The Epidermal Growth Factor Receptor System in Skin Repair and Inflammation

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The epidermal growth factor (EGF) family comprises multiple mediators such as transforming growth factor-α, amphiregulin, heparin binding-EGF, and epiregulin, which are crucially involved in the tissue-specific proliferation/differentiation homeostasis. Typically, they act in an autocrine and paracrine manner on their specific cell membrane receptor and mount an effective reparative response to any attack to biophysical integrity. In addition, the EGFR can be activated by transactivation from a variety of G-protein-coupled receptors, integrins, and cytokine receptors, so that it acts as the major transducer of disparate cell functions, including changes in proliferation rate, cellular shape, attachment and motility, and regulation of proinflammatory activation. However, numerous experimental observations indicate that the different EGFR ligands are not redundant, but may rather provide distinct and specific contributions to keratinocyte functions. Importantly, increasing evidence now suggests that the EGFR pathway has a major impact on the inflammatory/immune reactions of the skin, in the apparent effort of enhancing innate immune defense while opposing overactivation of keratinocyte pro-inflammatory functions. This review covers the molecular mechanisms and functions activated by this major growth factor system in the regulation of keratinocyte biology and focuses on the complex contribution of EGFR signaling to the inflammatory processes in the skin.

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Abbreviations: CCL, CC ligand, chemokine of the CC subfamily; CXCL, CXC ligand, chemokine of the CXC subfamily; CXCR, receptor for CXCL; ERK, extracellular signal-regulated kinase; GPCR, G-protein-coupled receptor; HB, heparin binding; TGF, transforming growth factor; TLR, Toll-like receptors; TNF, tumor necrosis factor

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INTRODUCTION

The epidermal growth factor (EGF) receptor signaling pathway regulates fundamental functions in mammalian cells including survival, migration, and proliferation. Consequently, it has been deeply investigated, both experimentally and computationally (Jost *et al.*, 2000; Oda *et al.*, 2005). A family of ligands binds to distinct subtypes of erythroblastic leukemia viral (v-erb-b) oncogene homolog (ErbB) receptors that activate multiple molecules in an extensive network of receptor complexes. These receptors are expressed on most human cell types, with the possible exception of mature hematopoietic cells. The binding of ligands induces homo- and heterodimerization of four ErbB family receptors: ErbB1 (also universally identified as EGFR), ErbB2, ErbB3, and ErbB4. EGFR, ErbB2, and ErbB3 are expressed in human skin, with evidence of a functional dominance of EGFR (Stoll *et al.*, 2001). Indeed, epidermal keratinocytes are a rich source of EGFR ligands, including transforming growth factor (TGF)-α (Coffey *et al.*, 1987), amphiregulin (Cook *et al.*, 1991), heparin binding (HB)-EGF (Hashimoto *et al.*, 1994), and epiregulin (Shirakata *et al.*, 2000). In unstimulated conditions, cultured keratinocytes display relevant levels of amphiregulin, whereas TGF-α, HB-EGF, and epiregulin are barely detectable at the transcript level (Shirakata *et al.*, 2000). EGFR immunoreactivity can be localized throughout the whole epidermis of normal skin, although it is more accentuated in the basal cell layer (Nanney *et al.*, 1984; Mascia *et al.*, 2003).

ErbB dimer binding by a specific ligand stimulates ErbB cytoplasmic kinase activity leading to auto- and transphosphorylation on tyrosine residues, which serve as docking sites for adaptor proteins and enzymes (Schlessinger, 2000). Between the two lobes of the kinase fold there is the ATP binding pocket, the focus of specific inhibitor design that exploits differences in kinase structure to achieve selectivity. In the last two decades, a number of effective small-molecule inhibitors have been synthesized (Noble *et al.*, 2004), representing invaluable tools for the investigation of EGFR-dependent cell events. Most importantly, they can be clinically effective in enhancing the response to cytotoxic drugs of EGFR-dependent tumors (Slichenmyer and Fry, 2001). The most selective compounds, which include gefitinib and erlotinib, possess a quinazoline nucleus (Noble *et al.*, 2004). Broad-spectrum anti-ErbB inhibitors, such as lapatinib or canertinib, may result in a greater efficacy and broad spectrum of antitumor activity (Arora and Scholar, 2005). Also mAbs, including cetuximab and ABX-EGF, show promise as chemotherapeutic agents (Kondapalli *et al.*,

2005). Notably, both small-molecule EGFR inhibitors and anti-EGFR antibodies typically exert peculiar inflammatory/toxic effects on the skin (Lacouture, 2006), with striking similarities with phenotypes of mice with reduced EGFR function (Roberts *et al.*, 2004).

DISPARATE MECHANISMS OF EGFR ACTIVATION

EGFR can be activated by several mechanisms under physiological or pathophysiological conditions. Apart from direct activation by specific ligands, heterologous ligand-dependent mechanisms are also at work, as demonstrated by the finding that stimulation of a number of G-protein-coupled receptors (GPCRs) results in EGFR activation via metalloproteinase-mediated cleavage of the mature form of EGFR ligands from membrane precursors (Figure 1). During wound healing, angiotensin II stimulates keratinocyte and fibroblast migration through a pathway initiated by the GPCR angiotensin II receptor that leads to HB-EGF shedding and consequent extracellular signal-regulated kinase (ERK) cascade activation (Yahata *et al.*, 2006). Not only specific antagonism to angiotensin II receptor, but also to EGFR, as well as inhibition of the extracellular metalloproteinases can efficiently abrogate angiotensin II pro-healing activity. Similarly, keratinocyte-released catecholamines can stimulate keratinocyte migration and wound re-epithelialization through a mechanism that requires both EGFR and ERK activation (Pullar and Isseroff, 2006). The existence of this ''triple-membrane-passing-signal'' mechanism (Gschwind *et al.*, 2001) underlying inter-receptor signal transmission is now well documented, and is certainly not confined exclusively to GPCR-triggered cell responses. We could recently demonstrate that both TNF-α and IFN-γ can activate this mechanism in human keratinocytes, with consequent induction of EGFR phosphorylation and EGFR-dependent ERK activation (Mascia *et al.*, 2003). EGFR can be activated also through EGFR ligand-independent mechanisms. Integrins form physical complexes at the cell membrane with growth factor receptors, giving rise to cooperative signaling platforms at the adhesive sites (Cabodi *et al.*, 2004). Active protein-tyrosine phosphatases can limit EGFR functions through its dephosphorylation (Xu *et al.*, 2005). In turn, the generation of reactive oxygen species secondary to EGFR activation leads to the reversible inactivation of crucial protein-tyrosine phosphatases by oxidizing the catalytic cysteine in their active site. Noteworthy, oxidative inhibition of protein-tyrosine phosphatase activity by reactive oxygen species underlies EGFR activation by UV irradiation (Xu *et al.*, 2006).

MOLECULAR EVENTS AND CELL FUNCTIONS DRIVEN BY EGFR

The EGFR–ERK pathway plays a crucial role in mediating both the pro-survival and proliferative programs of keratinocytes. Indeed, human epidermal keratinocytes maintain high steady-state levels of ERK activity essentially via their autocrine production of EGFR ligands (Iordanov *et al.*, 2002). The classical signal-transduction cascade that translates EGFR activation into the increased activity of ERK has been extensively investigated (Schlessinger, 2000; Wetzker and Böhmer, 2003). Sites of autophosphorylation in an

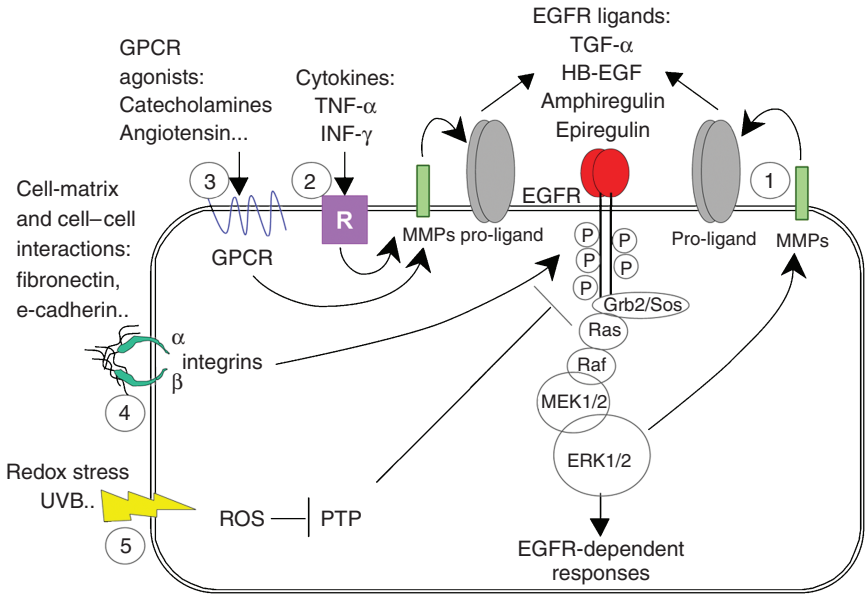

Figure 1. Mechanisms of EGFR activation in keratinocytes. In unstimulated conditions, EGFR contributes to stabilize its own activation via an autocrine/paracrine loop based on *de novo* synthesis and shedding of mature EGFR ligands (**1**). A variety of G-protein-coupled receptor (GPCR) ligands (**2**), but also pro-inflammatory cytokines binding their specific receptors (R) (**3**) promote a rapid metalloproteinase (MMP)-mediated shedding of EGFR ligands from membrane precursors. In addition, after cell–matrix adhesion, integrins associate with the EGFR to trigger downstream signaling through its ligand-independent phosphorylation (**4**). Finally, inactivation of protein tyrosine phosphatases (PTP) by intracellular generation of reactive oxygen species (ROS) in response to pro-oxidative stimuli (**5**) allows the maintenance of EGFR in its activated form, with eventual stimulation of ERK activity.

activated EGFR bind a number of other signaling proteins containing phosphotyrosine-binding domains, including phospholipase C-γ1 and the p85α subunit of phosphatidylinositol 3-kinase. In the phosphatidylinositol 3-kinase-dependent pathway, the phosphorylation of the serine–threonine kinase Akt results in the phosphorylation of a host of other proteins that improve cell survival (Cantley, 2002). The following subtitles will briefly overview major cell functions overtly under the control of EGFR signaling.

Pro-survival programs and protection from apoptosis

Blockade of EGFR signaling either by a specific antibody or by a small-molecule inhibitor does not induce keratinocyte cell death, but rather enhances susceptibility to cell death induction. Similar effects can be observed following ERK inhibition. By contrast, the antiapoptotic response triggered in keratinocytes after UVB is totally driven by EGFR-activated phosphatidylinositol 3-kinase–Akt pathway (Canguilhem *et al.*, 2005) and represents the successful effort to overcome the variety of UVB-induced pro-apoptotic signals. Indeed, rapid and sustained EGFR activation is a well-established event in response to UVB in keratinocytes (Peus *et al.*, 2000; Iordanov *et al.*, 2002), and plays a crucial role in UVB-induced epidermal hyperplasia (El-Abaseri *et al.*, 2006) and eventually in the onset of nonmelanoma skin cancer. An EGFR-dependent phosphatidylinositol 3-kinase-Akt-mediated pro-survival response can be observed in keratinocytes also in response to oxidative stress (Wang *et al.*, 2000; Ravid *et al.*, 2002). EGFR conveys critical survival signals that delay apoptotic death triggered by the loss of matrix interaction (Jost *et al.*, 2001). These ERK-dependent mechanisms may have particular relevance to disease states in which upregulated expression of the EGFR and/or its ligands is commonly observed, including wound healing (Marikovsky *et al.*, 1993) and hyperproliferative skin diseases (Elder *et al.*, 1989; Mascia *et al.*, 2003).

EGFR drives the prototypic mitogenic stimulation

In cultured human epidermal keratinocytes, activation of the EGFR by cognate ligands mediates the autonomous replicative capacity of these cells. Accordingly, specific inhibitors of EGFR signaling block the proliferation of human keratinocytes *in vitro* (Powell *et al.*, 1999). Amphiregulin provides by far the strongest autocrine stimulation to cell growth (Piepkorn *et al.*, 1994), in keeping with the observation that this ligand is the most abundantly expressed in unstimulated keratinocytes (Shirakata *et al.*, 2000), followed by HB-EGF (Hashimoto *et al.*, 1994). By contrast, TGF-α, a potent inducer of itself and other EGFR ligands, does not seem to have direct mitogenic effects on cultured keratinocytes (Pittelkow *et al.*, 1993), whereas there is no evidence that epiregulin stimulates keratinocyte proliferation *in vitro*.

In vivo, upregulated expression of EGFR ligands is a characteristic feature of both benign and malignant hyperproliferative conditions of the skin. In particular, psoriatic lesions overexpress EGFR (Nanney *et al.*, 1986), as well as TGF-α (Elder *et al.*, 1989), amphiregulin (Cook *et al.*, 1992; Piepkorn, 1996), HB-EGF (Stoll and Elder, 1998; Zheng *et al.*, 2003), and epiregulin (Shirakata *et al.*, 2007), with amphiregulin more represented than TGF-α when analyzed in the same lesions at the transcript level (Cook *et al.*, 1992). A humanized mAb capable of neutralizing human amphiregulin not only reduces epidermal thickness of human psoriatic lesions transplanted on the severe combined immunodeficient mouse, but also inhibits the hyperplastic response that develops in nonpsoriatic human skin after transplantation (Bhagavathula *et al.*, 2005). Transgenic overexpression of TGF-α leads to tissue hyperplasia and hyperkeratosis, with spontaneous squamous papillomas occurring at sites of wounding in adult animals in the absence of skin inflammation (Dominey *et al.*, 1993). By contrast, transgenic expression of amphiregulin in either basal or suprabasal epidermis causes severe psoriasis-like hyperplasia and skin inflammation (Cook *et al.*, 1997, 2004). On the whole, these observations point to a strong involvement of amphiregulin in both hyperplasia and inflammation of the psoriatic lesion.

EGFR activation underlies the hyperproliferative effects of retinoids on skin keratinocytes. Indeed HB-EGF is markedly induced in keratinocytes in response to retinoid treatment, both *in vitro* and *in vivo*, and retinoid-dependent hyperproliferation is impaired by EGFR inhibition (Stoll and Elder, 1998; Varani *et al.*, 2001). In normal human skin, the application of all *trans*-retinoic acids induces the specific transcripts of both HB-EGF and amphiregulin but no increase of TGF-α, and neutralization of either HB ligands impairs retinoic acid-dependent epidermal hyperplasia in the organ culture model (Rittié *et al.*, 2006).

Cell adhesion and migration

Integrins are adhesive receptors formed by α- and β-subunits, which anchor extracellular matrix proteins to the actin cytoskeleton and mediate the adhesion of cells to extracellular matrices and other cells. In the basal cells of the epithelia, the α6β4 integrin is concentrated at hemidesmosomes, junctional complexes that anchor cytokeratin intermediate filament networks and mediate adhesion of epithelial cells to the underlying basement membrane (Rezniczek *et al.*, 1998). Integrins trigger multiple signaling pathways, which, on the basis of differential expression and specific localization of the receptors, are involved in cell migration, proliferation, differentiation, and survival (Hynes, 2002). They form physical complexes at the cell membrane with growth factor receptors, and integrin-dependent adhesion triggers ligand-independent EGFR activation to transduce downstream signaling; conversely, integrin-induced signaling appears necessary for full EGFR-dependent transcriptional responses, including those depending on ERK (Cabodi *et al.*, 2004). Clearly, this bidirectional crosstalk suggests the requirement of the cooperation between cell matrix adhesion and EGFR to achieve full biological responses (Bill *et al.*, 2004).

Intercellular adhesion is strengthened by the clustering of cadherin receptors at junctions and by the association between cadherin complexes and the actin cytoskeleton. Cadherin-dependent adhesion is subject to regulation by cytoplasmic proteins, and recent work has focused on the

role of the small GTPases Rho and Rac in the stability of cadherins at cell–cell contacts. These two GTPases function in signal-transduction cascade downstream of a variety of cell surface receptors. In normal keratinocytes, cadherin receptor clustering is sufficient to activate Rac, and cadherin-dependent Rac activation does require EGFR signaling (Betson *et al.*, 2002).

EGFR ligands are potent pro-migratory factors for keratinocytes (Barrandon and Green, 1987). EGFR phosphorylation-dependent disruption of α6β4 at hemidesmosomes and their consequent disassembly is a prerequisite for normal keratinocyte migration and squamous cell carcinoma invasion (Mainiero *et al.*, 1996; Mariotti *et al.*, 2001). Indeed, wounding induce immediate shedding of EGFR ligands from keratinocyte membranes, and inhibition of this process by metalloproteinase inhibitors cause suppression of keratinocyte migration that can be rescued by recombinant EGFR ligands (Tokumaru *et al.*, 2000). In particular, TGF-α represents the major keratinocyte pro-motility factor present in human serum, being responsible of more than 80% of this activity during acute wound healing (Li *et al.*, 2006). In this condition, the basement membrane, which represents the fundamental scaffold for the orchestration of epithelial adhesion and migration, is lost, and keratinocytes must initiate their migration to a connective tissue matrix with a complex composition, dominated by type I collagen. Collagenase-1 (that is, matrix metalloproteinase1) activity is required for keratinocyte migration on a type I collagen matrix (Pilcher *et al.*, 1997), and its sustained production is dependent on autocrine EGFR activation as an obligatory intermediate step (Pilcher *et al.*, 1999). Importantly, ERK activation is involved also in collagen-triggered migration (Li *et al.*, 2004).

THE EGFR-LIGAND SYSTEM DURING WOUND HEALING OF THE SKIN

In cutaneous wound healing, the keratinocyte plays a central role not only as key structural cell type in the repairing skin but also as the source of growth factors, and EGFR-mediated proliferation and migration appear crucial in wound healing. Acute disruption of the permeability barrier by solvents or tape stripping in the mouse stimulates epidermal proliferation by increased expression of amphiregulin and nerve growth factor, but not by TGF-α (Liou *et al.*, 1997). In the model of regenerative wound healing provided by short-term organ culture of human skin biopsies, amphiregulin and HB-EGF, but not TGF-α, are both rapidly and markedly induced, with EGFR inhibition blocking the expression of EGFR ligands and epidermal cell outgrowth from the explants (Stoll *et al.*, 1997). In actual healing skin wounds, HB-EGF transcript appears by far the most strongly upregulated (Stoll *et al.*, 1997). The application of a specific inhibitor of the EGFR ligand shedding to wound sites in normal mice greatly retards re-epithelization as the result of a failure in keratinocyte migration, an effect that can be overcome by the application of recombinant soluble HB-EGF (Tokumaru *et al.*, 2000). In EGFR-null mice, re-epithelialization during the first 3 days is greatly impaired, although this partial block seems to be overcome by the intervention of other growth factor systems later on (Repertinger *et al.*, 2004). Remarkably, the wounds of these mice display an abnormally intense and diffuse inflammatory cell infiltrate especially rich in neutrophils, possibly due to an altered chemokine expression in epidermal keratinocytes. Persistence of an excess pro-inflammatory infiltrate at the wound site delays wound closure by the establishment of a hostile, unbalanced microenvironment, dominated by proteolytic rather than protective mechanisms (Eming *et al.*, 2007). A human wound-healing study also demonstrated that topical application of recombinant EGF enhanced the healing of split-thickness wounds (Brown *et al.*, 1989). In cancer patients, systemic administration of the EGFR inhibitor gefitinib decreases epithelial proliferation and stratification in response to corneal injury (Nakamura *et al.*, 2001). However, this drug does not impair wound healing following full-thickness incision (Govindan *et al.*, 2003), suggesting that redundant growth factor systems are set up in the skin to successfully replace missing EGFR functions.

EGFR-SPECIFIC EFFECTS ON THE INFLAMMATORY AND IMMUNE FUNCTIONS OF EPITHELIAL CELLS

Damage to epidermal integrity triggers a cascade of events that leads to rapid repair of the wound in association with a robust but transient inflammatory response, in which first neutrophils and then macrophages and mast cells emigrate from the nearby tissues or the circulation (Martin and Leibovich, 2005). The experimental clues suggesting that the EGFR-ligand system plays a relevant role in the defensive response mounted by the epithelial cells during wound healing or in chronic inflammatory skin disorders are quite recent, and in the following paragraphs the possible implications of the EGFR system in skin innate immunity and chronic inflammation are discussed.

Interplay between the mediators of the innate immunity and EGFR signaling

Epidermal cells can mount effective defenses against microorganisms invading the skin. Skin keratinocytes express a variety of Toll-like receptors (TLRs) (Baker *et al.*, 2003; Mempel *et al.*, 2003; Pivarcsi *et al.*, 2003), the most investigated germline-encoded pattern recognition receptors and probably the primary sensors of innate immunity (McInturff *et al.*, 2005). Cultured keratinocytes constitutively express TLR1-6, 9, and 10 mRNA, although only specific ligands for TLR3–5 and 9 appear functional and result in differential immune-associated responses (Lebre *et al.*, 2007). Notably, TGF-α can induce the expression of TLR5 and TLR9 and synergize with these receptors to upregulate the neutrophil-selective chemoattractant CXC ligand (CXCL)8/IL-8 and antimicrobial peptides (Miller *et al.*, 2005) (Table 1). Activation of the TLRs leads to enhanced expression of genes encoding for an array of antimicrobial peptides, including defensins and cathelicidins (Selsted and Ouellette, 2005). These small cationic peptides exert a broad range of actions against microorganisms, including Gram-positive and -negative bacteria, fungi, and viruses. A number of anti-

microbial peptides, which include human β-defensin 3, the neutrophil gelatinase-associated lipocalin, and secretory leukocyte protease inhibitor, can be induced by TGF-α in epidermal keratinocytes (Sørensen *et al.*, 2003) (Table 1). Even more relevant, upregulated expression of these peptides following skin wounding critically depends on EGFR signaling rather than the presence of microbial components (Sørensen *et al.*, 2006). A further level of complexity in the crosstalk between innate immunity and EGFR-driven events derives from the finding that the cathelicidin LL-37, abundantly released also by infiltrating neutrophils, can itself induce EGFR transactivation through a metalloproteinase-dependent release of EGFR ligands, leading to improved cell migration (Tokumaru *et al.*, 2005). Hence, a specific mediator of the innate immunity sustains intervention of EGFR-driven regenerative processes and eventually accelerates homeostatic recovery (Raz, 2007). Recently, Niyonsaba *et al.* (2007) confirmed that antimicrobial peptides do stimulate keratinocyte migration and proliferation via an EGFR-dependent mechanism. In addition, they found that antimicrobial peptides induce *de novo* expression of cytokines and T-cell chemoattractants (Niyonsaba *et al.*, 2007), hence promoting the development of an adaptive immune response in the skin (Oppenheim and Yang, 2005; Schauber and Gallo, 2007).

The contribution of keratinocytes to the pathogenesis of inflammatory skin disorders

A common feature of chronic inflammatory skin disorders, such as psoriasis, atopic dermatitis, and allergic contact dermatitis, is epidermal hyperplasia and thickening, a phenomenon attributed to leukocyte-derived cytokines such as TNF-α and IFN-γ, which are potent inducers of EGF family growth factors and EGFR (Valyi-Nagy *et al.*, 1992, Matsuura *et al.*, 1999). These cytokines also initiate a program of increased expression of inflammatory mediators, which include adhesion molecules, cytokines, and chemokines (Pastore *et al.*, 2005a) (Figure 2). Among the chemokines, the selective neutrophil chemoattractant IL-8 is also active as a mitogen for epithelial and endothelial cell proliferation (Tuschil *et al.*, 1992; Addison *et al.*, 2000; Gillitzer and Goebeler, 2001). In psoriasis, enhanced epidermal IL-8 expression is considered a secondary amplification mechanism leading to epidermal hyperplasia (Reich *et al.*, 2001). Indeed, IL-8 gene expression is actively induced by the EGFR ligands abundantly expressed and released in the psoriatic lesions, including TGF-α (Pastore *et al.*, 2005b) and amphiregulin (Chokki *et al.*, 2006). In turn, IL-8 could contribute to activate the metalloprotease-dependent release of EGFR ligands by acting on its specific GPCR, as already demonstrated in other epithelial cell systems (Tanida *et al.*, 2004). Under the effect of pro-inflammatory cytokines, keratinocytes also release a number of type 1 T-cell chemoattractants, including the CXCR3 ligands CXCL10/IP-10,

Table 1. Evidence of EGFR-driven activities on the immune/inflammatory functions of human epidermal keratinocytes

Molecules	Activity	References
Toll-like receptors	Upregulation	Miller *et al.* (2005)
TLR5, TLR9		
Antimicrobial peptides	Upregulation	Miller *et al.* (2005)
HBD2, HBD3, neutrophil gelatinase-associated lipocalin, secretory leukocyte protease inhibitor		Sørensen *et al.* (2003, 2006)
Chemokine	Upregulation	Mascia *et al.* (2003)
CXCL8/IL-8		Miller *et al.* (2005)
		Pastore *et al.* (2005b)
Chemokines	Downregulation	Mascia *et al.* (2003)
CCL2/MCP-1		Pastore *et al.* (2005b)
CCL5/RANTES		
CXCL0/IP-10		

CXCL, CXC ligand; MCP-1, monocyte chemotactic protein-1; RANTES, regulated upon activation, normal T-cell expressed.

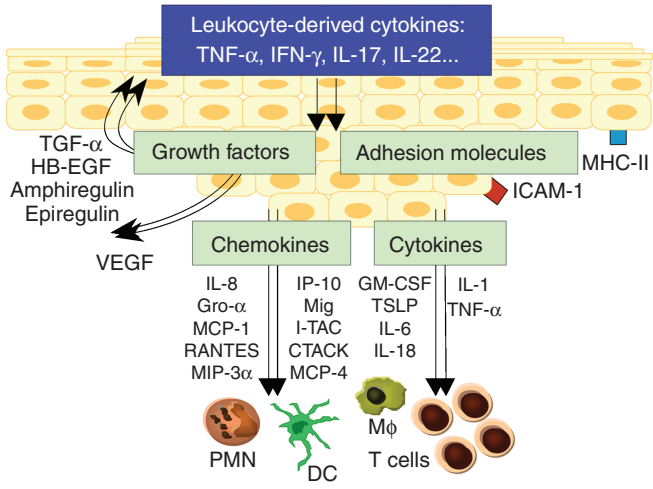

Figure 2. Keratinocytes amplify proinflammatory circuits in the skin. Leukocyte-released cytokines activate a program of enhanced (autocrine growth factors) or *de novo* expression of a plethora of chemokines and cytokines implicated in the recruitment and local activation of immune cells, including neutrophils (PMN), macrophages (Mϕ), dendritic cell precursors (DC), and T cells. The cytokines released by these cell populations, mainly represented by IL-1, TNF-α, IFN-γ, IL-17, and IL-22, are potent activators of keratinocyte pro-inflammatory functions, including the expression of membrane molecules (including ICAM-1 and MHC class II molecules) involved in the retention and further activation of T cells in the epidermis. EGFR ligands not only are involved in the protective response leading to epidermal proliferation, but may also perturb the proinflammatory response of resident and, possibly, infiltrating cell populations through complex molecular pathways only partially identified. EGFR blockade precipitates a condition of skin inflammation, whereas active EGFR signaling may favor an anti-inflammatory condition. However, upregulated expression of amphiregulin clearly correlates with enhanced skin inflammation. TSLP, thymic stromal lymphopoietin.

CXCL9/Mig, and CXCL11/I-TAC, whose expression in keratinocytes is boosted by IFN-γ. Keratinocytes also express potent chemokines with a broad-spectrum activity, which mainly include CCL2/monocyte chemotactic protein-1 and CCL5/RANTES (regulated upon activation, normal T-cell expressed). Epidermal monocyte chemotactic protein-1 is involved in the early response to injury or irritants, and can be found in different T-cell-mediated skin disorders, where it critically contributes to the recruitment of monocyte–macrophages, dendritic cells, and T cells. Active immigration of T cells, monocytes, and neutrophils is also supported by the increased expression of RANTES by epidermal keratinocytes (Pastore *et al.*, 2006).

The impact of EGFR-dependent mechanisms on skin inflammation

The need for an improved understanding of the impact of EGFR signaling on skin inflammation has become urgent since the introduction of the previously unknown anti-EGFR therapies as treatment options for epithelial cancers (Slichenmyer and Fry, 2001). Currently, a humanized anti-EGFR mAb (cetuximab) and two small-molecule EGFR tyrosine kinase inhibitors (gefitinib and erlotinib) have been approved for patients with colorectal and non-small-cell lung cancer refractory or intolerant to chemotherapy. In these patients, a common adverse effect is a papular pustular or acneiform eruption, which can be severe enough to lead to treatment modification or cessation (Pérez-Soler *et al.*, 2005; Agero *et al.*, 2006; Hu *et al.*, 2007). These skin lesions are frequently pruritic and affect mostly the face and the upper trunk. More important, data from a large number of clinical trials suggest that there is a direct relationship between rash incidence and response/survival rate, indicating rash as a possible marker of effective target inhibition and activity of EGFR-targeted agents (Pérez-Soler and Saltz, 2005). Although data on the mechanisms are limited, these lesions can reasonably derive from the impairment of the multiple EGFR-dependent homeostatic functions of the skin (Lacouture, 2006). Histopathologically, a moderate to severe inflammatory reaction dominated by neutrophils, which surround and then invade follicular infundibula, characterizes the eruption. In the epidermis, EGFR is strongly expressed in the basal layer of epidermal keratinocytes and in the outer root sheath of hair follicles. Accordingly, mice with an EGFR-dominant negative mutation have curled wiskers and short hair that become progressively sparse. Their hair follicles eventually disappear, accompanied by a macrophage- and multinucleated giant cell-driven inflammatory reaction (Murillas *et al.*, 1995; Hansen *et al.*, 1997). Indeed, focal keratinocyte necrosis due to persistent EGFR inhibition can *per se* activate and sustain immune cell recruitment and activation (Li *et al.*, 2001), and the consequent folliculitis. In human biopsy specimens taken from affected areas, the stratum corneum is thinner and more compact. Dry skin with diffuse fine scaling can be frequently observed after the onset of the papular pustular rash (Agero *et al.*, 2006) and, despite the sterile nature of the pustules, secondary bacterial infection with *Staphylococcus aureus* may supervene. Hence, an immature skin barrier, combined with a reduced contribution of EGFR-driven innate immune mechanisms of the epidermis, could contribute to this adverse skin reaction.

Animal models predicted a follicle-centered effect of EGFR inhibitor use. However, they can reasonably provide only a partial clue about the etiology of the inflammatory rash in humans treated with anti-EGFR drugs. Interestingly enough, these drugs can exacerbate skin lesions in psoriatic patients (Zorzou *et al.*, 2004), and in general they induce a rash that responds to anti-inflammatory drugs (Lacouture, 2006). We have recently demonstrated that EGFR activation is involved in the control of chemokine expression in human keratinocytes. In particular, EGFR activation not only by TGF-α (Mascia *et al.*, 2003) but also by HB-EGF and amphiregulin (S Pastore and F Mascia, unpublished observation) potently downregulates the levels of TNF-α- or IFN-γ-induced RANTES and monocyte chemotactic protein-1, thus potentially opposing the attraction of neutrophils, monocytes/macrophages, and dendritic cell precursors into lesional skin (Table 1). Also the immigration of T cells could be impaired due to EGFR-driven suppression of the CXCR3 ligand IP-10.In contrast, cultured keratinocytes display a massive upregulation in the levels of these chemokines when EGFR is effectively blocked (Pastore *et al.*, 2005b). The mechanism underlying upregulation of these chemokines is a dramatic stabilization of their transcripts, which is also detectable when ERK is selectively blocked (Pastore *et al.*, 2005b). Accordingly, in the mouse models of irritant contact dermatitis and allergic contact dermatitis, EGFR or ERK inhibition leads to aggravation of the skin inflammatory response, with upregulated chemokine expression and massive skin infiltration by T cells and macrophages (Figure 3). These data strongly suggest that pharmacological abrogation of EGFR/ERK signaling pathway worsens skin inflammation by increasing chemokine expression in keratinocytes. Direct or EGFR-dependent ERK inhibition could also contribute to skin inflammation by improving the function of the professional antigen-presenting cells of the skin, as suggested by the observation that mouse Langerhans cells enhance their expression of cell membrane costimulatory molecules, IL-1β, and distinct T-cell chemokines (CCL3/MIP1α and CCL17/TARC) following selective ERK inhibition (Fujita *et al.*, 2007). Finally, enhanced expression of a cluster of pro-inflammatory molecules, including the T-cell-selective chemoattractants Mig, CX3CL1/fractalkine, and CXCL18/MIP4, was observed in cervical carcinoma epithelial cells treated with small-molecule EGFR inhibitors (Woodworth *et al.*, 2005). Taken together, these independent observations strongly suggest that targeting EGFR should not be considered an attractive therapy in the inflammatory disorders associated with epithelial hyperproliferation, although its efficacy in the blockade of cell proliferation suggested otherwise (Ben-Bassat and Klein, 2000).

A relevant body of experimental evidence proofs that the upregulation of EGFR and itsEGFR ligands are characteristic features of chronic inflammatory skin disorders such as psoriasis, and suggests a pathogenic, rather than protective, role of the EGFR system in skin inflammation. This evidence

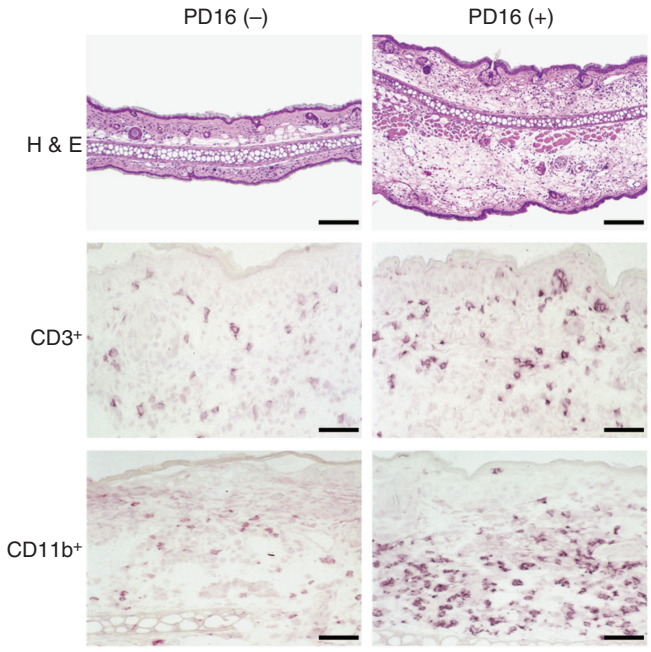

Figure 3. Enhanced inflammatory response in immune mice treated with the small-molecule EGFR inhibitor PD168393 (PD16). The inhibitor was painted on the ear surfaces 30 minutes before elicitation of contact hypersensitivity to 2,4-dinitrofluorobenzene. After 48 hours, the histology (hematoxylin and eosin, H&E) visualizes a more severe reaction and tissue damage, and the immunohistochemistry shows a stronger recruitment of CD3+ and CD11b+ cells (for details see Mascia *et al.*, 2003; Pastore *et al.*, 2005b). Bar: in H&E = 100 μm; in CD3+ and CD11b+ immunohistochemistry = 20 μm.

is particularly strong for amphiregulin, whose basal or suprabasal expression in the skin of transgenic mice leads to severe psoriasis-like hyperplasia, skin inflammation with a rich dermal and epidermal infiltration of neutrophils and lymphocytes, while in the case of suprabasal amphiregulin expression arthropathy is observed (Cook *et al.*, 1997, 2004). No clues about the underlying molecular mechanisms are so far available to explain such a strong neutrophil-rich inflammatory phenotype, although it seems to be strictly associated to amphiregulin-specific bioactivity. Indeed, epidermis targeted expression of TGF-α does not produce cutaneous inflammatory phenotypes (Vassar and Fuchs, 1991; Dominey *et al.*, 1993). By contrast, the deficiency of epiregulin results in chronic, epidermal cell-dependent dermatitis, which correlates with enhanced expression of the pro-inflammatory cytokine IL-18 by keratinocytes (Shirasawa *et al.*, 2004). On the whole, these observations suggest that the impact of EGFR-dependent mechanisms on inflammatory skin responses is a complex, still open issue in which each single EGFR ligand reasonably contributes with distinct, if not disparate, effects on resident and/or immigrated cells.

CONCLUDING REMARKS

Beyond its fundamental role for skin development, the EGFR system is now centrally implicated in all the aspects of skin homeostasis in the adult life, participating to its continuous regeneration in health and to the success of wound healing. Experimental evidence now indicates that its influence extends to inflammatory and immune functions of the epidermis, whereas clinical evidence suggests that the potentiation of the inflammatory response due to EGFR blockade could relevantly contribute to epithelial cancer therapy by boosting innate and adaptive antitumor immune response. Finally, these previously unknown acquisitions emphasize the importance of this system in skin pathophysiology and at the same time underline the limited knowledge currently available on its complexity.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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