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UVB radiation induces expression of HIF-1α and VEGF through the EGFR/PI3K/DEC1 pathway

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Abstract. EGFR is involved in the UV signal transduction pathway leading to skin cancer. UV radiation, mediated by EGFR, induces activation of PI3 kinase and AKT with a result of activation of a number of transcription factors. Transcription factor HIF-1α correlates with tumorigenicity and angiogenesis. Transcription factors DEC1 and DEC2 also play pivotal roles in multiple signaling pathways impacting various biological processes including development, cell differentiation, cell death, and oncogenesis. We investigated whether UV radiation and associated hypoxia induce expression of HIF-1α and its target genes such as VEGF and the signaling pathway mediating such responses. We found that UV radiation induced HIF-1α and VEGF protein expression in a dose- and time-dependent manner in cultured human keratinocytes. UV radiation also induced VEGF mRNA expression in a dose-dependent manner with maximum effect at 4 h post treatment, but did not affect HIF-1α mRNA expression. We also observed that UV radiation induced activation of EGFR in a time- and dose-dependent manner which was inhibited by EGFR inhibitor PD153035. In *egfr* (-/-) MEF cells, UV radiation did not induce HIF-1α and VEGF expression, in contrast, in *egfr* (+/+) MEF cells, UV radiation strongly induced HIF-1α and VEGF expression. EGFR kinase inhibitor, PD153035, inhibited UV-induced HIF-1α and VEGF protein expression in a dose-dependent manner. Further, we found that PI3K inhibitors, LY294002 and Wortmannin, inhibited HIF-1α and VEGF expression induced by UV radiation. In *DEC1* (-/-) HaCat cells, UV radiation did not induce HIF-1α and VEGF expression, in contrast, in *DEC1* (+/+) HaCat cells, UV radiation strongly enhanced HIF-1α and VEGF protein expression. We conclude that UV radiation induces HIF-1α and VEGF expression via the EGFR/PI3K/DEC1 signaling pathway.

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Introduction

UV radiation causes cellular damage in the form of premature skin photoaging and skin cancer (1,2). Depletion of ozone layer due to industrialization has unexpectedly increased the level of UV radiation that reaches the earth's surface, thus raising the incidence of human skin cancer. Mounting evidence indicates that activation of transcription factors by UV radiation results in altered gene expression, which appears to be as important as direct DNA damage in UV-induced carcinogenesis (3,4). UV radiation mimics the actions of receptor ligands in activating cell surface growth factor and cytokine receptors (5,6). Activation of EGFR is involved in the UV signal transduction pathway leading to skin cancer (7).

Studies in human skin *in vivo* and in cultured human keratinocytes *in vitro* indicate that UV radiation, mediated by EGFR, induces the activation of PI3 kinase and AKT (8). PI3 kinase is composed of two subunits, including p85 and p110. Receptor activation upon ligand binding leads to the phosphorylation of p85 subunit of PI3 kinase (9). Activation of PI3 kinase leads to PDK1/2 activation that is responsible for AKT activation (10). Activation of PI3 kinase or MAP kinases through EGFR is also involved in a complex network of downstream kinases including p70S6K and p90RSK. The activation of those kinases is associated with AP1 in tumor promoter-induced cell transformation (11). PI3 kinase is thus considered as an important cell-signaling component for carcinogenesis and invasiveness of cancer cells (12).

DEC1 and DEC2 are basic helix-loop-helix transcription factors. Both DEC1 and DEC2 genes are widely expressed in both embryonic and adult tissues. Expression of these genes is regulated in a cell type-specific manner by various extracellular stimuli, such as growth factors, serum starvation, hypoxia, hormones, nutrient, cytokines, UV radiation, and infection. Therefore, these transcription factors play pivotal roles in multiple signaling pathways that impact many biological processes including development, cell differentiation, cell growth, cell death, oncogenesis, immune systems, and circadian rhythmic homeostasis (13). DEC1 and DEC2,

recently found to interact with a transcription factor hypoxia inducible factor, HIF-1α, have been shown to be involved in cell proliferation and survival (14,15).

Hypoxia-inducible factor 1, or HIF-1 is a heterodimer of HIF-1α and HIF-1ß subunits, which contain basic helix-loop-helix PAS domains (16). HIF-1α is a unique subunit tightly regulated in response to hypoxia (16), whereas HIF-1ß is identical to aryl hydrocarbon nuclear translocator that forms heterodimers with aryl hydrocarbon receptor in cells (17). HIF-1 regulates the expression of various genes including vascular endothelial growth factor (VEGF), transferring receptor (TFR), erythropoietin, hemeoxygenase 1, aldolase, enolase, and lactate dehydrogenase A (18-21). The level or activity of HIF-1α in cells correlates with tumorigenicity and angiogenesis in nude mice (22). HIF-1 is overexpressed in many human cancers (23). Previous studies indicate that both the phosphatidylinositol 3-kinase (PI3K)/AKT and MAP kinase pathway are involved in HIF-1α expression induced by growth factors (24,25). In human prostate cancer cells, HIF-1α expression is modulated by the EGFR/PI3K/AKT pathway (26). HIF-1α interacts with tumor suppressor Von Hippel-Lindau protein. The mutation of Von Hippel-Lindau protein in human cancers results in constitutive expression of HIF-1α under nonhypoxic conditions (27). HIF-1α is degraded by the proteasome pathway (28). The regulation of HIF-1 by Von Hippel-Lindau protein and cellular oxygen has been shown to be mediated through the prolyl hydroxylation of HIF-1α at Pro^{564} by three mammalian PHD proteins (29,30). One of the major target genes regulated by HIF-1α is VEGF, which plays a key role in tumor progression and angiogenesis (31). There is a strong correlation between VEGF expression and blood vessel density in many tumor types (32). Inhibition of VEGF expression and its receptor function dramatically decreases tumor growth, invasion, and metastasis in animal models (18,33).

Given that both UV radiation itself and hypoxia associated with UV radiation (34) contribute to activation of genes in macrophages, neutrophils, and other cells, including those coding for VEGF that make blood vessels leaky (35), we undertook this study to investigate whether UV radiation induces HIF-1α and VEGF expression and to further examine the cell signaling pathway leading to UV-induced expression of HIF-1α and VEGF in cultured human keratinocytes.

Materials and methods

Cell cultures and UV irradiation. *egfr* (-/-), *egfr* (+/+) MEF cells were from the laboratory of Dr Zigang Dong at the Hormel Institute of the University of Minnesota, and human keratinocytes (HaCat cells) and *DEC1* (+/+) and (-/-) cells were from the University of Rhode Island. As previously reported (2), the UV irradiation apparatus used in this study consisted of four F36T12 ERE-VHO UV tubes. A Kodacel TA401/407 filter was mounted 4 cm in front of the tubes to remove wavelengths below 290 nm (UVC). Irradiation intensity was monitored using an IL443 phototherapy radiometer and an SED240/UV/W photodetector.

The complete culture medium was Dulbecco's modified Eagle's medium (D-MEM)(Sigma, USA) containing 10% fetal bovine serum (Hyclone, USA), penicillin 100 U/ml and streptomycin 100 μg/ml. Cells were plated at $3x10^5$ cells/well into 6-well plates and at $1x10^7$ in 100-mm dishes. Cells were placed in an incubator at 37˚C with 5% carbon dioxide. Cells at subconfluence of 80% were irradiated with different doses of UV (10-30 mJ/cm^2), no loss in cell viability was observed with the UV doses used within 24 h post UV irradiation. The cells were pretreated with PD153035, LY294002 and Wortmannin (Cell Signaling Technology, USA) for 1 h at the indicated concentrations. Prior to UV irradiation, the culture medium was replaced by phosphate-buffered saline (PBS). After UV irradiation, cells were returned to incubation in complete culture medium for various times prior to harvest.

Western blot analysis. Western blot analysis was carried out as described (36). Briefly, whole cell extract proteins (60 μg) were subject to SDS-PAGE. Proteins were transferred to PVDF membrane, blocked in 5% nonfat dry milk, 1% bovine serum albumin, and 0.1% Tween-20 in TBST. Membranes were probed with monoclonal antibody against human HIF-1α (1:250; BD Biosciences, USA) or monoclonal antibody against mouse HIF-1α (1 μg/ml; R&D Systems, USA). Subsequently, membranes were washed extensively and incubated with goat anti-mouse or rabbit IgG conjugated with peroxidase (1:5000; R&D Systems, USA) for 1 h at room temperature, followed by enhanced chemiluminescence detection or ECL.

Enzyme-linked immunosorbent assay (ELISA). ELISA was performed to detect VEGF protein level at different time points after treatments. Cells were plated in triplicate in six-well culture plates (Falcon, Becton Dickinson, Oxford, UK) at a density of $3x10^5$ and allowed to multiply to 80% confluence. Serum-free medium was added to the plates and incubated for 6, 12 and 24 h after UV irradiation. Cells were harvested at each time point for protein analysis and cell count. Media were collected, centrifuged to remove cellular debris, and stored at -70˚C until ELISA test for VEGF expression using the human or mouse VEGF sandwich ELISA kit (R&D Systems, USA). VEGF protein levels were expressed as pictograms of VEGF protein per 10^5 cells (or pg/ml).

Flow cytometry. HaCat cells of 80% confluence were irradiated with UV and subsequently cultured for the desired time. For EGFR detection, cells were collected by trypsinization and centrifugation. Cells were re-suspended in 1 ml PBS, and fixed in 1% formaldehyde for 10 min at 37˚C. Cells were kept on ice for 1 min and cell number was counted using a hemacytometer. Then, cells ($1x10^6$) were transferred into each assay tube, 2 ml of incubation buffer was added and cells were washed by centrifugation. Cells were maintained in incubation buffer for 10 min at room temperature. Anti-EGFR (0.5 μl) (1:200; Cell Signaling Technology, USA) was added to the assay tubes which were incubated for 30 min at room temperature. After washing with incubation buffer, cells were resuspended in FITC-conjugated secondary antibody (Caltag Laboratories, USA). For phosphor-EGFR detection, cells were collected and fixed with 0.3% formaldehyde in PBS for 10 min at 37˚C. After washing with PBS, cells were permeabilized by adding ice-cold 90% methanol slowly while vortexing. Cells were incubated for 30 min on ice. After washing, cells were re-suspended in PBS wash with cell

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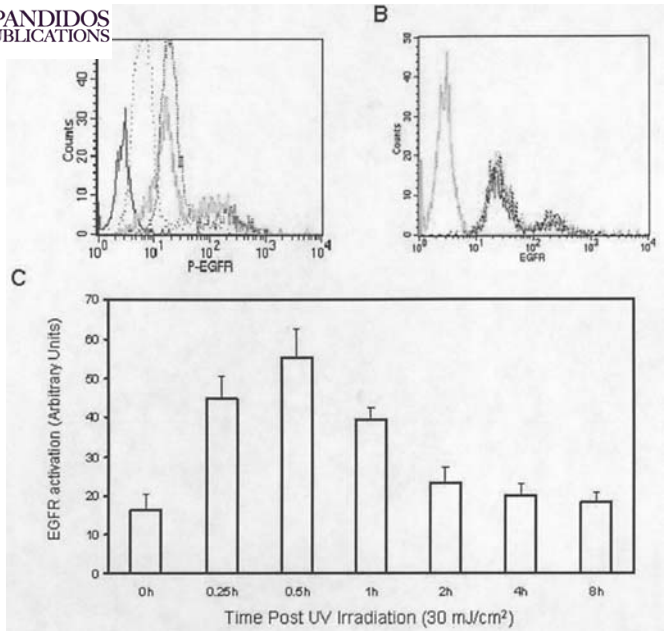


Figure 1. UV radiation induces EGFR phosphorylation in cultured human keratinocytes. HaCat cells (80% confluence) were starved by replacing the medium with 0.1% of FBS and cultured for 24 h. The cells were irradiated with UV (30 mJ/cm^2) and subsequently cultured for the indicated periods of time. Cells were collected for measurement of phosphorylated EGFR (A) and total EGFR (B) by FACS analysis. A time course of UV-induced EGFR phosphorylation expressed with arbitrary units is presented in C.

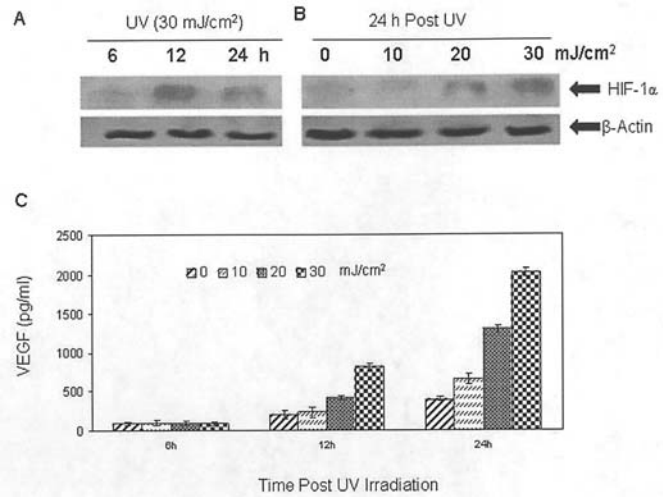


Figure 2. UV radiation induces expression of HIF-1α and VEGF at protein level in cultured human keratinocytes. HaCat cells (80% confluence) were starved by replacing the medium with 0.1% of FBS and cultured for 24 h. Cells were then irradiated with UV (30 mJ/cm^2), and cell lysates were collected (A). Alternatively, cells were irradiated with various doses of UV (0, 10, 20, 30 mJ/cm^2) and were colleted 24 h post UV radiation (B). Immunoprecipitation of cellular proteins in cell lysates with anti-HIF-1α was followed by Western blot analysis. Cell culture supernatants post UV treatments were collected for measurement of VEGF secretion by ELISA (C).

counts of 5x10^6/ml. Cells (100 μl) were added to each assay tube and incubated in anti-phospho-EGFR (1:1000; Cell Signaling Technology, USA) for 30 min at room temperature. After washing with PBS twice, cells were incubated in R-phycoerythrin-conjugated secondary antibody for 30 min at room temperature. After washing with PBS, cells were re-suspended in washing buffer and analyzed by a flow cytometer.

RNA isolation and real-time quantitative PCR. Total RNA was isolated from treated cells using a phenol/guanidine isothiocyanate/chloroform extraction technique (Trizol, Gibco BRL, Gaithersburg, MD). Complementary DNA was synthesized from isolated RNA using Taqman Gold reverse transcription reagents (Applied Biosystems, Foster City, CA). Reverse transcription was performed by using oligo dT primers at 25˚C for 10 min, 48˚C for 30 min, and 95˚C for 5 min. Quantitative PCR was performed in a GeneAmp 7300 sequence detection machine (Applied Biosystems, USA). A Taqman PCR core reagents kit and primers/probes for ß-actin (Applied Biosystems) were used. VEGF primer/probe sequences were: forward 5'-CTGCTGTCTTGGGTGCATTG-3', reverse 5'-ATGATTCTGCCCTCCTCCTTCT-3', and probe 5'-FAM-CTCCACCATGCCAAGTGGTCCCA-3'. HIF-1α primer/probe sequences were: forward 5'-TGGAAACGTGTAAAAGGATGCA-3', reverse 5'-TCCACTTTCATCCATTGATTGC-3', and probe 5'-FAM-CCCTCTGATTTAGCATGTAGA-3'. ß-actin primer/probe sequences were: forward 5'-CCGTCTTCCCCTCCATCG-3', reverse 5'-GTCCCAGTTGGTGACGATGC-3', probe 5'-FAM-CCAGGGCGTGATGGTGGGCAT-3'. The PCR reaction mixture contained AmpErase Uracil N-glycosylase to destroy any previously amplified product. The efficiency of ß-actin, VEGF, and HIF-1α amplification was shown to be approximately equal using a validation experiment as described by the sequence detection system manufacturer (Applied Biosystems, USA).

Statistical analysis. Experiments were repeated three times. The results were expressed as means ± SD. Statistical analysis of all data between the control and treated groups were performed by analysis of variance (ANOVA). P-values <0.05 were considered significant.

Results

UV radiation activates EGFR in cultured human keratinocytes. UV radiation activates cell surface receptors and a variety of cellular components and epidermal growth factor receptor (EGFR) plays a major role in UV-induced cell signal transduction pathways (5,8,37). To directly measure EGFR activation in response to UV radiation, human keratinocytes (cell line, HaCat cells) at 80% confluence were irradiated with UV (30 mJ/cm^2) and subsequently cultured for 0.25, 0.5, 1, 2, 4, and 8 h. FACS analysis was performed to detect tyrosine-phosphorylated EGFR and total EGFR respectively. The results showed that EGFR phosphorylation was detectable within 15 min after UV radiation, peaked at 30 min, and returned to basal level within 2 h after UV exposure (Fig. 1A). As expected, total EGFR remained unaltered in response to UV radiation (Fig. 1B). The maximum activity of EGFR in UV-radiated cells was ~4-fold within 30 min of treatment compared to untreated cells (Fig. 1C).

UV radiation induces expression of HIF-1α and VEGF at the protein level in cultured human keratinocytes. Cell surface

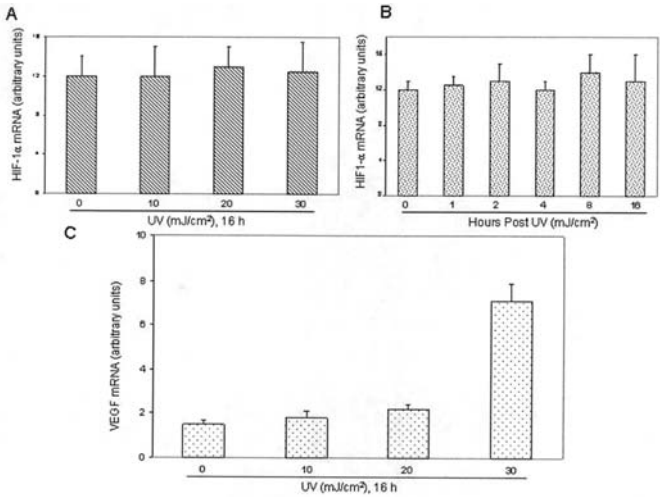


Figure 3. UV radiation induces expression of VEGF but not HIF-1α at mRNA level in cultured human keratinocytes. Total RNAs were prepared at different time points (1, 2, 4, 8, and 16 h) after UV irradiation and subject to real-time PCR. A, a dose response of HIF-1α mRNA expression. B, a time course of HIF-1α mRNA expression. C, a dose response of VEGF mRNA expression.

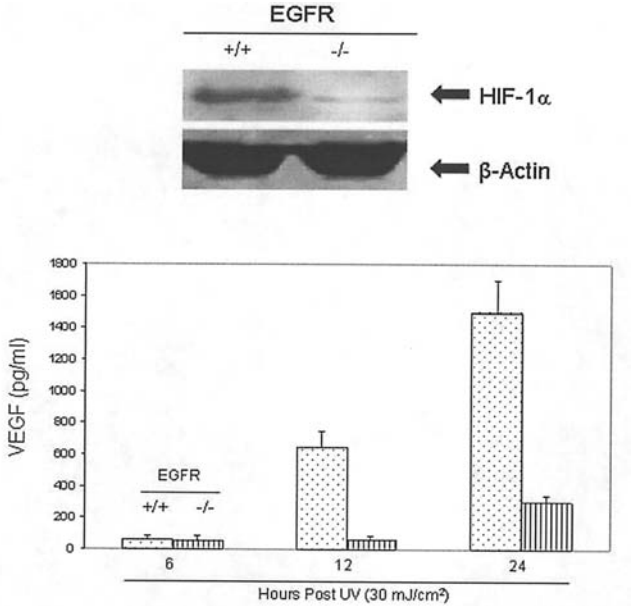


Figure 4. EGFR is required for UV-induced HIF-1α and VEGF expression. *egfr* (+/+) and *egfr* (-/-) MEF cells (80% confluence) were starved by replacing the medium with 0.1% of FBS and cultured for 24 h. A, cells were irradiated with UV (30 mJ/cm^2) and subsequently cultured for 12 h. Immunoprecipitation of cellular proteins in cell lysates with anti-HIF-1α was followed by Western blot analysis. B, cells were irradiated by UV (30 mJ/cm^2) or shammed at the time points (6, 12, 24 h) and VEGF in cell culture medium was determined by ELISA.

receptor activation leads to expression of HIF-1α and VEGF in response to growth factor treatment or hypoxia (26). The above data indicate that UV activates EGFR in cultured human keratinocytes. To determine whether UV radiation induces HIF-1α expression, HaCat cells were treated with UV (30 mJ/cm^2) and incubated and cell lysates were collected at various time points or treated with various doses of UV (10, 20 and 30 mJ/cm^2) and incubated for 12 h. The total proteins in cellular extracts were subject to SDS-PAGE and Western blot analysis. As shown in Fig. 2A, UV radiation induced HIF-1α protein expression in a time-dependent manner with the maximum expression at 12 h post UV radiation. UV induced HIF-1α expression in a dose-dependent manner with the maximum expression at 30 mJ/cm^2 of UV radiation (Fig. 2B). The prolonged exposure or higher dose of UV irradiation resulted in the decrease of HIF-1α protein levels, possibly because of apoptosis of the cells induced by UV. Existing data indicate that overexpression of HIF-1α increases transcription of a number of genes including VEGF in response to a number of stimulations. To further investigate whether induction of HIF-1α by UV radiation results in an increase of VEGF expression, cells were treated with different doses of UV radiation and cell culture supernatants were collected and VEGF level was measured using ELISA. The results showed that UV radiation induced VEGF expression in a time- and dose-dependent manner (Fig. 2C).

UV radiation induces VEGF transcription but does not alter HIF-1α mRNA expression in cultured human keratinocytes. To further study whether UV-induced HIF-1α and VEGF expression is at the transcriptional or translational level, we measured mRNA levels of HIF-1α and VEGF post UV treatment. HaCat cells were treated with various doses of UV (10, 20 and 30 mJ/cm^2) for 4 h or cells were treated with 30 mJ/cm^2 of UV and collected at various time points as indicated (1, 2, 4, 8, and 16 h), and cellular total RNA extracts were prepared for analysis of HIF-1α and VEGF mRNA levels by RT-PCR. The results showed that UV radiation did not alter HIF-1α mRNA expression ($P>0.05$) (Fig. 3A and B). However, UV radiation induced VEGF mRNA expression in a dose-dependent manner ($P<0.05$) (Fig. 3C).

EGFR is required for UV radiation-induced HIF-1α and VEGF expression. The above data indicate that UV radiation induces EGFR activation and HIF-1α and VEGF expression. To further investigate whether EGFR is required for UV-induced HIF-1α and VEGF expression, EGFR knockout cells were applied in the experiments. EGFR$^{+/+}$ and EGFR$^{-/-}$ MEF cells were treated with UV (30 mJ/cm^2) for 12 h and HIF-1α expression in cell lysates was measured by Western blot analysis, and VEGF expression in cell cultures was measured using ELISA. The results showed that UV radiation induced HIF-1α expression in EGFR$^{+/+}$ cells but not in EGFR$^{-/-}$ cells (Fig. 4A). UV radiation induced VEGF expression in EGFR$^{+/+}$ cells in a time-dependent manner but not in EGFR$^{-/-}$ cells ($p>0.05$) (Fig. 4B).

PI3K inhibitors inhibit UV-induced expression of HIF-1α and VEGF in cultured human keratinocytes. The above data indicate that UV induces EGFR activation and HIF-1α and VEGF expression. To further investigate cellular signaling pathways leading to UV-induced HIF-1α and VEGF expression, pharmacological inhibitors were applied in the experiments. HaCat cells were cultured in DMEM supplemented with 0.1% FBS for 24 h, pretreated for 1 h with PI3K inhibitors, LY294002 or Wortmannin. Cells were then treated with UV radiation (30 mJ/cm^2) for 12 h. Cellular

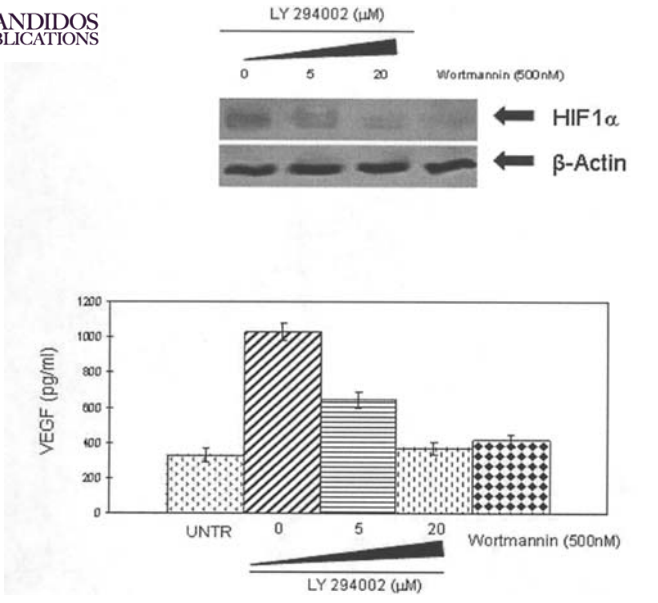


Figure 5. PI3 kinase inhibitors inhibit UV-induced expression of HIF-1α and VEGF in cultured human keratinocytes. HaCat cells (80% confluence) were starved by replacing the medium with 0.1% of FBS and cultured for 24 h. Cells were then pretreated with or without PI3 kinase inhibitors, LY294002, or Wortmannin for 1 h at indicated concentrations. A, cells were cultured for 12 h and immunoprecipitation of cellular proteins in cell lysates with anti-HIF-1α was followed by Western blot analysis. B, cells were irradiated with UV (30 mJ/cm^2) and subsequently cultured for 24 h and cell cultured media were collected for VEGF ELISA.

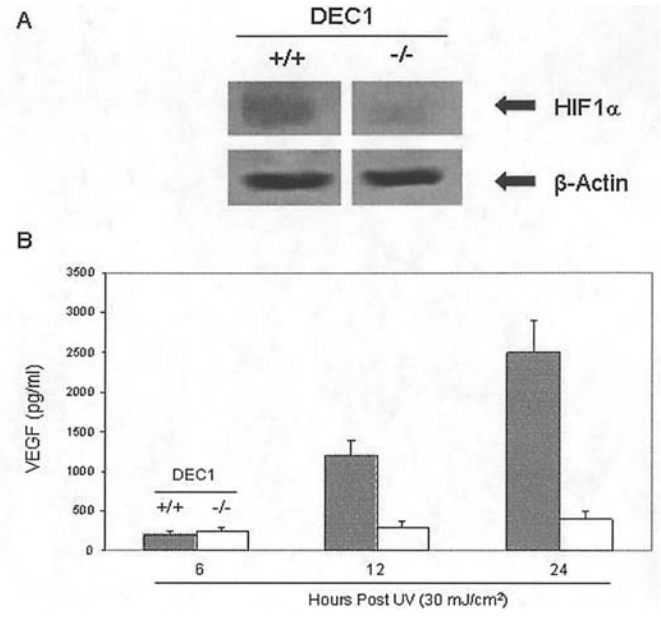


Figure 6. DEC1 is required for UV-induced expression of HIF-1α and VEGF. *DEC1* (+/+) and *DEC1* (-/-) cells (80% confluence) were starved by replacing the medium with 0.1% of FBS and cultured for 24 h. A, cells were irradiated with UV (30 mJ/cm^2) and subsequently cultured for 12 h. Immunoprecipitation of cellular proteins in cell lysates with anti-HIF-1α was followed by Western blot analysis. B, cells were irradiated by UV (30 mJ/cm^2) and cultured and media were collected at the time points (6, 12, 24 h) for VEGF ELISA.

proteins were subject to SDS-PAGE and Western blot analysis. As shown in Fig. 5A, both LY294002 and Wortmannin significantly inhibited UV-induced expression of HIF-1α protein. LY294002 and Wortmannin also inhibited UV-induced VEGF expression in a dose-dependent manner (Fig. 5B).

DEC1 is required for UV-induced HIF-1α and VEGF expression. DEC1 plays pivotal roles in multiple signaling pathways that impact many biological processes including development, cell differentiation, cell growth, cell death, and oncogenesis (15,38,39). To investigate whether DEC1 is involved in UV-induced HIF-1α and VEGF expression, we utilized DEC1 knockout cells. DEC1$^{+/+}$ and DEC1$^{-/-}$ cells (derived from HaCat cells) at 80% confluence were starved in a medium with 0.1% of FBS and cultured for 24 h. The cells were irradiated with UV (30 mJ/cm^2) and subsequently cultured for 12h. Immunoprecipitation of cellular proteins in cell lysates with anti-HIF-1α was followed by Western blot analysis. The results showed that UV induced HIF-1α expression in DEC1$^{+/+}$ but not in DEC1$^{-/-}$ cells (Fig. 6A). ELISA test results for VEGF in the supernatants of cultures indicated that UV induced VEGF expression in DEC1$^{+/+}$ but not in DEC1$^{-/-}$ cells (Fig. 6B).

Discussion

The results obtained from this study show that UV radiation induces HIF-1α and VEGF protein expression in a dose- and time-dependent manner in cultured human keratinocytes (Fig. 2). UV radiation also induced VEGF mRNA expression in a dose- and time-dependent manner (Fig. 3C), but did not affect HIF-1α mRNA expression (Fig. 3A and B). HIF-1α protein is rapidly degraded under normoxic condition by the ubiquitin-proteasome system (40), whereas hypoxia and $CoCl_2$ induce both stabilization and transactivation of HIF-1α (41). It has been shown that hypoxia is associated with UV radiation (42), it is likely that UV increases HIF-1α expression via a similar signal pathway as hypoxia. It is known that HIF-1α induces VEGF expression by directly binding to VEGF promoter in response to hypoxia (43,44). Similarly, induction of HIF-1α by UV results in an increased level of VEGF expression. There is a strong correlation between VEGF expression and cancer progression and metastasis (29). Inhibition of VEGF expression has a dramatic effect on tumor growth, invasion, and metastasis (45). Our data suggest that induction of HIF-1α and VEGF by UV radiation may play an important role in UV-induced carcinogenesis, and HIF-1α and VEGF may be targets for drug development for UV-induced skin cancer.

Previous studies indicate that PI3K/AKT and MAP kinase pathways are involved in HIF-1α expression induced by growth factors (24). In human prostate cancer cells, HIF-1α expression is modulated by the EGFR/PI3K/AKT pathway (26,46) and HIF-1α is considered as a candidate target of anticancer therapy (47,48). Activation of EGFR is also involved in the UV signal transduction pathway leading to skin cancer (7). UV radiation mimics the actions of receptor ligands in activating cell surface growth factor and cytokine receptors (5,6). UV radiation, mediated by EGFR, induces activation of PI3 kinase and AKT (6). Interestingly, hypoxia induces activation of the PI3 kinase/AKT cell survival pathway in PC12 cells. The respective roles of UV radiation and UV-induced hypoxia in expression of HIF-1α, and possible mediation by PI3 kinase/AKT, remain

to be clarified. Growth factor signaling, tightly coupled with PI3K/AKT, is also reportedly associated with cell survival and involved in HIF-1α expression in cancer cells (49,50). The present study also indicates that the EGFR/PI3K/AKT signaling pathway is required for induction of HIF-1α and VEGF expression induced by UV radiation. The following results support this conclusion. a) UV induces activation of EGFR in HaCat cells (Fig. 1). b) *egfr* (-/-) MEF cells are not capable of increasing VEGF and HIF-1α expression following UVB radiation. In contrast, *egfr* (+/+) MEF strongly enhanced VEGF and HIF-1α expression after UVB radiation. This confirms that EGFR is essential for VEGF and HIF-1α expression in response to UV radiation (Fig. 4). c) PI3K inhibitors, LY294002 or Wortmannin, inhibit HIF-1α and VEGF expression induced by UV radiation (Fig. 5). These data suggest that activation of the EGFR/PI3K signaling pathway and induction of HIF-1 and VEGF expression could be an important mechanism of UVB-induced carcinogenesis. DEC1 and DEC2, recently known to interact with HIF, have been shown to be involved in cell proliferation and survival. DEC1 and DEC2 are basic helix-loop-helix transcription factors (51,52). DEC1 is believed to be involved in the control of proliferation and/or differentiation of various types of cells (52). Hypoxia profoundly induces expression of DEC1 and the induction is abolished by Von Hippel-Lindau tumor suppressor protein (53), VHL, known to be one of the upstream regulators of HIF-1α. Our study also indicates that UV radiation induces HIF-1α and VEGF expression in a DEC1-dependent manner (Fig. 6). DEC1 is abundantly expressed in colon carcinoma but not in the adjacent normal tissues, suggesting that deregulated cell survival by DEC1 may have oncogenic significance (39). DEC1 negatively regulates the expression of DEC2 (38). DEC1 and HIF-1α protein expression are correlated in non-small cell lung cancer (54). DEC1 and DEC2 genes are direct targets of HIF1α, and DEC1 and DEC2, induced by HIF1α, may be crucial for the adaptation to hypoxia (55). Thus DEC1 may well be a valid target for UV-induced skin cancer treatment.

In conclusion, this work demonstrates that UV radiation induces HIF-1α and VEGF expression via the EGFR/PI3K/DEC1 signaling pathway. Our data provide insight into the molecular mechanisms of UV-induced skin cancer, and reveal DEC1 as a novel molecular target for eventual clinical management of UV-induced skin cancer.

Acknowledgments

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