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Ultraviolet irradiation induces keratinocyte proliferation and epidermal hyperplasia through the activation of the epidermal growth factor receptor

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Chronic exposure to ultraviolet (UV) irradiation induces skin cancer, in part, through epigenetic mechanisms that result in the deregulation of cell proliferation. UV irradiation also rapidly activates the epidermal growth factor receptor (EGFR). Since EGFR activation is strongly mitogenic in many cell types including keratinocytes of the skin, we hypothesized that UV-induced cutaneous proliferation results from EGFR activation. The role of EGFR activation in the response of the skin to UV was determined using *Egfr*-null and *Egfr*-wild-type skin grafted onto athymic nude mouse hosts, because *Egfr*-null mice survive only a few days after birth. EGFR was rapidly activated in mouse epidermis following exposure to UV, as detected by the phosphorylation of EGFR on tyrosine residues 992, 1045, 1068 and 1173. UV induced epidermal hyperplasia in *Egfr*-wild-type skin between 48 and 72 h post-UV. However, no epidermal hyperplasia occurred in *Egfr*-null skin. Baseline cell proliferation was similar in skin grafts of both genotypes. However, UV exposure increased cell proliferation, as measured by Ki67 immunohistochemistry and proliferating cell nuclear antigen immunoblotting, maximally at 48 h to a level more than three times higher in wild-type compared with *Egfr*-null skin. Apoptotic cell death, as measured by terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) analysis, was also increased in UV-exposed *Egfr*-null skin when compared with wild-type 1–2 days post-UV. These changes in cellular homeostasis after UV were accompanied by increased cyclin D expression in wild-type but not *Egfr*-null skin and increased expression of p53 and the cyclin-dependent kinase (CDK) inhibitor p21^{waf1} in *Egfr*-null skin when compared with wild-type. Collectively, these results demonstrate that the UV-induced activation of EGFR augments keratinocyte proliferation and suppresses apoptosis, leading to epidermal hyperplasia, associated with increased G_1 cyclin expression and suppression of CDK inhibitor expression.

Abbreviations: CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; ERK, extracellular-signal regulated kinase; JNK, NH_2-terminal Jun kinase; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor kappaB; p21, p21^{waf1}; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling; UV, ultraviolet.

Introduction

The primary cause of non-melanoma skin cancer is exposure to ultraviolet (UV) irradiation, often in the form of sunlight. The increased incidence of skin tumors following UV exposure is believed to stem from the combined influences of DNA damage and its epigenetic effects (reviewed in Ref. 1). The epigenetic effects of UV include changes in signaling and gene expression that regulate cell proliferation and survival in the short-term and contribute to skin cancer development in the long-term. Normally, the skin responds to UV exposure with cell cycle arrest to allow for the repair of damaged cells or apoptosis if repair is not possible. When cell cycle arrest and apoptosis mechanisms fail, however, further UV exposure results in the clonal expansion of cells with DNA damage to form tumors. The molecular mechanisms through which UV-induced epigenetic effects contribute to this process have not been well defined.

UV irradiation activates numerous signaling pathways that alter transcription. This process resembles the response to growth factors and is known as the UV response (2,3). Much of the UV response is due to the activation of mitogen-activated protein kinase (MAPK) family members and the nuclear factor kappaB (NFκB) pathway (2,4–8). Activation of extracellular-signal regulated kinase (ERK), p38 kinase and NH_2-terminal Jun kinase (JNK)—all MAPKs in response to UV depends, in large part, upon the epidermal growth factor receptor (EGFR) (3,9–12). Among other effects, the EGFR-activated MAPKs have been shown to phosphorylate p53 and prevent its activation (13,14). p53 activation following UV causes cell cycle arrest through induction of the cyclin-dependent kinase (CDK) inhibitor p21^{waf1} (designated p21 in this manuscript) and apoptosis through less well-defined mechanisms (15). The extent to which EGFR regulates the proliferative response of the skin to UV through these or other mechanisms is not known.

EGFR regulates the proliferation, differentiation and survival of many cell types, including keratinocytes in the skin (16–18). UV exposure results in the rapid activation of EGFR by a reactive oxygen intermediate-mediated mechanism (19–21). UV triggers receptor phosphorylation by damaging receptor associated phosphatases, thus blocking deactivation (19), altering receptor internalization and degradation (8,19,20,22), and increasing EGFR ligand expression at later time points (23,24). Thus, UV exposure results in both a short-lived and immediate as well as a more delayed and prolonged activation of EGFR.

Increased expression and activation of EGFR has been strongly implicated in human carcinogenesis at many organ sites (25–27). Mouse skin models have also provided evidence for the importance of EGFR-mediated signalingin skin cancer (11,28–30). Inhibition or genetic ablation of EGFR suppresses skin tumorigenesis in mice (11,29). Since cancer is essentially a disease of unregulated cell growth, we hypothesized that the

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activation of EGFR following UV exposure increases skin tumor growth by suppressing UV-induced cell cycle arrest and increasing UV-induced cell proliferation. This hypothesis was tested using *Egfr*-null and littermate control skin grafts because *Egfr*-null mice survive at most a few days after birth. We found that EGFR increases keratinocyte proliferation and epidermal hyperplasia following UV exposure, associated with the induction of cyclin D expression and the suppression of p21 expression. Therefore, chronic EGFR activation upon UV exposure may contribute to the deregulation of cell cycle progression during UV-induced skin carcinogenesis.

Materials and methods

Cells

Keratinocytes were prepared from newborn CD-1 mouse skin and cultured as described elsewhere (29). Some dishes were treated with 10 ng/ml TGFα before lysis.

Animals

CD-1 mice heterozygous for *Egfr* were mated to produce *Egfr*-null and *Egfr*-wild-type control mice, which were genotyped using polymerase chain reaction as described elsewhere (31). Pieces of full-thickness skin from newborn *Egfr*-null and wild-type littermates were grafted onto the backs of anesthetized athymic nude mice as described previously (18) and euthanized between 18 and 21 days post-grafting following UV exposure or sham-irradiation. Successful grafts were identified by the presence of haired skin. Mice were euthanized by CO_2 asphyxiation and portions of the skin were fixed in 70% ethanol or 10% neutral-buffered formalin or protein homogenate prepared.

UV irradiation

A bank of six FS40T12 fluorescent sunlamps (Westinghouse, NJ) was used for the UV exposure. UV intensity was measured using radiometric photodetector probes (Oriel, Startford, CT). The emitted radiation consisted of ~70% UVB, 30% UVA and <1% UVC, with a total output of 1.46 mW/cm^2. Mice were shaved at least 1 day prior to exposure to UV.

Immunohistochemistry

Following antigen retrieval in 10 mM citrate buffer, skin sections were incubated with an anti-Ki67 antibody (Novacastra, United Kingdom), a biotinylated secondary antibody (Jackson ImmunoResearch, West Grove, PA), a horseradish-peroxidase-conjugated ABC reagent (Vector Laboratories, Burlingame, CA), diaminobenzidine (Sigma, St. Louis, MO) and a hematoxylin counterstain. The number of Ki67-labeled cells per millimeter epidermis was determined by counting Ki67 labeled epidermal keratinocytes per millimeter of epidermis using stage and ocular micrometers. Measurements were performed in at least five randomly selected regions on each slide with the investigator blinded as to the identity of the samples.

Quantification of epidermal hyperplasia

For quantification of epidermal hyperplasia following UV exposure, the number of nucleated epidermal cell layers was counted in at least five randomly selected regions from each hematoxylin and eosin stained slide. The thickness of the epidermis from the epidermal–dermal junction to the distal edge of the stratum granulosum was measured in the same regions using ocular and stage micrometers. Measurements were performed with the investigator blinded as to the identity of the samples.

Immunoblotting

The epidermis was separated from the skin using the heat shock method, by the immersion of the skin in a 58°C water bath for 20 s followed by scraping off the epidermis, as described elsewhere (32). Epidermises were homogenized using a polytron (Brinkman, Westbury, NY), or cells lysed, in buffer containing 10 mM Tris (pH 7.4), 150 mM sodium chloride, 10% glycerol, 1% Triton X-100, 1 mM EDTA, Complete Protease Inhibitor tablets (Roche, Germany), 1 mM sodium orthovanadate, 1.5 μM EGTA and 10 μM sodium fluoride. Equal amounts of protein were resolved using SDS–PAGE and transferred to nitrocellulose. The efficiency of transfer and uniformity of loading were determined by Ponceau S (Sigma, St. Louis, MO) staining and actin (Cell Signaling, Beverly, MA) immunoblotting. Nitrocellulose membranes were also incubated with antibodies recognizing EGFR (Cell Signaling, Beverly, MA), EGFR phosphotyrosine-1068 (Cell Signaling, Beverly, MA), EGFR phosphotyrosine-1173 (Santa Cruz, Santa Cruz, CA), EGFR phosphotyrosine-992 (Cell Signaling, Beverly, MA), EGFR phosphotyrosine-1045 (Cell Signaling, Beverly, MA), p21 (Cell Signaling, Beverly, MA) or cyclin D1 (Upstate Biotech, Charlottesville, VA); the appropriate horseradish peroxidase-conjugated secondary antibody (Cell Signaling, Beverly, MA); and Chemiluminescence reagents (Pierce, Rockford, IL). Immunoblots were analyzed using densitometry and the signal normalized to actin expression.

TUNEL

Apoptotic cells were identified using terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) according to the manufacturer's directions (Promega, Madison, WI). The number of TUNEL-positive cells per millimeter epidermis was determined by counting labeled keratinocytes and using stage and ocular micrometers. Measurements were performed in at least five randomly selected regions on each slide with the investigator blinded as to the identity of the samples.

Results

EGFR is rapidly activated following UV exposure

Previous reports have documented the UV-induced activation of EGFR in keratinocytes in culture (33) and in human skin (34,35). The timing of EGFR activation and sites of phosphorylation were determined in mouse skin following UV irradiation. As reported previously in cultured human keratinocytes (33), UV exposure increased the phosphorylation of EGFR at tyrosines 992, 1045, 1068 and 1173 within the first hour after exposure (Figure 1). Maximal activation of EGFR, indicated by phosphorylation of the receptor on tyrosine 1068, was detected in the epidermis 5 min following exposure to UV. Elevated tyrosine 1068 phosphorylation persisted for 30 min after UV (Figure 1). Increased phosphorylation on tyrosines 1173, 992 and 1045 occurred 5 min after UV exposure and was sustained until 60 min post-UV, with two peaks of activation at 5–10 and 30 min post-UV (Figure 1). Phosphorylation of EGFR on tyrosine 1068, 1173, 992 and 1045 was maximally increased by 22-, 15-, 7- and 8-fold, respectively, after UV irradiation (Figure 1). The differences in the magnitude of EGFR phosphorylation at various sites were reproduced in multiple experiments although the causes and biological significance of these differences are not clear. Phosphorylation of EGFR on these residues recruits adaptors that activate multiple signaling pathways regulating both cell division and cell death.

EGFR is required for UV-induced epidermal hyperplasia

Both increased cell division and decreased cell death can contribute to hyperplasia, a characteristic feature of epigenetic or tumor promoting, carcinogenic stimuli. The role of EGFR in UV-induced epidermal hyperplasia was examined using *Egfr*-null and wild-type mouse skin. Because *Egfr*-null mice survive at most a few days after birth, newborn *Egfr*-null and wild-type mouse skin was grafted onto the backs of athymic nude mice and allowed to heal prior to UV irradiation. As reported previously (36), the epidermis of graft skin was slightly thickened compared with intact mouse skin (Figure 2, top panels). No difference in epidermal thickness or in the number of nucleated epidermal cell layers, two measures of epidermal hyperplasia, was detected in sham-irradiated *Egfr*-null compared with wild-type skin 3 weeks after grafting (Figure 2, top panels). No significant induction of epidermal hyperplasia was detected in the skin of either genotype 24 h post-UV (Table I and Figure 2). At later time points *Egfr*-wild-type, but not *Egfr*-null, skin exhibited epidermal hyperplasia (Table I and Figure 2). Epidermal hyperplasia was maximal in *Egfr*-wild-type skin 48 h after UV (Table I and Figure 2). By this time point, the number of nucleated epidermal cell layers had increased in sham-irradiated skin from 3–4 to 5–6 in UV-irradiated skin with a doubling of the epidermal thickness

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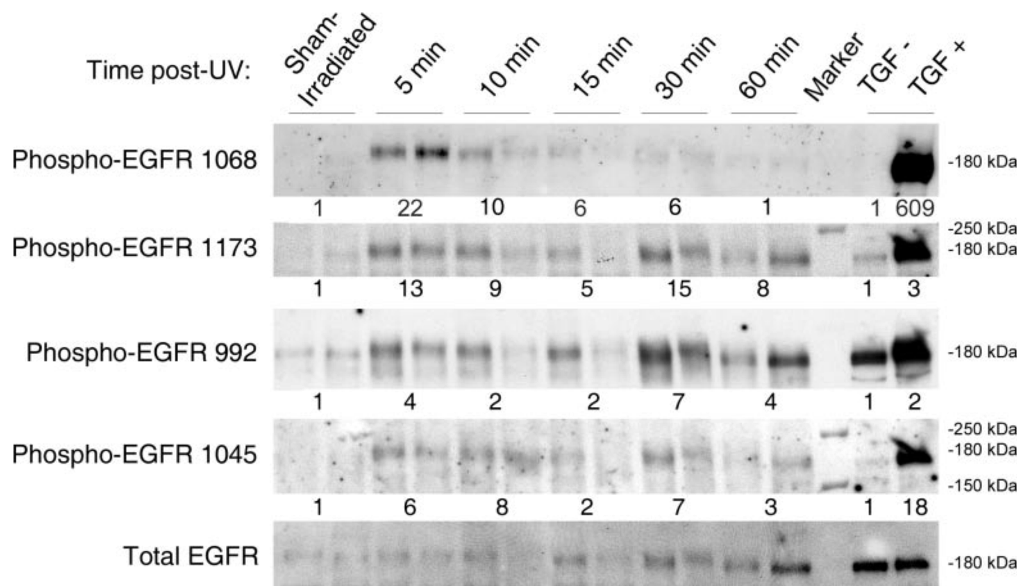

Fig. 1. Exposure of mouse skin to UV results in the rapid phosphorylation of EGFR. Epidermal homogenate was prepared from *Egfr*-wild-type mice at the indicated times following UV exposure. Protein extract was also prepared from cultured wild-type keratinocytes incubated with (+) or without (−) TGFα (far right lanes). Protein extracts were immunoblotted using the indicated phospho-specific EGFR and total EGFR antibodies. Mean signal relative to total EGFR for $N = 2$ mice is indicated below each panel. Each lane is homogenate from a different mouse. Experiment is representative of three experiments performed.

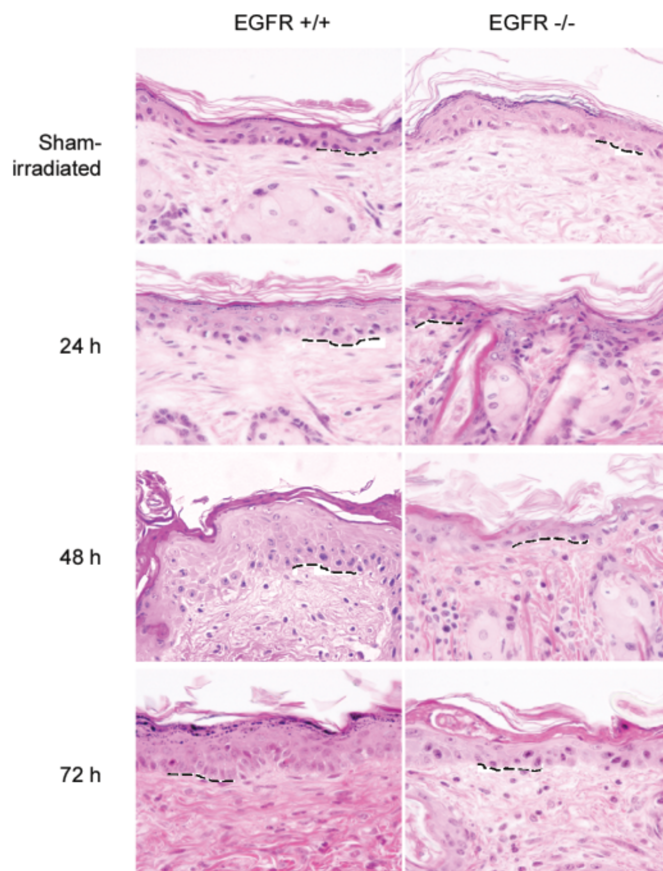

Fig. 2. EGFR increases epidermal hyperplasia following UV exposure. *Egfr*-null and wild-type skin grafts were exposed to 5 kJ/m^2 UV or sham-irradiated. Skin was removed 24, 48 or 72 h following UV- or sham-irradiation and sections stained with hematoxylin and eosin. Images are representative of sections from the four grafts of each genotype at each time point ($N = 4$). Dashed lines indicate the location of the dermal–epidermal junction.

Table I. Abrogation of EGFR prevents epidermal hyperplasia in response to UV

Time post-UV (h)	*Egfr*$^{+/+}$		*Egfr*$^{-/-}$	
	Cell layers	Epidermal thickness (μm)	Cell layers	Epidermal thickness (μm)
0	3–4	26.3 ± 2.1	3–4	23.0 ± 1.0
24	3–4	30.9 ± 5.7	3–4	31.9 ± 3.4
48	5–6	56.2 ± 9.1	2–3*	18.0 ± 2.9*
72	5–6	41.0 ± 4.4	3–4*	27.2 ± 2.8*

Epidermal hyperplasia was quantified in *Egfr*-null and *Egfr*-wild-type skin following irradiation with 5 kJ/m^2 UV by counting the number of nucleated epidermal cell layers and measuring the epidermal thickness in at least five randomly selected regions. Data from four mice in each group were averaged and are presented as mean ± SE ($N = 4$).
*Mean is significantly different from the corresponding wild-type control using a Student's *t*-test, where $P \leq 0.05$.

(Table I). UV-induced hyperplasia was sustained at 72 h post-UV in *Egfr*-wild-type skin (Table I). In contrast, no hyperplasia developed in *Egfr*-null skin by 72 h following exposure to UV (Figure 2 and Table I). The number of cell layers in *Egfr*-null skin was actually slightly decreased 48 h after UV exposure compared with sham-irradiated controls (Table I). In addition, *Egfr*-null skin exhibited decreased epidermal cellularity in some sections 48 and 72 h after UV irradiation when compared with the corresponding wild-type controls (Figure 2). Thus, EGFR is required for epidermal hyperplasia in response to a single exposure of the skin to UV.

UV-induced proliferation depends upon EGFR expression

To determine the effects of EGFR activation on cell division following UV exposure, two markers of proliferation, Ki67 and proliferating cell nuclear antigen (PCNA), were examined in UV-exposed and sham-irradiated *Egfr*-null and

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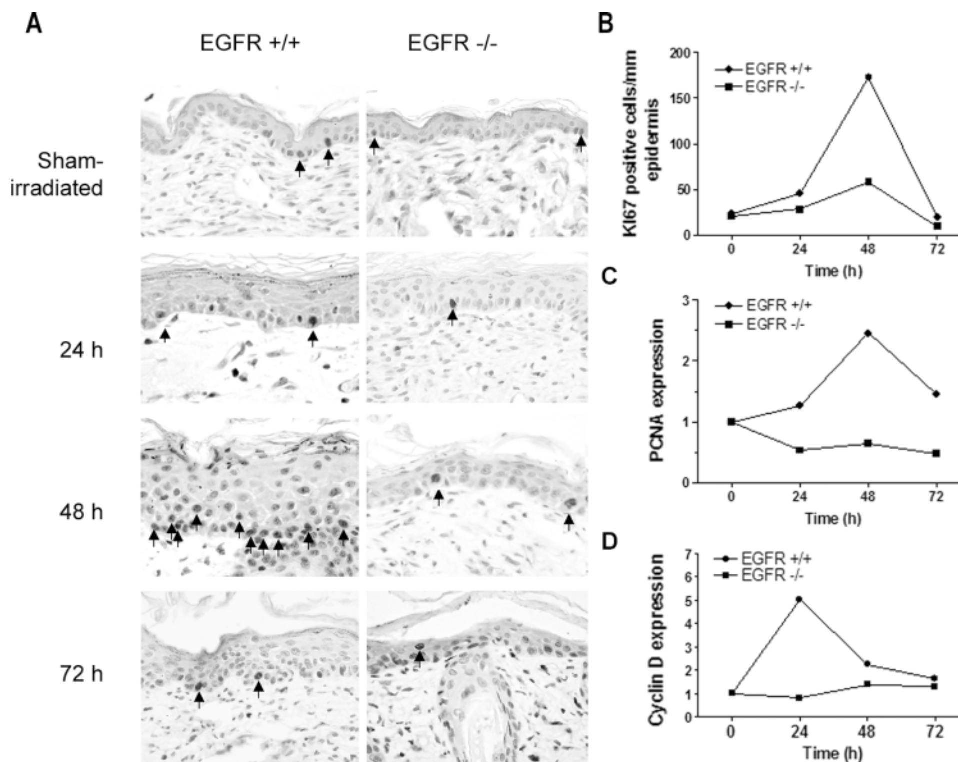

Fig. 3. UV-induced hyperplasia is dependent on EGFR. *Egfr*-null and wild-type skin was exposed to 5 kJ/m^2 UV or sham-irradiated, euthanized at the indicated timepoints, and Ki67 immunohistochemistry (**A** and **B**), PCNA immunoblotting **(C)** or cyclin D immunoblotting **(D)** performed. (A) Representative images from each group are shown. Arrows indicate Ki67-positive keratinocytes. (B) The number of Ki67-positive keratinocytes per millimeter epidermis was counted in at least five randomly selected regions from each slide. Data from four graft skins of each genotype at each time point were averaged and presented in (B). *, Mean is significantly different from the corresponding wild-type control using a Student's *t*-test where $P \leq 0.05$. PCNA (C) and cyclin D (D) expression relative to sham-irradiated controls is shown following normalization of the signal compared with actin. Each point is the mean of samples from three mice. (C,D).

-wild-type skin (Figure 3A-C). Ki67 labeling was similar in sham-irradiated *Egfr*-null and -wild-type skin grafts, consistent with previous results (18). UV exposure slightly increased Ki67 labeling in *Egfr*-wild-type but not *Egfr*-null epidermis at 24 h (Figure 3B). Ki67 labeling was maximally increased in wild-type skin 48 h after UV and returned to baseline levels by 72 h (Figure 3A and B). In *Egfr*-null skin, Ki67 labeling was also increased 48 h after UV exposure and declined to less than baseline levels by 72 h (Figure 3A and B). However, maximal Ki67 labeling in *Egfr*-null skin was less than one-third that of *Egfr*-wild-type skin (Figure 3A and B). PCNA levels largely paralleled the changes in Ki67 labeling, with maximal PCNA expression 48 h after UV in wild-type skin but no increase in PCNA expression after UV exposure of *Egfr*-null skin (Figure 3C). PCNA expression was actually decreased in *Egfr*-null skin after UV exposure (Figure 3C). Thus, although EGFR was not required for baseline epidermal proliferation in untreated skin grafts, UV-induced proliferation depended upon endogenous EGFR expression.

EGFR activation of MAPK signaling pathways induces the expression of cyclins D1 and D2, activating CDK4 and CDK6 and stimulating progression through the G_1 restriction point of the cell cycle. UV exposure strongly induced cyclin D expression with a maximum at 24 h in wild-type skin, which precedes maximum cell proliferation detected using Ki67 and PCNA antibodies (Figure 3D). Cyclin D remained increased 48 h post-UV, at the time of maximum cell proliferation (Figure 3D). In *Egfr*-null skin, in contrast, no induction of cyclin D expression was detected (Figure 3D). Thus, UV-induced proliferation associated with increased G_1 cyclin expression requires EGFR.

EGFR suppresses UV-induced apoptosis

The lack of hyperplasia in *Egfr*-null skin after UV exposure could be an indirect result of an increase in apoptosis, rather than a specific effect of EGFR on proliferation. EGFR has been shown to suppress UV-induced apoptosis through the activation of PI3K/AKT (37). For this reason, the timing and extent of UV-induced apoptosis was determined in *Egfr*-null and wild-type skin grafts. TUNEL analysis revealed increased apoptosis in wild-type controls by 24 h post-UV (Figure 4). Loss of EGFR expression further increased apoptosis by 26% at 24 h and 43% at 48 when compared with the corresponding wild-type controls (Figure 4). At 48 h this increase corresponds to an increase of 13 TUNEL positive cells/mm in *Egfr*-null compared with wild-type skin (Figure 4). However, the number of Ki67-labeled keratinocytes was 125 cells/mm lower in the *Egfr*-null skin compared with wild-type at 48 h (Figure 3), a much bigger effect compared with that of EGFR on apoptosis. This difference in the magnitude of effect on proliferation compared with apoptosis is consistent with a specific stimulation of cell proliferation by EGFR, in addition to its documented effect on apoptosis.

EGFR suppresses p53 and p21 expression following UV

The CDK inhibitor p21 is induced following UV irradiation (6). p21 blocks cell cycle progression through G_1 and S-phases by inhibition of cyclin/CDK complex activity leading to cell

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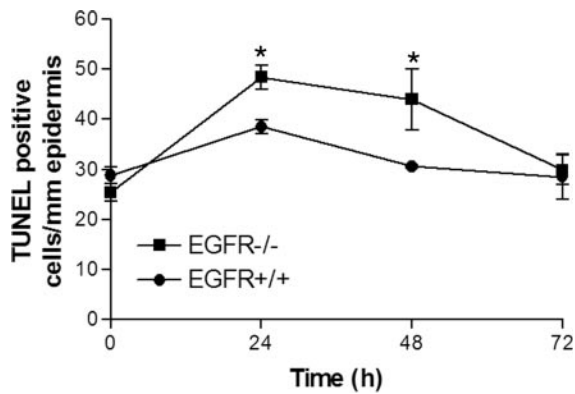

Fig. 4. EGFR suppresses UV-induced apoptosis. *Egfr*-null and wild-type skin was exposed to 5 kJ/m^2 UV or sham-irradiated, euthanized at the indicated timepoints and TUNEL performed. The number of TUNEL-positive keratinocytes per millimeter epidermis was counted in at least five randomly selected regions from each slide. Data from four grafts of each genotype at each time point were averaged and are presented as mean ± SE. *, Mean is significantly different from the corresponding wild-type control using a Student's *t*-test where $P \leq 0.05$.

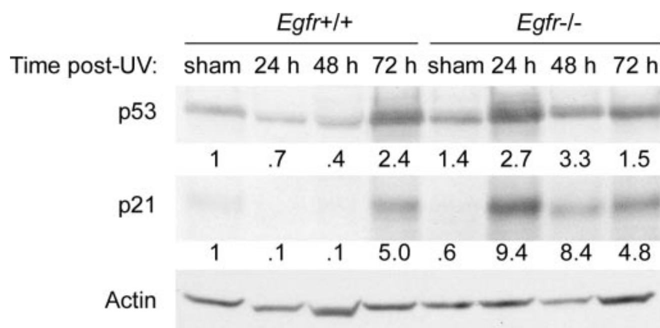

Fig. 5. EGFR suppresses p53 and p21 induction by UV. *Egfr*-null and wild-type mouse skin grafted to nude mouse hosts was immunoblotted for the indicated antibodies after sham-irradiation, or 24, 48 or 72 h post-UV. Signal relative to actin is shown under each panel. Each lane is homogenate from a separate graft. Experiment is representative of three experiments performed.

cycle arrest (reviewed in Ref. 38). To determine whether EGFR suppresses p21-dependent cell cycle arrest after UV exposure, p53 and p21 expression was examined in UV-exposed *Egfr*-null and wild-type skin grafts. Although p53 and p21 are reportedly increased in mouse skin 24 h post-UV (39), induction of both in wild-type skin did not occur until 72 h in our experiments (Figure 5). p53 and p21 expression was increased by ~2- and 5-fold, respectively, 3 days post-UV in *Egfr*-wild-type skin when compared with sham-irradiated controls (Figure 5). Both p21 and p53 expression were elevated several fold in *Egfr*-null compared with wild-type skin at both 24 and 48 h post-UV (Figure 5). These results are consistent with EGFR's suppression of p21 through a p53-dependent mechanism following UV and may reduce proliferation after UV exposure in *Egfr*-null skin.

Discussion

This research demonstrates that UV exposure rapidly activates EGFR in mouse skin, as previously reported in cultured keratinocytes and in human skin (33,34,40). The equivalent of minutes of exposure to summer sunlight in continental United States was sufficient to activate EGFR in mouse skin. Phosphorylation on tyrosines 992, 1045, 1068, and 1173 of the receptor was increased in response to UV. Although Iordanov *et al*. found EGFR phosphorylation on tyrosine residue 1086 was also increased following UV exposure of keratinocytes (33), we did not. This discrepancy may be due to technical differences in the sensitivity of the assays, species differences or differences in the response of the skin compared with cultured cells. Investigations of EGFR activation following UV exposure have not always yielded consistent results. In fact, one group has failed to detect any increase in the phosphorylation of EGFR after UV exposure (41,22).

The proliferative effects of UV (39) and of EGFR (16,18) in the skin have been independently documented. In *Egfr*-wild-type graft skin, proliferation, as quantified by Ki67 labeling, increased 6-fold following UV exposure, although the increase was less when measured using PCNA immunoblotting. Associated with the elevated proliferation was a hyperplastic response in the epidermis that peaked 48 h after UV. In the absence of EGFR, UV induced little proliferation and did not cause epidermal hyperplasia. Thus, UV-induced proliferation and epidermal hyperplasia depended on EGFR. As reported previously (11,37), EGFR suppressed UV-induced apoptosis although in this study the magnitude of EGFR's effect on apoptosis was less than on proliferation. Thus, this research demonstrates that UV stimulates cell proliferation through EGFR-dependent mechanisms. Because the induction of apoptosis precedes the stimulation of proliferation after UV, we propose that proliferation occurs in response to a wave of apoptotic cell death.

The UV response involves activation of numerous signal transduction pathways culminating in altered gene expression and in the regulation of cell division and death. Much of the UV response is believe to be due to the activation of MAPK and NFκB pathways. Many of these signaling pathways are known to be downstream of EGFR and, in fact, the activation of PI3K, ERK1/2 and JNK following UV irradiation of keratinocytes has previously been shown to be dependent on EGFR (33,37,43). Surprisingly, however, ERK activation was uncoupled from EGFR activation following UV in some reports (33). MAPK pathways upregulate cyclin D expression leading to progression through the G_1 phase of the cell cycle. Cyclin D1 has been shown to overcome UV-induced cell cycle arrest (44). UV induced cyclin D expression in wild-type but not *Egfr*-null keratinocytes. Cyclin D induction preceded maximal proliferation in *Egfr*-wild-type skin, consistent with the induction of cyclin D by EGFR activation of MAPK and PI3K pathways leading to cell division.

UV-activated ERK, JNK and p38 kinase also phosphorylate p53 and prevent its activation (13,14,45), although p38 also reportedly increases p53-dependent apoptosis (reviewed in Ref. 46). Since p53 is upregulated by UV exposure and it in turn induces the cyclin-dependent kinase inhibitor p21, it is another potential mechanism by which EGFR-dependent activation of MAPKs might regulate cell cycle progression following UV. UV induces p21 expression maximally 24–48 h post-UV (39,47). p21 upregulation has been implicated in DNA damage-induced cell cycle arrest by inhibiting PCNA in some but not all reports (48–52). Consistent with this hypothesis, abrogation of EGFR increased p53 and p21 expression. Surprisingly, however, no increase in p21 expression was observed in wild-type skin until 72 h post-UV, possibly reflecting differences between intact and graft skin. UV reportedly can induce p21 in a p53 dependent manner (6) although it

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can also be regulated independent of p53 (53,54). However, at low doses UV has also been shown to decrease p21 protein levels by upregulating ATR-dependent p21 ubiquitination and proteasomal degradation (55). This is a likely mechanism for the observed downregulation of p21 expression in *Egfr*-wild-type skin grafts 1–2 days following UV. The increase in p21 expression in *Egfr*-null skin in response to UV exposure may suggest a link between EGFR and ATR following UV.

EGFR-dependent activation of MAPK and PI3K signaling pathways in UV-exposed skin may well have other effects on the response of the skin to UV. UV-activated MAPKs have been shown to decrease p53-mediated apoptosis through the phosphorylation of p53 (13,14,45). ERK MAPKs activate IκBα kinase, a key step in the activation of NFκB, a regulator of UV-induced apoptosis (56,57). PI3K/AKT signaling is also an established EGFR-dependent mechanism for the suppression of apoptosis. In addition, long-term inhibition of EGFR increases apoptosis in cultured keratinocytes (58). Our data are consistent with suppression of apoptosis by EGFR in response to UV through these or other mechanisms.

This research demonstrates that EGFR is a major regulator of the UV response of the skin. Keratinocyte proliferation and epidermal hyperplasia following UV exposure were dependent on EGFR activation and associated with increased cyclin D expression and suppression of p21 expression. The stimulation of cell proliferation and hyperplasia followed a wave of apoptosis in response to UV that was suppressed by EGFR. Thus, EGFR is a central modulator of the UV response of the skin that regulates cell proliferation through both p53-dependent and independent mechanisms.

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Conflict of Interest Statement: None declared.

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