UVB-mediated activation of p38 mitogen-activated protein kinase enhances resistance of normal human keratinocytes to apoptosis by stabilizing cytoplasmic p53

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Human keratinocytes respond to UV rays by developing a fast adaptive response that contributes to maintaining their functions and survival. We investigated the role of the mitogen-activated protein kinase pathways in transducing the UV signals in normal human keratinocytes. We found that UVA, UVB or UVC induced a marked and persistent activation of p38, whereas c-Jun N-terminal kinase or extracellular signal-regulated kinase were less or not activated respectively. Inhibition of p38 activity by expression of a dominant-negative mutant of p38 or with SB203580 impaired cell viability and led to an increase in UVB-induced apoptosis. This sensitization to apoptosis was independent of caspase activities. Inhibition of p38 did not sensitize transformed HaCaT keratinocytes to UVB-induced apoptosis. In normal keratinocytes, expression of a dominant-negative mutant of p53 increased UVB-induced cell death, pointing to a role for p53. In these cells, UVB triggered a p38-dependent phosphorylation of p53 on Ser-15. This phosphorylation was associated with an SB203580-sensitive accumulation of p53, even in the presence of a serine phosphatase inhibitor. Accumulated p53 was localized mainly in the cytoplasm, independently of CRM1 nuclear export. In HaCaT cells, p53 was localized exclusively in the nucleus and its distribution and level were not affected by UVB or p38 inhibition. However, UVB induced an SB203580-insensitive phosphorylation on Ser-15 of mutated p53. Overall, our results suggest that, in normal human keratinocytes, protection against UVB depends on p38-mediated phosphorylation and stabilization of p53 and is tightly associated with the cytoplasmic sequestration of wild-type p53. We conclude that the p38/p53 pathway plays a key role in the adaptive response of normal human keratinocytes against UV stress.

Key words: adaptive response, cytotoxicity, signalling, UV rays.

INTRODUCTION

Because of their strategic location, keratinocytes constitute the first line of defence against environmental stresses, such as chemical pollutants and solar UV radiation (UVR). The proper responsiveness of keratinocytes to their surroundings is importantly implicated in regulating skin and general homoeostasis. For example, keratinocytes are actively involved in regulating systemic electrolytic balance and thermoregulation. The incapacity of keratinocytes to appropriately respond to their physical and chemical environment is associated with important pathological disorders. In particular, the inability of keratinocytes to cope with chronic exposure to UVR can lead to the development of non-melanocytic skin cancers. UV rays act as complete carcinogens favouring both tumour initiation and promotion [1]. UVR-mediated tumour initiation relies on DNA damage but the mechanisms of tumour promotion by UVR are still ill-defined and may involve alterations to the way in which keratinocytes sense and transduce the UV signal. Mitogen-activated protein (MAP) kinases are central components involved in transducing the signals elicited by growth factors and stressing stimuli. In mammalian cells, the three best-characterized MAP kinase pathways are the extracellular-signal regulated kinase (ERK), stress-activated protein kinase-1/c-Jun N-terminal kinase (herein called JNK) and stress-activated protein kinase-2/p38 α and β (herein referred to as p38) pathways [2]. ERK is mostly activated by growth factors and cytokines and is especially involved in transducing proliferating signals and in promoting cell survival by conferring protection against cell death [3,4]. JNK and p38 are also sensitive to growth factors but they are more specifically recognized as stress-sensitive pathways. These pathways are implicated in inducing homoeostatic response against stress and, depending on the cellular context, are important positive or negative modulators of the apoptotic cell death programme.

Apoptosis defines a type of regulated cell death that controls cell number during the development and adult life of multicellular organisms. Notably, apoptosis removes old or damaged cells during the shedding of the intestinal wall, the regression of mammary gland cells after lactation and the death of time-expired neutrophils. Apoptosis is also actively involved in removing abnormal pre-cancer and cancer cells. Dysregulation of UV-induced apoptosis of keratinocytes has been associated with various skin diseases, including atopic dermatitis and cancer [5,6]. Apoptosis is characterized by diverse morphological features that include membrane blebbing, cell shrinkage, chromatin condensation, nuclear/cytoplasmic fragmentation and formation of dense bodies that are quickly removed via phagocytosis by neighbouring cells. Once initiated, the apoptotic programme

Abbreviations used: UVR, UV radiation; MAP, mitogen-activated protein; MAPKAPK, MAP kinase-activated protein kinase; ERK, extracellular-signal regulated kinase; JNK, stress-activated protein kinase-1/c-Jun N-terminal kinase; p38, stress-activated protein kinase-2/p38 α and β; zVAD-fmk, *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone; LMB, leptomycin B; DEVD-AMC, acetyl-aspartyl-glutamyl-valyl-aspartyl-amino-4-methyl-coumarin; GST, glutathione S-transferase; DME, Dulbecco's modified Eagle's; DTT, dithiothreitol; ICE, interleukin-1b-converting enzyme.

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involves activation of a series of biochemical events that underlie the morphological hallmarks of apoptosis. This includes internucleosomal DNA fragmentation and proteolytic degradation of specific substrates such as poly(ADP-ribose) polymerase. Proteolysis is achieved through activation of cysteine proteases called caspases [7]. *In vitro* and *in vivo* studies showed that caspases act in a cascade to convey the apoptotic signal [8].

p38 modulates apoptosis induced by sodium salicylate in Rat-1 cells [9], glutamate in rat cerebellar granule cells [10] and withdrawal of trophic factors in Rat-1 fibroblasts and differentiated PC12 cells [11]. Suppression of Fas expression as a result of down-regulation of nuclear factor κB transcriptional activity is a consequence of p38 activation in melanoma cells and protects against UVC-induced apoptosis [12]. In U937 cells, activation of p38 by tumour necrosis factor-α is involved in cell survival, whereas it has no effect in Jurkat cells [13]. Fas induction of p38 activity requires the action of interleukin-1b-converting enzyme/*Caenorhabditis elegans* protein-3 (ICE/CED3) in Jurkat clone A3, whereas etoposide activation of p38-mediated-apoptosis relies on protease-independent mechanisms or on proteases unrelated to caspases-3 [7]. Intriguingly, in the case of *cis*-platinum, p38 contributes to nuclear condensation and fragmentation through both caspase-dependent and -independent mechanisms [14].

p53 is a tumour suppressor that is centrally involved in regulating the homoeostatic response to UVR and other DNA-damaging agents. Following DNA damage, p53 accumulates, leading to cell-cycle arrest, allowing more time for DNA repair or elimination of damaged cells through apoptosis. Several functions and features of p53 are regulated by phosphorylation. This includes its stability and capacity to bind DNA and trigger transcription [15–17]. The phosphorylation state of p53 may also modulate its cellular localization, possibly through masking/unmasking of nuclear import or export signals [18,19]. Several phosphorylation-dependent functions of p53 involve protein–protein interactions. For instance, tetramerization of p53, a step that is necessary for its transcriptional activity, is stabilized by phosphorylation on Ser-392 [20]. Moreover, phosphorylation of p53 on Ser-15, -20 and -37 impair its interaction with Mdm2, a negative regulator of p53 that binds to its N-terminal domain and targets it to proteasomes for degradation [16,21–23]. Most of the phosphorylation sites on p53 are found in the N-terminal transactivation domain [24]. These sites and especially Ser-15 are phosphorylated by a number of kinases, including ERK and p38 [25,26]. The p38-mediated phosphorylation of p53 is important in modulating apoptosis in transformed cells [25,27]. However, little is known concerning the role of the p38/p53 signalling pathway in regulating the apoptotic response of normal cells.

In this study, we investigated the role of the MAP kinase pathways in transducing the UV signals in normal human keratinocytes. We found that p38 is quickly and markedly activated by UV rays, which confers protection against apoptosis. UV-induced p38 activation increases the phosphorylation and stability of p53 and ensures its cytoplasmic localization through a mechanism unrelated to an increased nuclear export, but which requires the wild-type genotype of *p53*. These findings indicate for the first time that in normal human keratinocytes the p38/p53 pathway is a major component of the primary adaptive response against UV rays.

MATERIALS AND METHODS

Reagents

[γ-^{32}P]ATP (3000 Ci/mmol) was purchased from NEN Life Science Products (Boston, MA, U.S.A.). Recombinant HSP27 was purified from *Escherichia coli* transformed with a plasmid containing the coding sequence for Chinese hamster HSP27 [28]. Human recombinant glutathione S-transferase (GST)-p53 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Anisomycin, SB203580 and *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-fmk) were purchased from Calbiochem (La Jolla, CA, U.S.A.). These agents were diluted in DMSO in stock concentrations of 50 mg/ml, 40 mM and 20 mM, respectively. NaF (1 mM in water) and leptomycin B (LMB; 5 μg/ml in 70 % methanol) were purchased from Sigma (St. Louis, MO, U.S.A.). Acetyl-aspartyl-glutamyl-valyl-aspartyl-amino-4-methylcoumarin (DEVD-AMC) was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA, U.S.A.).

Antibodies and adenovirus

Anti-GST-MAPKAPK-2/3 is a polyclonal antibody raised in rabbit after injection of a GST fusion protein containing the 223 C-terminal amino acids of Chinese hamster MAP kinase-activated protein kinase (MAPKAPK)-2 [29]. This antibody immunoprecipitates both the p45 and p54 isoforms of human MAPKAPK-2, one of which may correspond to MAPKAPK-3 [28]. Anti-p38 is a polyclonal antibody raised in rabbit against the C-terminal sequence PPLQEEMES of murine p38 [30]. Mouse monoclonal anti-p53 (DO-1) and rabbit polyclonal anti-Bax (N-20) were purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-(phospho p53) (Ser15) was from New England Biolabs (Beverly, MA, U.S.A.).

The adenovirus expressing a dominant-negative mutant of p38 was prepared as follows: the kinase-inactive p38α cDNA (carrying the $T^{180}GY \rightarrow AGF$ mutation) [31] was cloned in an adenovirus transfer plasmid and made into a replication-incompetent Ad-5 (delE1, E3) virus, as described in [32,33]. The virus expresses p38α ($T^{180}GY \rightarrow AGF$) with a FLAG tag at its N-terminus. The adenovirus expressing a dominant-negative mutant of p53 (R175H) was kindly provided by Dr Bert Vogelstein (John Hopkins University, University Oncology Center, Baltimore, MD, U.S.A.). Cells were infected at a multiplicity of infection that gives 70 % of infection after 48 h.

Cell culture and irradiation protocol

Keratinocytes were isolated from normal human skin biopsies and cultured in Dulbecco's modified Eagle's (DME)/Ham's F12 (3:1) supplemented medium [34]. Cells were used at passage $\leqslant 3$. HaCaT cells were cultured in DME medium containing 10 % fetal bovine serum. These cells carry mutations characteristic of the UV signature in both alleles of the *p53* gene, one in codon 179 and the others in codons 281 and 282 [35]. All cultures were maintained at 37 °C in a humidified atmosphere containing 8 % CO_2. Before irradiation, the medium was replaced by a thin layer of irradiation medium (DME unsupplemented medium without Phenol Red). Cells were exposed to a single dose of UVA, UVB or UVC. In experiments with a post-irradiation period of > 2 h, irradiation medium was replaced with fresh supplemented medium for the indicated time.

UVA light was provided by two tubes of Blacklite blue F15T8/BLB 15W UVA-I (Sylvania, Danvers, MA, U.S.A.) with a narrow peak at 360 nm. The UVB light source consisted of two tubes of FS20T12/UVB/BP (Philips, Franklin Square Drive, NJ, U.S.A.) delivering 9.58 J/m^2 through a sheet of cellulose acetate (Kodacel TA-407 clear, 0.015 in; Eastman-Kodak Co., Rochester, NY, U.S.A.). This filter blocks contaminating wavelengths below 290 nm. The UVC light was provided by two germicidal tubes (TUV 15W G15T8; Philips). All the lamps were calibrated before each experiment with a radiometer fitted with

a UVA, UVB and UVC probe (model IL1700A; International Light, Newburyport, MA, U.S.A.).

Immunoprecipitation

Cells were scraped and extracted in lysis buffer containing 20 mM Mops, pH 7.0, 10% glycerol, 80 mM β-glycerophosphate, 5 mM EGTA, 0.5 mM EDTA, 1 mM Na_3VO_4, 5 mM $Na_4P_2O_7$, 50 mM NaF, 1% Triton X-100, 1 mM benzamidine, 1 mM dithiothreitol (DTT) and 1 mM PMSF. Extracts were centrifuged at 17000 ***g*** for 12 min at 4 °C. Clarified supernatants were stored at −80 °C until use. All the following steps were done at 4 °C. Clarified supernatants were diluted four times in buffer I (20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.1 mM EDTA, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM Na_3VO_4, 1% Triton and 1 mM PMSF). Undiluted anti-MAPKAPK-2/3 or anti-p38 antibodies were added in limiting concentrations and the mixtures were incubated for 1 h. Then 10 μl of Protein A–Sepharose [50% (v/v); 15 μl for the p38 kinase assay; Amersham Bioscience, Baie d'Urfé, Québec, Canada] in buffer I were added and the mixtures incubated for 30 min. Samples were centrifuged and washed three times with buffer I. Immunoprecipitates were directly used for kinase assays.

Protein kinase assays

Kinase activities were assayed in immune complexes using appropriate substrates. p38 activity was measured by assessing the activity of its substrate MAPKAPK-2. The activity of immunoprecipitated MAPKAPK-2 was measured using recombinant HSP27 as a substrate [28]. The assays were done in 25 μl of kinase buffer K (100 μM ATP, 3 μCi of [γ-^{32}P]ATP, 120 mM *p*-nitrophenyl phosphate, 30 mM Mops, pH 7.0, 10% glycerol, 15 mM $MgCl_2$, 1 mM DTT, 1 μM leupeptin and 0.1 mM PMSF). The kinase activity was assayed for 30 min at 30 °C and was stopped by adding 10 μl of SDS/PAGE loading buffer. Immunoprecipitated p38 was assayed analogously using recombinant p53 as a substrate. Phosphorylation of p53 by p38 was carried out at 30 °C for 60 min in the assay medium containing 50 mM Hepes, pH 7.4, 50 mM β-glycerophosphate, 50 mM $MgCl_2$, 0.2 mM Na_3VO_4, 4 mM DTT, 1 μg of recombinant p53 and 9 μCi of [γ-^{32}P]ATP. In the case of JNK activity, the cell extract was absorbed on GST-c-Jun beads and the kinase activity was tested using the same GST-c-Jun as substrate [36]. Briefly, the GST-c-Jun fusion proteins bound to glutathione–Sepharose beads were incubated for 30 min at 4 °C with the extracts in buffer I. The beads were then pelleted, washed with buffer I and incubated for 30 min at 30 °C with 3 μCi of [γ-^{32}P]ATP in buffer K containing 10 mM $MgCl_2$. The phosphorylated GST-c-Jun was boiled in SDS sample buffer to stop the reaction. The activities of the different kinases were quantified by measuring the incorporation of radioactivity into the specific substrate after SDS/PAGE and quantification using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA, U.S.A.).

Clonogenic assay

Long-term cell survival was analysed by clonogenic assay. After irradiation, cells were trypsinized and 10^2–10^6 keratinocytes in DME/Ham's F12 supplemented medium were plated in a 60 mm Petri dish containing 0.2×10^6 lethally irradiated 3T3 cells as feeder cells. After 10–12 days, colonies were fixed in 10% formaldehyde in PBS, rinsed and stained with 1% Rhodanile Blue. Colonies of 10 or more cells were counted under a Wild M3 Leitz Heerbrugg microscope (Leitz, Ontario, Canada). The survival data were corrected for the plating efficiency of the appropriate control.

Analysis of apoptosis by nuclear morphology and flow cytometry

Apoptosis was determined by microscopic examination of nuclear morphology or by flow cytometry. For nuclear morphology, cells were fixed in methanol/acetic acid (3:1, v/v) and the chromatin was stained with 0.5 μg/ml Hoechst 33258 for 30 min. Cells with condensed chromatin were counted under an Eclipse 600 epifluorescence microscope (Nikon, Melville, NY, U.S.A.). The percentages of apoptotic cells were determined from six fields chosen randomly (> 300 cells counted). For flow cytometry, floating and adherent cells were collected and apoptosis was determined using an annexin-V-fluos staining kit as recommended by the manufacturer (Boehringer Mannheim, Indianapolis, IN, U.S.A.). Flow-cytometric analysis were performed in a Beckman-Coulter EPICS Elite ESP (Beckman-Coulter, Miami, FL, U.S.A.).

DEVDase cleavage activity

DEVDase cleavage activity was determined according to Enari et al. [37] with some modifications. Briefly, floating and adherent cells were pooled, and cytosolic extracts were prepared by eight repeated cycles of freezing and thawing in 100 μl of DEVDase extraction buffer (50 mM Mops, pH 7.0, 50 mM KCl, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM DTT, 20 μM cytochalasin D, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin A and 50 μg/ml antipain). Extracts were clarified by centrifugation for 12 min in a microfuge at 4 °C. Protein extracts (or buffer only for the blank) were mixed with 500 μl of ICE standard buffer (100 mM Hepes/KOH, pH 7.5, 10% sucrose, 0.1% CHAPS, 10 mM DTT and 0.1 mg/ml ovalbumin) containing 1 μM DEVD-AMC and incubated at 30 °C for 30 min. The fluorogenic substrate-derived product AMC was detected by excitation at 380 nm and emission at 460 nm using a luminescence spectrometer (model LS50B; Perkin Elmer, Norwalk, CT, U.S.A.). DEVDase activities were corrected for protein concentrations and normalized to the activity of the control.

Western-blot analysis

Whole-cell extracts were prepared by lysing the cells in SDS sample buffer. Total proteins (35 μg) were resolved by SDS/PAGE (10% gels) and transferred on to nitrocellulose. Equal protein loading was controlled by Ponceau Red staining of the membrane. After 1 h 30 min of incubation in blocking solution (5% non-fat milk, 20 mM Tris/HCl, pH 7.4, 150 mM NaCl and 0.05% Tween 20 for anti-p53 and anti-Bax), the membrane was incubated overnight at 4 °C with anti-p53 (DO-1) or for 1 h at room temperature for anti-Bax, and probed with a horseradish peroxidase-conjugated donkey anti-mouse IgG and goat anti-rabbit IgG respectively (Jackson ImmunoResearch, West Grove, PA, U.S.A.). For anti-(phospho p53) (Ser15), the membrane was incubated for 1 h in blocking solution (5% non-fat milk, 20 mM Tris/HCl, pH 7.6, 137 mM NaCl and 0.1% Tween 20) and was thereafter incubated overnight at 4 °C with primary antibody followed by goat anti-rabbit IgG. The signal was detected using an ECL detection kit (Pierce, Rockford, IL, U.S.A.).

Immunofluorescence

p53 staining for immunofluorescence microscopy was performed as described previously by Ashcroft et al. [38] with some modifications. Cells were washed twice with PBS (pH 7.5) and were fixed in ice-cold 3.7% formaldehyde in PBS for 10 min at room temperature. After fixation, keratinocytes were permeabilized with cold 0.2% Triton X-100 in PBS for 5 min, blocked in 0.5% BSA in PBS for 30 min at room temperature and then

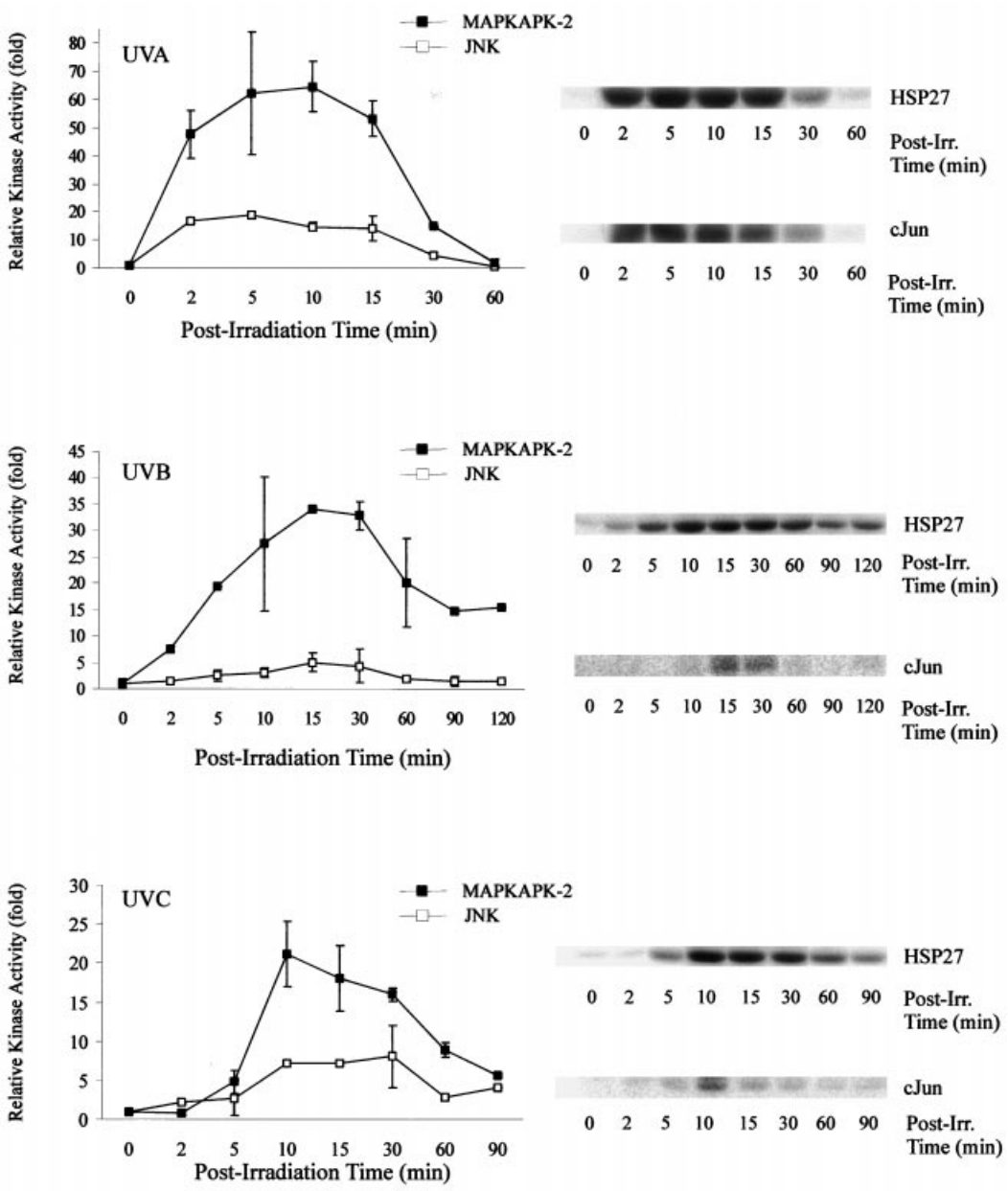

Figure 1 Activation of JNK and p38 in normal human keratinocytes exposed to UVA, UVB or UVC radiation

Quiescent keratinocytes were exposed to a single dose of UVA (3 J/cm^2; top panel), UVB (0.03 J/cm^2; middle panel) or UVC (0.003 J/cm^2; bottom panel). Cells were harvested in lysis buffer at different times after irradiation. JNK and MAPKAPK-2 activities were determined in immunocomplex kinase assays using GST-c-Jun and HSP27 as substrates respectively. Note that p38 activity was evaluated by measuring the activity of MAPKAPK-2, a direct downstream target of p38. Results are expressed as the ratio of kinase activities of stimulated cells over unstimulated cells. Representative results from three different experiments are shown. Data points represent means $\pm$ S.D. from duplicate samples. Representative autoradiograms are shown.

incubated overnight at 4 °C with anti-p53 (DO-1) in blocking solution. Cells were then washed three times with PBS and incubated for 2 h at room temperature with a rabbit anti-mouse Alexa 568-conjugated antibody (Jackson ImmunoResearch) in blocking solution containing 0.5 μg/ml Hoechst 33258. The cells were washed three times with PBS and mounted in glycine/glycerol mounting medium. Fluorescence was analysed on an Eclipse 600 epifluorescence microscope (Nikon, Melville, NY, U.S.A.), and pictures were taken with a Micromax CCD camera (Princeton Instruments, Trenton, NJ, U.S.A.). Images were processed with Metamorph software (Universal Imaging Corp., West Chester, PA, U.S.A.).

RESULTS

Exposure of normal keratinocytes to UV rays induces activation of p38 MAP kinase

The MAP kinase pathways are central components involved in transducing the signals elicited by physiological agonists and

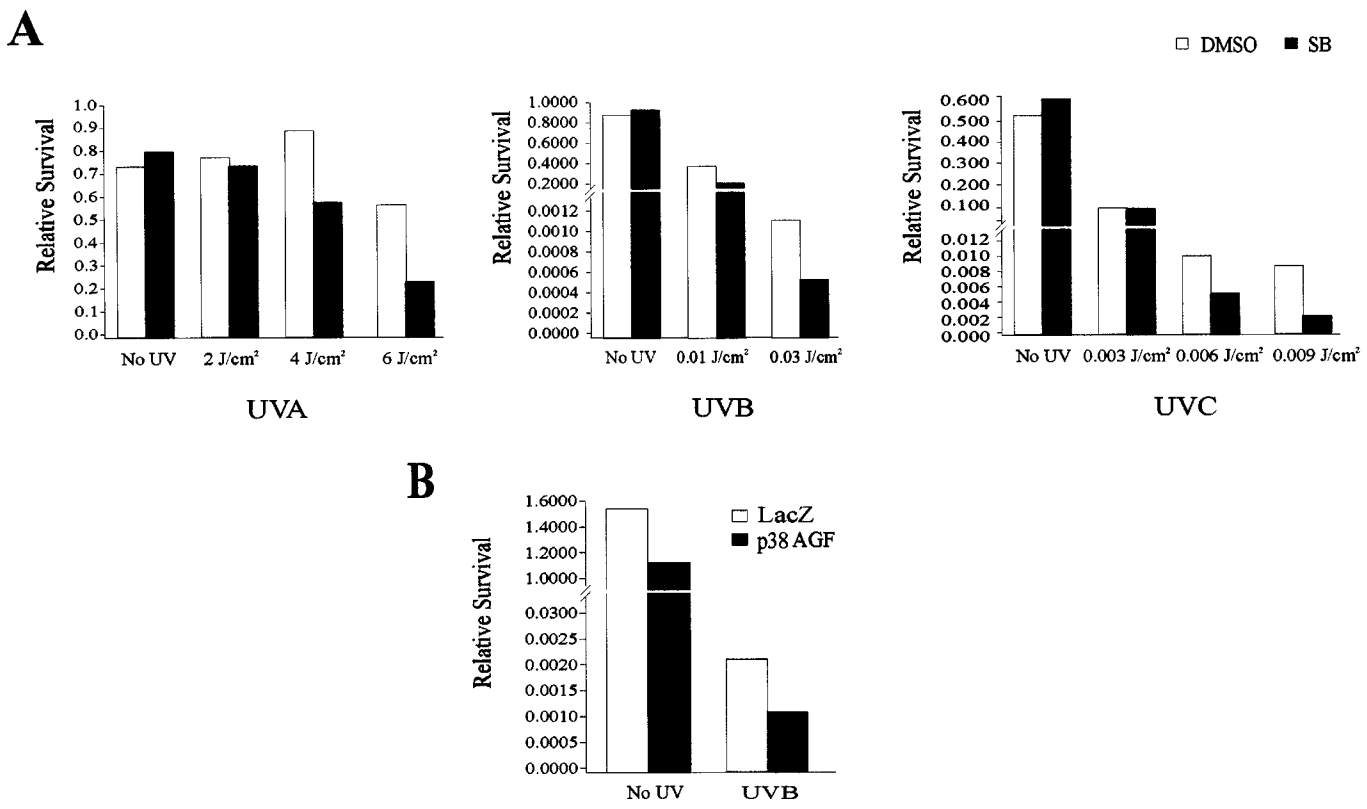

Figure 2 Inhibition of p38 sensitizes normal keratinocytes to UV-induced cell death

In (**A**) normal keratinocytes were incubated for 30 min with 10 μM SB203580 (SB) or vehicle alone (0.025% DMSO) and were then exposed to UVA (2, 4 and 6 J/cm^2; left-hand panel), UVB (0.01 and 0.03 J/cm^2; middle panel) or UVC (0.003, 0.006 and 0.009 J/cm^2; right-hand panel). The inhibitor SB203580 was withdrawn during irradiation and then added back for 2 h 30 min. In (**B**) normal keratinocytes were infected for 48 h with adenovirus expressing a dominant-negative mutant of p38 (p38AGF) or LacZ as a control, and were then exposed to 0.06 J/cm^2 UVB. Clonogenic assays were performed by seeding 10^2–10^6 keratinocytes on a feeder layer of lethally irradiated 3T3 cells in 60 mm dishes. After 10–12 days, colonies were stained with Rhodanile Blue and counted.

stressful stimuli. We investigated here the role of the ERK, p38 and JNK MAP kinase pathways in transducing the UV signals in normal human keratinocytes. Keratinocytes were exposed to single effective doses of UVA (3 J/cm^2), UVB (0.03 J/cm^2) and UVC (0.003 J/cm^2). Extracts were then prepared from these cells at different times post-irradiation and MAP kinase activities were assayed as described in the Materials and methods section. The activity of p38 was evaluated by determining the activity of MAPKAPK-2, a downstream physiological target of p38 [28]. Results showed that all three types of ray activate JNK and p38 with peaks of activation that occur 2–10 min after UVA irradiation, 15–30 min after UVB irradiation and 10–15 min after UVC irradiation (Figure 1). The activation of p38 was stronger and more persistent than that of JNK. In fact, MAPKAPK-2 was still activated 90 min after UVB and UVC irradiation. There was no significant activation of ERK by any type of UV ray (results not shown). Overall, these results suggested that p38 activation plays a major role in transducing the UV signal in normal human keratinocytes.

Inhibition of p38 activity sensitizes normal human keratinocytes to UV-induced cell death

p38 is an important modulator of the adaptive cell response to environmental stress [2]. We thus verified whether p38 was involved in regulating cell survival of keratinocytes submitted to UV stress. Cells were pre-treated for 30 min with SB203580 (10 μM), a potent inhibitor of p38 activity [28], and were then exposed to UV rays. To avoid modifications by UV rays, the inhibitor was withdrawn during irradiation and was added back for 2 h 30 min post-irradiation. After treatments, long-term cell survival was determined by clonogenic assays. The results revealed that UVA, UVB and UVC rays all decreased, in a dose-dependent manner, the efficiency of keratinocytes to form colonies (Figure 2A). Inhibiting p38 activity with SB203580 sensitizes keratinocytes to UVR-induced cell death (Figures 2A and 3A). This SB203580-sensitizing effect was accentuated at higher doses of UV (Figure 2A). Similar results were obtained in keratinocytes in which UVB-induced p38 activity has been inhibited following infection with an adenovirus vector carrying p38AGF, a dominant-negative mutant of p38 (Figures 2B and 3B). Also, a Trypan Blue-exclusion assay, performed 24 h after irradiation, showed an increase in UV-induced cell death when p38 activity was blocked by SB203580 (results not shown). We concluded that the p38 pathway is determinant in modulating survival of normal human keratinocytes exposed to UV stress.

Inhibition of p38 activity sensitizes normal human keratinocytes to UVB-induced apoptosis independently of caspase activity

UVA rays are less mutagenic than UVB and humans are not exposed to UVC. We thus decided to investigate further the

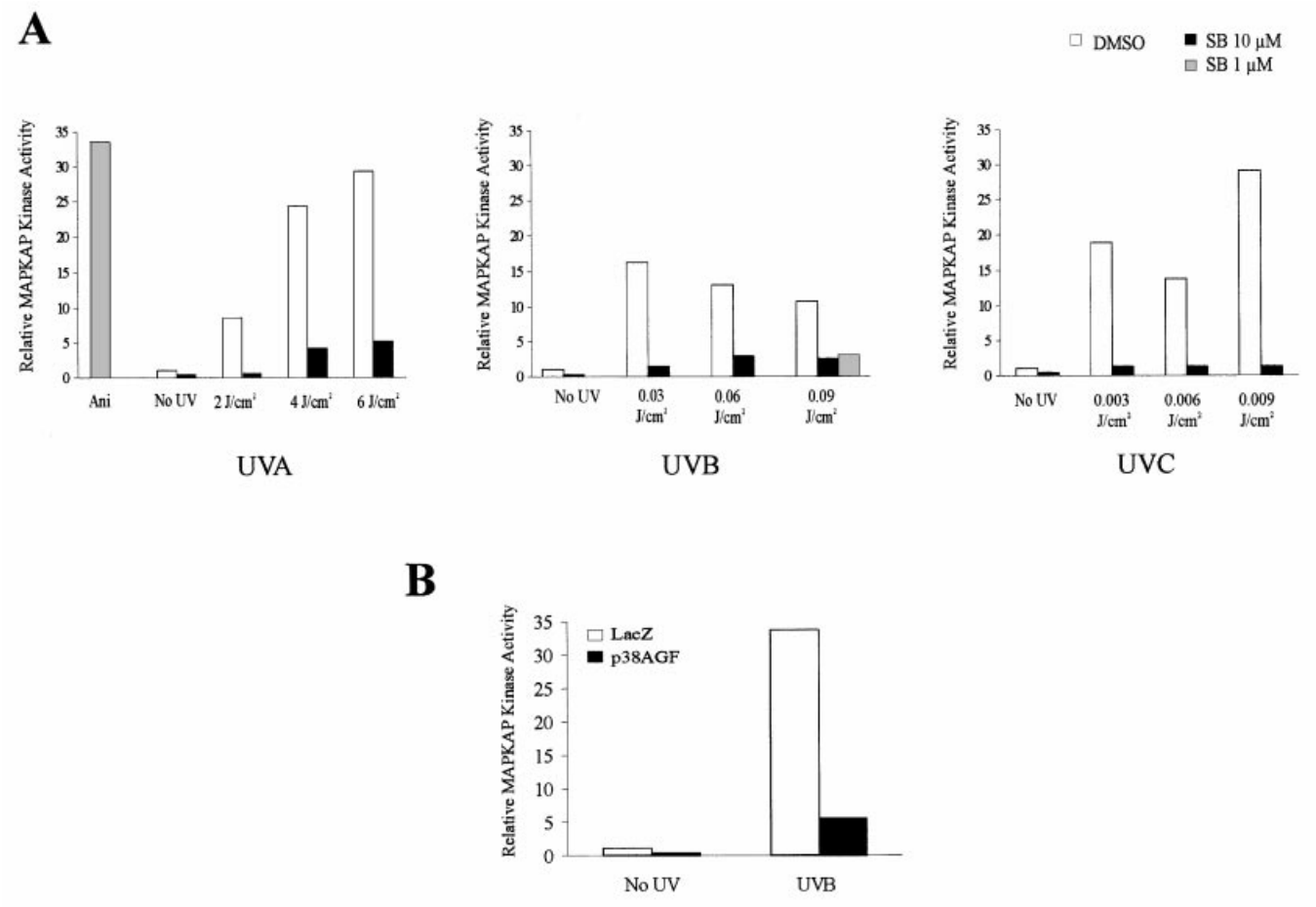

Figure 3 Inhibition of UV-induced p38 activity by SB203580 and a dominant-negative form of p38

(**A**) Normal keratinocytes were pre-treated for 30 min with 1 or 10 μM SB203580 (SB) or vehicle alone (0.025% DMSO) and were then exposed to UVA (2, 4 and 6 J/cm^2; left-hand panel), UVB (0.03, 0.06 and 0.09 J/cm^2; middle panel) or UVC (0.003, 0.006 and 0.009 J/cm^2; right-hand panel) in the absence of SB203580. Cells were harvested in lysis buffer after 2, 15 and 10 min respectively. As a control, cells were exposed to 10 μg/ml anisomycin (Ani) for 30 min. (**B**) Normal keratinocytes were infected for 48 h with adenovirus expressing a dominant-negative mutant of p38 (p38AGF) or LacZ as a control, and were then exposed to 0.06 J/cm^2 UVB. Cells were harvested in lysis buffer 15 min after irradiation. MAPKAPK-2 (p38) assays were performed using recombinant HSP27 as a substrate. Results are expressed as the ratio of kinase activities of stimulated cells to unstimulated cells. Representative results are shown.

mechanisms by which p38 modulated cell death and cell survival of normal human keratinocytes in response to UVB rays exclusively. We determined whether p38 affected UVB-induced cell death by regulating apoptosis. Keratinocytes were exposed to 0.06 J/cm^2 UVB, fixed at different times post-irradiation and then stained with Hoechst 33258 to label cell nuclei. Results revealed that UVB induced a time-dependent increase in the percentage of cells showing nuclear condensation and fragmentation [Figures 4A (arrows) and 4B]. Consistent with the fact that inhibition of p38 activity sensitized keratinocytes against UVB-induced apoptosis, we found that SB203580 increased by 1.5–2-fold the number of apoptotic cells with condensed nuclei (Figure 4B).

Apoptosis is frequently associated with induction of caspase activities that later cleave death substrates, such as poly(ADP-ribose) polymerase. We thus verified whether caspases were involved in mediating the apoptotic response that follows p38 inhibition in the presence of UVB. Following pretreatment or not with SB203580, cells were exposed to 0.06 J/cm^2 UVB, extracted at different times post-irradiation and then caspase activities were evaluated by measuring DEVDase cleavage activities. As shown in Figure 4(C), a time-dependent increase in DEVDase activities was observed following UVB irradiation but it was not affected by the inhibition of p38 with SB203580. This suggested that DEVDase activities were not regulated downstream of the p38 pathway activated by UVB. Caspase activities were not upstream of p38 either, since the pan-caspase inhibitor zVAD-fmk did not affect the activation of MAPKAPK-2 (Figure 4D), even at a concentration (50 μM for 60 min) that significantly inhibited DEVDase activities (Figure 4D, inset) [4]. These results indicated that the sensitization of keratinocytes to UVB-induced apoptosis subsequent to p38 inhibition is a mechanism that is caspase-independent.

p38 kinase does not mediate protection against UVB in transformed HaCaT keratinocytes

Interestingly, in the transformed HaCaT keratinocyte cell line, inhibition of p38 activity by SB203580 has no sensitizing effect on UVB-induced apoptosis, and depending on the pattern of administration it has even been shown to confer protection (Figure 5A) [39]. Yet, in these cells, as in normal keratinocytes,

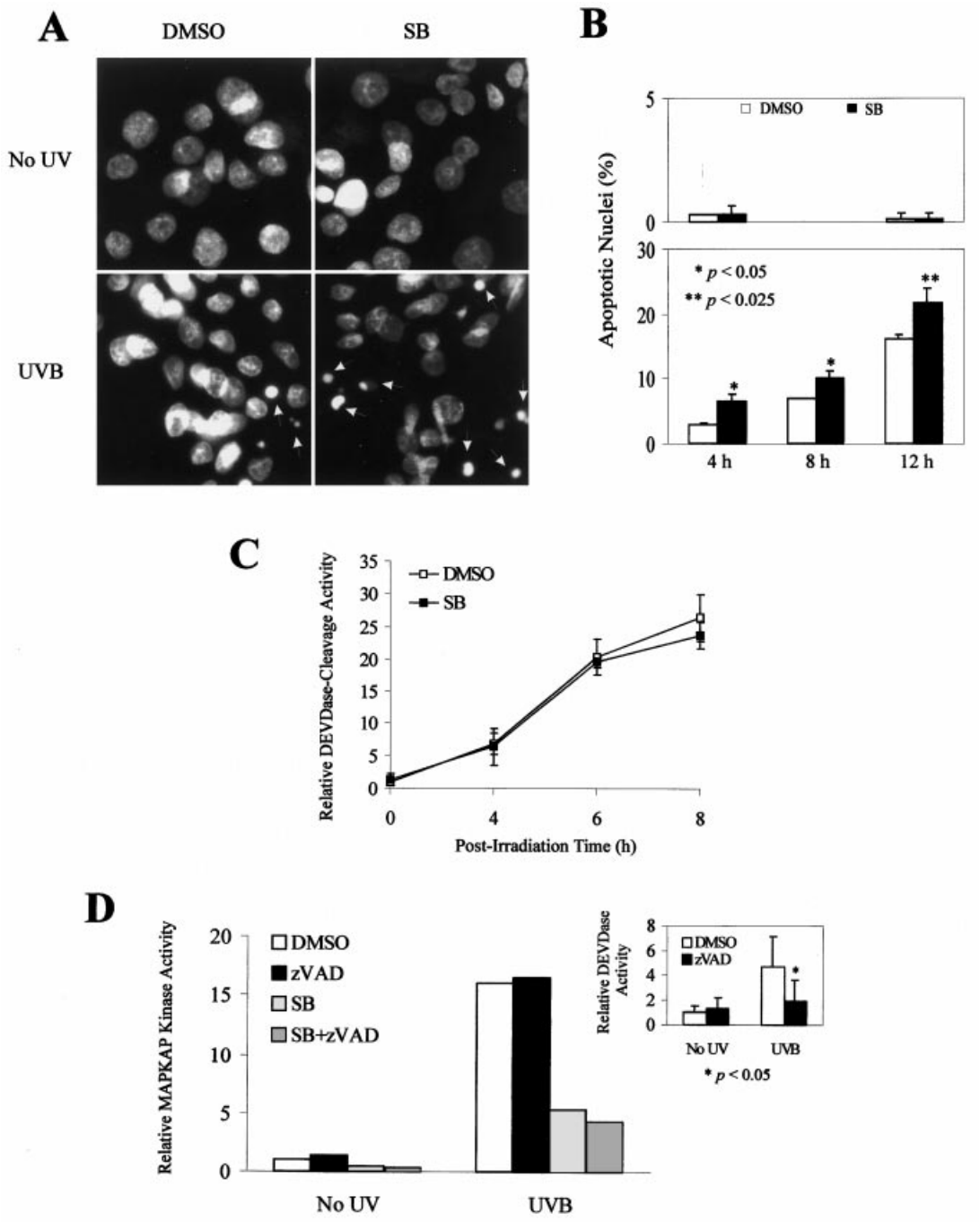

Figure 4 Inhibition of p38 activity sensitizes normal human keratinocytes to UVB-induced apoptosis independently of caspase activity

Normal keratinocytes were incubated for 30 min with 10 μM SB203580 (SB) or vehicle alone (0.025% DMSO) and were then exposed to UVB (0.06 J/cm^2). The inhibitor SB203580 was withdrawn during irradiation and then added back for 2 h 30 min. (**A**, **B**) After 4, 8 and 12 h, cells were fixed and nuclei stained with 0.5 μg/ml Hoechst 33258. Apoptotic nuclei were photographed and counted. (**A**) 8 h time point is shown. Arrowheads in the lower panels indicate cells with nuclear condensation and fragmentation. (**B**) Percentages of apoptotic nuclei in non-irradiated (upper panel) and irradiated (lower panel) cells; * and ** indicate statistical significances of $P < 0.05$ and $P < 0.025$ as determined by Student's t test. (**C**) At different times after irradiation, floating and adherent cells were pooled and processed for DEVDase activity assay as described in the Materials and methods section. Data represent means $\pm$ S.D. from triplicates. (**D**) Normal keratinocytes were pre-treated with vehicle (0.25% DMSO), SB203580 (30 min; 10 μM), zVAD-fmk (60 min; 50 μM) or SB203580 (30 min; 10 μM) plus zVAD-fmk (60 min; 50 μM) and were then exposed to UVB (0.06 J/cm^2). Cells were harvested in lysis buffer 15 min after irradiation and MAPKAPK-2 (p38) assays were performed using recombinant HSP27 as a substrate. Inset: inhibition of DEVDase by 50 μM zVAD assayed as described in the Materials and methods section. Data points represent means $\pm$ S.D. from triplicates. *$P < 0.05$, as determined by Student's t test.

p38 was activated by UVB, and SB203580 significantly impaired the activation of p38, as reflected by inhibition of MAPKAPK-2 (Figure 5B). However, the level and kinetics of p38 activation in HaCaT were different from normal human keratinocytes, being activated later and to a lesser extent (Figure 5B). From these results, we observed that UVB-induced p38 activity medi-

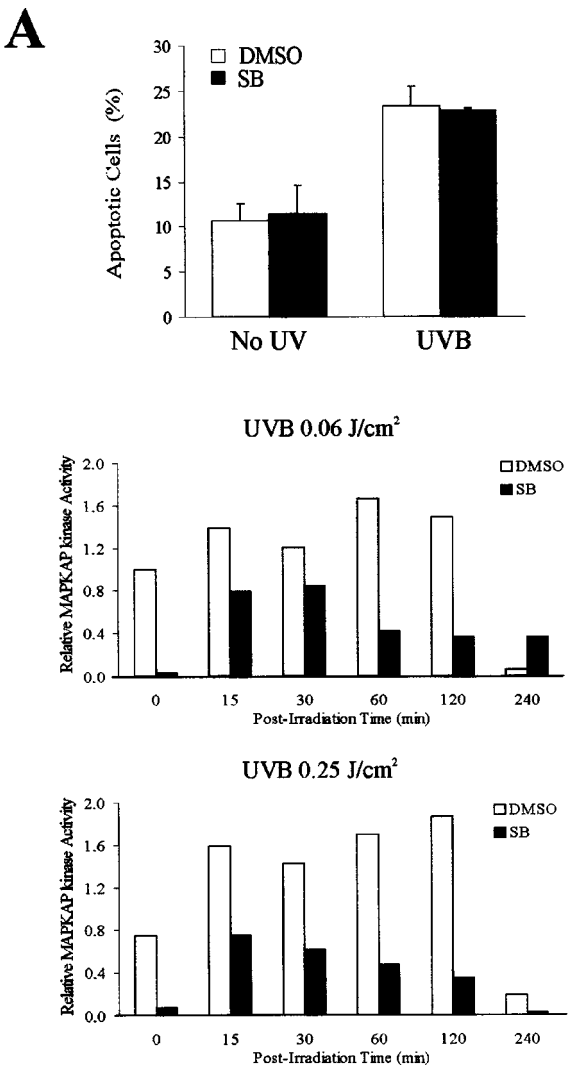

Figure 5 Activation of p38 kinase does not protect transformed HaCaT keratinocytes against UVB-induced apoptosis

HaCaT cells were pre-treated for 30 min with vehicle (0.025% DMSO) or with 10 μM SB203580 (SB), and then exposed to UVB. SB203580 was withdrawn during irradiation and then added back for 2 h 30 min. (**A**) 24 h after exposure to 0.25 J/cm^2 UVB, floating and adherent cells were pooled and apoptosis was evaluated by annexin-V/propidium iodide double staining and FACS analysis. Data represent means $\pm$ S.D. from triplicates. (**B**) Cells were harvested in lysis buffer 15 min after exposure to 0.06 J/cm^2 (top panel) or 0.25 J/cm^2 (bottom panel) UVB. MAPKAPK-2 (p38) assays were performed using recombinant HSP27 as a substrate.

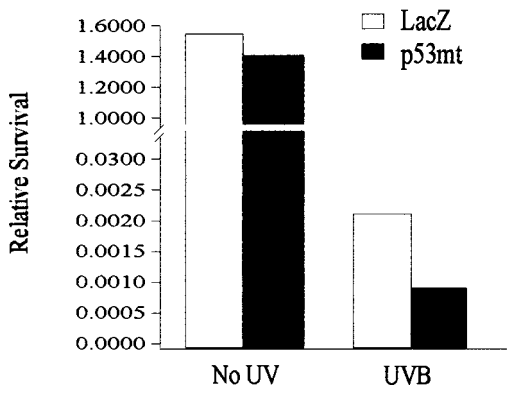

Figure 6 Expression of a dominant-negative form of p53 sensitizes normal keratinocytes to UVB-induced cell death

Keratinocytes were infected for 48 h with adenovirus expressing a dominant-negative mutant of p53 (R175H; p53mt) or LacZ as a control, and were then exposed to 0.06 J/cm^2 UVB. After irradiation, cells were trypsinized and 10^2–10^6 keratinocytes were seeded on feeder layers of lethally irradiated 3T3 cells in 60 mm dishes. After 10–12 days, colonies were stained with Rhodanile Blue and counted.

ates protective function in normal but not in transformed HaCaT keratinocytes.

UVB-induced p38 activation increases the phosphorylation and the stability of p53 in normal keratinocytes

The major difference between normal and HaCaT keratinocytes is that HaCaT cells express a mutated form of p53, the gene of which carries mutations that are characteristic of the UV signature [35]. The tumour suppressor p53 plays a central role in modulating the cellular response to DNA damage. p53 is also an important modulator of apoptosis and has been shown to induce distinct pro-apoptotic and anti-apoptotic signals in response to UV irradiation [40]. In the present study, we found that the expression of a dominant-negative form of p53 sensitized normal keratinocytes to UVB-induced cell death (Figure 6). The activation of p53 is regulated by several mechanisms, particularly by stabilization of the protein. Notably, the stability of p53 is controlled by post-translational modifications involving acetylation and phosphorylation [24]. We observed in normal keratinocytes that activation of p38 increased the phosphorylation of p53, both *in vitro* and *in vivo* (Figure 7). The p38-mediated phosphorylation of p53 in response to UVB involved Ser-15 (Figure 7B), an effect that was impaired by treatment with SB203580 even in the absence of NaF (Figures 7B and 7C).

It is known that phosphorylation of p53 on Ser-15 impairs the binding of Mdm2, which correlates with p53 accumulation following genotoxic stress [23,41]. In this context, we examined the role of UVB-mediated activation of p38 in regulating the cellular level of p53. Normal keratinocytes were treated or not with 10 μM SB203580 and were then exposed or not to 0.06 J/cm^2 UVB. Extracts were prepared at different times post-irradiation and the levels of p53 were determined by Western-blot analyses. Results showed that UVB markedly increased the amount of p53. The effect started at 4 h, reached a peak between 6 and 10 h post-irradiation, and then the level of p53 declined to basal levels (Figure 8A and results not shown). The inhibition of p38 by SB203580 or following infection of normal keratinocytes with the adenovirus carrying p38AGF impaired the UVB-induced enhancement of p53 level (Figure 8). This suggested that p38-mediated phosphorylation of p53 on serine residues was an important regulator of p53 stability in response to UVB. In accordance, pre-treating normal keratinocytes with NaF, a broad serine phosphatase inhibitor, was associated with an SB203580-sensitive increase in the amount of p53 in response to UVB rays (Figure 8A). The effect of SB203580 was maximal between 4 and 6 h and was followed by recovery. The results indicated that serines phosphorylated by p38 are important in maintaining p53 stability since phosphatase inhibitors could not restore p53 stability when p38 was inhibited. The recovery observed after 6 h could be explained by the fact that NaF was removed 2 h post-irradiation.

The phosphorylation of p53 is affected by its conformation which, in turn, is modified by mutation of the protein [42]. We then investigated whether or not p38 can phosphorylate mutated

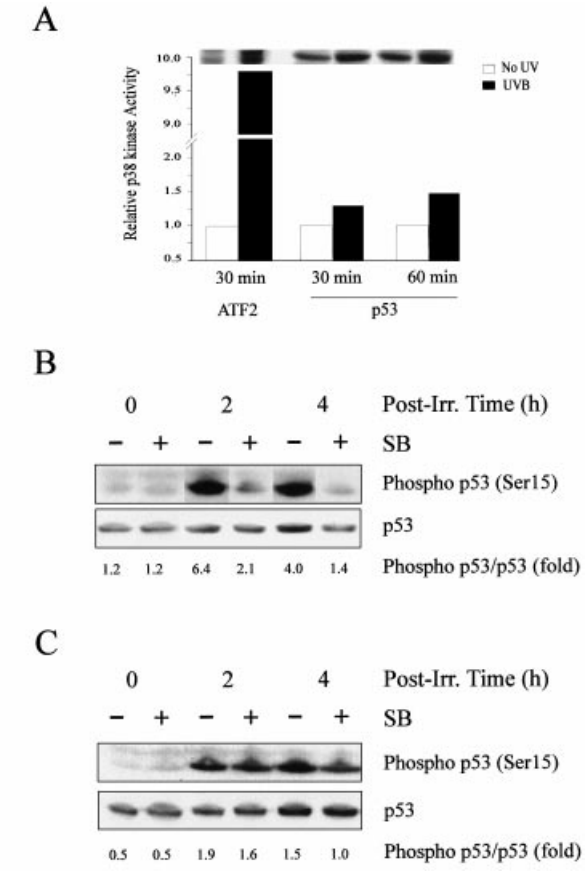

Figure 7 p38-mediated phosphorylation of p53 in normal keratinocytes exposed to UVB

(**A**) Normal keratinocytes were exposed to 0.06 J/cm^2 UVB and harvested in lysis buffer 15 min after irradiation. p38 kinase assay was performed at 30 °C for 30 and 60 min using recombinant human GST-p53 as a substrate. As a control, p38 was assayed at 30 °C for 30 min using activating transcription factor-2 (ATF2) as a specific substrate. Results are expressed as the ratio of kinase activities of stimulated cells to unstimulated cells. (**B**) Whole-cell extracts were collected at different times after irradiation from normal keratinocytes pre-treated with NaF (30 min; 5 mM) in the presence (+) or not (−) of 10 μM SB203580 (SB) and then exposed to UVB (0.06 J/cm^2). The inhibitors were removed for irradiation and then added back for 2 h. (**C**) Whole-cell extracts were collected at different times after irradiation from normal keratinocytes pre-treated with vehicle (0.025% DMSO) or SB203580 (30 min; 10 μM). The inhibitor was removed for irradiation and then added back for 2 h. In (**B**) and (**C**) phosphorylation of p53 on Ser-15 was analysed by Western blotting using anti-(phospho p53) (Ser15).

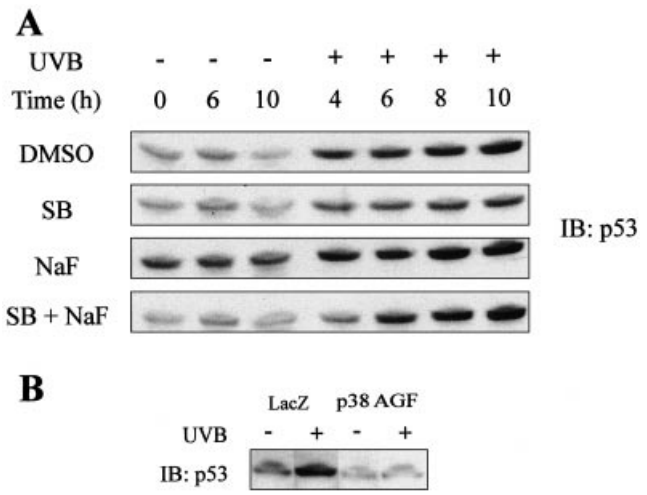

Figure 8 Inhibition of UV-induced p38 activity in normal human keratinocytes is associated with a decrease in p53 level

(**A**) Whole-cell extracts were collected at different times after irradiation from normal keratinocytes pre-treated with vehicle (0.025% DMSO), SB203580 (SB; 30 min; 10 μM), NaF (30 min; 5 mM) or SB203580 plus NaF and then exposed to UVB (0.06 J/cm^2). The inhibitors were removed for irradiation and then added back for 2 h. (**B**) Normal keratinocytes were infected for 48 h with adenovirus expressing a dominant-negative mutant of p38 (p38AGF) and then were exposed to 0.06 J/cm^2 UVB. Whole-cell extracts were collected 4 h after irradiation. p53 level was analysed by Western blotting using p53 (DO-1) antibody.

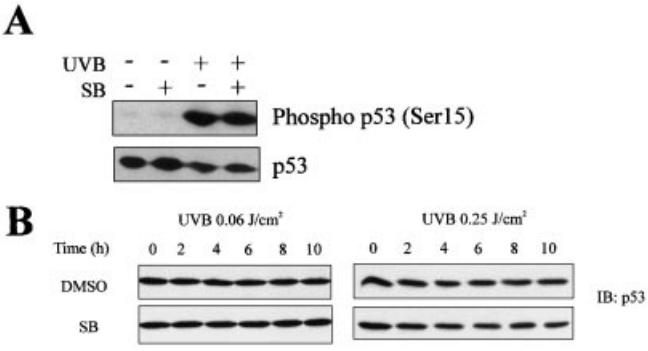

Figure 9 Phosphorylation of p53 on Ser-15 and p53 level are insensitive to inhibition of p38 activity in HaCaT keratinocytes exposed to UVB rays

HaCaT keratinocytes were pre-treated with vehicle (0.025% DMSO) or SB203580 (SB; 30 min; 10 μM). The inhibitor was removed for irradiation and then added back for 2 h 30. Whole-cell extracts were collected at different times after exposure to 0.06 J/cm^2 (**B**, left-hand panel) or 0.25 J/cm^2 (**B**, right-hand panel) UVB. (**A**) Phosphorylation of p53 on Ser-15 was analysed by Western blotting using anti-(phospho p53) (Ser15). (**B**) The p53 level was analysed by Western blotting using anti-p53 (DO-1).

p53 in transformed HaCaT keratinocytes. We observed an increase in Ser-15 phosphorylation that was not affected by SB203580 (Figure 9A). Moreover, with or without p38 inhibition, there was no change in the level of p53 following UVB exposure of HaCaT cells, even at a higher dose of UVB (0.25 J/cm^2; Figure 9B). These results suggest that p38-mediated phosphorylation and stabilization of p53 were dependent on wild-type p53 conformation.

p38-mediated protection against UVB depends on p53 cellular localization and requires its wild-type genotype

We next investigated whether p38, by increasing the phosphorylation and stability of p53, contributes to protecting normal keratinocytes from UVB-induced cell death. Apart from being inactivated by 'dead' mutations, p53 is rendered inactive by cytoplasmic sequestration [43]. We thus verified whether UVB-induced p38-mediated phosphorylation of p53 affected its nucleocytoplasmic distribution, thereby regulating cell death. Keratinocyte colonies were exposed to 0.06 J/cm^2 UVB, fixed 4 and 8 h post-irradiation, stained for p53 and examined using fluorescence microscopy. UVB irradiation of keratinocytes resulted in an important cytoplasmic accumulation of the protein, which became significant 8 h after exposure. This cytoplasmic accumulation was observed principally in the keratinocytes located at the margin of the colonies (Figure 10A). Nuclear staining was less pronounced. When p38-mediated phosphorylation of p53 was

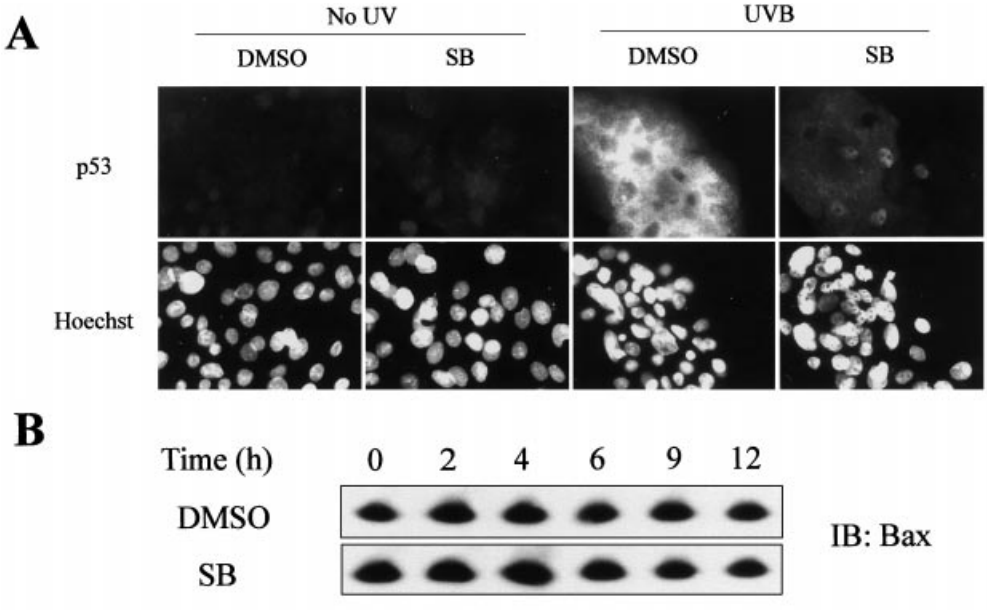

Figure 10 p38 activation mediates the nucleocytoplasmic distribution of p53 in normal human keratinocytes exposed to UVB and prevents Bax expression

(**A**) Normal keratinocytes were pre-treated for 30 min with vehicle (0.025% DMSO) or 10 μM SB203580 (SB) and then exposed to 0.06 J/cm^2 UVB. SB203580 was withdrawn during irradiation and then added for 2 h 30 min. Cells were fixed 8 h post-irradiation and p53 cellular localization was analysed by immunofluorescence using a monoclonal antibody against p53 (DO-1). Nuclei were stained with 0.5 μg/ml Hoechst 33258. (**B**) Whole-cell extracts were collected at different times after irradiation from normal keratinocytes pre-treated with vehicle (0.025% DMSO) or SB203580 (30 min; 10 μM) and then exposed to UVB (0.06 J/cm^2). The Bax level was analysed by Western blotting using Bax antibody (N-20).

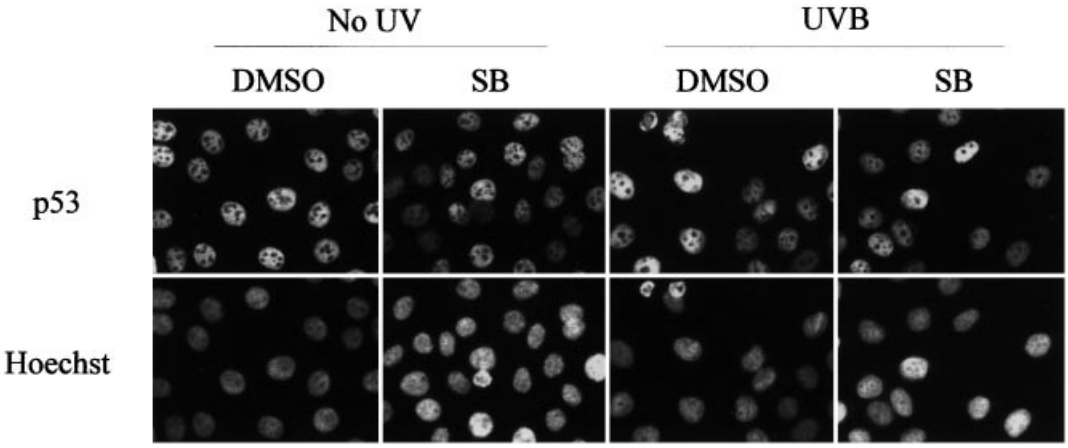

Figure 11 p38-mediated nucleocytoplasmic distribution of p53 requires its wild-type genotype

HaCaT cells were pre-treated for 30 min with vehicle (0.025% DMSO) or 10 μM SB203580 (SB), and then exposed to 0.06 J/cm^2 UVB. SB203580 was withdrawn during irradiation and added afterwards for 2 h 30 min. HaCaT cells were fixed at 8 h after irradiation and the cellular localization of p53 was analysed by immunofluorescence using a monoclonal antibody against p53 (DO-1). Nuclei were stained with 0.5 μg/ml Hoechst 33258.

inhibited by SB203580, the staining of the protein was weaker, which confirmed the results obtained by Western blot (Figure 8A), and remained confined to the nucleus (Figure 10A). In accordance, the cytoplasmic accumulation of p53 in response to UVB and the decreased levels of p53 found in the presence of SB203580 both correlated with a lack of p21^{WAF-1} and Bax accumulation, two p53 target genes (Figure 10B and results not shown).

These findings raised the possibility that UVB-induced activation of p38 in normal human keratinocytes increased the cytoplasmic distribution of p53 by enhancing its nuclear export. To test this hypothesis, keratinocytes were irradiated and were treated with LMB (4 ng/ml) to block cellular export receptor CRM1 (exportin-1)-dependent nuclear export. In non-irradiated cells, LMB treatment resulted in a nuclear accumulation of p53 after 8 h, suggesting that the basal level of p53 was regulated by CRM1-dependent nuclear export (results not shown). In contrast, in irradiated cells, the cytoplasmic staining of p53 was not reduced by LMB. This suggested that following UV exposure, p53 accumulates in the cytoplasm by a mechanism independent

of CRM1. Following inhibition of p38 with SB203580, UVB exposure was associated with the same low nuclear staining of p53 (results not shown). We observed that LMB alone had no effect on MAPKAPK-2 activity, excluding the possibility that LMB induced an unspecific stress signal (results not shown). From these results, we concluded that the cytoplasmic accumulation of p53 in response to UVB exposure did not result from an increase in its nuclear export whether it was mediated or not by p38.

Since the major difference between normal and HaCaT keratinocytes is that HaCaT cells express a mutated form of p53, we thus hypothesized that the wild-type *p53* genotype was necessary for its p38-dependent cytoplasmic sequestration and thus for p38-mediated protective function. We found that, in contrast to normal human keratinocytes, non-irradiated HaCaT cells exhibited a strong basal level of p53 in the nucleus. Following UVB exposure, and independently of p38 inhibition, p53 remained confined to the nucleus (Figure 11). The results suggest that p38 has no other protective function than via wild-type p53 and that the nuclear localization of p53 in HaCaT may be attributed to its mutated genotype, presumably through alterations of its conformation. The subcellular location of p53 is indeed tightly associated with its conformational phenotype [44]. By comparing the results obtained with normal and transformed HaCaT keratinocytes, we conclude that p38 protects normal keratinocytes against UVB by contributing to the sequestration and stabilization of p53 in the cytoplasm and that this protection requires the wild-type form of p53.

DISCUSSION

The ability of normal keratinocytes to quickly and efficiently cope with adverse stimuli is essential to protect themselves against cell toxicity and cell death. This requires the balanced integration of the stress signals that are sensed by the cell surface and that are transduced within the cells to generate the appropriate adaptive response. Here we show for the first time that activation of the p38 MAP kinase pathway, by leading to stabilization of the tumour suppressor p53, is a major determinant of the homoeostatic response developed by normal keratinocytes to resist apoptotic cell death induced by UVB. We also obtained results that support the point that the p38/p53-mediated protection is associated with the cytoplasmic accumulation of p53 and requires the structural conformation conferred by wild type *p53*. In accordance, in transformed HaCaT keratinocytes expressing a mutated form of p53, UVB-induced apoptosis was insensitive to p38 inhibition by SB203580.

The finding that the p38 pathway is a major component of the primary adaptive response of normal human keratinocytes against UV toxicity is supported by the fact that it was quickly and strongly activated by all three types of UV ray. In contrast, ERK MAP kinase was not significantly activated by any of these rays in normal keratinocytes, whereas JNK was mostly activated by UVA and to a lesser extent by UVB and UVC. Interestingly, previous findings have shown that ERK and JNK are weakly implicated in transducing signals involved in the response of skin to biologically relevant solar radiation [45]. The fact that p38 activation contributes to protection against UV exposure is supported firmly by the observation that its inhibition sensitized keratinocytes to cell death induced by all three types of radiation. In the case of UVB, to which humans are more specifically exposed, cell death was associated with typical features of apoptosis, including nuclear condensation and fragmentation. Apoptosis-associated nuclear fragmentation and condensation generally result from activation of caspases, which results in cleavage of death substrates, such as lamins, poly(ADP-ribose) polymerase and DNases [14]. Accordingly, we found that UVB increased caspase activities, as evaluated by measuring DEVDase activities. Nuclear condensation can also proceed in the absence of caspase activity [46]. In the present study, we found that inhibition of p38 sensitized normal keratinocytes to UVB-induced apoptosis but that the process was caspase-independent, since DEVDase cleavage activity remained unchanged in the presence of p38 inhibition by SB203580. Reciprocally, p38 activity was unchanged in the presence of the pan-caspase inhibitor zVAD-fmk. One proposed mechanism underlying caspase-independent apoptotic cell death involved apoptosis-inducing factor, a mitochondrial oxidoreductase which, after leaking out from the mitochondria, can induce nuclear condensation independently of caspase activation [46]. Whether p38 contributes to prevent the release of apoptosis-inducing factor from mitochondria in normal keratinocytes remains to be determined.

Intriguingly, inhibition of UV-induced p38 activation did not sensitize transformed HaCaT keratinocytes to UVB-induced apoptosis. Moreover, p38 activation by UV rays rather promotes apoptosis in various types of transformed cells [25]. These findings confirm the possibility raised by other studies that the p38-mediated effects in response to UV rays depend on the cellular context. In particular, the availability of specific targets at the moment of p38 activation, as well as the way cells utilize the phosphorylated targets, could be a determinant. Along these lines, different kinetics and level of p38 activation were observed between normal and transformed HaCaT keratinocytes. Moreover, we found that following UVB exposure p53 accumulates in the cytoplasm of normal keratinocytes but not of transformed HaCaT cells.

Following genotoxic stresses, accumulation of p53 results mainly from post-translational modifications, since its mRNA level remains unchanged [47], and phosphorylation of p53 is associated with its stabilization [16,41]. Accordingly, we found that UVB irradiation increased the level of p53 phosphorylation on Ser-15 and enhanced its stability in normal keratinocytes. This phosphorylation and accumulation were p38-dependent, being blocked by SB203580. Moreover, the inhibition of serine phosphatases could not restore the p53 stability that was impaired by the inhibition of p38. These results suggest that p38 directly phosphorylates p53 on Ser-15. In HaCaT cells, we found that UVB induced phosphorylation of p53 on Ser-15 but that this phosphorylation was unaffected by SB203580. These findings introduce the further possibility that phosphorylation of Ser-15 is p38-dependent but is not directly phosphorylated by p38 but by other kinases that are activated following UVB exposure. In this context, Bulavin et al. [25] have demonstrated that UV-induced phosphorylation of Ser-15 is p38-dependent but is not directly phosphorylated by p38 but by another kinase whose access to Ser-15 requires the prior phosphorylation of Ser-33 and Ser-46 by p38. As shown recently, phosphorylation of the fragment of p53 corresponding to amino acids 1–39 led to a more open conformation of this peptide [48].

Phosphorylation of p53 on Ser-15 is associated with a decrease in Mdm2 (murine double minute-2) binding and proteasome mediated-degradation [23,41], which could explain the accumulation of p53 following UV irradiation. Consistent with this interpretation, we observed that inhibition of p38 was associated with a lower level of p53 in the cytoplasm of irradiated cells. When p53 is phosphorylated by p38, this will stabilize p53 and

confine it to the cytoplasm. The retention of p53 in the cytoplasm will thus prevent the expression of the p53 target genes, many of which, such as Bax, Fas/APO1 and Killer/DR5, are involved in regulating apoptosis [49]. Incidentally, we found that UVB failed to increase the expression of Bax and also of p21, two classical targets of p53.

In various types of cells, the nucleocytoplasmic distribution of p53 depends on a CRM1-mediated export from the nucleus [19]. In the present study, we obtained evidence that the UVB-induced cytoplasmic accumulation of p53 was independent of CRM1-mediated nuclear export, since the CRM1 functional inhibitor LMB had no effect on the cellular distribution of p53 following exposure of normal keratinocytes to UVB. However, the cytoplasmic accumulation of p53 was tightly associated with the wild-type genotype of *p53*. This is supported by the fact that p53 did not accumulate in the cytoplasm of transformed HaCaT keratinocytes in which *p53* is mutated in both alleles. It is thus possible that, in HaCaT cells, a mutated non-functional p53 may account for its nuclear sequestration irrespectively of p38 activation. It remains also possible that the mutated conformation of p53 in HaCaT cells made it insensitive to p38-mediated regulation, as suggested by the finding that SB203580 did not inhibit phosphorylation of Ser-15 in these cells. Overall, these findings strengthen the point that different mutations of p53 each differently affect its conformation, modifying its pattern of phosphorylation and its localization [42,44]. Interestingly, the cytoplasmic staining of p53 in normal keratinocytes was mostly observed in the more differentiated cells located at the margin of the colonies. It is well known that the conformation of p53 can change with the differentiation state of the cells, being phenotypically wild type in proliferative cells and mutant in differentiated cells [50,51]. We have shown in a previous study that UVB irradiation enhances the differentiation process of human keratinocytes [52]. It is thus possible that during the UV-mediated transition from the proliferative to the differentiating state there is a modification in the conformation of p53 that favours its cytoplasmic retention in response to p38 activation. Sabapathy et al. [51] have shown that this conformational switch in embryonic stem cells is associated with reduced sensitivity to UV rays, which is consistent with our results.

In summary, our study revealed that p38 activation by UVB confers a fast adaptive response by which normal keratinocytes resist apoptosis. The p38-mediated protection results from the cytoplasmic retention of p53 following its phosphorylation and stabilization, which prevents its nuclear functions, as suggested by the lack of expression of pro-apoptotic genes like Bax. In transformed HaCaT keratinocytes expressing a mutated form of p53 that probably affects its configuration, p53 remains confined to the nucleus and cell survival is not affected by inhibition of p38. The identification of the p38/p53 pathway as an important regulator of the homoeostatic response of normal keratinocytes to UV rays will help us to better understand photocarcinogenesis, a process that might in part result from dysregulation of this pathway.

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