Mol. Cells *28*, 537-543, December 31, 2009
DOI/10.1007/s10059-009-0148-2

Molecules and Cells

Involvement of HIF-1α in UVB-Induced Epidermal Hyperplasia

Young-Suk Cho[1], Chan-Hyung Kim[2], and Jong-Wan Park[1,*]

Keratinocyte overgrowth after UVB exposure is believed to contribute to skin photoageing and cancer development. However, little is known about the transcription factors that epigenetically regulate keratinocyte response to UVB. Recently, HIF-1α was found to play a role in epidermal homeostasis by controlling the keratinocyte cell cycle, and thus, we hypothesized that HIF-1α is involved in UVB-induced keratinocyte growth. In cultured keratinocytes, HIF-1α was found to be down-regulated shortly after UVB exposure and to be involved in UVB-induced proliferation. In mice repeatedly treated with UVB, the epidermis became hyperplasic and keratinocytes lacked HIF-1α in nuclei. Based on these results, we suggest that the deregulation of HIF-1α is associated with UVB-induced hyperplasia of the epidermis. This work provides insight of the molecular mechanism underlying UV-induced photoageing and skin cancer development.

INTRODUCTION

Solar ultraviolet radiation (UV) is the most important cause of skin diseases, such as, photoaging and cancer. The primary event in UV-induced skin carcinogenesis is DNA injury and the subsequent formation of abnormal DNA adducts, which leads to cell cycle arrest, DNA mutation, and transformation (Melnikova and Ananthaswamy, 2005). In addition, many lines of evidence support the notion that UV dysregulates the expressions and activities of many transcription factors and by so doing alters gene expression (Matsumura and Ananthaswamy, 2002). Therefore, both DNA mutation and altered gene expression are believed to be responsible for UV-induced skin diseases.

Ultraviolet B (UVB, 290-320 nm) is known to be primarily responsible for breaking DNA strands. UVB causes keratinocyte cell cycle arrest, which allows time for the repair of damaged cells, or for apoptosis, if the damage is beyond repair (Marrot and Meunier, 2008). However, several studies have demonstrated that mouse skin thickens and the epidermal expression of PCNA increases at 48 h after UVB exposure (El-Abaseri et al., 2006; Lee et al., 2003; Ouhtit et al., 2000). This temporal switch from growth arrest to proliferation is likely to be necessary for the replacement of dead cells. However, if keratinocytes repeatedly undergo death and regeneration due to repeated UVB exposure, the coupling response to UVB could be deregulated and contribute to skin cancer development. However, little is known about the transcription factors that induce epigenetically regulated temporal changes in the cell cycle.

HIF-1α is a key transcription factor and is responsible for the expressions of a variety of genes required for cellular adaptation to hypoxia. HIF-1α belongs to the basic-helix-loop-helix Per-Arnt-Sim family, and is regulated at the post-translational level in an O_2-dependent manner (Iyer et al., 1998; Wang and Semenza, 1995). In an aerobic environment, HIF-1α is prolyl-hydroxylated by PHD1-3 enzymes, ubiquitinated by the E3 complex including von Hippel-Lindau protein (pVHL), and finally degraded through 26S proteasomes (Schofield and Ratcliffe, 2004). Since this hydroxylation process requires molecular oxygen, it is inhibited in hypoxic environments, and then HIF-1α, in association with HIF-1β (aryl-hydrocarbon receptor nuclear translocator, ARNT), targets and transactivates hypoxic genes (Park, 1999). In addition, HIF-1α participates in controlling cell cycle by antagonizing the p21 gene-repressive action of c-Myc and subsequently inducing p21 (Koshiji et al., 2004).

Recently, we found that HIF-1α plays a role in epidermal homeostasis by controlling the keratinocyte cell cycle through p21 expression (Cho et al., 2008). At high cell densities, keratinocytes underwent cell cycle arrest in the G1 phase. Furthermore, HIF-1α and p21 in keratinocytes were both substantially induced in a cell density-dependent manner, and were also highly expressed in the epidermal layers of normal human and mouse skin. Moreover, treatment with HIF-1α siRNAs released keratinocytes from growth arrest and induced epidermal hyperplasia in mice. Accordingly, we hypothesized that HIF-1α mediates UVB-induced changes in keratinocyte growth. In the present study, keratinocyte HIF-1α was found to be down-regulated after UVB exposure and to be involved in UVB-induced keratinocyte regrowth. When mice were repeatedly treated with UVB, their epidermises became hyperplasic, and HIF-1α was not expressed in keratinocyte nuclei. Based on these results, we suggest that the deregulation of HIF-1α is associated with UVB-induced epidermal hyperplasia.

[1]Department of Pharmacology, Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 110-799, Korea, [2]Department of Pharmacology, Chungbuk National University College of Medicine, Cheongju 361-763, Korea
*Correspondence: parkjw@snu.ac.kr

Received June 25, 2009; revised September 10, 2009; accepted September 14, 2009; published online November 19, 2009

Keywords: hyperplasia, hypoxia-inducible factor-1alpha, keratinocyte, skin, ultraviolet

MATERIALS AND METHODS

Materials

Desferrioxamine (DFO), cycloheximide (CHX), LY294002, rapamycin and other chemicals were purchased from Sigma-Aldrich Corp (USA). Culture media and fetal bovine serum (FBS) were purchased from Invitrogen Corp. (USA). Anti-human HIF-1α antiserum was generated in rabbits against bacterially expressed fragments encompassing amino acids 418-698 of human HIF-1α, as previously described (Chun et al., 2000). Antibodies against ARNT, PCNA, β-tubulin, and HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology Inc. (USA).

Cell culture and UV irradiation

HaCaT (normal human keratinocyte) cells were cultured in RPMI-1640 medium, supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were grown in a humidified 5% CO_2 atmosphere at 37°C. When plating cells one day before experiments, cell numbers were adjusted to reach 80-100% of confluence at harvest. Cultured cells were exposed to UVB for 3-12 seconds in a chamber (Vilber Lourmat, France) equipped with five UV lamps (peak wavelength of 312 nm, 8 Watt) and stainless steel mirrors. Lids of culture dishes were opened and media were replaced with phosphate-buffered saline (PBS). Immediately after cells were irradiated with 5-20 mJ/cm^2 of UVB, PBS was replaced with RPMI-1640 plus 10% FBS medium, which had been preincubated in a CO_2 chamber.

UV irradiation to mice

Male nude (BALB/cAnNCrj–nu/nu) mice were purchased from Charles River Japan Inc. (Shin-Yokohama, Japan), and housed in a specific pathogen-free room under controlled temperature and humidity. All animal procedures were performed according to the procedures stipulated in the Seoul National University Laboratory Animal Maintenance Manual. During UVB irradiation, the left backs of mice were open and the right backs were screened with aluminum foil as the control skins. Mice were irradiated once a day for four days with 460 mJ/cm^2 of UVB and further kept in cages for two days. Left and right back skins were excised, fixed with 4% paraformaldehyde, and embedded into paraffin blocks.

Immunohistochemistry

HIF-1α and PCNA (proliferating cell nuclear antigen) were analyzed by immunohistochemistry staining in paraffin-embedded specimens of rat skins. Six-micrometer serial sections were cut from each paraffin block, and immunostained following deparaffinization and rehydration through a graded alcohol series. Antigens were retrieved by heating the sections in a microwave for 5 min in 10 mM sodium citrate (pH 6.0). After blocking nonspecific sites, sections were incubated overnight at 4°C with anti-HIF-1α (1:100) or anti-PCNA (1:100) antibodies. To visualize HIF-1α or PCNA, the sections were immunostained with secondary antibodies (1:400), as previously described (Cho et al., 2008). Avidin-biotin-horseradish peroxidase complex was used to localize bound antibodies, and diaminobenzidine was used as the final chromogen.

Immunoblotting

Total proteins were separated on 8% or 10% SDS/polyacrylamide gels, and transferred to Immobilon-P membranes (Millipore, USA), which were then blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 (TTBS) at room temperature for 1 h, and incubated overnight at 4°C with primary antibodies (1:1000) in 5% nonfat milk in TTBS. Membranes were then incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies (1:5000) in 5% milk-containing TTBS. Antigen-antibody complexes were visualized using an Enhanced Chemiluminescence Plus kit (Amersham Biosciences, USA).

Reporter gene construction and luciferase assay

To evaluate the cap-dependent translation of HIF-1α mRNA, we cloned the 5′-UTR (1-284) segment of HIF-1α mRNA using RT-PCR, and then inserted the cloned DNA between thymidine kinase promoter and luciferase in GL3 promoter plasmid (Kim et al., 2009). To evaluate the IRES-mediated translation of HIF-1α mRNA, we inserted the 5′-UTR segment between the GFP gene (5′ side) and the luciferase gene (3′ side) in the pcDNA plasmid. HaCaT cells were co-transfected with 3 μg each of the luciferase reporter plasmid and the β-galactosidase plasmid using Lipofectamine (Invitrogen). Transfected cells were split into several aliquots and incubated for 24 h. After stabilized, the cells were exposed to UVB and further incubated for 12 h. Luciferase activities were determined using a luminometer. Differences in transfection efficiencies and sample preparations were corrected by normalizing luciferase activities to β-gal activities.

Cell proliferation and cell cycle analysis

To analyze cell proliferation, BrdU incorporation assays were performed using a FITC BrdU Flow Kit purchased from BD PharMingen (USA). Total DNAs were stained with 7-amino-actinomycin D (7AAD). For cell cycle analysis, cells were fixed in 75% ethanol and labeled with propidium iodide (0.05 mg/ml) for 30 min. FITC, 7-AAD and propidium iodide were detected using a FACStar flow cytometer (BD Biosciences).

HIF-1α knock-down using siRNA

To inhibit HIF-1α expression, a synthesized siRNA duplex was obtained from Samchully Pharm. (Korea). The RNA sequence silencing HIF-1α (NM_001530) corresponded to nucleotides 360-384 of the coding region and Stealth RNAi negative control was used as the control RNA (si-Con). To put siRNA into HaCaT cells, about 40% confluent cells in 60-mm cell culture dishes were transfected with 40 nM siRNA using Lipofectamine (Invitrogen). Cells were allowed to stabilize for 48 h before being used in experiments. The gene-silencing efficacy of siRNA was evaluated by checking the HIF-1α protein level.

Statistical analysis

All data were analyzed using Microsoft Excel 2002 software, and results are expressed as means and standard deviations. The Mann-Whitney U test (http://faculty.vassar.edu/lowry/utest.html) was used to compare cell cycle phases and reporter activities. Differences were considered statistically significant at the $P < 0.05$ level. All statistical tests were two-sided.

RESULTS

HIF-1α is suppressed by UVB

As was observed previously (Cho et al., 2008), HIF-1α was stably expressed even under normoxic conditions in HaCaT cells cultured subconfluently. After HaCaT cells had been transiently exposed to UVB, HIF-1α levels were noticeable reduced in a UVB dose-dependent manner, whereas ARNT and β-tubulin levels were not (Fig. 1A). To determine whether UVB affects the transcription or mRNA stability of HIF-1α, we analyzed HIF-1α mRNA levels in HaCaT cells using a highly sensitive RT-PCR method. However, we found that HIF-1α mRNA

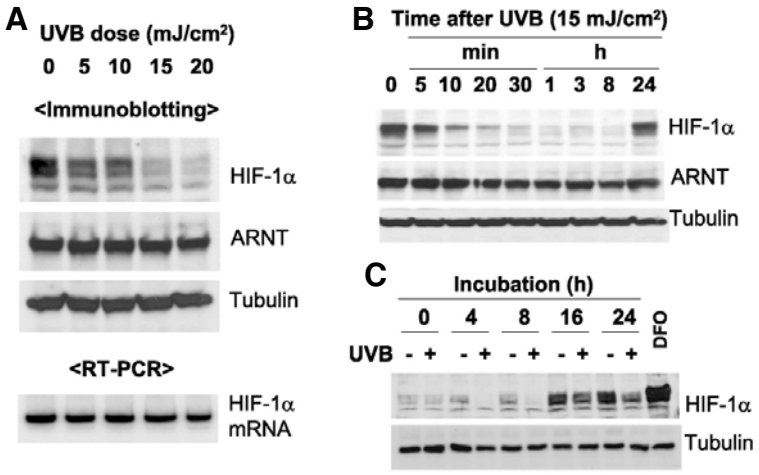

Fig. 1. UVB down-regulates HIF-1α. (A) UVB dose-dependent suppression of HIF-1α. After HaCaT cells had been cultured to reach 80-100% of cell density, the cells were transiently irradiated with various doses of UVB. Two hours later, cells were harvested and subjected to immunoblotting for HIF-1α, ARNT, and β-tubulin. Alternatively, total RNAs were extracted from the UVB-treated cells and HIF-1α mRNA levels were determined by a semiquantitative RT-PCR and autoradiography. (B) Time course of UVB-induced HIF-1α suppression. After exposed to 15 mJ/cm^2 of UVB, HaCaT cells at 80% confluence were harvested at the indicated time, and then subjected to immunoblotting. (C) HIF-1α expression is not recovered above the control level. HaCaT cells were cultured to reach 30% of cell density, and exposed to 15 mJ/cm^2 of UVB. Untreated or UVB-treated cells were further cultured for the indicated time, and then harvested for immunoblotting. As a positive control for HIF-1α expression, HaCaT cells were treated with 130 μM of desferrioxamine (DFO) for 8 h.

expression was not altered by UVB (Fig. 1A). To determine the time of onset and the duration of UVB-induced HIF-1α suppression, we checked HIF-1α levels at various times after UVB treatment. HIF-1α suppression was found to start from 5 min after treatment completion and to be maintained for at least 8 h. Also, we found that HIF-1α expression had substantially recovered at 24 h after UVB treatment (Fig. 1B). Since HIF-1α had been reported to be overexpressed by UVB irradiation (Rezvani et al., 2007; Wunderlich et al., 2008), it should be examined if the post-UV expression of HIF-1α is just a recovery or an induction over the control level. As we previously reported (Cho et al., 2008), cell density increased incubation time-dependently, and this increase was followed by HIF-1α expression. In addition, UVB attenuated the cell density-dependent expression of HIF-1α. However, HIF-1α levels in UVB-treated cells were not higher than those in untreated cells (Fig. 1C), which suggests that HIF-1α expression was recovered, rather induced, from the UVB-induced suppression.

UVB represses HIF-1α at the translational level

To understand how UVB suppresses HIF-1α, we first examined the possibility that UVB stimulates the degradation of HIF-1α in HaCaT cells. However, the degradation rate of HIF-1α was unchanged after UVB exposure (Fig. 2A). Next, we checked HIF-1α translation, and found that the de novo synthesis of HIF-1α was substantially impaired after UVB exposure (Fig. 2B). These results suggest that UVB suppresses HIF-1α by blocking its protein synthesis.

UVB inhibits the cap-dependent translation of HIF-1α

With respect to the regulation of HIF-1α translation, two distinct mechanisms have been reported, namely, cap-dependent translation and IRES-dependent translation (Hudson et al., 2002; Lang et al., 2002). Furthermore, the HIF-1α 5′-UTR segment has been identified as the main target site for both of these mechanisms during translational regulation. In addition, in many cell types, the cap-dependent pathway is positively regulated by the PI3K/AKT/mTOR pathway (Arsham et al., 2002). To determine which translational mechanism is targeted by UVB, we constructed two reporter plasmids, as shown in Fig. 3. The activity of HIF-1α 5′-UTR-Luc reporter, which reflects cap-dependent translation, was found to be significantly inhibited after UVB exposure. Moreover, its activity was repressed by LY294002 (a

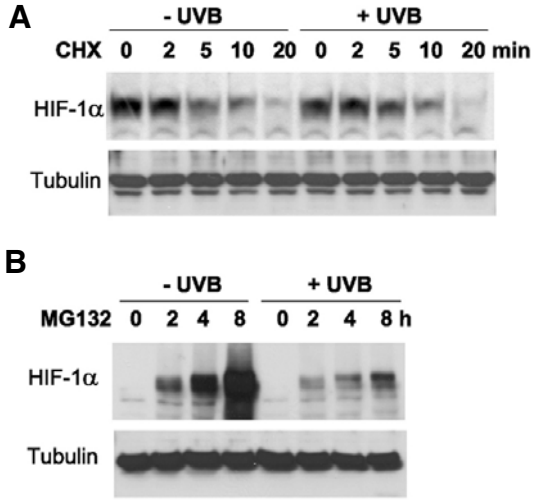

Fig. 2. UVB inhibits de novo synthesis of HIF-1α. (A) HIF-1α protein stability. HaCaT cells were cultured to reach 80% confluence. Immediately after irradiated with 15 mJ/cm^2 of UVB, cells were treated with 60 μg/ml of cycloheximide (CHX), and HIF-1α levels were analyzed at the indicated time by immunoblotting. (B) HIF-1α protein synthesis. HaCaT cells at 80% confluence were treated with 60 μg/ml of cycloheximide for 30 min to remove remaining HIF-1α. To start HIF-1α synthesis, cells were washed with PBS and incubated in fresh media containing 10 μM MG132 (used to prevent the degradation of newly synthesized HIF-1α, and HIF-1α levels were determined at the indicated time by immunoblotting.

PI3K inhibitor) and by rapamycin (a mTOR inhibitor) in control cells, whereas its activity was not further inhibited by these agents in UVB-treated cells (Fig. 3A). In contrast, the IRES activity of HIF-1α 5′-UTR was unaffected by UVB or by PI3K/mTOR inhibitors (Fig. 3B). These results indicate that UVB inhibits the cap-dependent translation of HIF-1α.

UVB induces keratinocyte proliferation by suppressing HIF-1α

Previously, we found that HIF-1α was expressed cell density-dependently in epidermal keratinocytes, and that it mediated

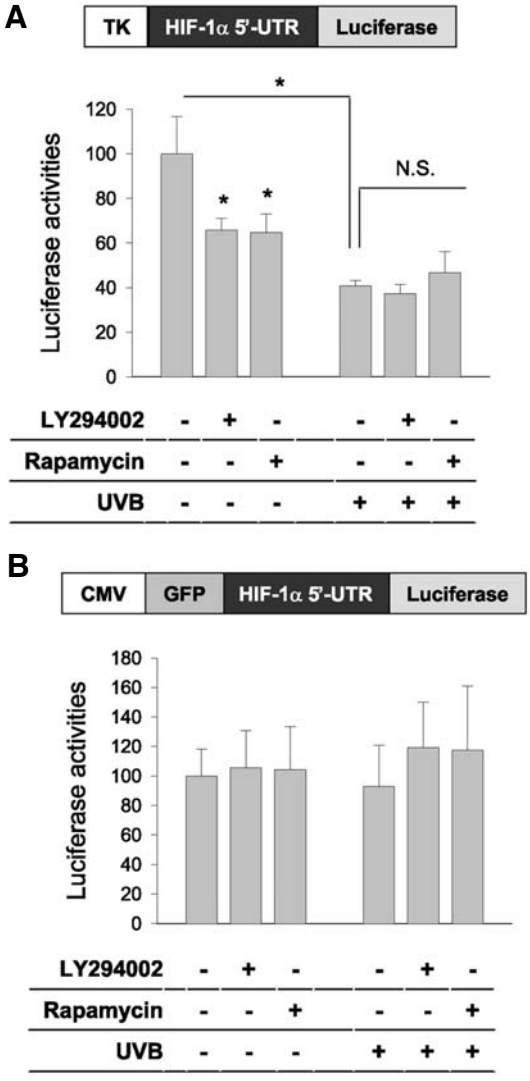

Fig. 3. UVB inhibits the cap-dependent translation of HIF-1α mRNA. (A) Cap-dependent translational activity of HIF-1α 5′-UTR. The luciferase reporter plasmid contains the HIF-1α 5′-UTR segment between the tk promoter and the luciferase gene. HaCaT cells were co-transfected with the reporter plasmid (5 μg per 100-mm dish) and the β-gal plasmid (5 μg per 100-mm dish). The cells at 80-100% confluence were treated with 10 μM LY294002 or 50 nM rapamycin for 1 h, and then irradiated with 15 mJ/cm^2 of UVB. After 8 hours, cells were lysed and subjected to luciferase assay. The reporter activities were analyzed by luminometry and normalized to the β-galactosidase activity. Each bar represents the mean and S.D. from 4 experiments. *denotes the significant difference ($p < 0.05$) and N.S. no significance. (B) IRES-dependent translational activity of HIF-1α 5′-UTR. The luciferase reporter plasmid contains the HIF-1α 5′-UTR segment between the GFP gene and the luciferase gene. HaCaT cells were co-transfected with the reporter plasmid (5 μg per 100-mm dish) and the β-gal plasmid (5 μg per 100-mm dish). The reporter activities were analyzed in cells at 80-100% confluence, as described previously.

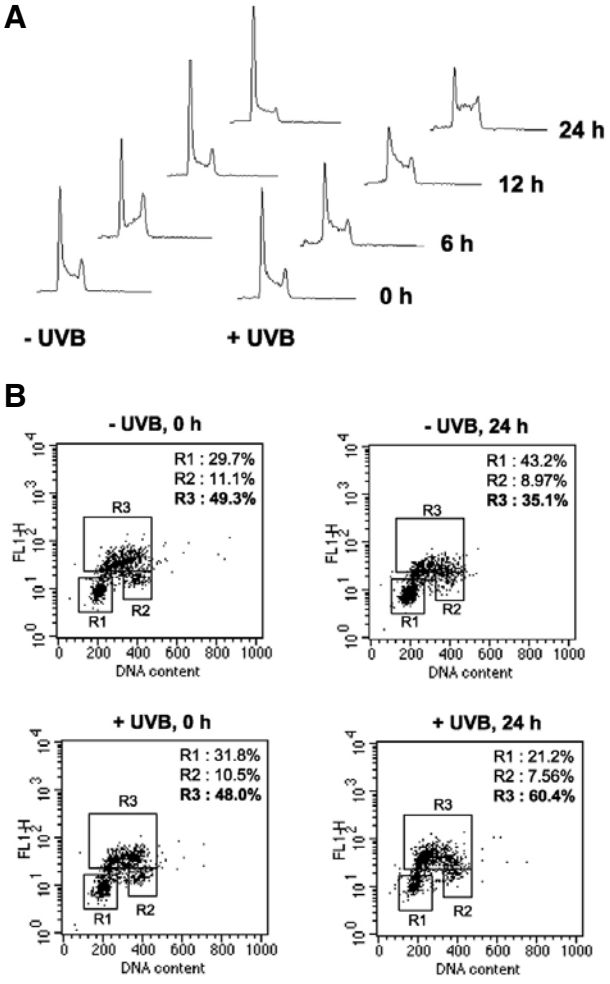

Fig. 4. UVB releases the cell density-dependent G1 arrest of HaCaT cells. (A) Cell cycle. After HaCaT cells at 80% confluence were irradiated with 15 mJ/cm^2 of UVB, total DNAs were labeled with propidium iodide at the indicated time and analyzed by flow cytometry. (B) Cell proliferation. Cell proliferation was analyzed by assaying the DNA incorporation of bromodeoxyuridine (BrdU). After irradiated with 15 mJ/cm^2 of UVB, HaCaT cells at 80-100% confluence were treated with 10 μM BrdU for 30 min. The incorporated BrdU was reacted with an FITC-conjugated anti-BrdU antibody and total DNAs were stained with 7-amino-actinomycin D. BrdU and DNA contents were analyzed by flow cytometry. Bivariate distributions of BrdU incorporation (*y* axis) versus DNA content (*x* axis) were plotted. The boxes (R3) indicate BrdU-incorporated cells in the S-phase. The R1-3 populations with the squares were the mean values from three experiments.

G0/G1 phase arrest at high cell densities (Cho et al., 2008). Therefore, we hypothesized that UVB releases keratinocytes from growth arrest by removing HIF-1α. As was expected, in subconfluent cultures of untreated HaCaT cells, the proportion of cells in the G0/G1 phase increased in a time-dependent manner, and this was accompanied by a reduction in the S proportion. However, in UVB exposed HaCaT cells, the G0/G1 proportion was noticeably lower and the S proportion higher (Fig. 4A). These results suggest that keratinocytes in the G0/G1 phase reenter the S phase after being exposed to UVB. Next, to examine whether this UVB-induced cell cycle restart is connected with proliferation, de novo synthesized DNA was stained with Brdu. When HaCaT cells were cultured at > 80% of confluence for 24 h, the Brdu (+)/S-phase population de-

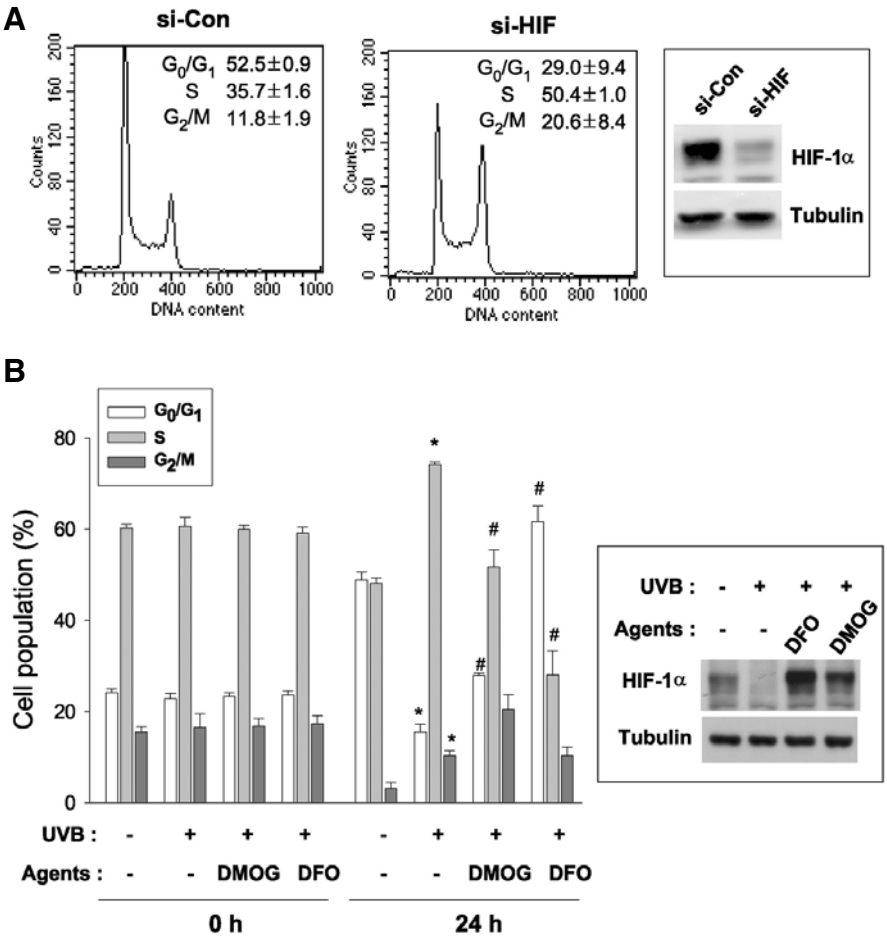

Fig. 5. HIF-1α negatively regulates cell cyle in keratinocytes. (A) Cell cycle change by HIF-1α knock-down. HaCaT cells were transfected with 40 nM of a HIF-1α-targeting siRNA (si-HIF) or a scrambled RNA control (si-Con). The gene-silencing effect of siRNA was verified by immunoblotting (right panel). After 48 h, the cells at 80% confluence were harvested, and cell cycle was analyzed by pro-0pidium iodide staining and flow cytometry (left panel). Cell populations (%) of G0/G1, S, and G2/M phases were presented as the mean and S.D. from 4 experiments, and significantly different between two groups ($p < 0.05$). (B) The UVB-in-duced proliferation is reversed by HIF-1α inducers. HaCaT cells at 80% confluence were pretreated with HIF-1α inducers, DMOG (1 mM) and DFO (130 μM) 4 h before UVB irradiation. After irradiated with 15 mJ/cm^2 of UVB, cell cycle was analyzed by DNA staining with propidium iodide and flow cytometry. Each bar represents the mean and S.D. from 4 experiments. * denotes $p < 0.05$ vs. the untreated control; #, $p < 0.05$ vs. the UVB-treated group.

creased by 14.2%. However, after UVB treatment, the Brdu (+)/S-phase population increased by 12.4% (Fig. 4B, R3 values). Therefore, exposure to UVB is likely to induce keratinocyte growth by releasing cells from G0/G1 arrest.

To determine whether UVB-induced HIF-1α suppression is responsible for keratinocyte reentry into the cell cycle, we first examined the role of HIF-1α in the cell cycle. In HIF-1α knocked-down keratinocytes (Fig. 5A, right panel), the G0/G1 population was decreased and the S and G2/M populations increased significantly (Fig. 5A, left panel), which suggests that HIF-1α is responsible for cell cycle arrest in keratinocytes. We next examined if the effect of UVB on the cell cycle is reversed by HIF-1α induction in UVB-treated cells. We confirmed that HIF-1α was expressed in UVB-treated HaCaT cells by DMOG (a 2-oxoglutarate analogue) and desferrioxamine (an iron chelator), which are known HIF-1α inducers (Fig. 5B, right panel). After HaCaT cells had been treated with these HIF-1α inducers, we found that the UVB effect on the cell cycle was abolished, that is, the Go/G1 population increased but the S population decreased (Fig. 5, left panel). These findings suggest that UVB induces keratinocyte proliferation by suppressing HIF-1α.

UVB suppresses epidermal HIF-1α and induces skin hyperplasia *in vivo*

To examine the *in vivo* effect of UVB on epidermal homeostasis, nude mice were transiently exposed to UVB and their back skins were prepared for immunohistochemistry. When skin slides were stained with H&E, the epidermal layers in UVB-exposed skins were found to be substantially thicker than contra-lateral non-exposed skins (Fig. 6A). Also, keratinocytes in UVB-exposed skins robustly expressed PCNA in their nuclei (Fig. 6B), which indicates that the skin had become hyperplasic. More importantly, the intensity of HIF-1α staining in the epidermis faded after UVB exposure (Fig. 6C). These *in vivo* findings concur with the observed effects of UVB on HIF-1α expression (Fig. 1) and cell proliferation (Fig. 4) in cultured HaCaT cells.

DISCUSSION

In the present study, we tested the hypothesis that HIF-1α is involved in development of post-UVB epidermal hyperplasia. Initially, HIF-1α was found to be suppressed in keratinocytes shortly after UVB exposure and to recover 16 h later. Mechanistically, UVB was found to impair the de novo synthesis of HIF-1α protein by inhibiting the cap-dependent translation of HIF-1α mRNA. In addition, UVB was found to initiate the transition from G0/G1 to the S phase in growth-arrested keratinocytes, which in turn increased DNA synthesis. Furthermore, when keratinocytes were treated with HIF-1α inducers to overcome HIF-1α suppression by UVB, the effect of UVB on the cell cycle was reversed. To examine the *in vivo* effect of UVB on epidermal homeostasis, nude mice were exposed to UVB and their skins were examined immunohistochemically. After UVB exposure, mouse skins were found to be hyperplasic and keratinocytes lost HIF-1α nuclear expression. These results suggest that the deregulation of HIF-1α is associated with UVB-induced skin hyperplasia.

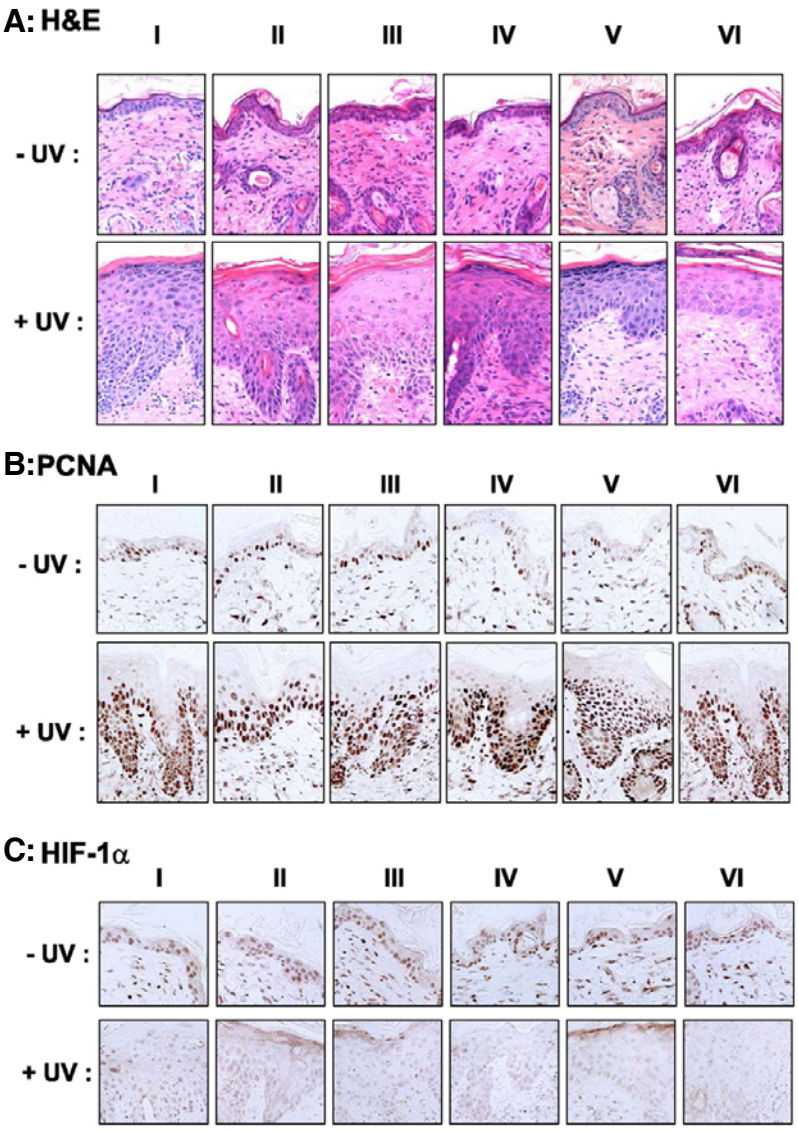

Fig. 6. *In vivo* effects of UVB on epidermal growth and HIF-1α expression. During UVB irradiation, the left backs of mice were open as the UVB-treated skins (+UV) and the right backs were screened with aluminum foil as the control skins (-UV). Six mice were irradiated with 460 mJ/cm^2 of UVB once a day for four days. After two days had passed, back skins were excised, fixed, and embedded into paraffin blocks. (A) Epidermal thickening by UVB. Skin specimens were stained with H&E dyes, and the thickness of the epidermis was analyzed at a magnification of 100X. The results were obtained from six mice (animal number, I to VI). (B) PCNA expression in mouse epidermal keratinocytes. Mouse skin sections were incubated with anti-PCNA antiserum, and nuclear PCNA expression was identified at a magnification of 100X. (C) HIF-1α expression in mouse epidermal keratinocytes. Mouse skin sections were incubated with anti-HIF-1α antiserum, and nuclear HIF-1α expression was identified at a magnification of 100X.

In addition to its role in hypoxic gene regulation, HIF-1α plays a pivotal role in determining cell fate. Given the information currently available, HIF-1α appears to inhibit tumor growth under hypoxic conditions by directly binding and inhibiting transcription factors related to cell proliferation. For example, HIF-1α antagonizes the function of c-Myc by sequestering MAX, and thus, repressing the expression of Myc-targeted genes, and c-Myc inhibition also increases the transcription of $p21^{WAF1/Cip1}$, which arrests the cell cycle by inhibiting cyclin-dependent kinases (Huang, 2008). In addition, HIF-1α dissociates β-catenin/TCF4 complex by directly interacting with β-catenin. Furthermore, because the c-Myc gene is transactivated by β-catenin/TCF4 complex, the HIF-1α interaction with β-catenin results in c-Myc down-regulation and subsequent p21 up-regulation (Kaidi et al., 2007). Recently, it was also demonstrated that HIF-1α blocks the ARD1 acetylation/activation of β-catenin by sequestering ARD1, and by so doing inhibits the proliferation of cancer cells (Lim et al., 2008). Likewise, HIF-1α has been reported to control the proliferation of normal cells. In a subconfluent culture of primary keratinocytes, the cell cycle was arrested at the G0/G1 phase, and this arrest was attributed to the inductions of HIF-1α and p21. Furthermore, this effect of HIF-1α was demonstrated in normal human and mouse skin (Cho et al., 2008). These findings suggest that the cell cycle control exercised by HIF-1α is likely to be shared by normal and cancer cells.

UV stimulates various signaling pathways, some of which are known to alter gene expression by regulating transcription factors. Of these signaling pathways, epidermal growth factor receptor (EGFR) has been intensively studied and found to play important roles in cellular responses to UV. Indeed, EGFR is acutely activated by UV, and subsequently positively regulates keratinocyte proliferation and survival (El-Abaseri et al., 2006). Also, UV-induced oxidative stress is regarded to activate EGFR (Peus et al., 1999). Therefore, oxidative stress and EGFR activation plausibly contribute to the UV-induced suppression of HIF-1α, and conversely, EGFR stimulation and oxidative stress have both been reported to up-regulate HIF-1α by stabilizing HIF-1α protein due to the inhibition of prolyl-hydroxylation or due to increased HIF-1α synthesis through the PI3K and MAPK signaling pathways (Gerald et al., 2004; Zhong et al., 2000). According to this scenario, HIF-1α expres-

sion could be induced in keratinocytes or mouse skin by UV. However, in the present study, keratinocyte HIF-1α was found to be down-regulated immediately after UV exposure. Therefore, HIF-1α suppression by UV appears to occur independently of EGFR activation or oxidative stress. The detailed mechanism responsible for the effect of UVB on HIF-1α regulation remains an open question.

In terms of the effect of UVB on HIF-1α expression, two recent studies demonstrated a biphasic HIF-1α response (Rezvani et al., 2007; Wunderlich et al., 2008). HIF-1α suppression in keratinocytes was found to start as early as 0.5-1 h after UVB exposure and to be maintained until 5-6 h. However, in these studies HIF-1α expression was found to increase above the control level after 10 h, which suggests that HIF-1α is induced by UVB. We also found that HIF-1α levels fluctuated after UVB exposure, i.e., HIF-1α disappeared as early as 10 min after treatment and reappeared after 16 h. However, HIF-1α levels at 16 and 24 h post-UV were lower than in the control level, indicating that HIF-1α expression during the late phase is due to recovery from UVB exposure, rather than being due to HIF-1α induction by UVB. We also found that HIF-1α expression in mouse skin was consistently suppressed, not induced, at 24 and 48 h post-UV. Therefore, we focused on UVB-induced suppression of HIF-1α, and represented little to the HIF-1α recovery.

In conclusion, UVB was found to suppress HIF-1α acutely in cultured keratinocytes and mouse skins, which we believe may be associated with restart of the keratinocyte cell cycle and epidermal thickening in mice. This work provides insight of the molecular mechanism underlying UV-induced hyperproliferative skin diseases, such as, premature photoageing and skin cancer. We also propose that HIF-1α be viewed as therapeutic target for preventing skin hyperplasia, and in particular, that its induction offers a possible means of preventing UVB-induced skin diseases.

ACKNOWLEDGMENT

This work was supported by a grant (#A080139) from Korea Health Industry Development Institute.

REFERENCES

Arsham, A.M., Plas, D.R., Thompson, C.B., and Simon, M.C. (2002). Phosphatidylinositol 3-kinase/Akt signaling is neither required for hypoxic stabilization of HIF-1 alpha nor sufficient for HIF-1-dependent target gene transcription. J. Biol. Chem. *277*, 15162-15170.

Cho, Y.S., Bae, J.M., Chun, Y.S., Chung, J.H., Jeon, Y.K., Kim, I.S., Kim, M.S., and Park, J.W. (2008). HIF-1alpha controls keratinocyte proliferation by up-regulating p21(WAF1/Cip1). Biochim. Biophys. Acta *1783*, 323-333.

Chun, Y.S., Choi, E., Kim, G.T., Lee, M.J., Lee, M.J., Lee, S.E., Kim, M.S., and Park, J.W. (2000). Zinc induces the accumulation of hypoxia-inducible factor (HIF)-1alpha, but inhibits the nuclear translocation of HIF-1beta, causing HIF-1 inactivation. Biochem. Biophys. Res. Commun. *268*, 652-656.

El-Abaseri, T.B., Putta, S., and Hansen, L.A. (2006). Ultraviolet irradiation induces keratinocyte proliferation and epidermal hyperplasia through the activation of the epidermal growth factor receptor. Carcinogenesis *27*, 225-231.

Gerald, D., Berra, E., Frapart, Y.M., Chan, D.A., Giaccia, A.J., Mansuy, D., Pouysségur, J., Yaniv, M., and Mechta-Grigoriou, F. (2004) JunD reduces tumor angiogenesis by protecting cells from oxidative stress. Cell *118*, 781-794.

Huang, L.E. (2008). Carrot and stick: HIF-alpha engages c-Myc in hypoxic adaptation. Cell Death Differ. *15*, 672-677.

Hudson, C.C., Liu, M., Chiang, G.G., Otterness, D.M., Loomis, D.C., Kaper, F., Giaccia, A.J., and Abraham, R.T. (2002). Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol. Cell. Biol. *22*, 7004-7014.

Iyer, N.V., Kotch, L.E., Agani, F., Leung, S.W., Laughner, E., Wenger, R.H., Gassmann, M., Gearhart, J.D., Lawler, A.M., Yu, A.Y., et al. (1998). Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. *12*, 149-162.

Kaidi, A., Williams, A.C., and Paraskeva, C. (2007). Interaction between beta-catenin and HIF-1 promotes cellular adaptation to hypoxia. Nat. Cell Biol. *9*, 210-217.

Kim, H.L., Cho, Y.S., Choi, H., Chun, Y.S., Lee, Z.H., and Park, J.W. (2009). Hypoxia-inducible factor 1alpha is deregulated by the serum of rats with adjuvant-induced arthritis. Biochem. Biophys. Res. Commun. *378*, 123-128.

Koshiji, M., Kageyama, Y., Pete, E.A., Horikawa, I., Barrett, J.C., and Huang, L.E. (2004). HIF-1alpha induces cell cycle arrest by functionally counteracting Myc. EMBO J. *23*, 1949-1956.

Lang, K.J., Kappel, A., and Goodall, G.J. (2002). Hypoxia-inducible factor-1alpha mRNA contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia. Mol. Biol. Cell *13*, 1792-1801.

Lee, J.K., Kim, J.H., Nam, K.T., and Lee, S.H. (2003). Molecular events associated with apoptosis and proliferation induced by ultraviolet-B radiation in the skin of hairless mice. J. Dermatol. Sci. *32*, 171-179.

Lim, J.H., Chun, Y.S., and Park, J.W. (2008). Hypoxia-inducible factor-1alpha obstructs a Wnt signaling pathway by inhibiting the hARD1-mediated activation of beta-catenin. Cancer Res. *68*, 5177-5184.

Marrot, L., and Meunier, J.R. (2008). Skin DNA photodamage and its biological consequences. J. Am. Acad. Dermatol. *58*, S139-S148.

Matsumura, Y., and Ananthaswamy, H.N. (2002). Molecular mechanisms of photocarcinogenesis. Front. Biosci. *7*, d765-d783.

Melnikova, V.O., and Ananthaswamy, H.N. (2005). Cellular and molecular events leading to the development of skin cancer. Mutat. Res. *571*, 91-106.

Ouhtit, A., Muller, H.K., Davis, D.W., Ullrich, S.E., McConkey, D., and Ananthaswamy, H.N. (2000). Temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. Am. J. Pathol. *156*, 201-207.

Park, H. (1999). Aromatic hydrocarbon nuclear translocator as a common component for the hypoxia- and dioxin-induced gene expression. Mol. Cells *9*, 172-178.

Peus, D., Meves, A., Vasa, R.A., Beyerle, A., O'Brien, T., and Pittelkow, M.R. (1999). H_2O_2 is required for UVB-induced EGF receptor and downstream signaling pathway activation. Free Radic. Biol. Med. *27*, 1197-1202.

Rezvani, H.R., Dedieu, S., North, S., Belloc, F., Rossignol, R., Letellier, T., de Verneuil, H., Taïeb, A., and Mazurier, F. (2007). Hypoxia-inducible factor-1alpha, a key factor in the keratinocyte response to UVB exposure. J. Biol. Chem. *282*, 16413-16422.

Schofield, C.J., and Ratcliffe, P.J. (2004). Oxygen sensing by HIF hydroxylases. Nat. Rev. Mol. Cell Biol. *5*, 343-354.

Wang, G.L., and Semenza, G.L. (1995). Purification and characterization of hypoxia-inducible factor 1. J. Biol. Chem. *270*, 1230-1237.

Wunderlich, L., Paragh, G., Wikonkál, N.M., Bánhegyi, G., Kárpáti, S., and Mandl, J. (2008). UVB induces a biphasic response of HIF-1alpha in cultured human keratinocytes. Exp. Dermatol. *17*, 335-342.

Zhong, H., Chiles, K., Feldser, D., Laughner, E., Hanrahan, C., Georgescu, M.M., Simons, J.W., and Semenza, G.L. (2000). Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/ FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res. *60*, 1541-1545.