Chemoprevention of UV Light-Induced Skin Tumorigenesis by Inhibition of the Epidermal Growth Factor Receptor

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Abstract

The epidermal growth factor receptor (EGFR) is activated in skin cells following UV irradiation, the primary cause of nonmelanoma skin cancer. The EGFR inhibitor AG1478 prevented the UV-induced activation of EGFR and of downstream signaling pathways through c-Jun NH2-terminal kinases, extracellular signal-regulated kinases, p38 kinase, and phosphatidylinositol 3-kinase in the skin. The extent to which the UV-induced activation of EGFR influences skin tumorigenesis was determined in genetically initiated v-rasHa transgenic Tg.AC mice, which have enhanced susceptibility to skin carcinogenesis. Topical treatment or i.p. injection of AG1478 before UV exposure blocked the UV-induced activation of EGFR in the skin and decreased skin tumorigenesis in Tg.AC mice. AG1478 treatment before each of several UV exposures decreased the number of papillomas arising and the growth of these tumors by ~50% and 80%, respectively. Inhibition of EGFR suppressed proliferation, increased apoptotic cell death, and delayed the onset of epidermal hyperplasia following UV irradiation. Genetic ablation of Egfr similarly delayed epidermal hyperplasia in response to UV exposure. Thus, the UV-induced activation of EGFR promotes skin tumorigenesis by suppressing cell death, augmenting cell proliferation, and accelerating epidermal hyperplasia in response to UV. These results suggest that EGFR may be an appropriate target for the chemoprevention of UV-induced skin cancer. (Cancer Res 2005; 65(9): 3958-65)

Introduction

UV irradiation is the major cause of the >1 million cases of nonmelanoma skin cancer arising each year. UV causes both DNA damage and epigenetic effects in response to DNA damage (1). The importance of UV-induced DNA damage, such as frequent p53 mutations and occasional rasHa mutations in the resulting skin tumors, is clear (reviewed in ref. 1). The influence of the epigenetic effects of chronic UV exposure on skin carcinogenesis, however, is less understood. Skin responds to UV by activating numerous signal transduction pathways, such as the mitogen-activated protein kinase (MAPK) signaling cascades that coordinate cell cycle arrest, up-regulation of DNA damage repair pathways, and apoptosis (2–4). Whereas extracellular signal-regulated kinases (ERK) are critical in response to mitogenic stimuli under normal conditions, p38 kinase and c-Jun NH2-terminal kinases (JNK) are activated in response to stress, such as exposure to UV irradiation (5).

The epidermal growth factor receptor (EGFR), an activator of MAPK signaling pathways, is rapidly activated following exposure to UV. EGFR activation following UV exposure is a result of both the induction of EGFR ligands and the inactivation of phosphatases that would otherwise inactivate the receptor tyrosine kinase (6–8). UV-induced activation of EGFR up-regulates several MAPK (4, 9) and phosphatidylinositol 3-kinase (PI3K)/AKT (10) signaling pathways that control epidermal cell division and cell death, processes whose deregulation is critical during tumorigenesis. EGFR has been implicated previously in mouse skin carcinogenesis, because genetic ablation of the receptor reduces skin tumor growth (11). Conversely, overexpression of EGFR ligands leads to spontaneous mouse skin tumorigenesis (12). The receptor is also up-regulated in many human tumors, including epithelial cancers, through gene amplification, constitutively activating mutations, or increased ligand expression (13, 14). Because of these data, we hypothesized that EGFR activation in response to UV may be important in the epigenetic events, or tumor promotion, of UV-induced skin carcinogenesis.

To determine the effects of EGFR activation on the promotion of UV-induced skin tumors, an EGFR inhibitor was applied before UV exposure of mice harboring an initiating mutation in the skin. Both practical and scientific considerations led to the selection of v-rasHa transgenic Tg.AC mice for this experiment. This genetically initiated mouse model rapidly develops skin tumors in response to both mutagenic and nonmutagenic human carcinogens (15–17). The use of Tg.AC mice, with their uniform initiating mutation and rapid response to skin carcinogens, allows for an examination of the influence of EGFR activation in the promotion process independent of any initiating effects. rasHa mutations occur in a low but variable number of human nonmelanoma skin cancers (18), supporting the relevance of this model for skin cancer research.

In this mouse model, the EGFR inhibitor AG1478 blocked the UV-induced activation of EGFR and of EGFR-dependent signaling pathways in the skin. Both the number and the size of skin tumors arising in UV-exposed Tg.AC mice were reduced on inhibition of EGFR, consistent with a role for the UV-induced activation of EGFR in the promotion of skin tumors. Inhibition of EGFR suppressed tumorigenesis by decreasing cell proliferation, enhancing apoptotic cell death, and delaying epidermal hyperplasia in response to UV. We propose that abrogation of EGFR activity through pharmacologic means may be useful in the prevention of tumorigenesis in skin chronically exposed to UV. EGFR inhibitors have been used in various clinical trials, usually for the treatment of late-stage cancers rather than chemoprevention trials (reviewed in ref. 19). However, chemoprevention offers obvious advantages compared with treatment of existing cancer, including the avoidance of cancer morbidity and mortality. Although additional
research needs to be done, our data suggest that the application of EGFR inhibitors before UV exposure may be a potentially useful and novel strategy for the prevention of UV-induced skin tumorigenesis.

Materials and Methods

Chemicals. Fetal bovine serum was purchased from Gemini Bioproducts (Woodland, CA) and AG1478 from Calbiochem (San Diego, CA).

Animals. The dorsal hair of mice was trimmed with electric clippers at least 1 day before UV exposure. Some mice were topically treated or injected i.p. with 150 mg/kg AG1478 in DMSO or the vehicle DMSO alone 2 hours before UV exposure. FS40T12 sunlamps (Westinghouse, Pittsburgh, PA) emitted ~70% UVB, 30% UVA, and <1% UVC, with a total output of 1.46 mW/cm², as measured with radiometric photodetector probes (Oriel, Stratford, CT). Tumors in age-matched homozygous female Tg.AC mice were counted and tumor volume was measured using calipers. Epidermis null and wild-type newborn mice were genotyped (20), and full-thickness skin was grafted onto athymic nude mice (21).

Microscopy. Following antigen retrieval, sections were incubated with antibodies to Ki-67 (Novocastra, United Kingdom), keratin 1 (Covance, Richmond, CA), or keratin 6 (Covance); Alexa Fluor 488–conjugated secondary antibody (Molecular Probes, Eugene, OR); and 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). Apoptotic cells were identified by nuclear deoxyribonucleotides using fluorescence microscopy. The number of nucleated epidermal cell layers was counted in randomly selected regions from each H&E-stained sample. The thickness of the epidermis from the epidermal-dermal junction to the distal edge of the stratum granulosum was measured in the same regions of each slide using ocular and stage micrometers. Measurements were done in at least four randomly selected regions on each slide, with the investigator blinded as to the identity of the samples.

Immunoprecipitation and immunoblotting. The epidermis was separated from the skin using the heat shock method (22). Epidermis were homogenized or cells were lysed in buffer containing 10 mM/L Tris (pH 7.4), 150 mM/L NaCl, 1% glycerol, 1% Triton X-100, 1 mM/L EDTA, complete protease inhibitor (Roche, Germany), 1 mmol/L Na 3VO4, 1.5 mM/L EGTA, and 10 mM/L NaF, and protein was measured using the Bio-Rad assay (Hercules, CA). Some samples were immunoprecipitated using an anti-phosphorysine antibody conjugated to protein A agarose–conjugated beads. Immunoblotting with antibodies recognizing EGFR (Life Technologies, Carlsbad, CA); phospho-1068 EGFR, phospho-AKT, AKT, phospho-ERK1/2, ERK1/2, phospho–p38 kinase, JNK, p38 kinase, phospho-JNK, and the appropriate horse radish peroxidase–conjugated secondary antibody (all from Cell Signaling, Beverly, MA); and chemiluminescence reagents (Pierce, Rockford, IL) was done.

Flow cytometry. Keratinocytes were isolated from the skin as described in ref. 23 by digesting the skin 1 hour in trypsin at 32°C, separating the epidermis and dermis using a cell scrapper, and resuspending the epidermis in 10 μg/mL RNase A, 0.1% NP40, and 85 μg/mL propidium iodide in PBS. At least 10,000 cells from each sample were analyzed by flow cytometry.

Results

AG1478 inhibits the UV-induced activation of epidermal growth factor receptor in vivo. Exposure of mouse skin to UV activated EGFR within minutes as measured by its phosphorylation (Fig. 1A and B). This activation occurred as early as 2 minutes post-UV and persisted for 1 hour (Fig. 1A). By 24 hours after exposure, EGFR activity had returned to less than constitutive levels (Fig. 1A). A second increase in EGFR activation was observed 48 hours post-UV (Fig. 1A) likely due to the up-regulation of EGFR ligands that occurs with a slower time course (8). Because of these data and the growth-promoting effects of EGFR in other skin tumorigenesis models, we hypothesized that inhibition of the UV-induced activation of EGFR would prevent UV-induced skin tumorigenesis. To test this hypothesis, we developed a method for the pharmacologic inhibition of EGFR in the skin. Topical or i.p. injection of the tyrosphostin EGFR inhibitor AG1478 prevented the UV-induced activation of EGFR (Fig. 1B). The effects of the inhibitor were transient, substantially suppressing EGFR phosphorylation in the first 24 hours post-UV (data not shown).

Inhibition of epidermal growth factor receptor suppresses UV-induced skin tumorigenesis. To test our hypothesis that the inhibition of EGFR prevents skin tumor promotion by UV, the effects of AG1478 on UV-induced skin tumorigenesis were determined in v-ras1 transgenic Tg.AC mice. Tg.AC mice were topicaly treated with AG1478 or the vehicle DMSO alone before each of three exposures to 5 kJ/m² UV (15 kJ/m² cumulative exposure), 10 kJ/m² UV (30 kJ/m² cumulative exposure), or sham irradiation. Tumors first arose 3 to 4 weeks after the first UV exposure (Fig. 2A). Tumor multiplicity was reduced by ~50% in mice pretreated with AG1478 (Fig. 2A). The time until plateau of tumor multiplicity in AG1478-treated and UV-exposed mice was 1 week later than that of the vehicle-treated mice in both 15 and 30 kJ/m² UV exposure groups (Fig. 2A). Similar to other Tg.AC tumorigenesis experiments (24), one sham-irradiated group developed a low number of tumors (Fig. 2A and B).

The effects of AG1478 on tumor size were as striking as its effects on tumor numbers. By the end of the experiment, mean tumor volume was 80% lower in mice treated with AG1478 before exposure to 30 kJ/m² when compared with vehicle-treated mice with the same UV exposure (Fig. 2B). In mice exposed to 15 kJ/m², AG1478 treatment decreased tumor volume by 40% (Fig. 2B). The experiment was ended at 12 weeks because the number of tumors had plateaued and some mice had very high tumor burdens. All tumors of a representative sample examined histologically at 12 weeks were benign squamous papillomas (Fig. 2C).

To confirm that the effects of topical AG1478 on UV-induced skin tumorigenesis were not due to a nonspecific sunblock effect, a second tumor experiment was done using i.p. injection rather than topical treatment of AG1478. For this experiment, groups of Tg.AC mice were injected i.p. with AG1478 or the vehicle DMSO alone 2 hours before a single exposure to 10 kJ/m² UV. The experimental design was modified for this experiment, from three UV exposures using topical inhibitor application to a single exposure with injection of the inhibitor, because of the toxicity of i.p. injection of the vehicle DMSO. Although the mice developed fewer tumors than in the previously described experiment due to the lower cumulative UV exposure, AG1478 was also effective in preventing skin tumorigenesis via i.p. delivery. Vehicle-treated and UV-exposed mice developed an average of 4.0 papillomas per mouse by 4 weeks after UV exposure, whereas none of the inhibitor-treated mice developed any tumors during the 10 weeks of the experiment (Table 1). Collectively, these experiments show that inhibition of UV-induced EGFR activation decreases both skin tumor multiplicity and tumor growth.

Epidermal growth factor receptor activity is not suppressed in skin tumors arising following AG1478 treatment. To determine how long-lasting the effects of EGFR inhibition were, EGFR phosphorylation in tumors from AG1478- and DMSO-treated mice was examined. As shown in Fig. 1C, AG1478-treated and sham-irradiated skin had similar levels of EGFR phosphorylation compared with vehicle-treated skin at the end of the tumor
Experiment, ~10 weeks after the last inhibitor treatment. EGFR phosphorylation, although variable, was not increased in UV-induced skin tumors when compared with sham-irradiated mouse skin (Fig. 1C). EGFR activation was not significantly decreased in tumors arising from inhibitor-treated mice when compared with vehicle-treated mice (Fig. 1C). Consistent with these results, proliferation and apoptotic cell death were similar in tumors arising from vehicle- and inhibitor-treated mice as shown by Ki-67 and TUNEL labeling (Fig. 2C). Expression of the keratin differentiation marker keratin 1, which is frequently lost during skin tumor progression, and hyperproliferation-associated keratin 6, which is up-regulated during skin tumorigenesis, was also examined as early markers of tumor progression. K1 and K6 expression was similar in tumors from vehicle- and inhibitor-treated mice (Fig. 2C). These results are consistent with a transient inhibition of EGFR in response to AG1478 treatment. They further suggest that the short-lived, UV-induced activation of EGFR is a powerful promoter of skin tumorigenesis in initiated keratinocytes.

AG1478 inhibits the UV-induced activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase/AKT signaling pathways. Numerous signal transduction pathways are activated in keratinocytes in response to UV. Among these, MAPKs and PI3K/AKT signaling are downstream from EGFR (4, 9, 25). To determine the effects of EGFR inhibition on signal transduction in response to UV, MAPK and PI3K/AKT activity was examined in inhibitor- or vehicle-treated and UV-exposed mouse skin. Similarly to previously published results using keratinocytes in culture (26), a robust phosphorylation, and thus presumably activation, of JNK was detected within minutes following exposure of mouse skin to UV, coincident with the timing of EGFR activation (Fig. 1D). Although pretreatment with AG1478 slightly increased the basal level of phosphorylated JNK, it prevented the UV-induced activation of JNK (Fig. 1D). The activation of p38 kinase, as measured by immunoblotting with a phospho-specific p38 kinase antibody, was increased 5 minutes following UV exposure (Fig. 1D). Inhibition of EGFR prevented the UV-induced activation of p38 kinase (Fig. 1D). ERK MAPKs are regulators of proliferation, which are phosphorylated weakly after UV in some reports (25, 27, 28). ERK1/2 phosphorylation was only slightly increased 5 minutes after UV exposure (Fig. 1D). This response was inhibited by AG1478 (Fig. 1D). UV-induced phosphorylation of AKT (Fig. 1D), reported previously in human skin (29), was blocked by AG1478 (Fig. 1D).
apoptotic cell death following UV irradiation was examined at response to UV exposure.

The effect of inhibition of EGFR on apoptotic cell death following UV irradiation was examined at several time points. Although apoptosis reportedly peaks 16 hours after a single UV exposure (30), we found that it occurred somewhat earlier following the second of two weekly exposures of vehicle-treated mice to 10 kJ/m² UV (Fig. 4A). The percentage of sub-G₁ apoptotic cells, as detected by DNA content flow cytometry, was increased by ~70% in vehicle-treated epidermis 8 hours after UV when compared with vehicle-treated and sham-irradiated skin at the same time point (Fig. 4A). Apoptosis was similarly increased in AG1478-treated skin 8 hours following UV (Fig. 4A). However, although apoptosis declined to control levels at later time points in UV-exposed, vehicle-treated skin, it increased between 8 and 16 hours on inhibition of EGFR (Fig. 4A). Apoptosis in UV-exposed and AG1478-treated skin was increased ~5-fold 16 hours post-UV when compared with UV-exposed and vehicle-treated skin and remained significantly elevated at 24 hours (Fig. 4A). TUNEL labeling revealed a similar 4-fold increase in apoptosis in AG1478-treated epidermis when compared with vehicle-treated, UV-exposed skin at 16 hours (Fig. 4B and C). By 40 hours post-UV, apoptosis declined to basal levels in inhibitor-treated skin (Fig. 4B).

Inhibition of epidermal growth factor receptor delays UV-induced epidermal hyperplasia. Alterations in the balance between cell division and cell death in response to UV can result in epidermal hyperplasia, which is strongly associated with tumor promotion. The influence of EGFR on UV-induced epidermal hyperplasia was determined using both pharmacologic and genetic means to abrogate receptor function. Consistent with our analysis of cell proliferation following UV irradiation, a single exposure to 10 kJ/m² UV did not result in epidermal hyperplasia.
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and wild-type mouse skin were grafted onto the backs of athymic

A thickness (Fig. 5

number of nucleated cell layers and increased epidermal compared with inhibitor-treated skin as indicated by an increased vehicle-treated skin was significantly more hyperplastic when UV-exposed mice, they were both smaller and fewer than in the vehicle-treated and UV-exposed mice. These results are consistent with previous evidence of the oncogenic function of EGFR in tumorigenesis in other mouse skin models (11, 32). However, they are particularly striking given the transient effects of the EGFR inhibitor. The effects of AG1478 on EGFR activity were pronounced only during the first day after UV exposure, indicating that short-lived EGFR activation in response to multiple UV exposures can have a profound effect on skin tumorigenesis.

Abrogation of EGFR signaling increased UV-induced apoptotic cell death in the skin. Earlier studies revealed that the inhibition within 3 days of exposure (data not shown). Because of this, hyperplasia was examined following the second of two weekly exposures to UV. Seven days following a single exposure to 10 kJ/m² UV, at the time of the second UV exposure, epidermal hyperplasia was observed in both AG1478- and vehicle-treated skin (Fig. 5A, 0 hour). Eight hours following the second UV exposure, the number of nucleated epidermal cell layers decreased in both inhibitor- and vehicle-treated epidermis (Fig. 5B), consistent with increased cell death due to apoptosis (Fig. 4A). Epidermal thickness was reduced in inhibitor-treated but not vehicle-treated skin at 8 hours as well (Fig. 5B). At both 8 and 16 hours post-UV, vehicle-treated skin was significantly more hyperplastic when compared with inhibitor-treated skin as indicated by an increased number of nucleated cell layers and increased epidermal thickness (Fig. 5A and B). In the vehicle-treated but not AG1478-treated mice, the number of nucleated epidermal cell layers recovered by 40 hours to a level similar to that at the time of the second irradiation (Fig. 5A and B). The effects of the inhibitor diminished over time, although the trend remained the same as late as 60 hours after UV (Fig. 5A and B).

UV-induced epidermal hyperplasia is delayed in epidermal growth factor receptor null skin. Like all pharmacologic inhibitors, AG1478 may have nonspecific effects. To determine whether genetic ablation of Egfr would have similar effects, Egfr null and wild-type skin was examined following UV. Because Egfr null mice survive for only a few days after birth, newborn Egfr null and wild-type mouse skin were grafted onto the backs of athymic nude mice and allowed to heal before UV irradiation. Because of the sensitivity of the athymic nude mice to UV, the mice were exposed to less UV per irradiation but were irradiated thrice, for a cumulative exposure of 15 kJ/m². As reported previously in graft skin (31), the number of nucleated epidermal cell layers and the epidermal thickness was increased in Egfr null compared with Egfr wild-type graft skin (Fig. 6A, top). Egfr wild-type skin, but not Egfr null skin, exhibited increased epidermal hyperplasia 24 hours after the third UV exposure (Fig. 6A and B). Epidermal hyperplasia did not significantly increase in UV-exposed Egfr null skin until 2 days after UV, when it reached the same level as in wild-type controls (Fig. 6A), although the fold increase when compared with controls is less than that of Egfr wild-type skin (Fig. 6B). Interestingly, epidermal hyperplasia in Egfr null skin exceeded that of wild-type controls by 3 days post-UV (Fig. 6A). However, because of the increased thickness and number of cell layers in sham-irradiated Egfr null skin compared with wild-type, the fold increase in hyperplasia 3 days post-UV is similar in skin from both genotypes (Fig. 6B). Thus, the appearance of UV-induced epidermal hyperplasia was delayed in the absence of the receptor, consistent with our results using the EGFR inhibitor AG1478. Both methods for abrogation of EGFR activity revealed a requirement for the receptor in epidermal hyperplasia at early time points in response to UV.

Discussion

The research presented herein shows that the UV-induced activation of EGFR promotes UV-induced skin tumorigenesis. Our data support a model in which the activation of EGFR by UV activates MAPK and PI3K signaling pathways, which in turn stimulate cell proliferation and suppress apoptosis, cumulatively resulting in epidermal hyperplasia and eventual promotion of skin tumors. Although tumors arose in inhibitor-treated and UV-exposed mice, they were both smaller and fewer than in the vehicle-treated and UV-exposed mice. These results are consistent with previous evidence of the oncogenic function of EGFR in tumorigenesis in other mouse skin models (11, 32). However, they are particularly striking given the transient effects of the EGFR inhibitor. The effects of AG1478 on EGFR activity were pronounced only during the first day after UV exposure, indicating that short-lived EGFR activation in response to multiple UV exposures can have a profound effect on skin tumorigenesis.

Abrogation of EGFR signaling increased UV-induced apoptotic cell death in the skin. Earlier studies revealed that the inhibition

<table>
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<tr>
<th>Treatment/application method</th>
<th>Mean no. papillomas per mouse</th>
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<tr>
<td>Sham-irradiated 10 kJ/m² UV</td>
<td>2.3 ± 1.1</td>
</tr>
<tr>
<td>DMSO/topical</td>
<td>ND</td>
</tr>
<tr>
<td>DMSO/l.p. injection</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>AG1478/topical</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>AG1478/l.p. injection</td>
<td>0 ± 0.0</td>
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NOTE: Mice were treated with AG1478 in DMSO or the vehicle alone via i.p. injection or topical application as described in Materials and Methods 2 hours before a single UV irradiation or sham irradiation, and papillomas were counted weekly. Mean number of papillomas per mouse at 10 weeks. ND, not determined.
of EGFR sensitized keratinocytes to apoptosis following UV (33, 34). UV irradiation activates both death receptor and mitochondrial apoptotic pathways in keratinocytes in culture. Our data suggest that EGFR suppresses cell death through mitochondrial-mediated mechanisms, such as the activation of PI3K/AKT signaling (29). The suppression of UV-induced cell death would be expected to increase skin tumorigenesis. Consistent with this hypothesis, deficiency of the PTEN phosphatase, an inhibitor of PI3K/AKT pathway, enhances epidermal hyperplasia and skin tumor formation (35).

Inhibition of UV-induced EGFR activation also decreased proliferation following UV. Our data suggest that the suppression of proliferation by abrogation of EGFR activity may be mediated by the loss of activation of MAPK signaling pathways, because AG1478 blocks ERK, JNK, and p38 kinase activation in response to UV. Although increased proliferation has been reported after a single UV exposure (30), we found no increase in keratinocyte proliferation in the first few days after a single exposure to UV. This discrepancy is likely a result of differing UV exposures and/or strain-dependent differences. For this reason and also because the focus of the research was on the later end point of tumorigenesis, the influence of inhibition of EGFR on UV-induced proliferation was examined after multiple UV exposures. Increased cell death and decreased cell proliferation on inhibition of EGFR combined to suppress epidermal hyperplasia following multiple UV exposures.

The magnitude of the effects of AG1478 on cell death (a 4– to 5-fold increase) was greater than its effect on cell proliferation (a 65% decrease) following UV. Thus, increased cell death seems to be the dominant mechanism for suppression of UV-induced epidermal hyperplasia by inhibition of EGFR. However, a greater influence of EGFR inhibition on cell proliferation might be revealed using a lesser UV exposure in which proliferation would be expected to increase within 24 hours post-UV (30). Because epidermal hyperplasia is tightly linked to tumor promotion in the skin, this is likely the mechanism by which inhibition of EGFR decreases UV-induced skin tumorigenesis. We propose that the effects of suppression of proliferation and enhancement of cell death by inhibition of UV-induced EGFR activation increase with each exposure to UV. Thus, the cumulative effects of EGFR inhibition after many exposures could substantially suppress tumorigenesis.

EGFR phosphorylation, apoptotic cell death, and cell proliferation were similar in tumors from vehicle- and inhibitor-treated mice, consistent with a transient inhibition of EGFR by AG1478. These data support our conclusion that the brief UV-induced activation of EGFR plays an early and critical role in promoting tumorigenesis in initiated keratinocytes following UV exposure. Because abrogation of EGFR activity both reduces proliferation and increases apoptotic cell death, inhibition of EGFR before UV exposure may also allow time for repair of DNA damage and thus decrease the number of mutations following UV irradiation. Whether EGFR inhibition does, in fact, decrease the number of cells harboring initiating mutations following UV irradiation remains to be determined.

Although the transient effects of AG1478 on UV-induced cell proliferation, cell death, and hyperplasia are consistent with a short-lived inhibition of EGFR, genetic ablation of Egfr resulted in a similar delay in epidermal hyperplasia following UV. Compensatory mechanisms independent of EGFR must thus be responsible for the increased proliferation and hyperplasia in inhibitor-treated and Egfr null skin at later time points. Tumorigenesis experiments in skin-targeted Egfr null mice, currently in development, will provide additional information about the influence of EGFR in UV-induced carcinogenesis. The use of a pharmacologic inhibitor, however, has allowed for an examination of the role of EGFR in UV-induced skin cancer that would have been difficult otherwise. Although the transgenic mice used for these experiments do not perfectly model human skin carcinogenesis, Tg.AC mice were selected because they (a) are a genetically initiated model that allows for isolation of the promotion phase of carcinogenesis, (b) have enhanced sensitivity to skin tumorigenesis, and (c) carry an oncogenic rasH mutation relevant to human skin carcinogenesis.

rasH mutations occur in a variable but low percentage of human nonmelanoma skin cancers (18). This variability is due in part to clear methodologic differences between studies but may also reflect heterogeneity among the populations examined. For
example, \(ras^{Ha}\) mutations occurred in the majority (73%) of squamous cell carcinomas from psoriasis patients receiving psoralen-UVA therapy (36). In addition, deficiency of the xeroderma pigmentosum gene XPA results in a high frequency of \(ras^{Ha}\) mutations in skin tumors following low-level UV exposure of mice (37), suggesting that \(ras^{Ha}\) mutations might be more common under some environmental conditions in patients with DNA repair pathway defects. Further support for the relevance of \(ras^{Ha}\) mutations in the pathogenesis of nonmelanoma skin cancer comes from research in which the introduction of an activated \(ras^{Ha}\) together with either nuclear factor-\(κ\)B blockade or the expression of exogenous CDK4 produced squamous cell carcinomas in primary human epidermal cells grafted onto immunocompromised mice (38, 39). This work suggests that increased activity of \(ras^{Ha}\) effectors, such as the activating B-raf mutations found in a small subset of human squamous cell carcinomas (40), might also contribute to squamous cell carcinoma development. Thus, although \(ras^{Ha}\) mutations are relatively infrequent overall in human squamous cell carcinomas, mutations in \(ras^{Ha}\) and its effectors may well be important in the development of a subset of these cancers, for which mutant \(ras^{Ha}\)-containing models would be especially relevant.

We propose the use of EGFR inhibitors as a potential strategy for the chemoprevention of UV-induced skin cancer. The effectiveness of clinically relevant EGFR inhibitors in suppressing UV-induced skin tumorigenesis, the toxicity of these agents at the concentrations necessary to effectively inhibit the UV-induced activation of EGFR, the efficacy of more clinically useful...
cutaneous delivery systems, and any potential undesirable side effects of this regimen, however, remain to be determined. EGFR inhibitors have, in fact, been reported to induce cutaneous inflammation, a potential problem for their use in chemo-prevention. This reaction might also be exacerbated by the proinflammatory effects of UV, decreasing the utility of the inhibitors for this purpose. Because several approaches for inhibiting EGFR expression or its activity in several preclinical studies and clinical trials were effective in treating several solid tumors (41–44), our data may have practical implications for EGFR-targeted therapeutic strategies for human tumors either alone or in combination with other antitumor therapies. Increased understanding of these and other effects of EGFR inhibitors may prove useful in the prevention and treatment of UV-induced pathologies.

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