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Epidermal Growth Factor Receptor Is a Critical Mediator of Ultraviolet B Irradiation-Induced Signal Transduction in Immortalized Human Keratinocyte HaCaT Cells

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Epidermal growth factor receptor (EGFR) is a critical mediator of several types of epithelial cancers. Skin cancer arising from exposure to ultraviolet B irradiation (UVB) from the sun is a prominent form of human cancer. Recent data indicate that in addition to cognate ligands, EGFR is activated by UVB irradiation. We used pharmacological and genetic approaches to investigate the function of EGFR in mediating UVB-induced signal transduction in human skin keratinocyte HaCaT cells. Pharmacological inhibition of EGFR tyrosine kinase significantly inhibited UVB-mediated induction of ERK, p38, and JNK MAP kinases, and their effectors, transcription factors c-Fos and c-Jun. Inhibition of UVB activation of EGFR also suppressed activation of AKT-, PKC-, and PKA-dependent signal transduction pathways. B82 mouse L cells devoid of EGFR were used to further investigate EGFR dependence of UVB-induced signal transduction. UVB failed to induce ERK, and JNK activation was reduced 60% in B82 cells compared to B82K$^+$ cells, which express EGFR. In addition, UVB induced both c-Fos and c-Jun proteins in B82K$^+$ cells, whereas neither were induced in B82 cells. Taken together, these data demonstrate that EGFR is required for UVB-mediated induction of multiple signaling pathways that are known to mediate tumor formation in skin. ***(Am J Pathol 2006, 169:823–830; DOI: 10.2353/ajpath.2006.050449)***

Ultraviolet (UV) irradiation is a potent carcinogen, capable of causing cell transformation and promotion of tumor formation. The shorter wavelength UVB region (290 to 310 nm) of the UV spectrum (290 to 400 nm) contains the most highly energetic photons. UVB irradiation causes DNA damage that can result in mutations stemming from imperfect DNA repair. In addition, accumulating evidence indicates that UVB-induced cellular responses lead to skin damage, promoting an environment conducive to tumor formation.[1–3] The mammalian UV response comprises UV activation of cell surface growth factor and cytokine receptors and their attendant downstream signal transduction machinery. UVB activation of four major families of growth factor receptors has been demonstrated: epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor, fibroblast growth factor receptor, and insulin receptor.[4–8] In addition, UVB activates receptors for the primary cytokines interleukin-1 and tumor necrosis factor-α and the death receptor Fas.[9–11]

UVB activation of these diverse cell surface receptors results in concomitant activation of multiple receptor-coupled signal transduction pathways, including the three MAP kinase signaling modules (ERK, JNK, and p38), Jak/STAT pathways, protein kinase-C pathways, integrin-coupled focal adhesion kinase pathways, and PI-3 kinase/AKT pathways.[7,12–14] UVB stimulation of these signal transduction pathways directly stimulates activation of transcription factors, which in turn regulate target gene expression. UVB-inducible transcription factors include Ets family members, EGR-1, AP-1 components (c-Jun and c-Fos), and nuclear factor (NF)-κB.[15–19]

A prominent feature of the mammalian UV response is induction of AP-1 and NF-κB-regulated genes including numerous cytokines, adhesion molecules, cyclooxygenase-2 (cox-2), nitric-oxide synthase, and matrix metalloproteinases. In human skin, these UVB-induced gene products cause an inflammatory response characterized by vasodilation, recruitment of circulating immune cells into the skin, and breakdown of skin connective tis-

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sue.[19–24] Recent evidence indicates that UVB-induced inflammation provides a microenvironment that promotes tumor formation by cells harboring permissive UVB-induced mutations.[25]

The activation of these diverse cell surface receptors by UVB irradiation has been confirmed by several research groups.[9–11] What remains unclear is the relative contribution of each receptor type to specific downstream signaling pathways. Studies designed to address this question will be helpful to dissect the interconnections among UVB-induced signal pathways and to build a detailed map of the signal relay mechanisms.

Binding of EGF family ligands to EGFR triggers a complex network of signaling pathways, culminating in responses ranging from cell division to death, and motility to adhesion proteolysis.[26–29] Dysregulation of EGFR family protein tyrosine kinases (HER, erbB) has been reported in multiple epithelial human cancers.[30–36] Accumulating evidence has expanded the role of EGFR from solely mediating responses to EGF-like ligands to being a major transducer of diverse signaling systems and a switch point for cellular communication networks.[26] EGFR is an essential participant in signal transduction pathways engaged by G-protein-coupled receptors, cytokine receptors, integrins, ion channels, and other tyrosine kinase receptors.[26,27,37,38] Various mechanisms have been proposed for this nonclassical transmodulation of the EGFR. These mechanisms include activation by intracellular, soluble tyrosine kinases such as those of the Src family and release of membrane-bound EGFR ligands by proteolysis.[26–28] Irrespective of the mechanisms of activation, it is becoming increasingly clear that EGFR functions not only to transduce signals in response to its cognate ligands but is also critical for activation of a diverse array of other cell surface receptors.[38]

UVB induction of EGFR tyrosine phosphorylation has been observed in several cell types. Although UVB-irradiation is known to activate EGFR, the role of this activation in UVB-induced signal transduction is not well understood. Given the central participation of EGFR in other diverse signaling pathways, we have used both pharmacological and genetic approaches to investigate the role of EGFR in UVB-induced signal transduction pathways.

Materials and Methods

Materials

HaCaT cells were generously provided by Dr. N.E. Fusenig (German Cancer Research Center, Heidelberg, Germany). Dulbecco's modified Eagle's medium, heat-inactivated fetal bovine serum, Dulbecco's phosphate-buffered saline (PBS), and trypsin 2.5% were obtained from Life Technologies, Inc. (Grand Island, NY). The B82 cell line and its derived EGFR-expressing cell line B82K$^+$ cells were generous gifts from Dr. Gordon Gill of University of California, San Diego, CA. EGFR, ERK, JNK, and p38 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phosphotyrosine antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Phospho-ERK, phospho-JNK, phospho-p38, phospho-AKT, PKC, and PKA substrate antibodies were purchased from Cell Signaling Technology (Beverly, MA). c-Fos and c-Jun antibodies were purchased from Oncogene Research Products (Cambridge, MA). Sodium orthovanadate was purchased from Sigma Chemical (St. Louis, MO). PD169540, a highly specific irreversible inhibitor of EGFR and ErbB2 tyrosine kinases, was provided by Dr. David Fry (Pfizer, Ann Arbor, MI).

Preparation of Whole Cell Lysate

Cells were washed twice with ice-cold PBS, scraped from the dishes in WCE buffer (25 mmol/L HEPES, pH 7.2, 75 mmol/L NaCl, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L ethylenediaminetetraacetic acid, 0.1% Triton X-100, 0.5 mmol/L dithiothreitol, 20 mmol/L β-glycerophosphate), supplemented with 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 1 mmol/L phenylmethyl sulfonyl fluoride, and 1 mmol/L orthovanadate. The cell homogenates were centrifuged at 14,000 $\times$ *g* for 15 minutes, and supernatants were collected and used as whole cell lysate.

Cell Culture

Human keratinocyte HaCaT cells and mouse L cell lines B82 and B82K$^+$ cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum under 5% CO_2 at 37°C.

UVB Irradiation Source and Irradiation

An Ultralite Panelite lamp (Daavlin, Bryan, OH) containing six FS24T12 UVB-HO bulbs was used as the UVB irradiation source. A Kodacel filter (Kodak, Rochester, NY) was used to eliminate wavelengths below 290 nm (UVC). The spectral output was 70% UVB (290 to 320 nm) and 30% UVA2 (320 to 340 nm). Because the majority of the energy was in the UVB range, we refer to this UV irradiation as UVB. Irradiation intensity was monitored and calculated with an IL1443 phototherapy radiometer and a SED240/UVB/W photodetector (International Light, Newbury, MA), positioned at the same distance from the source as the cells.

For UVB irradiation, human keratinocyte HaCaT cells and B82 cells were seeded in 10-cm dishes, grown to ~80% confluence, and then exposed to UVB while covered with a thin film (6 ml) of Dulbecco's PBS. Media were then replaced and plates were returned to the incubator for the indicated times. Sham-irradiated cells were also kept in PBS for equal amount of time without UV irradiation.

Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Measurements of c-Fos and c-Jun mRNA

Human keratinocyte HaCaT cells were harvested, and total cellular RNA was purified using a Miniprep RNA isolation kit according to the manufacturer's instruction (Qiagen, Chatsworth, CA). Reverse transcription of total

RNA was performed using a TaqMan reverse transcription kit (Applied Biosystems, Foster City, CA). Real-time RT-PCR was performed using a TaqMan Universal PCR Master Mix kit (Applied Biosystems) and a 7700 Sequence Detector (Applied Biosystems) was used to run the PCR. Primer/probe combinations were produced by the Custom Oligonucleotide Synthesis Service (Applied Biosystems). Target gene mRNA levels (number of molecules/10 ng total RNA) were normalized to mRNA levels of *36B4* (internal control housekeeping gene).

UVB-Induced Phosphorylation of AKT, PKC, and PKA Substrate Proteins

Antibodies that specifically recognize the phosphorylated forms of substrates for AKT, PKC, and PKA were used to determine the role of these protein kinase pathways. The phospho-AKT substrate antibody recognizes R/K-X-R/K-X-X-S/T motif when the serine (S) or threonine (T) residue is phosphorylated.[39,40] The phospho-PKC substrate antibody recognizes the R/K-X-S-Hydrophobic-R/K motif when the serine (S) is phosphorylated.[41,42] The phospho-PKA substrate antibody recognizes the R-X-X-T-X-X-X/R-R-X-S-X-X motif when the threonine (T) and serine(S) are phosphorylated.[41]

Western Blot

Equal amounts of whole cell lysate were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Immobilon-P filter paper (Millipore, Bedford, MA). Immunoreactive proteins were visualized by enhanced chemifluorescence according to the manufacturer's protocol (Amersham Biosciences, Piscataway, NJ). Quantification was performed using a STORM PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Results

UVB-Induced Tyrosine Phosphorylation of EGFR in Human HaCaT Cells Is Blocked by PD169540

EGF (10 ng/ml) and UVB irradiation (40 mJ/cm^2) induced tyrosine phosphorylation of EGFR in intact human keratinocyte HaCaT cells (Figure 1). However, pretreatment of cells with 200 nmol/L PD169540, which specifically inhibits EGFR and related ErbB tyrosine kinase activities through covalent modification of a cysteine residue in the ATP binding site of EGFR,[43] nearly completely blocked tyrosine phosphorylation of EGFR by both EGF and UVB irradiation (Figure 1).

UVB Activation of ERK, JNK, and p38 MAP Kinases Is Differentially Suppressed by EGFR Inhibitor PD169540

UVB irradiation induced a sevenfold increase in phosphorylation of p42 ERK and a fivefold increase in p44

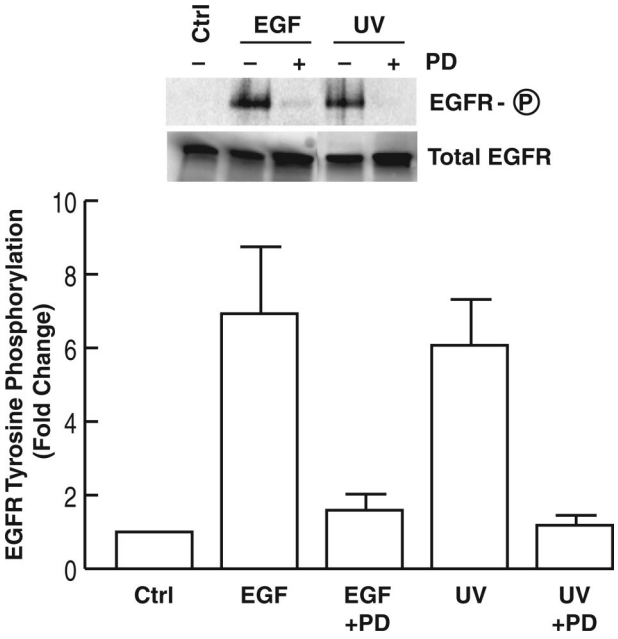

Figure 1. PD169540 inhibits both EGF and UVB activation of EGFR in human keratinocytes. HaCaT cells were pretreated with 200 nmol/L PD169540 (+PD) or with vehicle DMSO (−PD) for 16 hours before treatment with EGF (10 ng/ml) or UVB irradiation (40 mJ/cm^2). Whole cell lysates were prepared from cells treated with 10 ng/ml of EGF for 10 minutes or 15 minutes after UVB irradiation (40 mJ/cm^2). Untreated cells were used as control. EGFRs were immunoprecipitated from the whole cell lysates and subjected to SDS-PAGE and Western blot using anti-phosphotyrosine antibody. A duplicate gel was probed with anti-EGFR antibody. Results are representative of three experiments.

ERK phosphorylation in HaCaT keratinocytes (Figure 2A). Pretreatment of HaCaT cells with PD169540 almost completely blocked UVB activation of both p42 and p44 isoforms of ERK. Treatment of cells with PD169540 alone, in the absence of UVB irradiation, did not alter basal ERK phosphorylation (Figure 2A).

UVB irradiation induced a 13-fold increase in the level of p54 JNK phosphorylation and a 20-fold increase in the level of p46 JNK phosphorylation relative to levels in untreated, nonirradiated controls. Pretreatment of cells with PD169540 inhibited UVB-induced phosphorylation of p54 and p46 JNK by 50 and 30%, respectively (Figure 2B). Treatment of cells with PD169540 alone did not alter either p54 JNK or p46 JNK phosphorylation (Figure 2B). UVB irradiation induced a threefold increase in the level of p38 phosphorylation relative to untreated, nonirradiated HaCaT keratinocytes. Pretreatment of cells with PD169540 inhibited UVB-induced phosphorylation of p38 by 50% (Figure 2C). Pretreatment with PD169540 alone did not alter p38 phosphorylation (Figure 2C).

UVB Induction of c-Fos and c-Jun mRNA Is Dependent on EGFR Tyrosine Kinase Activity

Because UVB activation of the MAP kinase pathways results in induction of c-Fos and c-Jun expression, we investigated whether EGFR activation by UVB irradiation is required for stimulation of c-Fos and c-Jun gene expression. Human HaCaT keratinocytes were pretreated with vehicle or PD169540 (200 nmol/L) and irradiated

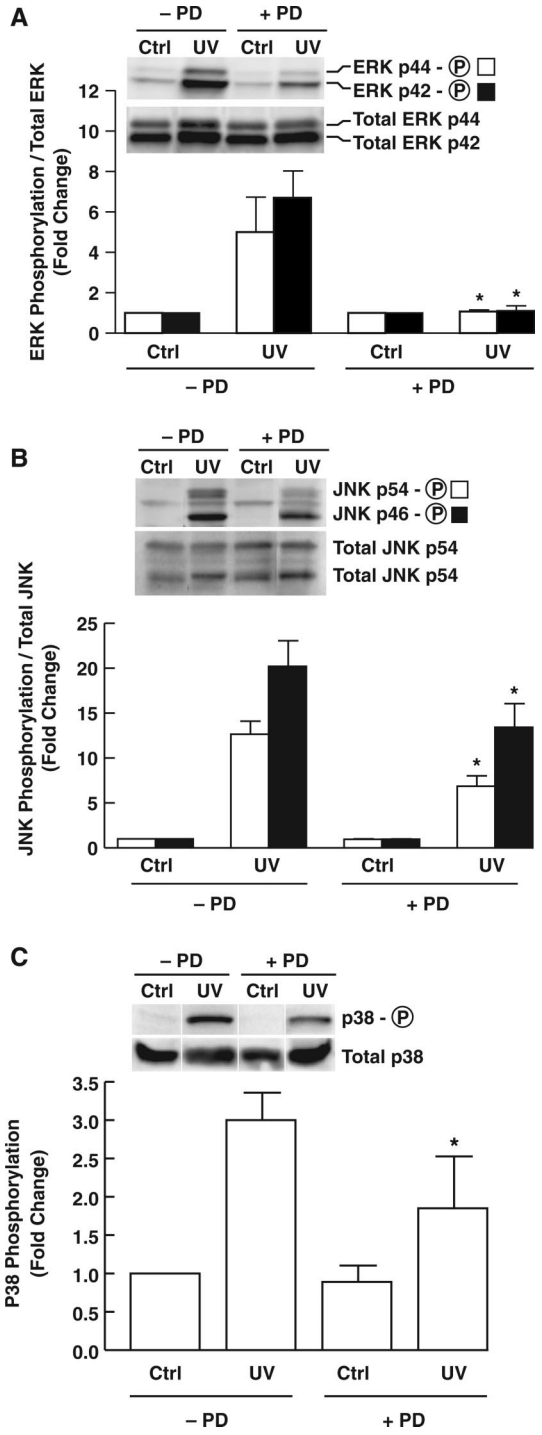

Figure 2. EGFR mediates UVB activation of ERK, JNK, and p38 in human keratinocytes. HaCaT cells were pretreated with PD169540 (+PD, 200 nmol/L) or with DMSO (−PD) for 16 hours, UVB-irradiated (40 mJ/cm^2), and whole cell lysates were prepared 15 minutes later. **A:** UVB activation of p44 and p42 ERK was analyzed by Western blot using total and phospho-ERK antibodies and quantified by STORM PhosphorImager. Data expressed as fold change relative to levels in nonirradiated, untreated control cells (CTRL). $N = 4$, $^*P < 0.05$ when compared with UV-irradiated −PD samples (Student's *t*-test, same below). **B:** UVB activation of p54 and p46 JNK were analyzed by Western blot using total and phospho-JNK antibodies and quantified by STORM PhosphorImager. Data are presented as fold change relative to levels in nonirradiated, untreated control cells (CTRL). $N = 5$, $^*P < 0.05$. **C:** UVB activation of p38 was analyzed by Western blot using total and phospho-p38 antibodies and quantified by STORM PhosphorImager. Data are presented as fold change relative to levels in sham-irradiated, untreated control cells (CTRL). $N = 5$, $^*P < 0.05$.

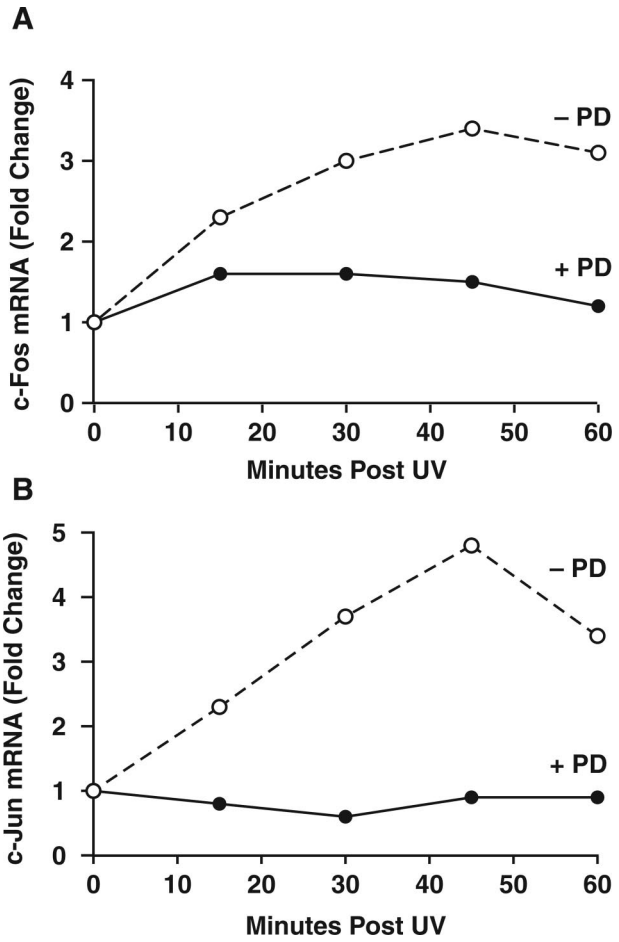

Figure 3. EGFR mediates UVB induction of *c-Fos* and *c-Jun* mRNA in human keratinocytes. Human HaCaT cells were treated with PD169540 (+PD, 200 nmol/L) or DMSO vehicle (−PD) for 16 hours before UVB irradiation (50 mJ/cm^2) and total RNA was isolated. Cells were harvested at the indicated time points after UVB irradiation. *c-Fos* (**A**) and *c-Jun* (**B**) mRNA analysis was performed by real-time RT-PCR. Data are expressed as fold change relative to levels in untreated, sham-irradiated controls (0 time). A representative graph from three independent experiments is shown.

with UVB (40 mJ/cm^2). Cells were harvested for real-time RT-PCR analysis of *c-Fos* and *c-Jun* mRNA at 15 to 60 minutes after UVB irradiation. UVB irradiation induced a marked increase in the level of *c-Fos* mRNA as early as 15 minutes after UVB, peaking at 3.5-fold the level in untreated controls at 45 minutes after UVB and remaining elevated for at least 60 minutes (Figure 3A). Pretreatment with PD169540 before UVB irradiation inhibited *c-Fos* mRNA induction to levels in untreated, nonirradiated controls at all time points after exposure to UVB.

UVB irradiation also induced a marked increase in the level of *c-Jun* mRNA as early as 15 minutes after UVB, peaking at fivefold at 45 minutes after UVB and remaining elevated for at least 60 minutes (Figure 3B). PD169540 pretreatment of human HaCaT keratinocytes before UVB irradiation also reduced UVB induction of *c-Jun* mRNA to levels observed in nonirradiated cells at all time points after exposure to UVB (Figure 3B).

We used overnight treatment for logistical reasons. To address the specificity of overnight incubation of HaCaT cells with PD169540, we determined its effect on UV

irradiation induction of *TNF-α* gene expression. Induction of *TNF-α* gene expression is a well-characterized EGFR-independent response to UV irradiation. We found similar levels of *TNF-α* mRNA induction (approximately eightfold over the mock-irradiated cells) in vehicle- and PD169540-treated HaCaT cells (data not shown).

UVB Induction of AKT, PKC, and PKA Pathway Is Suppressed by EGFR Inhibitor PD169540 in Human HaCaT Keratinocytes

In addition to MAP kinases, EGFR effectors include PI3K/AKT, PLC/PKC, and PKA. To determine the role of EGFR in UVB activation of these effector pathways, we used antibodies that specifically recognize the phosphorylated forms of substrate proteins, which have been phosphorylated by AKT, PKC, or PKA protein serine/threonine kinases. Human HaCaT keratinocytes were pretreated with vehicle or PD169540 (200 nmol/L), and sham- or UVB-irradiated. Whole cell lysates were prepared 10 and 20 minutes after UVB irradiation and subjected to SDS-PAGE/Western blot using phospho-AKT substrate, phospho-PKC substrate, or phospho-PKA substrate antibody.

UVB irradiation induced phosphorylation of at least four AKT substrate proteins. Treatment of keratinocytes with EGFR inhibitor PD169540 before UVB irradiation markedly decreased phosphorylation of these four AKT substrate proteins (Figure 4A) ranging in size from 32 to 120 kd. The identities of these phosphor-proteins are not known.

UVB irradiation also induced phosphorylation of two major PKC protein substrates of molecular weights 34 kd and 200 kd (Figure 4B). Phosphorylation of both of these protein bands was substantially reduced by pretreatment of cells with EGFR kinase inhibitor PD169540 (Figure 4B). UVB irradiation stimulated phosphorylation of at least five protein substrates for PKA, ranging in molecular weight from 30 to 150 kd (Figure 4C). UVB-induced phosphorylation of these PKA substrates was markedly reduced by EGFR inhibition by PD169540. Although the identities of the phosphorylated substrate proteins are not known at this time, the above data demonstrate that EGFR is a critical mediator of MAP kinase-, AKT-, PKC-, and PKA-mediated UVB-induced signal transduction in cultured human HaCaT keratinocytes.

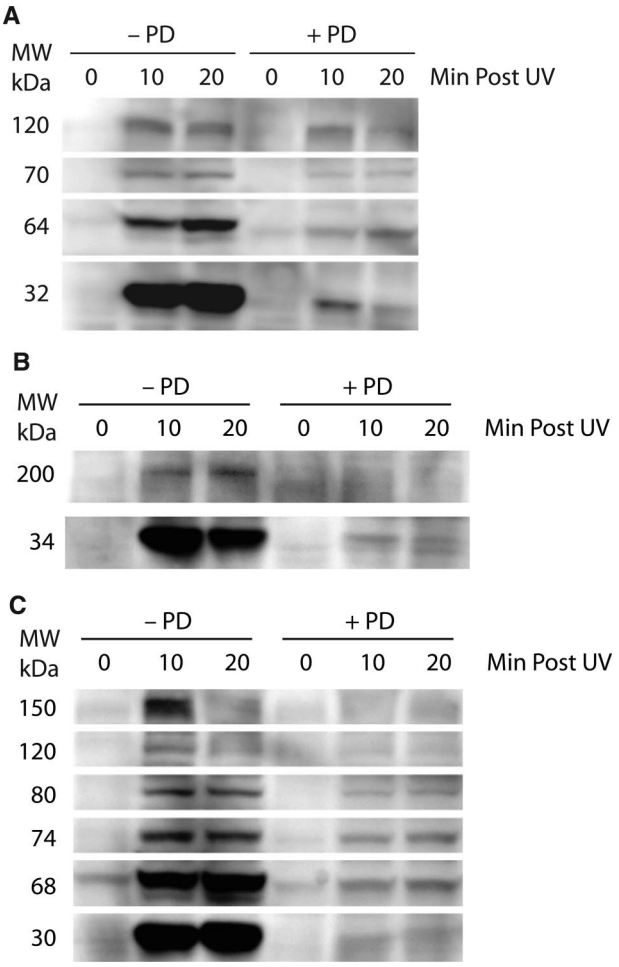

Figure 4. EGFR mediates UVB-induced phosphorylation of AKT, PKC, and PKA substrates in human keratinocytes. Human HaCaT cells were treated with PD169540 (+PD, 200 nmol/L) or DMSO vehicle (−PD) for 16 hours before UVB irradiation (50 mJ/cm^2). Cells were harvested at the indicated time points, and whole cell lysates were subjected to Western blot probed with phospho-AKT substrate antibody (**A**), phospho-PKC substrate antibody (**B**), and phospho-PKA substrate antibody (**C**). Apparent molecular weights (MW) are indicated to the left of each band. Data are representative Western blots from three independent experiments.

UVB Irradiation Activation of ERK and JNK Is Dependent on EGFR

To further investigate the importance of EGFR in the UVB-induced signaling, we used B82 cells, which do not express EGFR, and B82K$^+$ cells, which are B82 cells made to express EGFR by stable transfection.[44,45] B82 and B82K$^+$ cells were UVB-irradiated, and whole cell lysates were subjected to Western analysis for phosphorylated and total ERK and phosphorylated and total JNK. UVB irradiation caused substantial activation of p42 ERK in EGFR-expressing B82K$^+$ cells (Figure 5A). In contrast, no p42 ERK activation was observed after UVB irradiation of EGFR-negative B82 cells, although the level of p42 ERK expression in B82 was similar to that of EGFR-expressing B82K$^+$ cells (Figure 5A). UVB irradiation also induced p46 and p54 JNK phosphorylation in B82K$^+$ cells. Activation of both p46 and p54 JNK by UVB irradiation was ~50% less in B82 cells compared to B82K$^+$ cells (Figure 5B), and total p46 and p54 JNK in B82 and B82K$^+$ cells did not differ.

UVB Induction of c-Fos and c-Jun Proteins Is Dependent on EGFR

Induction of AP-1 proteins c-Fos and c-Jun is downstream of ERK and JNK pathways. Because UVB activation of ERK and JNK was reduced in B82 cells lacking EGFR, we examined induction of c-Fos and c-Jun protein by UVB irradiation in B82 and B82K$^+$ cells. In EGFR-expressing B82K$^+$ cells, UVB irradiation induced c-Fos (Figure 6A) and c-Jun (Figure 6B) proteins approximately threefold, compared to nonirradiated B82K$^+$ cells. In EGFR-negative B82 cells, UVB irradiation had a negligi-

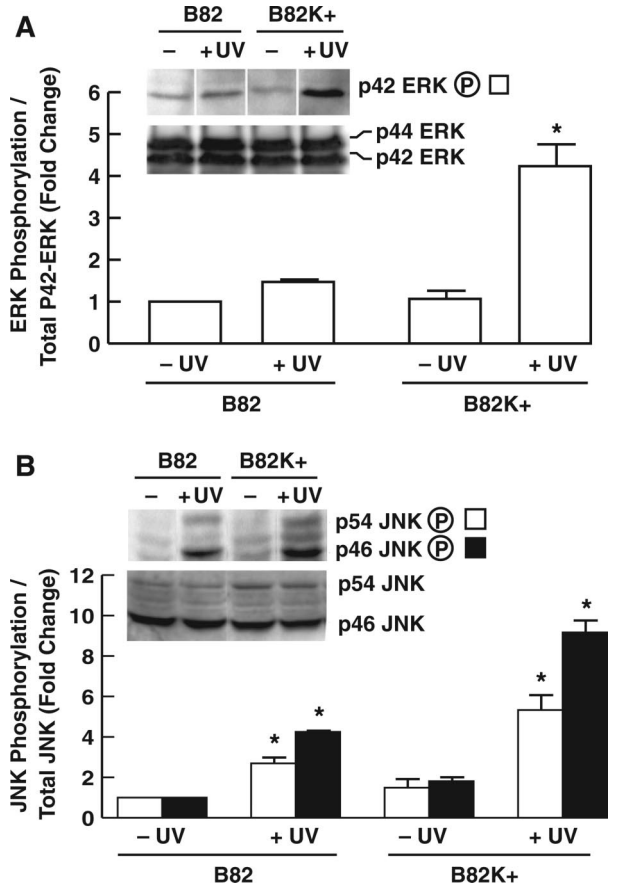

Figure 5. EGFR-dependent ERK JNK activation in B82 cells. B82 cells lacking EGFR and B82K$^+$ cells expressing EGFR were UVB (50 mJ/cm^2)- or sham-irradiated, and whole cell lysates were prepared 15 minutes after treatment. **A:** p42 and p44 ERK were analyzed by Western blot using phospho-ERK antibody and quantified by STORM PhosphorImager. Data are presented as fold change relative to sham-irradiated cells. $N = 3$, $^*P < 0.05$. **B:** p54 and p46 JNK were analyzed by Western blot using phospho-JNK antibody and quantified by STORM PhosphorImager. Data are presented as fold change relative to levels in sham-irradiated cells. $N = 3$, $^*P < 0.05$.

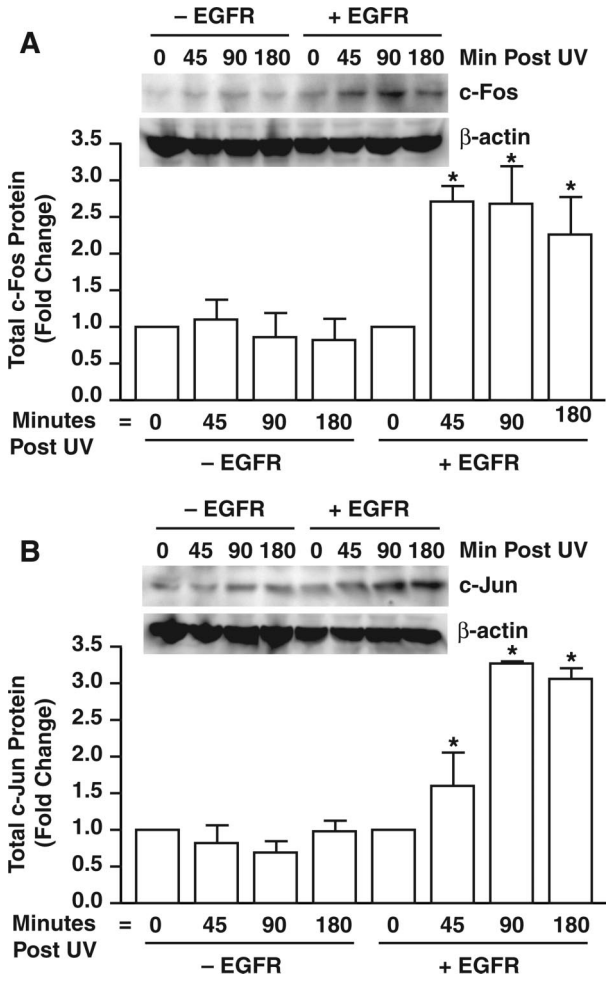

Figure 6. UVB induction of c-Fos and c-Jun protein requires EGFR. B82 lacking EGFR and B82K$^+$ EGFR-expressing cells were UVB-irradiated (50 mJ/cm^2) or sham-irradiated, and whole cell lysate was prepared at the indicated time points after UVB irradiation. c-Fos (**A**) and c-Jun (**B**) protein levels were quantified by SDS-PAGE/Western blot at the indicated times after UVB. Data are presented as fold change relative to levels in sham-irradiated cells. $N = 3$, $^*P < 0.05$.

ble effect on c-Fos (Figure 6A) and c-Jun (Figure 6B) protein levels at all time points examined.

Discussion

Rosette and Karin[10] reported that UV irradiation induced activation of EGF, tumor necrosis factor-α (TNF-α), and interleukin-1 (IL-1) receptors and that consequent downstream signaling resembled the sum of the three individual receptor-stimulated pathways in HeLa cells. We have previously reported that EGF, TNF-α, and IL-1 receptors are also activated by UV irradiation in human skin *in vivo*.[46] From these results, one would predict that activation of each of the three cell surface receptors should contribute approximately equally to the activation of MAP kinase and other downstream pathways, in human skin *in vivo*. Surprisingly, however, our current data indicate that EGFR plays a preeminent role in UVB irradiation-induced activation of multiple signal transduction pathways. This finding suggests that interconnections among cell surface receptor activation and downstream signaling pathways that are triggered in response to UV irradiation may differ depending on cell type and cellular context. It should be noted that our studies were conducted with human immortalized keratinocyte HaCaT cells, which may differ in their responsiveness to UV irradiation compared to normal human keratinocytes *in vivo*.

Increased tyrosine phosphorylation of EGFR occurs within 5 minutes after UVB exposure in human skin *in vivo* and cultured human skin cells.[47] EGFR activation results from autophosphorylation of specific tyrosine residues within its intracellular C-terminal domain. These phosphorylated tyrosine residues function as docking sites for a variety of signaling molecules, which initiate membrane-proximal steps of signal transduction cascades that ultimately bring about cellular responses.[26–28,37,48]

It has previously been reported that EGFR mediates several responses to UVB irradiation including induction of transcription factor EGR-1, induction of cyclooxygenase-2 (cox-2), activation of the PI-3 kinase/AKT pathways, and induction of matrix metalloproteinases.[4,6,19,38,49–51] In the present study, we observed that EGFR was critical for UVB-induced activation of

AKT, PKC, PKA, and MAP kinase signal transduction pathways. In addition, UVB induction of AP-1 components c-Fos and c-Jun was dependent on EGFR activation in human keratinocytes. AP-1 has been shown to be necessary for tumor formation in a mouse skin carcinogenesis model.[52,53] These data support the concept that EGFR is a critical initiator of many cellular responses to UVB irradiation. Taken together, these EGFR-dependent UVB-induced responses promote cell survival, cell growth, inflammation, and extracellular matrix degradation. These alterations create a tissue milieu that is favorable for expansion of cells harboring transforming mutations and thereby promote tumor formation. Thus, EGFR emerges as a key mediator of UVB-induced skin cancer.

From the above discussion, it follows that prevention of UVB activation of EGFR may be beneficial in reducing skin cancer. A number of modalities to accomplish this inhibition could be imagined. In principle, conscientious use of sunscreens/blocks or protective clothing should prevent EGFR activation by restricting UVB exposure of the skin. Unfortunately, practical considerations encompassing use of inadequate amounts of sunscreen and the popularity of tanning appear to limit the success of approaches aimed at reducing skin sun exposure.[54,55]

A more direct strategy for preventing EGFR activation is the use of topical or systemic EGFR tyrosine kinase inhibitors. For example, topical application of the EGFR kinase inhibitor genistein (which also has an antioxidant property) has been shown to inhibit UVB irradiation activation of EGFR in human skin[56] and to reduce skin cancer in mice.[57,58] Synthetic EGFR tyrosine kinase inhibitors are currently under investigation for treatment of certain types of epithelial cancer.[59,60] Given the role of EGFR in normal skin physiology, daily, long-term use of EGFR inhibitors to reduce skin cancer may cause undesirable side effects. The efficacy and practicality of this approach remains to be investigated.

A third approach to preventing EGFR activation is to target the activation process. The mechanism by which UVB irradiation activates EGFR is unknown. However, it has been proposed that EGFR activation is mediated by UVB-induced reactive oxygen species, which directly inhibit protein tyrosine phosphatase activities.[61–65] In the presence of reduced protein tyrosine phosphatase activities, EGFR tyrosine phosphorylation accumulates, thereby resulting in activation. If this mechanism is operative, then antioxidants that prevent UVB-induced protein tyrosine phosphatase oxidation may also protect against UVB-induced EGFR activation and skin cancer.

Acknowledgments

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