Apoptosis rates for samples treated with 20 μmol/l Vitamin E and not upon UVB exposure to 1 J/cm^2 at 24, 48 and 72 h; (**B**) the mitochondrial transmembrane potentials reflected as Rhodamine-123 fluorescent intensities for samples treated with 20 μmol/l Vitamin E and not after UVB exposure to 1 J/cm^2 at 24, 48 and 72 h.

A vast number of antioxidants, including Vitamin E, can have a protective role against UVB-induced apoptosis in human keratinocytes through their ability to scavenge reactive oxygen species (Chirico et al., 2007; Cho et al., 2007; Tsoyi et al., 2008). Our results showed that 20 μmol/l Vitamin E significantly reduced apoptotic rates, an effect that displayed a time-lapse increase. MOMP (mitochondrial outer membrane permeabilization) occurs in both caspase-dependent and -independent cell death, followed by the formation of PTPC (permeability transition pore complex) by VDAC (voltage-dependent anion channel) and ANT (adenine nucleotide translocator), leading to the release of apoptotic factors, including cytochrome *c* and a drop in mitochondrial transmembrane potential (Green and Reed, 1998; Zamzami and Kroemer, 2001; Chipuk et al., 2006). In contrast to previous assumptions, the results suggest that mitochondrial transmembrane potential decreases with the existence of Vitamin E, being especially obvious at 72 h. It is possible that Vitamin E temporarily prevents transmembrane potential from dropping; however, it might eventually depolarize through other mechanisms. In response to apoptotic-inducing stimulus, p53 could be activated and block cell cycle at G_1/S checkpoint or S/G_2 checkpoint (Levine, 1997). UVB induces a G_2 arrest of human melanocytes in a Gadd45a-dependent manner (Fayolle et al., 2006). In murine epidermis, UVB irradiation at 90 mJ/cm^2 induces a rapid and sustained increase in S phase by 18 h. By suppressing cyclin D1 expression, UVB could arrest the cell cycle at the G_0/G_1 transition in mouse embryonic fibroblasts (Song et al., 2010). UV light could, according to our results, block the cell cycle at S/G_2 checkpoint, the effects of which could be counteracted to a great extent by the presence of Vitamin E. But it remains unclear why the S phase population totally vanished in the group treated with Vitamin E that was not exposed to UVB.

The anti-apoptotic protein Bcl2 is overexpressed in many malignant cells of keratinocyte origin, but the expression is at low levels and restricted to follicular and interfollicular basal cells of normal human skin, and the deficiency of bcl-xL, another member in Bcl-2 family, can sensitize epidermal keratinocytes to UVB-induced apoptosis (Rossiter et al., 2001; Dae et al., 2009). In HaCaT cells, bcl2 decreased slightly upon exposure to UVB, 400 mJ/cm^2, whereas in primary melanoma cells, reduction of bcl-xL (but not bcl-2) expression is involved in UVB-induced apoptosis (Zhang and Rosdahl, 2006; Dae et al., 2009). In human prostate cancer cells, exposure to Vitamin E and selenium increases Bax, Bak and Bid proteins and decreases Bcl-2 protein; Bax knockdown and Bcl-2 overexpression resulted in a rescue of the cells from apoptosis (Reagan-Shaw et al., 2008). In melanocytes, both Bcl-2 and Bax mRNA were up-regulated with only a slight increase in apoptosis being found 24 h after UVB (λ>305 nm), and increasing UVB between 280 and 305 nm enhanced apoptosis and up-regulated Bcl-2, whereas Bax mRNA remained unaltered, indicating that a redistribution of Bax protein might be responsible for the acceleration of apoptosis (Bivik et al., 2005). Our data show that Bcl-2 is up-regulated after exposure to UVB with and without Vitamin E treatment. It is noteworthy that Bax was up-regulated upon exposure to UVB except at 4 J/cm^2, and with Vitamin E treatment, Bax levels were reduced to comparable levels in the corresponding samples exposed to UVB at the same intensities except as that at 4 J/cm^2. This presumably resulted from a flexible pathway choice of cell death following different levels of exogenous stimuli, i.e. upon the receipt of moderate exogenous stimuli, the cells may be subjected to apoptosis, while after a more drastic insult, they would probably switch to a different cell death mechanism than apoptosis, possibly necrosis, in which Bax plays only a minor role. According to the RT-PCR results treatment in which Vitamin E was given after exposure to 4 J/cm^2 UVB, apoptosis predominates again as the main mechanism of cell death. UVB irradiation can induce apoptosis

Table 2 Cell cycle analysis for groups at 48 h treated with 20 μmol/l vitamin E and not after UV radiation of 1 J/cm^2

			UV radiation	
Phase	**Medium control (%)**	**20 μmol/l (%)**	**0 μmol/l (%)**	**20 μmol/l (%)**
G_0/G_1	64.62	80.93	66.92	65.45
S	18.82	0.00	20.78	19.41
G_2/M	16.56	19.07	12.30	15.14

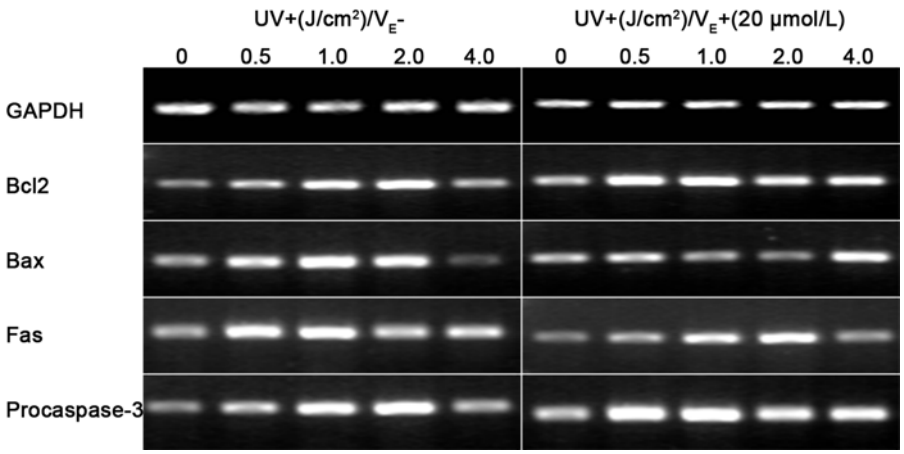

Figure 6 RT-PCR data at 48 h
GAPDH was used as internal control. Similar results were obtained in three replicates.

at lower intensities and necrosis at higher intensities in HaCaT keratinocytes (Mammone et al., 2000). By implication, Vitamin E has significant effects on the mitochondrial pathway through the alteration of Bcl-2/Bax ratio at lower UVB intensities, whereas at a higher intensity (4.0 J/cm^2), multiple cell death mechanisms might be involved. *In vivo* exposure of human epidermal cells to UVB induces an increased expression and clustering of Fas, and the polypeptide from *Chlamys farreri* was shown to inhibit the expression of Fas and FADD (Fas-associated death domain), as well as the activation of caspase-8 in HaCat cells in a dose-dependent manner (Bang et al., 2003; Li et al., 2008). In contrast to earlier work on the effects of UV light on human skin, and perhaps due to the variation of intensities, our results suggest that Fas expression is augmented after radiation for 0.5 and 1 J/cm^2 (Jung et al., 2008). Fas was down-regulated after being treated with Vitamin E, indicating possible anti-apoptotic effects of Vitamin E on the death receptor pathway. As reflected by caspase-3 activation, exposure to lower intensity UVB (300 mJ/cm^2) is not pro-apoptotic, in contrast with keratinocytes exposed to a higher dose (2400 mJ/cm^2) (Bertrand-Vallery et al., 2010). Expression of procaspase-3 was elevated after exposure to UVB and was similar with and without Vitamin E in this report, yet their activation might have been retarded to a certain degree.

In summary, an apoptotic model using chicken embryonic fibroblasts has been successfully established and could contribute to the studies concerning unknown apoptotic mechanisms by expanding the species spectrum of research. The results from FACS, TUNEL, Ca^{2+} dynamics and RT-PCR provided possible explanations for the anti-apoptotic effects of Vitamin E against UV light. However, the detailed molecular mechanisms require further investigation.

Author contribution

Dapeng Jin is the major researcher of this investigation, responsible for most experiments as well as manuscript preparation. Chunying Li was responsible for the experimental design and a co-worker for most experiments. Yimei Cong was the technician support for flow cytometry. Hongjian Yang was responsible for the statistic analysis and experimental direction. W.X. Zhang was responsible for cell culture and preparation of experimental cells. Weijun Guan provided guidance for molecular experiments and manuscript revision. Yuehui Ma was responsible for the experimental design, data analysis and manuscript revision.

Acknowledgements

We thank BioMedES and Dr X.Ch. Li at Chinese Academy of Agricultural Sciences for giving constructive revision comments in the manuscript preparation.

Funding

This research was supported by the ''863'' National Major Research Program [grant number 2007AA10Z170], the National Key Technology R&D Program [grant numbers2006BAD13B08, 2008BADB2B01] and the National Scientific Foundation of China [grant number 30671539].

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Received 9 October 2009/12 July 2010; accepted 5 November 2010

Published as Immediate Publication 5 November 2010, doi 10.1042/CBI20090285