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Research Article

EGFR Activation and Ultraviolet Light-Induced Skin Carcinogenesis

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The epidermal growth factor receptor (EGFR) regulates the proliferation of keratinocytes through multiple mechanisms that differ depending on the localization of the cell within the skin. Ultraviolet (UV) irradiation, the main etiologic factor in the development of skin cancer, also activates the receptor. In this review, we discuss how the UV-induced activation of EGFR regulates the response of the skin to UV. UV-induced EGFR activation increases keratinocyte proliferation, suppresses apoptosis, and augments and accelerates epidermal hyperplasia in response to UV. Pharmacological inhibition of the UV-induced activation of EGFR in a genetically initiated mouse skin tumorigenesis model suppresses tumorigenesis and the activation of mitogen-activated protein (MAP) kinases and phosphatidyl inositol-3-kinase (PI3K)/AKT signaling pathways. EGFR has pleiotropic, complex, and cell-type-specific functions in cutaneous keratinocytes; suggesting that the receptor is an appropriate target for the development of molecularly targeted therapies for skin cancer and other pathologies.

1. INTRODUCTION

Ultraviolet (UV) irradiation is the main etiological factor for nonmelanoma skin cancer. UV has a wide variety of effects on the skin that contribute to the induction of skin cancer. Our laboratory has for the last several years focused on the role of the epidermal growth factor receptor (EGFR) in UV-induced skin cancer.

EGFR is the prototypic member and the first receptor identified of the ErbB family of receptor tyrosine kinases (RTKs). The ErbB receptor family includes EGFR as well as three other receptors, ErbB2, ErbB3, and ErbB4. EGFR is a single transmembrane glycoprotein of approximately 170 kd that was discovered in the mid-1970s as a binding site for EGF (epidermal growth factor). EGF is now known to be part of a large family of ligands for ErbB receptors. These growth factors are of three types: those that bind to EGFR including EGF, amphiregulin, and transforming growth factor-α (TGF-α); those that bind to both EGFR and ErbB4 including betacellulin, epiregulin, and heparin-binding EGF-like growth factor, and those that bind to erbB3 and erbB4 including Neu differentiation factors (heregulins) [1].

In naïve skin, EGFR stimulates proliferation, regulates differentiation, and promotes cell survival [2–4]. Upon trauma to the skin, the receptor increases cell adhesion and the migration of keratinocytes during wound reepithelialization [5]. In addition, the effects of EGFR activation depend on the localization of the cell within the skin [4]. EGFR's localization in basal and suprabasal keratinocytes of the epidermis and in the outer root sheath of the hair follicle allows its wide diversity of action within the skin and the potential for multiple roles during skin carcinogenesis.

Increased activation of EGFR family members has been implicated in the development of a variety of human carcinoma (reviewed in [6, 7]). Studies using transgenic mouse models also provide evidence of the importance of EGFR in tumorigenesis. Mice overexpressing cutaneous TGF-α exhibit epidermal hyperplasia, hyperkeratosis, and increased sensitivity to the chemical induction of skin tumors [8–10]. v-*ras*^{Ha}-initiated *Egfr* null skin grafts develop smaller tumors when compared to wild-type control grafts as a result of the premature migration of S-phase cells into the suprabasal differentiating compartment [11, 12].

Moreover, the development of spontaneous papillomas in transgenic mice expressing a dominant form of Son of Sevenless (SOS-F), a guanine nucleotide exchange factor associated with Grb2 adaptor protein that transmits signals from EGFR to *ras*, is reduced in mice bred onto an *Egfr* null background [13, 14]. Pharmacological inhibition of EGFR using specific kinase inhibitors also results in decreased tumor volume in athymic nude mice grafted with EGFR overexpressing cell lines [15]. Because EGFR is highly expressed in many human tumors, two robust anti-EGFR strategies have been used in clinical trials. These studies target EGFR using either small-molecule tyrosine kinase inhibitors or monoclonal antibodies and have been approved for the treatment of non-small cell lung carcinoma, head and neck cancers, and colorectal cancer [7, 16].

From these data and our experiences in characterizing the phenotype of *Egfr* null mice, we hypothesized that UV-induced EGFR activation stimulates keratinocyte proliferation following UV irradiation. We further hypothesized that the repeated activation of EGFR upon exposure of the skin to UV contributes to the development of skin cancer. The present review will describe the results of our research investigating the role of EGFR in the response of the skin to UV.

2. UV IRRADIATION ACTIVATES EGFR

UV irradiation rapidly activates EGFR and a number of other cell surface receptors through reactive oxygen species-mediated inactivation of the cytoplasmic protein tyrosine phosphatases that maintain low basal levels of phosphorylated EGFR [17–21]. Increased EGFR activation is observed within minutes after UV irradiation of cultured keratinocytes or skin [22, 23]. This initial pulse of receptor activation subsides within an hour after UV irradiation, while the induction of EGFR ligands at later time points produces a second later wave of receptor activation as well [17]. The phosphorylation of EGFR family members Erbb2 and Erbb3, indicative of receptor activation, similarly increases following UV irradiation of cultured keratinocytes or skin [24]. UV-activated EGFR in turn activates a number of signaling cascades, including phosphatidyl inositol-3-kinase (PI3K), leading to AKT activation and a suppression of apoptosis [25, 26]. EGFR is also at least partly responsible for the activation of extracellular regulated kinases (ERKs), p38 kinase, and c-Jun NH2-terminal kinase (JNK) following UV exposure [22, 23, 27], all of which are known regulators of cell division. Accordingly, we examined the importance of EGFR in the regulation of proliferation following UV irradiation.

3. EGFR INCREASES CELL CYCLE PROGRESSION THROUGH MULTIPLE MECHANISMS

Genetic ablation of *Egfr* significantly reduces proliferation in the interfollicular epidermis of the skin [3]. Because of EGFR's activation following UV exposure, we hypothesized that EGFR-dependent signaling stimulates keratinocyte proliferation following UV irradiation. To test this hypothesis, genetic ablation of EGFR or an EGFR inhibitor was used in both in vitro and in vivo model systems. Proliferation is decreased in *Egfr* null epidermis and in EGFR inhibitor-treated epidermis [2, 4, 22, 23]. Cultured keratinocytes with abrogated EGFR function arrest in multiple cell cycle phases, depending on the manipulation of the culture conditions. Growth factor stimulation reveals a G_1 phase cell cycle arrest due to loss of EGFR's activation of ERK1/2 and JNK pathways (unpublished observations and [15]). Without exogenous addition of growth factor, ablation of EGFR causes a G_2/M arrest (unpublished observations). Further investigation is required to explain the mechanisms of its regulation of the G_2/M cell cycle checkpoint. However, our data suggest that the cellular environment determines which EGFR-dependent signaling pathways will be dominant.

The in vitro cell cycle data are consistent with our analysis of the role of EGFR in vivo. The role of physiological levels of EGFR in keratinocytes depends quite clearly on the cell's localization within the skin. EGFR is necessary for the normal organization and differentiation of the follicular epithelium but not of the interfollicular epidermis [2, 4]. In contrast, within the interfollicular epidermis, EGFR signaling maintains proliferation while proliferation of follicular keratinocytes is independent of the receptor [3, 4]. Roles for EGFR at multiple cell cycle checkpoints would increase the plasticity of the skin's response to environmental insults such as wounding or UV exposure. Because cell cycle checkpoints provide time for DNA repair after exposure to a mutagen, the control of multiple cell cycle checkpoints by EGFR underscores the potential importance of the receptor in controlling cell cycle progression in response to UV.

In response to UV-induced activation, EGFR increases cell proliferation, suppresses cell death, and augments and accelerates epidermal hyperplasia. In addition, EGFR increases G_1 cyclin expression and suppresses the expression of the CDK inhibitor, $p21^{waf1}$ (see [23] and unpublished observations). The UV-induced activation of ERK1/2, p38 kinase, and JNK MAP kinases as well as PI3K/AKT is largely dependent on EGFR [21]. EGFR's influence on proliferation following UV exposure was mediated by its activation of both JNK and PI3K/AKT [22, 23]. EGFR-mediated activation of MAP kinases may also increase keratinocyte survival. Together with evidence of the antiapoptotic effect of EGFR-dependent PI3K/AKT signaling in response to UV [22, 25, 26], these data reveal the importance of multiple EGFR-activated pathways in cutaneous responses to UV. They also suggest specificity of the various EGFR-dependent signaling pathways in differentially regulating the biological functions of the skin. These data further suggested to us that repeated activation of EGFR upon frequent exposure to UV might promote skin tumor development.

4. EGFR PROMOTES UV-INDUCED SKIN TUMORIGENESIS

To test our hypothesis that repeated activation of EGFR upon chronic exposure to UV contributes to UV-induced skin carcinogenesis, we used a genetically initiated mouse model with enhanced susceptibility to skin tumor development.

This mouse is the v-*ras*^{Ha} transgenic Tg · AC mouse, which rapidly develops skin tumors following UV irradiation. Groups of Tg · AC mice were treated with the EGFR inhibitor AG1478, a tyrphostin that blocks its kinase activity, or the vehicle alone 2 hours prior to UV exposure. Inhibition of the UV-induced activation of EGFR blocks the development of about half of the tumors, with an even larger reduction in tumor growth [21]. These results are especially striking given the relatively brief period of receptor inhibition over the course of the tumor experiment. The reduction in tumor development upon inhibition of EGFR is associated with a suppression or delay in the development of epidermal hyperplasia after UV irradiation, an effect observed in UV-exposed *Egfr* null skin grafts as well [21]. EGFR increases UV-induced epidermal hyperplasia by both increasing cell proliferation and suppressing cell death [21]. These effects of EGFR on cell division and cell death further implicate EGFR in modulating DNA repair and the acquisition of mutations after UV. Whether deregulated EGFR signaling inhibits DNA damage repair and increases mutagenesis remains to be investigated.

The suppression of UV-induced skin tumorigenesis by inhibition of EGFR suggests that EGFR might serve as a molecular target for chemoprevention or treatment of UV-induced skin cancer. This strategy could prove useful in patient populations with increased susceptibility to UV-induced skin carcinogenesis. For example, organ transplant recipients on immunosuppressive therapy have extraordinarily high rates of very aggressive squamous cell carcinomas associated with UV exposure. Many of these patients develop multiple skin tumors as well. In this population, methods for the prevention of skin cancer are much needed. However, adverse skin reactions, including skin rashes, develop in cancer patients treated with EGFR inhibitors or monoclonal antibodies to the receptor [28], a response predicted by the *Egfr* null mouse phenotype [4, 28]. Additional preclinical and clinical studies to determine creative alternatives to decrease the adverse effects of the inhibitors for future clinical studies targeted to the skin are clearly needed. For example, novel topical drug delivery systems could restrain drug delivery to the epidermis, thus eliminating the dermal side effects of these inhibitors. These trials should also identify the additive or synergistic effect of inhibition of EGFR in combination with the inhibition of other EGFR family members.

5. SUMMARY

In summary, we have demonstrated multiple functions for EGFR in the skin, which depend on the localization of the cell within the skin. Upon UV-induced activation of the receptor, EGFR increases proliferation, suppresses apoptosis, augments hyperplasia, and increases UV-induced skin tumorigenesis. Taken together, these data provide insights into the mechanisms by which EGFR enhances keratinocyte proliferation under normal growth conditions and during UV-induced skin tumorigenesis, as illustrated in Figure 1, which may be important for the treatment or prevention of skin cancer. EGFR's regulation of the response of the skin to UV suggests that interruption of EGFR-dependent signaling prior to UV exposure may be an effective means to prevent UV-induced skin tumorigenesis. Moreover, understanding EGFR-dependent signal transduction after UV exposure will aid in investigations of the role of EGFR in other UV-induced skin pathologies such as sunburn and photoaging. We propose that molecular targeting of EGFR may be an effective strategy in skin carcinogenesis treatment of prevention, particularly in highly susceptible populations such as immune-compromised organ transplant recipients.

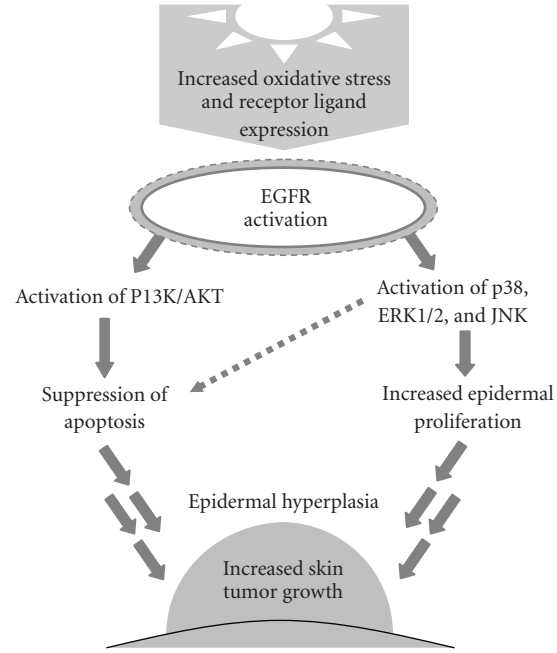

Figure 1: Mechanisms through which the UV-induced activation of EGFR contributes to skin carcinogenesis.

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