Showa Univ J Med Sci **27**(3), 193～203, September 2015

Original

Apoptosis-induced Proliferation in UV-Irradiated Human Conjunctival Epithelial Cells

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Abstract: A pterygium is a benign growth that develops on the conjunctiva and, in some cases, extends to the cornea and interferes with vision. Excessive exposure to ultraviolet (UV) light is one of the causes of pterygium development. We previously reported that UV-induced apoptosis is led by production of reactive oxygen species (ROS) that activate p38 mitogen-activated protein kinase (MAPK) in human conjunctival epithelial (HCE) cells. Also, ROS-dependent induction of interleukin-11 (IL-11) has been reported to upregulate MAPK pathways, which results in compensatory proliferation. In this study, we examined the effect of UV exposure on HCE cells, in terms of change in apoptosis, ROS generation, phosphorylation of c-Jun N-terminal kinase (JNK), levels of IL-11 (a key cytokine in tissue repair and compensatory proliferation), production of activator protein 1 (AP-1), and expression of c-myc, c-fos and c-jun (which provides evidence of healthy cell proliferation). Apoptosis in HCE cells was induced by UV light irradiation (312 nm, 4.94 mW/cm^2). Apoptosis was measured using the Muse Annexin V and Dead Cell Assay Kit. ROS generation was measured by using 5-(and 6-) chloromethyl-2'7'-dichlorodihydrofluorescein diacetate, acetyl ester. JNK phosphorylation, IL-11 levels and AP-1 production were measured by enzyme-linked immunosorbent assays (ELISAs). Imnunocytochemical staining was used to measure c-myc, c-fos and c-jun expression. UV irradiation increased ROS generation, phosphorylation of JNK, and apoptotic cell count. IL-11 levels and AP-1 production were significantly increased by UV irradiation. The irradiated cells had increased expression of c-myc, c-fos and c-jun, and treatment of the cells with IL-11 significantly increased expression of c-myc, c-fos and c-jun. These results suggest that the release of IL-11 from UV-induced apoptotic HCE cells and surrounding healthy cells could promote proliferation to maintain homeostasis.

Key words: Ultraviolet (UV), apoptosis, compensatory proliferation, interleukin-11 (IL-11), conjunctiva

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Introduction

The keratoconjunctiva of the ocular surface is directly irradiated with solar ultraviolet (UV) light and exposed to many stresses compared with organ tissue, so various UV-induced diseases, such as pterygium, pinguecula, and cataract, affect the keratoconjunctiva[1–3]. A pterygium is a fibrovascular neoformation characterized by a triangular or wing-shaped overgrowth of abnormal conjunctiva onto the cornea. The mechanism of pterygium development is unclear. Many interpretations have been proposed for the pathogenesis of pterygium. Inflammation, fibrovascular proliferation, and cell apoptosis are known to play important roles in human pterygium pathogenesis[4–6]. Some researchers have reported that not only oxidative stress but also increases in inflammatory cytokines induced by UV irradiation cause the pathogenesis of pterygium[7]. However, the cellular and molecular mechanisms of pterygium development are poorly understood.

An increase in reactive oxygen species (ROS) generation has been implicated in the pathogenesis of numerous forms of ocular surface disease[8–11]. We previously reported that ROS generation activated the p38 mitogen-activated protein kinase (MAPK) and promoted apoptosis mediated in mitochondria when cultured human conjunctival epithelial (HCE) cells were irradiated with UV at 312 nm, a relatively long wavelength[12].

ROS are produced as byproducts of mitochondrial electron transport and induce significant damage to cell structures. However, ROS have important roles in cellular processes including cell proliferation[13, 14]. ROS may have both harmful and beneficial effects[15].

Coordination of cell death and proliferation is critical for the maintenance of tissue homeostasis. Excessive cell loss in tissue can be compensated for by the division of remaining cells. A phenomenon termed 'compensatory proliferation' plays an important and beneficial role in histogenesis and organogenesis, maintenance of homeostasis, tissue regeneration, wound healing, and cancer development and progression[16, 17]. Recently, Sakurai *et al* reported that cells adjacent to apoptotic or damaged cells in the liver undergo compensatory proliferation and lead to cancer[14]. Apoptosis and necrosis are tightly associated with ROS that activate various signaling pathways, and oxidative stress is involved in compensatory proliferation[18].

Growth factors are released from apoptotic cells to repair tissue[19], and the repair mechanism is associated with MAPK and interleukins (ILs)[20]. Recent analysis of gene expression induced by enhanced ROS production accompanying cell death identified IL-11, one of the IL-6 family of cytokines, as having a key role in tissue repair and compensatory proliferation[18].

We thus assumed that apoptosis-induced proliferation helps to maintain the homeostasis of keratoconjunctive cells through rapid tissue turnover induced by negative feedback in conditions of severe stress such as UV exposure.

In this study, we investigated the apoptosis-induced proliferation in UV-irradiated HCE cells, in terms of change in apoptosis, generation of ROS, phosphorylation of c-Jun N-terminal kinase (JNK), levels of IL-11, activation of activator protein 1 (AP-1), and expression of c-myc, c-fos and c-jun (which provides evidence of healthy cell proliferation).

Materials and methods

Culture of HCE cells

A human eyeball-derived conjunctival epithelial cell line, Clone-1-5c-4 (HCE cells; Wong-Kilbourne derivative of Chang conjunctiva clone) was purchased from DS Pharma Biomedical (Osaka, Japan) and cultured in 2 mM glutamine, 10% fetal bovine serum, and penicillin-streptomycin-containing Medium 199 (Sigma-Aldrich, St Louis, Mo, USA) at 37℃ in 5 % CO_2[21].

UV irradiation

Using a UV lamp (TFX-20MC, Vilber Lourmat, Marne La Vallée, France), UV was applied at a central wavelength of 312 nm, intensity of 4.94 mW/cm^2, and dose of 30, 99 or 296 mJ/cm^2. To apply the UV, the lamp made contact with the bottom of the culture dish.

Apoptosis analysis

Cells were adjusted to 5×10^5 cells/ml and seeded in six-well plates, and cultured for 24 hours. Cells were irradiated with UV of 30, 99 or 296 mJ/cm^2, then cultured for 24 hours, and then extracted. Apoptosis was measured using the Muse Annexin V and Dead Cell Assay Kit (EMD Millipore, Billerica, Mass, USA). The rate of apoptotic cells with Annexin V-PE binding to phosphatidylserine that moved to the cell surface in the early phase of apoptosis was determined, and the ratio of apoptotic cells to cells that lost cell membrane integrity was determined.

Measurement of ROS generation

HCE cells were adjusted to 1×10^5 cells/ml, cultured for 24 hours and UV irradiated at 99 mJ/cm^2. ROS were measured 30 minutes after irradiation using 5- (and 6-) chloromethyl-2'7' -dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA, Invitrogen, La, USA). CM-H_2DCFDA is degraded to 2'7'-dichlorodihydrofluorescein by esterase and oxidized by ROS (mainly hydrogen peroxide) in cells, producing fluorescent 2'7'-dichlorodihydrofluorescein. CM-H_2DCFDA was dissolved in dimethyl sulfoxide and adjusted to 100 μM, and then 8 μl of this solution was added to UV-irradiated cells in 96-well microplates, followed by incubation at 37℃ for 15 minutes. The medium was changed to 100 μl of phosphate-buffered saline, and fluorescence was measured at 488 nm excitation and 525 ± 10 nm emission using a Twinkle LB 970 fluorometer (Berthold Technologies, Bad Wildbad, Germany).

Measurement of JNK phosphorylation ability

Cells were adjusted to 1×10^5 cells/ml, cultured in 96-well plates for 24 hours, pretreated with or without 10 μM SP600125 (Sigma-Aldrich, St Louis, Mo, USA) — a c-Jun N-terminal kinase inhibitor (JNKi) — and then irradiated with UV. At 1 hour after irradiation, phosphorylation was measured in cell lysates using the Cell-Based JNK (Thr183/Tyr185) Phosphorylation ELISA kit (Ray Bio®, Ray Biotech, Norcross, Ga, USA.), with horseradish peroxidase (HRP)-labeled anti-JNK and anti-phosphorylated JNK antibodies. Following incubation with the appropriate

substrate for color development, absorbance at 450 nm was measured using a microplate reader.

Measurement of IL-11 levels and AP-1 activity

After culturing cells adjusted to 5×10^5 cells/ml in 6-well plates or 1×10^5 cells/ml in 96-well plates for 24 hours, 10 μM JNKi was added 1 hour before UV irradiation. For all samples, the medium was changed to normal culture medium before irradiation. Immediately after irradiation, the medium was changed to normal medium with or without JNKi, and cell lysates from each cell group were collected after 1, 3, 6, 15 and 24 hours of culture. Separately, we collected supernatant after centrifuging the samples at 3000 × g for 5 minutes, and the isolated residual cells were subjected to extraction of cytoplasm and nucleus using the Nuclear/Cytosol Fraction Kit (BioVision, Milpitas, Calif, USA). The culture supernatant fraction was subjected to measurement of time-course changes in IL-11 production. IL-11 was detected by employing the sandwich ELISA method, using the IL-11 Human ELISA Kit (Abcam®, Cambridge, UK) and HRP-labeled anti-IL-11 antibody, and absorbance at 450 nm was measured using a microplate reader.

The nuclear fraction was subjected to measurement of time-course changes in nuclear AP-1 activity using the Transcription Factor ELISA Kit (Panomics®, Affymetrix, Santa Clara, Calif, USA). AP-1 was detected employing the sandwich ELISA method using HRP-labeled anti-AP-1 antibody, and absorbance at 450 nm was measured using a microplate reader.

Immunocytochemical staining for c-myc, c-fos and c-jun

HCE cells were adjusted to 3×10^5 cells/well, and cultured on Chamber Slides™ for 24 hours. To determine the effect of UV irradiation (99 mJ/cm^2), cells were cultured for 24 hours after UV irradiation. To determine the effect of IL-11 treatment, cells were cultured with 10 nM IL-11 (recombinant human IL-11, PeproTech, Rocky Hill, NJ, USA) for 24 hours. Cells were fixed with formalin at each time point and reacted with primary antibodies: c-myc (c-8) mouse monoclonal IgG_{2a} (sc-41, Santa Cruz Biotechnology, Dallas, Tex, USA), c-fos (Ab-2) rabbit polyclonal IgG (PC05-100UG, Oncogene Research Products, La Jolla, Calif, USA) and Phospho-c-jun (Ser63) rabbit monoclonal IgG (54B3, Cell Signaling Technology, Danvers, Mass, USA) for 2 hours, followed by reactions with secondary antibodies and peroxidase-conjugated dextran polymer reagent to stain the nucleus and cytoplasm, using the EnVision staining system.

Statistical analysis

Results are presented as mean ± standard error (n = 3–12). The parameters in the UV group were analyzed using the Student's t-test for comparison between two groups or ANOVA followed by the Bonferroni test for repeated measurements, and $P < 0.05$ was regarded as significant.

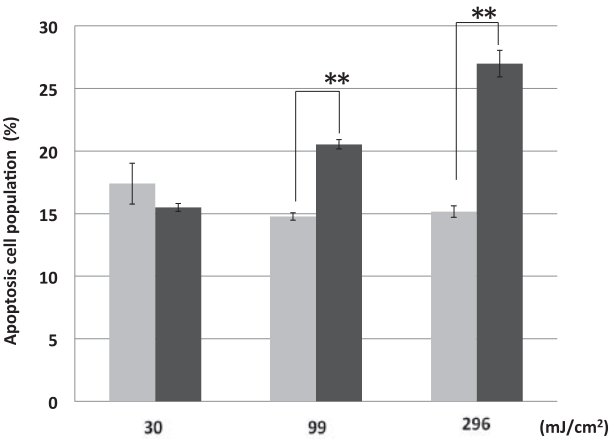

Fig. 1. Effects of UV irradiation on apoptosis in HCE cells
HCE cells were exposed to 30, 99 or 296 mJ/cm^2 of UV and incubated for 24 hours. Apoptosis was determined using the MUSE™ Cell Analyzer and UV-irradiated cells (dark column) were compared with non-irradiated cells (white column). **: $P < 0.01$ versus non-irradiated HCE cells. Values are mean ± SEM (n = 3).

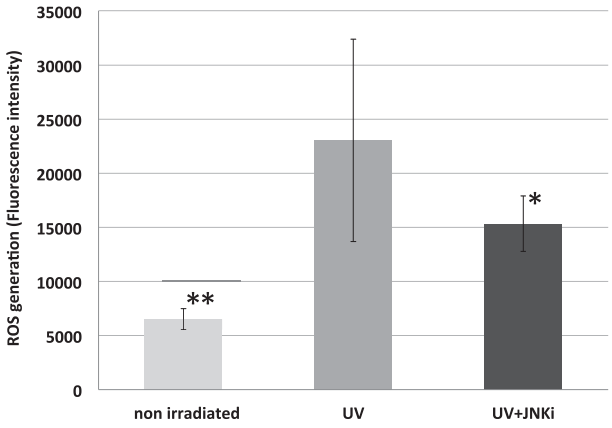

Fig. 2. Effects of UV irradiation on ROS generation in HCE cells
ROS generation in HCE cells was evaluated by using CM-H_2DCFDA. In the case of JNKi pretreatment, HCE cells were pretreated with JNKi for 1 hour, exposed to 99 mJ/cm^2 UV irradiation, and then incubated with JNKi for 30 minutes. Values are mean ± SEM, and represent the average fluorescence intensity/well (n = 6). *: $P < 0.05$, **: $P < 0.01$ versus UV-irradiated HCE cells from two independent experiments.

Results

UV irradiation-induced apoptosis in HCE cells

Fig. 1 shows the results of our apoptosis analysis of living HCE cells exposed to 30, 99 or 296 mJ/cm^2 UV irradiation. The percentage of apoptotic cells was significantly increased: 20.5% ± 0.4% after 99 mJ/cm^2 irradiation and 27.0% ± 1.1% after 296 mJ/cm^2 irradiation, when compared with non-irradiated cells (14.8% ± 0.3%, n = 3, $P < 0.01$ and 15.2% ± 0.5%, n = 3, $P < 0.01$, respectively).

UV irradiation-induced ROS generation

Fig. 2 shows ROS generation in HCE cells presented as the fluorescence intensity at 30 minutes after 99 mJ/cm^2 UV irradiation. The level of ROS significantly increased in the UV group (23030 ± 9361.3 fluorescence intensity [FI], n = 6) compared with the non-UV group (6518.3 ± 968.5 FI, n = 6; $P < 0.01$), confirming the induction of oxidative stress by UV irradiation. Pretreatment with JNKi significantly inhibited the UV-induced ROS generation (15333.6 ± 2558.0 FI, n = 6; $P < 0.05$).

UV irradiation-induced JNK phosphorylation ability

Fig. 3 shows the JNK phosphorylation ability in HCE cells 24 hours after 99 mJ/cm^2 UV irradiation presented as the phosphorylated JNK/total JNK ratio. JNK phosphorylation ability was

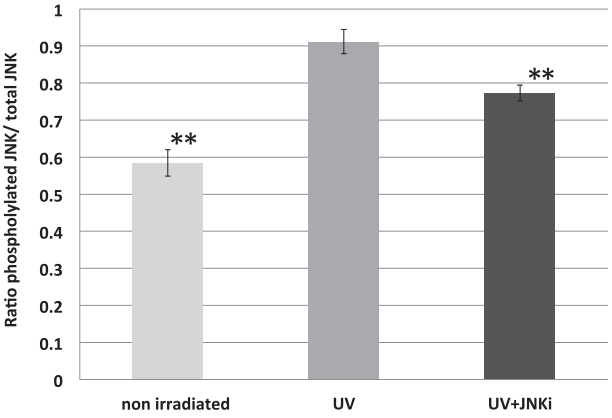

Fig. 3. Effects of UV irradiation on JNK phosphorylation in HCE cells
The levels of phosphorylated JNK and total JNK in non-irradiated HCE cells, UV-radiated HCE cells and UV-irradiated HCE cells that were treated with JNKi were compared by ELISA. The UV-irradiated HCE cells that were treated with JNKi cells were pretreated with JNKi for 1 hour, exposed to 99 mJ/cm^2 UV irradiation, and then incubated with JNKi for 1 hour. Values are mean ± SEM and represent the phosphorylated JNK/ total JNK ratio (n = 12). **: P < 0.01 versus UV-irradiated HCE cells from three independent experiments.

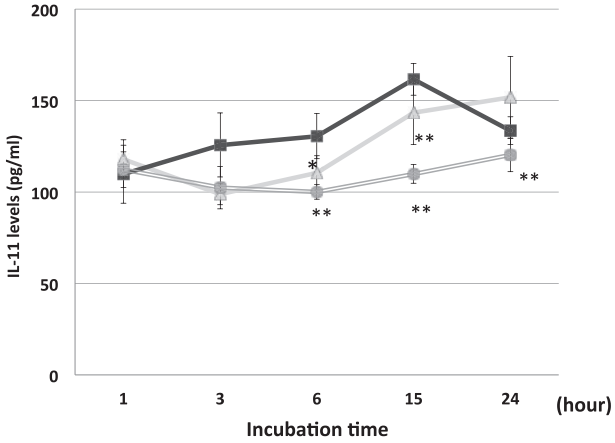

Fig. 4. Effects of UV irradiation on IL-11 levels in HCE cells
IL-11 levels were measured by ELISA using culture medium from HCE cells after UV irradiation. HCE cells were pretreated with JNKi for 1 hour, exposed to 99 mJ/cm^2 UV irradiation, and then incubated with JNK inhibitor for 1, 3, 6, 15 or 24 hours. Values are mean ± SEM (pg/ml, n = 6). *: $P < 0.05$, **: $P < 0.01$ versus UV-irradiated HCE cells from two independent experiments. △: non-irradiated, ■: UV-irradiated, ○: UV-irradiated+ JNKi.

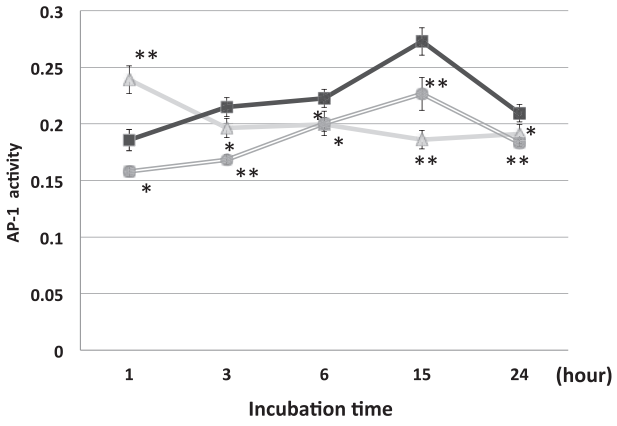

Fig. 5. Effects of UV irradiation on AP-1 production in HCE cells
AP-1 was detected by ELISA using nuclear extracts from HCE cells after UV irradiation. UV-irradiated HCE cells that were treated with JNKi were pretreated with JNKi for 1 hour, exposed to 99 mJ/cm^2 UV irradiation, and then incubated with JNKi for 1, 3, 6, 15 or 24 hours. Values are mean ± SEM and represent AP-1 activity/ ml (n = 6). *: $P < 0.05$, **: $P < 0.01$ versus UV-irradiated HCE cells from two independent experiments. △: non-irradiated, ■: UV-irradiated, ○: UV-irradiated + JNKi.

significantly increased by UV irradiation (0.91 ± 0.03, n = 6, $P < 0.01$) and significantly decreased by JNKi pretreatment (0.77 ± 0.02, n = 6; $P < 0.01$).

UV irradiation-induced IL-11 levels

Fig. 4 shows the sequential change in IL-11 production after 99 mJ/cm^2 UV irradiation. IL-11 levels were significantly increased by UV irradiation (153.8 ± 4.7 pg/ml, n = 6, $P < 0.01$) and significantly decreased by JNKi pretreatment (105.6 ± 3.5 pg/ml, n = 6, $P < 0.01$) at 15 hours after UV irradiation. This suggests involvement of JNK in increasing IL-11 production.

UV irradiation-induced AP-1 activation

Fig. 5 shows sequential changes in the levels of AP-1 activity in HCE cells after 99 mJ/cm^2 UV irradiation. AP-1 activitywas significantly increased by UV irradiation (0.27 ± 0.01 n = 6, $P < 0.01$) and decreased by JNKi pretreatment (0.22 ± 0.01, n = 6, $P < 0.01$) at 15 hours after UV irradiation.

Immunocytochemical staining for c-myc, c-fos and c-jun

Nuclear expression of c-myc, c-fos and c-jun were increased in the 99 mJ/cm^2 UV group (Fig. 6) and the IL-11-treated group (Fig. 7) at 15 and 24 hours. After UV irradiation, the expression of c-myc, c-fos and c-jun stain was increased at 15 and 24 hours; similar increases in expression of c-myc, c-fos and c-jun were seen after IL-11 stimulation. In contrast, cells that were not UV irradiated or not treated with IL-11 did not express these proteins.

Discussion

The maintenance of tissue homeostasis is a crucial biological mechanism. Cell differentiation, proliferation, wound healing, and cell death are strictly controlled by intercellular communication. It is well known that ROS generation and oxidative stress are causes of UV-induced cell damage. As the conjunctiva is directly exposed to oxygen and UV[11], we investigated the relationship between proliferation, oxidative stress and IL-11 in UV-irradiated HCE cells.

We applied UV irradiation at 312 nm to HCE cells at low, intermediate and high doses (30, 99, and 296 mJ/cm^2, respectively), with the high UV dose roughly corresponding to the daily exposure level in Tokyo in fine weather. At 24 hours after irradiation, we measured the dose-dependent increase in apoptotic cells using Annexin V staining.

Recent studies on cell death have shown that dying cells are not only phagocytosed, but are also involved in the maintenance of tissue homeostasis by releasing various factors[19]. The residual dying cells release signals or growth factors to surrounding living cells — these signals and growth factors induce tissue repair, inflammation, and even autoimmune disease[22]. Both exogenous and endogenous ROS may function as signals to promote cell proliferation[11, 23]. Increased levels of ROS lead to the activation of MAPK and Akt signaling pathways[24–26], and then the signals transfer to the nucleus and induce the expression of various genes which determine whether a cell will live or die — for example, by cell cycle regulation and apoptosis promotion.

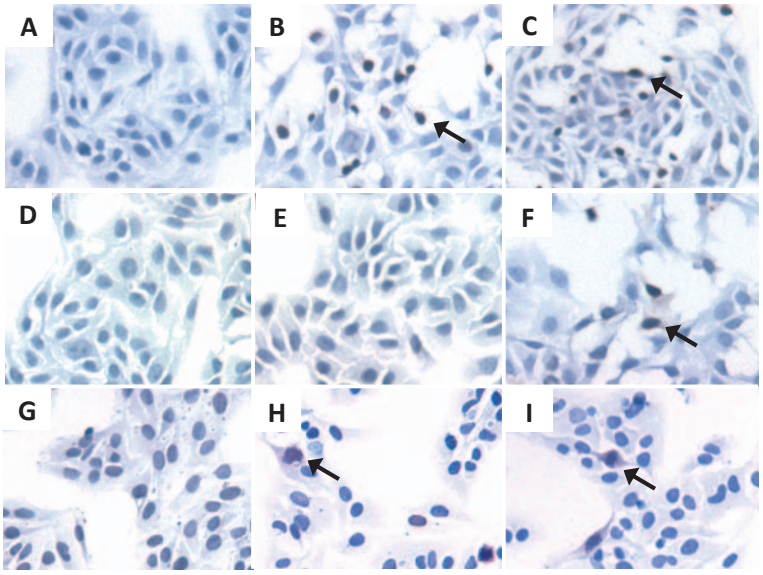

Fig. 6. Immunocytochemical staining for c-myc, c-fos and c-jun in HCE cells after UV irradiation
The upper panels show expression of c-myc, the middle panels show expression of c-fos, and the lower panels show expression of c-jun. (A), (D) and (G): HCE control for 24-hour cells (non-irradiated); (B), (E) and (H): 15 hours after UV irradiation; (C), (F) and (I): 24 hours after UV irradiation (original magnification, × 200).

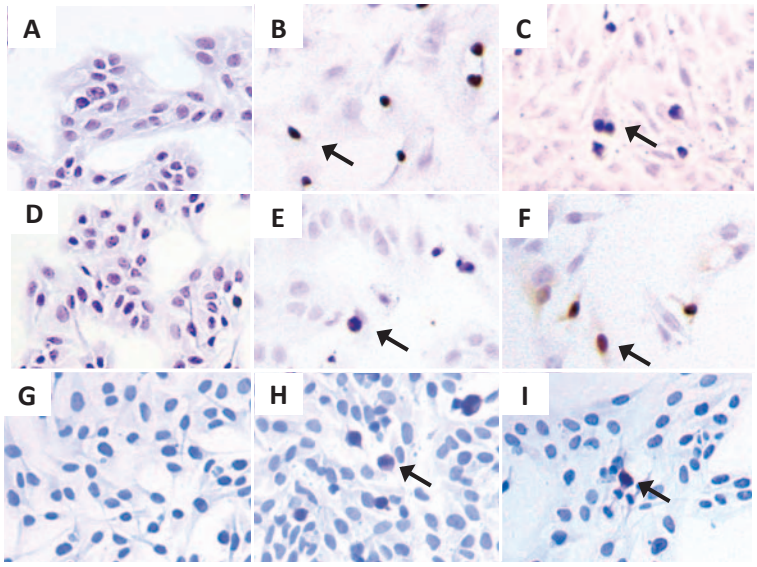

Fig. 7. Immunocytochemical staining c-myc, c-fos and c-jun in HCE cells after IL-11 treatment
The upper panels show expression of c-myc, the middle panels show expression of c-fos, the lower panels show expression of c-jun. (A), (D) and (G): HCE control for 24 hours cells (without IL-11); (B), (E) and (H): 15 hours after IL-11 treatment; (C), (F) and (I): 24 hours after IL-11 treatment (original magnification, × 200).

Studies on compensatory proliferation in the *Drosophila* wing disc recently demonstrated that apoptotic cells induce proliferation of surrounding living cells to maintain tissue homeostasis[25-28]. Cell proliferation is induced by growth factors released through a caspase-initiated (apoptosis signaling) JNK pathway[29]. In a zebrafish tail injury-healing model, NADPH-dependent oxidase (NOX)-dependent ROS generation induced in the injured tissues[30] activates neutrophil infiltration which is important for wound healing[31]. In mammals, damage-associated molecular patterns (DAMPs) are released from damaged tissues and induce the production of cytokines and chemokines[32-34], leading to compensatory proliferation through STAT3 activation[33]. Furthermore, ROS induced members of the IL-6 family of cytokines, which includes IL-6, IL-11, IL-27 and IL-31, in an acetaminophen-induced liver injury model. IL-11 induction resulted in compensatory proliferation by activating STAT3 in surrounding cells and stimulating the pathway of compensatory proliferation[18]. By forming a complex with IL-11 receptor alpha chain (IL-11RA) and gp130, IL-11 activates the JAK-STAT and Ras-ERK pathways in cells[36] and promotes compensatory proliferation. In this study, ROS generation and JNK activation significantly increased after 30 minutes and 1 hour of intermediate-dose UV irradiation, respectively. The mitochondria is an important source of ROS[34, 35]. Previously, we described increases in ROS generation and mitochondrial membrane damage resulting from UV irradiation, which induced apoptosis[12]. Furthermore, we investigated sequential changes in the production of IL-11 (which promotes vicarious growth) and AP-1 after 15 hours of intermediate-dose UV irradiation, and suppression of JNKi. These findings suggest that JNK is involved in intracellular production of ROS, IL-11 and AP-1.

Fifteen and 24 hours after the intermediate-dose UV irradiation, c-myc, c-fos and c-jun immnocytochemical stain was observed in HCE cells. Expression of c-myc , c-fos and c-jun was particularly high at 24 hours (Fig. 6, B-I). Expression of c-myc and c-fos was localized to the nucleus, and expression of c-jun was observed in cytoplasm. Expression of these proteins was confirmed by IL-11 treatment of HCE cells (Fig. 7, B-I). These findings suggest that JNK-related IL-11 production activated AP-1, and then induced the expression of genes involved in cell proliferation, such as c-myc and c-fos. In a recent study, treatment with lipopolysaccharide did not induce IL-11 in primary cultures of conjunctival epithelial cells[32]. Our study suggests that the increase in IL-11 expression was caused by UV irradiation-induced apoptosis and ROS production. Pterygium causing inflammatory, infiltrating, and proliferative lesions on the ocular surface is a representative eye disease induced by UV. The overexpression of matrix metalloproteinases (MMPs) in human tissue has frequently been reported in UV-irradiated tissues[21, 37-39]. AP-1 has also been implicated in this MMP-associated proliferation mechanism[40], suggesting that compensatory proliferation is partially involved in the pathogenesis. In conclusion, intermediate-dose UV irradiation causes the apoptosis of HCE cells due to ROS generation. Also, this condition may increase JNK activity and related IL-11 production. It may lead to AP-1 activation and promote cell proliferation through c-myc, c-fos and c-jun. These findings suggest that conjunctival epithelial cells of the ocular surface which are constantly exposed to UV stress possess an apoptosis-induced proliferation mechanism. This mechanism seems to be involved in tissue repair and maintenance of cell growth through turnover.

Conflict of interest disclosure

The authors declare no conflict of interest.

References

1) Longstreth J, de Gruijl FR, Kripke ML, *et al*. Health risks. *J Photochem Photobiol B*. 1998;**46**:20–39.
2) Nolan TM, DiGirolamo N, Sachdev NH, *et al*. The role of ultraviolet irradiation and heparin-binding epidermal growth factor-like growth factor in the pathogenesis of pterygium. *Am J Pathol.* 2003;**162**:567–574.
3) Cullen AP. Photokeratitis and other phototoxic effects on the cornea and conjunctiva. *Int J Toxicol*. 2002;**21**:455–464.
4) Golu T, Mogoanta L, Streba CT, *et al*. Pterygium: histological and immunohistochemical aspects. *Rom J Morphol Embryol*. 2011;**52**:153–158.
5) Bianchi E, Scarinci F, Grande C, *et al*. Immunohistochemical profile of VEGF, TGF-β and PGE_2 in human pterygium and normal conjunctiva: experimental study and review of the literature. *Int J Immunopathol Pharmacol.* 2012;**25**:607–615.
6) Cimpean AM, Sava MP, Raica M. DNA damage in human pterygium: one-shot multiple targets. *Mol Vis*. 2013;**19**:348–356.
7) Bradley JC, Yang W, Bradley RH, *et al*. The science of pterygia. *Br J Ophthalmol*. 2010;**94**:815–820.
8) Matsumura Y, Ananthaswamy HN. Toxic effects of ultraviolet radiation on the skin. *Toxicol Appl Pharmacol*. 2004;**195**:298–308.
9) Pauloin T, Dutot M, Joly F, *et al*. High molecular weight hyaluronan decreases UVB-induced apoptosis and inflammation in human epithelial corneal cells. *Mol Vis.* 2009;**15**:577–583.
10) Demir U, Demir T, Akpolat N. The effect of octreotide against oxidative damage in photosensitized conjunctiva and cornea of rabbits. *Doc Ophthalmol*. 2005;**110**:193–201.
11) Bachelor MA, Bowden GT. UVA-mediated activation of signaling pathways involved in skin tumor promotion and progression. *Semin Cancer Biol*. 2004;**14**:131–138.
12) Murayama M, Udaka Y, Tsuji M, *et al*. Role of MAPK on UV-induced early cytotoxicity in HCE cells. *Jpn Pharmacol Ther.* 2012;**40**:265–273. (in Japanese).
13) Hultqvist M, Olsson LM, Gelderman KA, *et al*. The protective role of ROS in autoimmune diseases. *Trends Immunol*. 2009;**30**:201–208.
14) Sakurai T, He G, Matsuzawa A, *et al*. Hepatocyte necrosis induced by oxidative stress and IL-1α release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. *Cancer Cell*. 2008;**14**:156–165.
15) Matsuzawa A, Ichijo H. Stress-responsive protein kinases in redox-regulated apoptosis signaling. *Antioxid Redox Signal*. 2005;**7**:472–481.
16) Kato K, Awasaki T, Ito K. Neuronal programmed cell death induces glial cell division in the adult Drosophila brain. *Development*. 2009;**136**:51–59.
17) Maeda S, Kamata H, Luo JL, *et al*. IKKβ couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell*. 2005;**121**:977–990.
18) Nishina T, Komazawa-Sakon S, Yanaka S, *et al*. Interleukin-11 links oxidative stress and compensatory proliferation. *Sci Signal* (Internet). 2012;**5**(**207**):ra5. [doi:10.1126/scisignal.2002056] (January 17, 2012) Available from: http://stke.sciencemag. org/content/5/207/ra5
19) Li F, Huang Q, Chen J, *et al*. Apoptotic cells activate the "phoenix rising" pathway to promote wound healing and tissue regeneration (Internet). *Sci Signal*. 2010;**3**(**110**):ra13. [doi:10.1126/scisignal.2000634] (February 13, 2010) Available from: http://stke.sciencemag.org/content/3/110/ra13
20) West XZ, Malinin NL, Merkulova AA, *et al*. Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands. *Nature.* 2010;**467**:972–976.

21) Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. *Cell.* 2010;**140**:619-630.
22) Niethammer P, Grabher C, Look AT, *et al*. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. *Nature.* 2009;**459**:996-999.
23) Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol.* 2000;**279**:L1005-L1028.
24) Ryoo HD, Gorenc T, Steller H. Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. *Dev Cell.* 2004;**7**:491-501.
25) Perez-Garijo A, Martin FA, Morata G. Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in Drosophila. *Development.* 2004;**131**:5591-5598.
26) Kondo S, Senoo-Matsuda N, Hiromi Y, *et al*. DRONC coordinates cell death and compensatory proliferation. *Mol Cell Biol.* 2006;**26**:7258-7268.
27) Huh JR, Guo M, Hay BA. Compensatory proliferation induced by cell death in the Drosophila wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. *Curr Biol.* 2004;**14**:1262-1266.
28) Fuchs Y, Steller H. Programmed cell death in animal development and disease. *Cell.* 2011;**147**:742-758.
29) Degterev A, Yuan J. Expansion and evolution of cell death programmes. *Nat Rev Mol Cell Biol*. 2008;**9**:378-390.
30) Yoo SK, Starnes TW, Deng Q, *et al*. Lyn is a redox sensor that mediates leukocyte wound attraction *in vivo*. *Nature*. 2011;**480**:109-112.
31) Zitvogel L, Kepp O, Kroemer G. Decoding cell death signals in inflammation and immunity. *Cell*. 2010;**140**:798-804.
32) Gamache DA, Dimitrijevich SD, Weimer LK, *et al*. Secretion of proinflammatory cytokines by human conjunctival epithelial cells. *Ocul Immunol Inflamm.* 1997;**5**:117-128.
33) Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer.* 2009;**9**:798-809.
34) Murphy MP. Modulating mitochondrial intracellular location as a redox signal (Internet). *Sci Signal*. 2012;**5**(**242**): pe39. [doi:10.1126/scisignal.2003386] (September 18, 2012) Available from: http://stke.sciencemag.org/content/5/242/pe39
35) Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* 2009;**417**:1-13.
36) Campbell CL, Jiang Z, Savarese DM, *et al*. Increased expression of the interleukin-11 receptor and evidence of STAT3 activation in prostate carcinoma. *Am J Pathol.* 2001;**158**:25-32.
37) Di Girolamo N, Coroneo M, Wakefield D. Epidermal growth factor receptor signaling is partially responsible for the increased matrix metalloproteinase-1 expression in ocular epithelial cells after UVB radiation. *Am J Pathol.* 2005;**167**:489-503.
38) An MX, Wu KL, Lin SC. Detection and comparison of matrix metalloproteinase in primary and recurrent pterygium fibroblasts. *Int J Ophthalmol.* 2011;**4**:353-356.
39) Tsai YY, Chiang CC, Yeh KT, *et al*. Effect of TIMP-1 and MMP in pterygium invasion. *Invest Ophthalmol Vis Sci.* 2010;**51**:3462-3467.
40) Park SY, Kim YH, Kim Y, *et al*. Frondoside A has an anti-invasive effect by inhibiting TPA-induced MMP-9 activation via NF-kB and AP-1 signaling in human breast cancer cells. *Int J Oncol*. 2012;**41**:933-940.

[Received June 16, 2015 : Accepted July 15, 2015]