PREVENTION OF ULTRAVIOLET B RADIATION-INDUCED EPIDERMAL DAMAGE BY EXPRESSION OF HEAT SHOCK PROTEIN 70*  

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Irradiation with ultraviolet (UV) light, especially UVB, causes epidermal damage via the induction of apoptosis, inflammatory responses and DNA damage. Various stressors, including UV light, induce heat shock proteins (HSPs) and the induction, particularly that of HSP70, provides cellular resistance to such stressors. The anti-inflammatory activity of HSP70, such as its inhibition of nuclear factor kappa B (NF-κB), was recently revealed. These in vitro results suggest that HSP70 protects against UVB-induced epidermal damage. Here we tested this idea by using transgenic mice expressing HSP70 and cultured keratinocytes. Irradiation of wild-type mice with UVB caused epidermal damage such as induction of apoptosis, which was suppressed in transgenic mice expressing HSP70. UVB-induced apoptosis in cultured keratinocytes was suppressed by overexpression of HSP70. Irradiation of wild-type mice with UVB decreased the cutaneous level of IκB-α (an inhibitor of NF-κB) and increased the infiltration of leukocytes and levels of pro-inflammatory cytokines and chemokines in the epidermis. These inflammatory responses were suppressed in transgenic mice expressing HSP70. In vitro, the overexpression of HSP70 suppressed the expression of pro-inflammatory cytokines and chemokines and increased the level of IκB-α in keratinocytes irradiated with UVB. UVB induced an increase in cutaneous levels of cyclobutane pyrimidine dimers and 8-hydroxy-2′-deoxyguanosine, both of which were suppressed in transgenic mice expressing HSP70. This study provides genetic evidence that HSP70 protects the epidermis from UVB-induced radiation damage. The findings here also suggest that the protective action of HSP70 is mediated by anti-apoptotic, anti-inflammatory and anti-DNA damage effects.

The skin can be structurally classified into several layers, including the most apical layer, the epidermis, containing large numbers of keratinocytes, and a second layer, immediately under this, the dermis, which has a high fibroblast content (1). Skin provides a major interface between the environment and the body and is constantly exposed to an array of physical and chemical stressors. Therefore, in addition to intrinsic causes, harmful exogenous causes are involved in the process of skin damage. Among exogenous harmful agents, ultraviolet (UV) irradiation is the most relevant to skin damage (photo-damage). UV light can be separated, based on wavelength, into three categories: UVA (320-400 nm), UVB (290-320 nm) and UVC...
(100-290 nm). Of these, the cell damaging effect of UVA is relatively weak, while most UVC is absorbed by the ozone layer (2). Thus, UVB seems to play the central role in photo-damage, such as clinical sunburn, hyperpigmentation, erythema, plaque-like thickening, loss of skin tone, deep furrowing and fine wrinkle formation, all of which constitute both clinical and cosmetic problems. Furthermore, UVB irradiation induces the development of skin cancer (photo-carcinogenesis) (3). UVB-induced photo-damage and photo-carcinogenesis both involve epidermal damage (such as induction of apoptosis), immunosuppression, inflammation (activation of pro-inflammatory cytokines and chemokines) and DNA damage (4). Since most UVB radiation is absorbed at the epidermis, keratinocytes become a major target of its deleterious effects. For example, the UVB-induced disruption of collagen and elastin (deep furrowing and fine wrinkle formation in the skin) involves inhibition of their synthesis in fibroblasts and stimulation of their degradation by matrix metalloproteinases and other proteases, both of which are triggered by pro-inflammatory cytokines and chemokines released from UVB-irradiated keratinocytes (4,5). Therefore, suppression of UVB-induced damage (apoptosis) of keratinocytes is beneficial for the prevention of photo-damage. However, since such protection may actually aid in the survival of DNA-damaged cells, resulting in promotion of photo-carcinogenesis, a mechanism that not only suppresses UVB-induced apoptosis but also UVB-induced DNA damage is important to establish protocols to prevent photo-damage without promoting photo-carcinogenesis.

UVB irradiation damages the epidermis both directly and indirectly. For example, in addition to UVB-induced direct damage of nucleic acids, proteins and lipids, UVB irradiation stimulates the production of reactive oxygen species (ROS), which also damage these molecules by oxidization. In this way, direct absorption of UVB by DNA causes DNA damage through the formation of covalent linkages, resulting in products such as cyclobutane pyrimidine dimers (CPDs). On the other hand, UVB-produced ROS also damage DNA by producing damaged nucleotides such as 8-hydroxy-2’-deoxyguanosine (8-OHdG) (6). Supporting this notion, it was reported that anti-oxidant molecules prevent UVB-induced epidermal DNA damage (7). Thus, mechanisms that protect the epidermis from both UVB and ROS are important to establish ways in which to suppress photo-damage efficiently.

When cells are exposed to stressors, a number of so-called stress proteins are induced in order to confer protection against such stressors. Heat shock proteins (HSPs) are representative of these stress proteins and their cellular up-regulation of expression, especially that of HSP70, provides resistance given that HSPs re-fold or degrade denatured proteins produced by stressors such as ROS (8,9). Since stressor-induced tissue damage is involved in various diseases, HSPs and HSP-inducers have received much attention for their therapeutic potential. It is known that various HSPs are constitutively expressed in keratinocytes and their expression, especially that of HSP70, is up-regulated by different stressors (10-13). UVB irradiation of keratinocytes induces the expression of HSP70 not only in vitro but also in vivo (11,13-17). Furthermore, artificial expression of HSP70 in keratinocytes confers protection against UVB and ROS in vitro (8,16,18,19). The protective role of HSP70 against UVB-induced epidermal damage was also suggested by in vivo studies: the whole-body hyperthermia of mice prevented UVB-induced sunburn cell formation and HSP70-null mice showed a sensitive phenotype to UVB-induced epidermal damage (20-22). Protection of the skin against UVB by expression of HSP70 has been suggested to occur in human skin (21). These previous results suggest that HSP70 expression suppresses UVB-induced epidermal damage, although no genetic evidence has been reported showing that overproduction of HSP70 prevents...
UVB-induced epidermal damage.

The potential benefit of HSP70-inducers as medicines for UVB-related skin diseases and cosmetics was also supported by a number of previously reported observations. For example, HSP70 has an anti-inflammatory activity by means of its inhibition of nuclear factor kappa B (NF-κB) and a resulting suppression of pro-inflammatory cytokine and chemokine expression (23-26). HSP70 has been reported to stimulate base excision repair, possibly by activation of human AP endonuclease and DNA polymerase β (27-29). We also recently found that artificial overexpression of HSP70 in mouse melanoma cells suppresses melanin production (Hoshino et al., submitted). Although we showed in that study that the UVB-induced production of melanin in the skin is suppressed in transgenic mice expressing HSP70, the anti-inflammatory and protective effects against DNA damage of HSP70 in UVB-irradiated skin have not been proved genetically. In this study, we examined the protective role of HSP70 against photo-damage by using transgenic mice expressing HSP70. The results obtained here suggest that expression of HSP70 protects the epidermis against UVB-induced damage via anti-inflammatory and anti-apoptotic effects and suppression of DNA damage. Based on these findings, we propose that non-toxic HSP70-inducers could be beneficial for use in cosmetics and medicines for the treatment of UVB-related skin diseases.

EXPERIMENTAL PROCEDURES

Materials and animals - Paraformaldehyde, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), peroxidase standard and fetal bovine serum (FBS) were obtained from Sigma Aldrich (St. Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits for interleukin (IL)-1β and IL-6 were from Pierce Chemical (Rockford, IL). Mayer’s hematoxylin, 1% eosin alcohol solution and malinol were from Muto Pure Chemicals (Tokyo, Japan). Terminal nucleotidyltransferase (TdTase) was obtained from Toyobo (Osaka, Japan). The Envision kit was from Dako (Carpinteria, CA). Biotin-14-ATP and Alexa Fluor 488-conjugated streptavidin were purchased from Invitrogen (Carlsbad, CA). VECTASHIELD was from Vector Laboratories. 4’, 6-diamidino-2-phenylindole (DAPI) was from Dojindo Laboratories (Kumamoto, Japan). The RNase Fibrous Tissue Mini kit was obtained from Qiagen Inc (Valencia, CA). The first-strand cDNA synthesis kit was from Takara Bio (Ohtsu, Japan) and IQ SYBR Green Supermix was from Bio-Rad (Hercules, CA). Lipofectamine (TM2000) and pcDNA3.1 plasmid were obtained from Invitrogen (Carlsbad, CA). Antibodies against IκB-α and actin were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). An antibody against HSP70 was from Stressgen (Ann Arbor, MI). Antibody against CPDs was from Kamiya Biomedical Company (Seattle, WA), while another against 8-OHdG was from Nikken SEIL (Shizuoka, Japan). α-(4-pyridyl-1-oxide)-N-tert-butyl nitronate (POBN) was from Alexis (San Diego, CA). Transgenic mice expressing HSP70 and their wild-type counterparts (6-8 weeks old, male) were gifts from Drs. C. E. Angelidis and G. N. Pagoulatos (University of Ioannina, Ioannina, Greece) and were prepared as described previously (30). Homozygotic transgenic mice expressing HSP70 were used in experiments. The experiments and procedures described here were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health, and were approved by the Animal Care Committee of Kumamoto University.

UV irradiation - Animals and cultured cells were exposed to UVB irradiation with a double bank of UVB lamps (peak emission at 312 nm, VL-215LM lamp, VILBER LOUMAT). The UV energy was monitored by a radiometer sensor (UVX-31, UV Products). Animals were placed under deep anesthesia with chloral hydrate (250 mg/kg) and fur was removed with electric
clippers prior to the irradiation.

**Myeloperoxidase (MPO) activity** - MPO activity in the skin was measured as described previously (30). Animals were placed under deep ether anesthesia and killed. The skin was dissected, rinsed with cold saline, and cut into small pieces. Samples were homogenized in 50 mM phosphate buffer, freeze-thawed and centrifuged. The protein concentrations of the supernatants were determined using the Bradford method (31). MPO activity was determined in 10 mM phosphate buffer with 0.5 mM \( \text{o-dianisidine} \), 0.00005% (w/v) hydrogen peroxide and 20 \( \mu \)g of protein. MPO activity was obtained from the slope of the reaction curve, and its specific activity was expressed as the number of hydrogen peroxide molecules converted per min/mg of protein.

**Immunoblotting analysis** - Whole cell extracts were prepared as described previously (32). The protein concentration of each sample was determined by the Bradford method (31). Samples were applied to 9% (HSP70 and actin) or 12% (IkB-\( \alpha \)) polyacrylamide SDS gels and subjected to electrophoresis, after which the proteins were immunoblotted with appropriate antibodies.

**Real-time RT-PCR analysis** - Total RNA was extracted from skin tissues using the RNaseasy Fibrous Tissue Mini kit according to the manufacturer’s protocol. Samples (2.5 \( \mu \)g of RNA) were reverse-transcribed using the first-strand cDNA synthesis kit according to the manufacturer’s instructions. Synthesized cDNA was used in real-time RT-PCR (Chromo 4 system, Bio-Rad) experiments using iQ SYBR Green Supermix and analyzed with Opticon Monitor software according to the manufacturer’s instructions. Specificity was confirmed by electrophoretic analysis of the reaction products and by inclusion of template- or reverse transcriptase-free controls. To normalize the amount of total RNA present in each reaction, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as an internal standard. The primers used were, \( \text{hsp70} \), \( 5'-\text{tggtgctgacagatgaag-3'} \) (forward) and \( 5'-\text{aggtgaaagatggcgtt-3'} \) (reverse); \( \text{il-1}\beta \), \( 5'-\text{gtcccaaggcatactcataaa-3'} \) (forward) and \( 5'-\text{gggaaactctgagactcata-3'} \) (reverse); \( \text{il-6} \), \( 5'-\text{ctggagtccagaggtggttg-3'} \) (forward) and \( 5'-\text{gttggcagagctactcata-3'} \) (reverse); monocyte chemoattractant protein-1 (mpc-1), \( 5'-\text{ctcactgtgctacattc-3'} \) (forward) and \( 5'-\text{gctgtgggggtcgttggaga-3'} \) (reverse); macrophage inflammatory protein-2 (mip-2), \( 5'-\text{acccagcaaggtgtgctc-3'} \) (forward) and \( 5'-\text{ggcactacaggtagctacg-3'} \) (reverse); \( \text{gapdh} \), \( 5'-\text{aactttggcattgtggaagg-3'} \) (forward) and \( 5'-\text{acacattgggggtagaaca-3'} \) (reverse).

**Histological and immunohistochemical analyses and terminal deoxynucleotidyltransferase-mediated biotinylated UTP nick end labeling (TUNEL) assay** - Skin samples were fixed in 4% buffered paraformaldehyde and embedded in paraffin before being cut into 4 \( \mu \)m-thick sections which were then deparaffinized and washed in phosphate-buffered saline (PBS).

For histological examination (hematoxylin and eosin (H & E) staining), sections were stained first with Mayer’s hematoxylin and then with 1% eosin alcohol solution. Samples were mounted with malinol and inspected using a BX51 microscope (Olympus).

For immunohistochemical analyses, sections were incubated with 0.1% (for 8-OHdG) or 0.3% (for CPDs and HSP70) hydrogen peroxide in methanol for removal of endogenous peroxidase. Sections were incubated with 0.125% trypsin in PBS for 10 min and then with 1N HCl for 30 min for DNA denaturation. Sections were blocked with 2.5% goat serum for 10 min, incubated for 12 h with antibody against HSP70 (1:200 dilution), 8-OHdG (1:100 dilution) or CPDs (1:500 dilution) in the presence of 2.5% bovine serum albumin, and then incubated for 1 h with peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulins. 3', 3'-diaminobenzidine was applied to the sections, which were then incubated with Mayer’s hematoxylin (hematoxylin-staining was omitted for
8-OHdG. Samples were mounted with malinol and inspected using a BX51 microscope (Olympus). The intensity of 8-OHdG-staining in the epidermis was measured by LuminaVision (MITANI).

For TUNEL assay, sections were incubated first with proteinase K (20 μg/ml) for 15 min at 37 °C, then with TdTase and biotin-14-ATP for 1 h at 37 °C, and finally with Alexa Fluor 488-conjugated streptavidin and DAPI (5 μg/ml) for 2 h. Samples were mounted with VECTASHIELD and inspected using a BX51 fluorescence microscope (Olympus).

**Cell culture and apoptosis analysis** - PAM212 cells were cultured in DMEM medium supplemented with 10% FBS in a humidified atmosphere of 95% air with 5% CO₂ at 37 °C. Transfection of PAM212 cells with pcDNA3.1 containing the hsp70 gene (33) was carried out using Lipofectamine (TM2000) according to the manufacturer's protocol. The stable transfectants expressing HSP70 were selected by immunoblotting and real-time RT-PCR analyses. Positive clones were maintained in the presence of 200 μg/ml G418. Cell viability was determined by the MTT method as previously described (34) and the measurement of caspase-3-like activity and fluorescence activated cell sorting (FACS) analysis for measurement of apoptotic cells (cells in sub-G1) were performed as described in (34).

**Immunostaining of 8-OHdG and CPDs in cultured cells** - Cells were cultured on 8-well Lab-Tek II Chamber slides (Nunc). They were then fixed in methanol for 20 min after UVB irradiation. Cells were permeabilized with 0.5% Triton X-100 for 5 min, treated in a microwave oven with 0.01 M citric acid buffer for antigen activation and then treated with 1N HCl for 20 min for DNA denaturation. Cells were blocked with 5% goat serum for 10 min, incubated for 2 h with antibody against 8-OHdG (1:10 dilution) or CPDs (1:2000 dilution) in the presence of 2.5% bovine serum albumin, and finally incubated with Alexa Fluor 488 goat anti-mouse immunoglobulin G. Cells were simultaneously stained with DAPI (5 μg/ml) for 2 h. Samples were mounted with VECTASHIELD and inspected with the aid of a BX51 fluorescence microscope (Olympus). The fluorescence intensity of 8-OHdG- or CPD-staining was measured by LuminaVision (MITANI).

**Determination of ROS production in vivo by electron spin resonance (ESR) analysis** - In vivo ESR analysis was performed as described (35) with some modifications. Immediately after UVB exposure, animals were placed under deep anesthesia with chloral hydrate (250 mg/kg) and injected with POBN (a spin trap reagent) (36,37) intraperitoneally (4 mmol/kg). After 1 h, mice were sacrificed, the skins were dissected and the lipid phase extracted. After evaporating the sample, ESR spectra were immediately recorded at room temperature using a quartz flat cell (160 μl) in a JES-TE200 spectrometer (JEOL). The operating conditions of the ESR apparatus were: 9.43 GHz, field 335.2 ± 5 mT, 40 mW microwave power, 100 kHz modulation frequency, 0.25 field modulation width, 0.3 s time count and sweep time 2 min.

**Statistical Analysis** - All values are expressed as the mean ± standard error of the mean (SEM). Two-way analysis of variance followed by the Tukey test was used to evaluate differences between more than three groups. Differences were considered to be significant for values of P<0.05.

**RESULTS**

**Effect of expression of HSP70 on UVB-induced epidermal apoptosis** - Overexpression of HSP70 in the transgenic mice that we used in this study has been shown in various organs (9,30,38-40). We examined HSP70 expression in the skin of these animals as this has not been determined to date. Transgenic mice expressing HSP70 and wild-type mice were irradiated or not with 180 mJ/cm² UVB. The dorsal skin was removed 24 h after completion of the irradiation and subjected to immunoblotting.
analysis. As shown in Fig. 1A and B, the level of HSP70 was significantly higher in transgenic mice than in wild-type mice in both the presence and absence of UVB irradiation. However, under these conditions, UVB irradiation did not up-regulate the expression of HSP70 in either type of mice (Fig. 1A and B), a finding that differs from previous reports (20). Although we examined the effect of UVB on expression of HSP70 under various conditions (various doses of UVB and time-course of the induction periods), we could not detect the UVB-dependent up-regulation of expression of HSP70 under any conditions by immunoblotting analysis (supplemental Fig. S1). We consider that this is due to the UVB-dependent increase in total amount of proteins (we applied same amount of proteins in each lane in immunoblotting analysis). Supporting this notion, immunohistochemical analysis with an antibody against HSP70 demonstrated that the expression of HSP70 was induced by UVB irradiation at the skin (the top panels in supplemental Fig. S2). Immunohistochemical analysis also demonstrated that the expression of HSP70 is higher in the epidermis than in the dermis, as described previously (11) and that expression in the epidermis is further heightened in transgenic mice (Fig. 1C). The results in Fig. 1 suggest that these transgenic mice could be useful for examining the protective role of HSP70 against UVB-induced epidermal damage.

Histological observations revealed extensive infiltration of leukocytes and epidermal disruption in skin sections prepared from UVB-irradiated wild-type mice, whereas the extent of cutaneous damage was not so apparent in transgenic mice expressing HSP70 (Fig. 2A). MPO activity, an indicator of the inflammatory infiltration of leukocytes, was increased in wild-type mice in response to the UVB irradiation. This activity was lower in UVB-irradiated transgenic mice expressing HSP70 compared to wild-type mice (Fig. 2B). The overexpression of HSP70 in transgenic mice did not affect the background level of MPO activity (Fig. 2B). These results show that UVB-induced epidermal damage and the resulting infiltration of leukocytes are suppressed in transgenic mice expressing HSP70.

The extent of epidermal cell apoptosis was determined by TUNEL assay. An increase of TUNEL-positive (apoptotic) cells in the epidermis of wild-type mice was observed after the UVB irradiation and this increase was clearly suppressed in transgenic mice expressing HSP70 (Fig. 2C and D). The overexpression of HSP70 in transgenic mice did not affect the background level of epidermal apoptosis (Fig. 2C and D). These results suggest that the expression of HSP70 protects epidermal cells (keratinocytes) from UVB-induced apoptosis.

To identify cells expressing HSP70 in transgenic mice and wild-type mice irradiated with UVB, we performed co-immunostaining assay. As shown in supplemental Fig. S2, strong co-staining of HSP70 with CD11b (a marker of macrophage) and pan cytokeratin (a marker of keratinocyte) was observed at the skin of transgenic mice expressing HSP70 or wild-type mice irradiated with UVB. A relatively weak co-staining of HSP70 with MPO (a maker of neutrophil) and vimentin (a maker of fibroblast) was also observed, (supplemental Fig. S2). These results suggest that the transgenic mice and wild-type mice irradiated with UVB express HSP70 in various types of cells at the skin.

We also tried to examine the effect of expression of HSP70 induced by geranylgeranylacetone (GGA), a leading anti-ulcer drug on the Japanese market and an HSP-inducer (41). However, as shown in supplemental Fig. S3, GGA did not induced expression of HSP70 by any route of administration (oral, intraperitoneal and percutaneous administrations). Thus, we used heat treatment to induce expression of HSP70. As shown in supplemental Figs. S3 and S4, heat treatment induced the expression of HSP70 at the skin and we found that this heat treatment protects the skin from UVB-induced damage (epidermal disruption, increase in MPO activity and
epidermal apoptosis).

To test the idea that the expression of HSP70 protects epidermal cells (keratinocytes) from UVB-induced apoptosis in vitro, we constructed a stable transfection of a mouse keratinocyte cell line (PAM212) that continuously overexpresses HSP70 (Clone 2). As shown in Fig. 3A, the level of HSP70 in Clone 2 was higher than mock transfectant control cells in both presence and absence of UVB irradiation. We also found that UVB irradiation up-regulated the expression of HSP70 in both types of cells (Fig. 3A). Exposure of cells to UVB irradiation decreased cell viability in a dose-dependent manner; this effect was suppressed in HSP70-overexpressing cells (Fig. 3B). To detect UVB-induced apoptosis, we counted cells in sub-G1 (apoptotic cells) by FACS analysis. UVB irradiation increased the number of apoptotic cells and this increase was suppressed in HSP70-overexpressing cells (Table 1). We also monitored apoptosis by measuring caspase-3-like activity using fluorogenic peptide substrates and obtained similar results to those for the FACS analysis (Table 1). The results in Fig. 3 and Table 1 suggest that the expression of HSP70 helps to protect keratinocytes from UVB-induced apoptosis.

Effect of HSP70 expression on UVB-induced epidermal inflammation – As described above, HSP70 was reported to suppress the activation of NF-κB through various mechanisms such as suppression of the inflammatory stimuli-induced degradation of IκB-α (an inhibitor of NF-κB) (26). We therefore examined the effect of UVB irradiation and/or expression of HSP70 on the level of IκB-α both in vivo and in vitro. As shown in Fig. 4A and B, UVB irradiation decreased the cutaneous level of IκB-α both in wild-type mice and in transgenic mice expressing HSP70, though the level remained significantly higher in the latter. We also compared the mRNA expression of pro-inflammatory cytokines (IL-1β and IL-6) and chemokines (MIP-2 and MCP-1) between UVB-irradiated transgenic mice expressing HSP70 and wild-type mice. The mRNA expression of il-1β, il-6, mip-2 and mcp-1 was increased by UVB irradiation, but this increase was much lower in skin samples prepared from transgenic mice expressing HSP70 compared to samples from wild-type mice (Table 2A). The expression of HSP70 in transgenic mice did not affect the background levels of mRNA expression (Table 2A). Similar results were observed for the protein levels of cytokines (IL-1β and IL-6) determined by ELISA (Table 2B). The results in Fig. 4 and Table 2 suggest that expression of HSP70 in the skin suppresses the UVB-induced expression of cytokines and chemokines via the inhibition of IκB-α degradation and the resulting suppression of NF-κB activity.

In order to test this notion in vitro, we examined the effect of UVB irradiation and/or expression of HSP70 in cultured keratinocytes on the degradation of IκB-α and expression of just one pro-inflammatory cytokine (IL-6; it has been reported that IL-1β is not expressed in mouse keratinocytes (42,43)) and chemokines (MIP-2 and MCP-1). As shown in Fig. 5A, UVB irradiation transiently (at 6-12 h after the UVB irradiation) decreased the level of IκB-α and this level was higher in HSP70-overexpressing cells than in mock transfectant control cells at any period after the UVB irradiation. Expression of the pro-inflammatory cytokine and chemokine genes tested (il-6, mip-2 and mcp-1) was up-regulated by the UVB irradiation, although the expression was suppressed in UVB-irradiated HSP70-overexpressing cells compared to mock transfectant control cells (Fig. 5B). Overexpression of HSP70 suppressed the background expression of mcp-1 but not il-6 and mip-2 genes (Fig. 5B). The results in Fig. 5 support the notion that the expression of HSP70 in keratinocytes suppresses the UVB-induced expression of cytokines and chemokines via the inhibition of IκB-α degradation and the resulting
suppression of NF-κB activity.

**Effect of HSP70 expression on UVB-induced epidermal DNA damage** – As described in the Introduction, UVB irradiation damages DNA (formation of photo-products) directly (formation of products such as CPDs) and indirectly via the production of ROS (formation of products such as 8-OHdG). In order to examine the effect of HSP70 expression on UVB-induced DNA damage in the epidermis, we compared the time-course profile of the level of CPDs and 8-OHdG after irradiation with UVB between transgenic mice expressing HSP70 and wild-type mice. As shown in Fig. 6A and B, the level of 8-OHdG, judged from the intensity of immunohistochemical staining, was significantly lower in the epidermis of UVB-irradiated transgenic mice expressing HSP70 than in wild-type mice 1 h after the UVB irradiation (45 mJ/cm²), suggesting that the UVB-induced formation of 8-OHdG is suppressed in the transgenic mice. Although the level of 8-OHdG 1 h after the irradiation was similar between wild-type mice irradiated with 45 mJ/cm² UVB and transgenic mice irradiated with 55 mJ/cm² UVB, the level was significantly lower in transgenic mice than in wild-type mice 48 h after the irradiation (Fig. 6A and B), suggesting that the repair process of 8-OHdG is stimulated in transgenic mice expressing HSP70.

We also measured the level of CPDs in a similar manner. As shown in Fig. 6C and D, the number of CPD-positive cells was similar between wild-type mice and transgenic mice 1 h after the UVB-irradiation. On the other hand, the number was significantly lower in transgenic mice than in wild-type mice 24 h or 48 h after the UVB irradiation (Fig. 6C and D). The results suggest that the repair rather than the formation of CPDs is affected by the expression of HSP70.

We then tested whether or not the effect of HSP70 expression on the formation and repair of 8-OHdG and CPDs can be reproduced in vitro. HSP70-overexpressing PAM212 cells and mock transfectant control cells were irradiated with UVB and the nuclear levels of 8-OHdG and CPDs were monitored by immunostaining. As shown in Fig. 7A and B, HSP70-overexpressing cells showed a lower level of 8-OHdG than mock transfectant control cells 5 min after the UVB irradiation (50 mJ/cm²), suggesting that the formation of 8-OHdG is suppressed by the expression of HSP70. Furthermore, comparing the level of 8-OHdG between HSP70-overexpressing cells irradiated with 65 mJ/cm² UVB and mock transfectant control cells irradiated with 50 mJ/cm² UVB, the initial (5 min after the UVB irradiation) levels were indistinguishable; however, the level was lower in HSP70-overexpressing cells than in mock transfectant control cells 24 h after the irradiation (Fig. 7A and B), suggesting that the repair process of 8-OHdG is stimulated by the expression of HSP70. In other words, the protective effect of HSP70 against UVB-induced formation of 8-OHdG and its simulative effect on the repair process can be reproduced in vitro. On the other hand, the level of CPDs was indistinguishable between HSP70-overexpressing cells and mock transfectant control cells both 5 min and 24 h after the UVB irradiation, suggesting that neither the formation nor repair of CPDs is affected by the expression of HSP70. That is to say, the effect of HSP70 on the repair of CPDs was not reproduced in vitro.

The results in Fig. 6A and B suggest that UVB-induced ROS production in the skin is suppressed in transgenic mice expressing HSP70. On this basis, we measured the level of ROS in the skin by monitoring the lipid-derived free radical spin adduct with ESR spectroscopy and spin trap POBN, which reacts with ROS to form a radical spin adduct. As shown in Fig. 8A, a radical spin adduct of ESR spectrum similar to that reported in other organs was obtained (35-37,44). The hyperfine coupling constants for the POBN radical adducts were \(a^N = 14.91 \pm 0.08\) G and \(a^H = 2.45 \pm 0.04\) G, which are similar to data previously reported for other organs (35-37,44), suggesting that this ESR spectrum is derived from lipid-derived free radicals. As shown in Fig.
8B, the level of ROS in the skin was elevated by UVB irradiation in wild-type mice and this increase was suppressed in transgenic mice expressing HSP70. This finding suggests that the expression of HSP70 suppresses UVB-induced ROS production in the skin.

**DISCUSSION**

An ameliorative effect of HSP70 due to its cytoprotective, anti-inflammatory and molecular chaperone (quality control of proteins) properties has been reported for various diseases. For example, we have shown using transgenic mice that HSP70 protects against irritant-produced lesions in the stomach and small intestine and inflammatory bowel disease-related experimental colitis (30,38-40). The potential therapeutic applicability of HSP70 for use in other diseases, such as neurodegenerative diseases, ischemia-reperfusion damage and diabetes has also been suggested (9,45). Interestingly, GGA, a leading anti-ulcer drug on the Japanese market, has been reported to be an HSP-inducer, up-regulating various HSPs not only in cultured gastric mucosal cells but also in various tissues in vivo (41). It was reported that GGA suppresses not only gastric lesions but also lesions of the small intestine, inflammatory bowel disease-related experimental colitis (39,40,46,47). On the other hand, the use of HSP70-inducers in cosmetics and medicines to aid in the treatment of UVB-related skin diseases has not been fully evaluated. A number of in vitro studies suggested that HSP70 protects keratinocytes from UVB irradiation; however, the protective role of HSP70 against UVB-induced functional and structural alterations of the epidermis has not been proved genetically. In this study, using transgenic mice overexpressing HSP70, we have shown that the expression of HSP70 suppresses UVB-induced epidermal apoptosis, inflammatory responses, ROS production and DNA damage, suggesting that HSP70-inducers could be beneficial for use as agents in medicines and cosmetics to alleviate the symptoms and/or cure UVB-related skin diseases. These effects of HSP70 should be mutually dependent. For example, ROS stimulate NF-κB activity and DNA damage, and both ROS and DNA damage induce apoptosis (48,49). Our results also suggest that the high level of constitutive expression of HSP70 in keratinocytes could play an important role in protecting the skin against UVB irradiation.

We showed here that UVB-induced apoptosis was suppressed in the epidermis of transgenic mice and in cultured keratinocytes overexpressing HSP70. These results are basically consistent with previous results (8,16,18,20-22). It was suggested that HSP70 suppresses various steps in the molecular pathways governing apoptosis, including p53 activation, which plays an important role in UVB-induced apoptosis (50,51). In addition to this anti-apoptotic (cytoprotective) effect of HSP70, an anti-inflammatory effect (suppression of NF-κB activity) was recently revealed and thought to be important for HSP70 function (24-26). However, it was not clear whether HSP70 suppresses the activity of NF-κB in vivo. In this study, we confirmed that expression of HSP70 increases the level of IκB-α (an inhibitor of NF-κB) in vitro and found that a UVB-induced decrease in the level of IκB-α in the skin is suppressed in transgenic mice expressing HSP70. We also showed that the UVB-induced expression of pro-inflammatory cytokines and chemokines is suppressed in transgenic mice expressing HSP70. These results suggest that HSP70 expression in the skin suppresses inflammation via the inhibition of NF-κB activity and the resulting inhibition of pro-inflammatory cytokine and chemokine expression. Considering the adverse effects of inflammation on various skin diseases, these properties of HSP70 could make the development of HSP70-inducers an important advance in the search for medicines to cure UVB-related skin diseases.

We also showed that the UVB-induced increase in levels of both
CPDs (UVB-induced direct DNA damage) and 8-OHdG (UVB-dependent indirect DNA damage via the production of ROS) is suppressed in transgenic mice expressing HSP70. This is the first in vivo evidence of the protective effect of HSP70 against UVB-induced DNA damage to the skin. This finding is particularly important because UVB-induced DNA damage plays an important role in UVB-induced skin diseases, especially carcinogenesis. Since HSP70 protects epidermal cells from UVB-induced apoptosis, it could also in fact stimulate skin carcinogenesis by aiding the survival of DNA-damaged cells. However, a further beneficial effect of HSP70 (suppression of UVB-induced DNA damage) may circumvent this problem. The formation and repair of 8-OHdG have been suggested to be suppressed and stimulated, respectively, in transgenic mice expressing HSP70. We also reproduced those findings in cultured keratinocytes, suggesting that HSP70 expressed in these cells is directly responsible for these phenomena. HSP70 seems to suppress the formation of 8-OHdG by decreasing the level of ROS, because the UVB-induced increase in the level of ROS was suppressed in transgenic mice expressing HSP70. A decrease in the level of ROS due to the increased expression of HSP70 was also reported in vitro (52). HSP70 stimulates base excision repair, possibly via the activation of human AP endonuclease and DNA polymerase β (27-29). This effect may be involved in a HSP70-dependent stimulation of the 8-OHdG repair process, since the base excision repair system plays a major role in the repair of 8-OHdG (6). On the other hand, although our in vivo results suggested that the repair process of CPDs is stimulated by the expression of HSP70, we could not reproduce these results in vitro. However, a slight up-regulation of CPD repair by the expression of HSP70 was reported elsewhere (53). Furthermore, in E. coli, an HSP70 homologue (DnaK) stimulates the nucleotide excision repair of damaged DNA (54), which plays a major role in the repair of CPDs (6).

We recently found that the artificial expression of HSP70 in cultured melanoma cells suppresses melanin production (Hoshino et al., submitted), suggesting that HSP70-inducers could be beneficial for use as hypopigmenting cosmetics and medicines. A number of compounds that inhibit melanin production have been discovered, however most of their cosmetic and pharmaceutical applications have not been successful due to the occurrence of skin irritation (55), which is caused by the fact that UV-induced mild melanogenesis has a protective role against UVB-induced skin damage, especially DNA damage. Melanin also acts as a scavenger of the UVB-induced production of ROS (56). Therefore, the findings in this study that HSP70 expression suppresses both UVB-induced epidermal DNA damage and the increase in the cutaneous level of ROS are important for the development of HSP70-inducers as hypopigmenting cosmetics and medicines. The anti-inflammatory effects of HSP70 may help in this manner because UVB-induced inflammation actually stimulates pigmentation (57). Based on these results, we propose that HSP70-inducers could have numerous cosmetically and pharmaceutically beneficial applications. We have already screened for HSP70-inducers from Chinese herbal extracts and found that their HSP70-inducing activities were more potent than GGA (Yamashita et al., submitted). We hope to develop some of these extracts as hypopigmenting (whitening) cosmetics or as drugs to combat melanin-related diseases.

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245-254
751-761
6, 348-353
Ther. 315, 624-630

**FOOTNOTES**

*We thank Drs. Angelidis CE and Pagoulatos GN (University of Ioannina, Greece) for
generously providing transgenic mice expressing HSP70. This work was supported by
Grants-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare of
Japan, as well as the Japan Science and Technology Agency, Grants-in-Aid for Scientific
The abbreviations used are; CPDs, cyclobutane pyrimidine dimers; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride; ELISA, enzyme-linked immunosorbent assay; ESR, electron spin resonance; FACS, fluorescence activated cell sorting; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GGA, geranylgeranylacetone; H & E, hematoxylin and eosin; HSP, heat shock protein; IL, interleukin; IκB-α, an inhibitor of NF-κB; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein-2; MPO, myeloperoxidase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide; NF-κB, nuclear factor kappa B; 8-OHdG, 8-hydroxy-2’-deoxyguanosine; POBN, α-(4-pyridyl-1-oxide)-N-tert-butyl nitrotrone; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SEM, standard error of the mean; TUNEL, terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling; UV, Ultraviolet.

FIGURE LEGENDS

Figure 1. Expression of HSP70 in the dorsal skin after UVB irradiation. Transgenic mice expressing HSP70 (HSP70 Tg) and wild-type mice (WT) were irradiated with or without 180 mJ/cm² UVB and the dorsal skin was removed after 24 h. A, whole cell extracts were analyzed by immunoblotting with an antibody against HSP70 or actin. B, the band intensity of HSP70 was determined and expressed relative to the control sample (n = 6) (one of two gels is shown in panel A). Values are mean ± SEM. **P<0.01; *P<0.05. C, sections of dorsal skin were prepared and subjected to immunohistochemical analysis with an antibody against HSP70. Brown staining indicates HSP70 expression. Scale bar, 50 μm.

Figure 2. UVB-induced skin damage and apoptosis in wild-type mice and transgenic mice expressing HSP70. Transgenic mice expressing HSP70 (HSP70 Tg) and wild-type mice (WT) were irradiated with or without the indicated doses of UVB and the dorsal skin was removed after 48 h (A), 24 h (B) or 12 h (C, D). A, sections of dorsal skin were prepared and subjected to H & E staining. B, MPO activity was measured as described in the experimental procedures. Values are mean ± SEM. (n = 8-12). **P<0.01. C, sections of dorsal skin were subjected to TUNEL assay and DAPI staining. D, the ratio of TUNEL-positive cells in the epidermis was counted (400 - 1000 cells in total). Values are mean ± SEM (n = 3). **P<0.01; *P<0.05. Scale bar, 50 μm.

Figure 3. Effect of HSP70 expression on UVB-induced apoptosis in vitro. HSP70-overexpressing PAM212 cells (Clone 2) and mock transfectant control cells (Mock) were irradiated with or without indicated doses of UVB and cultured for indicated periods (A) or 24 h (B). A, whole cell extracts were subjected to immunoblotting with an antibody against HSP70 or actin. The relative HSP70 band intensity is shown under the band. B, cell viability was determined by the MTT method. Values are mean ± SEM (n = 3). **P<0.01.

Figure 4. UVB-induced decrease in the level of IκB-α. Transgenic mice expressing HSP70 (HSP70 Tg) and wild-type mice (WT) were irradiated with or without 180 mJ/cm² UVB. A, the dorsal skin was removed after 48 h and whole cell extracts were analyzed by immunoblotting with an antibody against IκB-α or actin. B, the band intensity of IκB-α was determined and expressed relative to the control sample (one of two gels is shown in panel A). Values are mean ± SEM (n = 6). *P<0.05.

Figure 5. Effect of HSP70 expression on UVB-induced decrease in the level of IκB-α and
expression of pro-inflammatory cytokines and chemokines in vitro. HSP70-overexpressing PAM212 cells (Clone 2) and mock transfectant control cells (Mock) were irradiated with or without 50 mJ/cm² UVB and incubated for indicated periods (A) or 6 h (mip-2), 12 h (il-6) or 24 h (mcp-1) (B). A, the expression of IκB-α was estimated by immunoblotting and shown as described in the legend of Fig. 3. B, mRNA expression of each gene was monitored as described in the legend of Table 2. Values are mean ± SEM (n = 3). **P < 0.01.

Figure 6. UVB-induced epidermal DNA damage. Transgenic mice expressing HSP70 (HSP70 Tg) and wild-type mice (WT) were irradiated with or without (CTRL) indicated doses (A, B) or 45 mJ/cm² (C, D) of UVB. Sections of the dorsal skin were prepared after indicated periods and subjected to immunohistochemical analysis with an antibody against 8-OHdG (A) or CPDs (C). The intensity of 8-OHdG-staining (B) and the percentage of CPD-positive cells (400 – 600 cells in total) (D) were measured. B, D, values are mean ± SEM (n = 6). **P < 0.01; *P < 0.05; n.s., not significant. Scale bar, 50 μm.

Figure 7. Effect HSP70 expression on UVB-induced DNA damage in vitro. HSP70-overexpressing PAM212 cells (Clone 2) and mock transfectant control cells (Mock) were irradiated with or without (CTRL) indicated doses (A, B) or 50 mJ/cm² (C, D) of UVB and cultured for indicated periods. Cells were subjected to immunostaining analysis with an antibody against 8-OHdG (A) or CPDs (C). The fluorescence intensity of 8-OHdG (B) and CPDs (D) staining was measured. B, D, values are mean ± SEM (n = 6). **P < 0.01; *P < 0.05; n.s., not significant. Scale bar, 100 μm.

Figure 8. UVB-induced increase in the epidermal ROS level. Transgenic mice expressing HSP70 (HSP70 Tg) and wild-type mice (WT) were irradiated with 45 mJ/cm² UVB. A, POBN was administered and the dorsal skin was removed after 1 h and subjected to radical adduct ESR spectrum analysis. B, the intensity of the ESR signal of the radical adduct (shown by the bar in A) was determined, expressed relative to the control sample, and given as the mean ± SEM (n = 6-10). **P < 0.01.
Table 1. Effect of HSP70 expression on UVB-induced apoptosis in vitro.

<table>
<thead>
<tr>
<th>UVB (mJ/cm²)</th>
<th>Cells in sub-G1 (%)</th>
<th>Caspase-3-like activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mock Clone 2</td>
<td>Mock Clone 2</td>
</tr>
<tr>
<td>0</td>
<td>2.8 ± 0.37</td>
<td>14.2 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>1.6 ± 0.24</td>
<td>27.6 ± 5.7</td>
</tr>
<tr>
<td>50</td>
<td>19.2 ± 0.26</td>
<td>762.9 ± 19.0</td>
</tr>
<tr>
<td></td>
<td>10.3 ± 0.18 **</td>
<td>601.7 ± 29.3 **</td>
</tr>
<tr>
<td>75</td>
<td>65.2 ± 1.40</td>
<td>1316.9 ± 7.4</td>
</tr>
<tr>
<td></td>
<td>24.4 ± 0.28 **</td>
<td>700.7 ± 12.6 **</td>
</tr>
</tbody>
</table>

HSP70-overexpressing PAM212 cells (Clone 2 in Fig. 3) and mock transfectant control cells (Mock) were irradiated with or without indicated doses of UVB and cultured for 24 h. Apoptotic cells (cells in sub-G1) were counted by FACS. Caspase-3-like activity was measured. Values are mean ± SEM (n = 3). **P<0.01.

Table 2. UVB-induced expression of pro-inflammatory cytokines and chemokines.

A  Relative expression

<table>
<thead>
<tr>
<th>UVB (mJ/cm²)</th>
<th>WT</th>
<th>HSP70 Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>*il-1β</td>
<td>0</td>
<td>1.0 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>24.7 ± 9.19</td>
</tr>
<tr>
<td>*il-6</td>
<td>0</td>
<td>1.0 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>10.6 ± 1.66</td>
</tr>
<tr>
<td>*mip-2</td>
<td>0</td>
<td>1.0 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>16.7 ± 1.13</td>
</tr>
<tr>
<td>*mcp-1</td>
<td>0</td>
<td>1.0 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>36.7 ± 0.40</td>
</tr>
</tbody>
</table>

B  ng/g tissue

<table>
<thead>
<tr>
<th>UVB (mJ/cm²)</th>
<th>WT</th>
<th>HSP70 Tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>*IL-1β</td>
<td>0</td>
<td>0.48 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>27.9 ± 2.71</td>
</tr>
<tr>
<td>*IL-6</td>
<td>0</td>
<td>1.2 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>67.4 ± 1.66</td>
</tr>
</tbody>
</table>

Transgenic mice expressing HSP70 (HSP70 Tg) and wild-type mice (WT) were irradiated with or without 180 mJ/cm² UVB. A, the dorsal skin was removed after 12 h (mip-2), 24 h (il-6, mcp-1) or 48 h (il-1β) and total RNA was extracted. Samples were subjected to real-time RT-PCR using a specific primer set for each gene. Values were normalized to the gapdh gene, expressed relative to the control sample. B, the dorsal skin was removed after 48 h and skin homogenates were prepared. The amount of IL-1β and IL-6 was determined by ELISA. Values are mean ± SEM (n = 6-9). **P<0.01; *P<0.05.
Matsuda et al., Figure 1

A

<table>
<thead>
<tr>
<th>UVB (mJ/cm²)</th>
<th>WT</th>
<th>HSP70 Tg</th>
<th>WT</th>
<th>HSP70 Tg</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

HSP70

Actin

B

[Graph showing relative intensity with UVB exposure levels 0 and 180 mJ/cm² for WT and HSP70 Tg groups]

C

[Images showing skin tissue samples with UVB exposure levels 0 and 180 mJ/cm² for WT and HSP70 Tg groups]
Matsuda et al., Figure 2

A

UVB (mJ/cm²)  WT  HSP70 Tg

B

MPO activity (μmol H₂O₂/min/mg protein)

C

UVB (mJ/cm²)  WT  HSP70 Tg

D

TUNEL-positive cells (%)

---

Matsuda et al., Figure 2

A

UVB (mJ/cm²)  WT  HSP70 Tg

B

MPO activity (μmol H₂O₂/min/mg protein)

C

UVB (mJ/cm²)  WT  HSP70 Tg

D

TUNEL-positive cells (%)

---

Matsuda et al., Figure 2

A

UVB (mJ/cm²)  WT  HSP70 Tg

B

MPO activity (μmol H₂O₂/min/mg protein)

C

UVB (mJ/cm²)  WT  HSP70 Tg

D

TUNEL-positive cells (%)

---

Matsuda et al., Figure 2

A

UVB (mJ/cm²)  WT  HSP70 Tg

B

MPO activity (μmol H₂O₂/min/mg protein)

C

UVB (mJ/cm²)  WT  HSP70 Tg

D

TUNEL-positive cells (%)

---

Matsuda et al., Figure 2

A

UVB (mJ/cm²)  WT  HSP70 Tg

B

MPO activity (μmol H₂O₂/min/mg protein)

C

UVB (mJ/cm²)  WT  HSP70 Tg

D

TUNEL-positive cells (%)

---

Matsuda et al., Figure 2

A

UVB (mJ/cm²)  WT  HSP70 Tg

B

MPO activity (μmol H₂O₂/min/mg protein)

C

UVB (mJ/cm²)  WT  HSP70 Tg

D

TUNEL-positive cells (%)

---

Matsuda et al., Figure 2

A

UVB (mJ/cm²)  WT  HSP70 Tg

B

MPO activity (μmol H₂O₂/min/mg protein)

C

UVB (mJ/cm²)  WT  HSP70 Tg

D

TUNEL-positive cells (%)

---
Matsuda et al., Figure 3

A

<table>
<thead>
<tr>
<th>UVB (mJ/cm²)</th>
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<th>50</th>
</tr>
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<tr>
<td>Time (h)</td>
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<tr>
<td></td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Mock</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Clone 2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>HSP70</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Actin</td>
<td>5.1</td>
<td>6.6</td>
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</table>

B

![Bar chart showing UVB (mJ/cm²) vs. Cell viability (%).](chart.png)
Matsuda et al., Figure 4

A

<table>
<thead>
<tr>
<th>UVB (mJ/cm²)</th>
<th>0</th>
<th>180</th>
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<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSP70 Tg</td>
<td></td>
<td></td>
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</tbody>
</table>

**IκB-α**

**Actin**

B

![Graph showing relative intensity of WT and HSP70 Tg over UVB exposure](image)

- **WT**: No significant change
- **HSP70 Tg**: Decreased intensity with UVB exposure

*Significant difference*
Matsuda et al., Figure 5

A

<table>
<thead>
<tr>
<th>UVB (mJ/cm²)</th>
<th>0</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
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<tr>
<td>Mock</td>
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<tr>
<td>Clone 2</td>
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<tr>
<td>Intensity</td>
<td>1.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Actin</td>
<td>[image]</td>
<td></td>
</tr>
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</table>

B

- [Graph A1: Mock vs Clone 2 for Il-6 expression after UVB exposure]
- [Graph A2: Mock vs Clone 2 for Mip-2 expression after UVB exposure]
- [Graph A3: Mock vs Clone 2 for Mcp-1 expression after UVB exposure]
Matsuda et al., Figure 7

A

<table>
<thead>
<tr>
<th>CTRL</th>
<th>Mock 50 mJ/cm²</th>
<th>Clone 2 50 mJ/cm²</th>
<th>Clone 2 65 mJ/cm²</th>
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<td>5 min</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

Graph showing relative intensity (%) over time (CTRL, 5 min, 24 h).

C

<table>
<thead>
<tr>
<th>CTRL</th>
<th>Mock</th>
<th>Clone 2</th>
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<tr>
<td>5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D

Graph showing relative intensity (%) over time (CTRL, 5 min, 24 h).
Matsuda et al., Figure 8

A

UVB (mJ/cm²)

0

HSP70 Tg

WT

HSP70 Tg

WT

B

Relative intensity (%)

250

200

150

100

50

0

0

45

0

45

UVB (mJ/cm²)

WT

HSP70 Tg

**
Prevention of ultraviolet B radiation-induced epidermal damage by expression of heat shock protein 70

Minoru Matsuda, Tatsuya Hoshino, Yasuhiro Yamashita, Ken-ichiro Tanaka, Daisuke Maji, Keizo Sato, Hiroaki Adachi, Gen Sobue, Hironobu Ihn, Yoko Funasaka and Tohru Mizushima

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