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Mechanisms underlying skin disorders induced by EGFR inhibitors

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Abbreviations: ADAM, disintegrin and metalloproteinase; AMP, antimicrobial peptide; AREG, amphiregulin; BD, betadefensin; CRC, colorectal cancer; DC, dendritic cell; DETC, dendritic epidermal T-cells; EGFR-I, EGFR inhibitor; EREG, epiregulin; GEMM, genetically modified mouse model; GMCSF, granulocyte-macrophage colony-stimulating factor; HBEGF, heparin-binding EGF; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MyD88, myeloid differentiation primary response gene 88; SALT: Skin associated lymphoid tissue; TGM, transglutaminase; TKI, tyrosine kinase inhibitor; TRPV, transient receptor potential cation channel, subfamily V; wa, waved.

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is frequently mutated or overexpressed in a large number of tumors such as carcinomas or glioblastoma. Inhibitors of EGFR activation have been successfully established for the therapy of some cancers and are more and more frequently being used as first or later line therapies. Although the side effects induced by inhibitors of EGFR are less severe than those observed with classic cytotoxic chemotherapy and can usually be handled by outpatient care, they may still be a cause for dose reduction or discontinuation of treatment that can reduce the effectiveness of antitumor therapy. The mechanisms underlying these cutaneous side effects are only partly understood. Important questions, such as the reasons for the correlation between the intensity of the side effects and the efficiency of treatment with EGFR inhibitors, remain to be answered. Optimized adjuvant strategies to accompany anti-EGFR therapy need to be found for optimal therapeutic application and improved quality of life of patients. Here, we summarize current literature on the molecular and cellular mechanisms underlying the cutaneous side effects induced by EGFR inhibitors and provide evidence that keratinocytes are probably the optimal targets for adjuvant therapy aimed at alleviating skin toxicities.

Introduction

Our understanding of the molecular mechanisms at the basis of cancer has substantially progressed over the past decades, to a large extent due to the study of genetically engineered mouse models (GEMMs). More recently, the advent of automated large-scale analysis of cancer patient material has boosted this field and paved the way for new avenues toward understanding this debilitating disease. It is, however, still a challenge to effectively translate results from basic research into clinical applications.

One of the first molecules to be targeted based on its mutation, high level of expression, and involvement in tumor cell proliferation and survival was the epidermal growth factor receptor (EGFR, also known as ErbB1). Numerous *in vitro* studies have evaluated the antiproliferative potential of different EGFR inhibitors (EGFR-I) such as anti-EGFR antibodies or tyrosine kinase inhibitors (TKIs),[1,2] and inhibition of angiogenesis and metastasis has been shown using *in vivo* models.[3,4] Although the promising results from preclinical studies did not entirely hold true in the clinic there is no doubt that anti-EGFR therapy results in a significant benefit for specific cancer patients when applied either alone or in combination with radiation therapy or chemotherapy. However, a large number of patients experience adverse events that, although usually moderate, in some cases necessitate dose reduction or termination of therapy. Additionally, in the course of therapy tumors may upregulate other tyrosine kinases to escape anti-EGFR therapy.[5] Future therapeutic strategies will aim at targeting several tyrosine kinases simultaneously, with the disadvantage of potentially increased side effects. Therefore, understanding the mechanisms underlying the side effects and their management, and also how these side effects correlate with the efficacy of the therapy, will be important for improving the effectiveness of anti-EGFR therapy. This review will give an overview of current knowledge of the pathomechanisms underlying adverse events in the skin of EGFR-I–treated patients.

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The Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR, also known as ErbB1) is a receptor tyrosine kinase of the ErbB family that

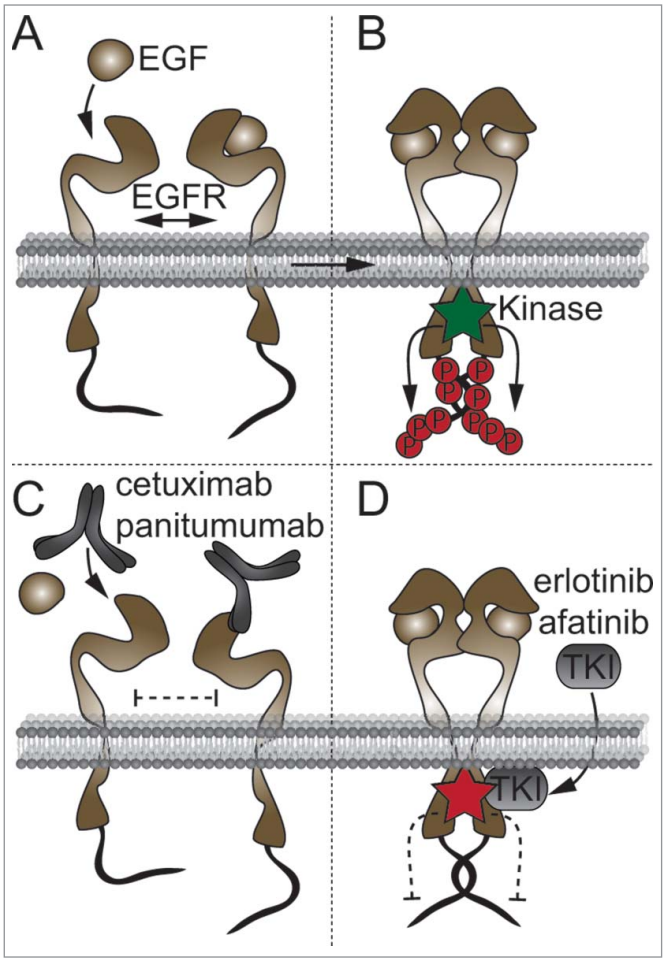

Figure 1. Principles of EGFR activation and inhibition. (**A**) In the absence of ligand, EGFR remains in a conformation that inhibits dimerization. (**B**) Upon ligand binding, the resultant structural change allows homo- or hetero-dimerization with members of the ErbB family, resulting in autophosphorylation of the intracellular tyrosine kinase domain. Kinase activity induces phosphorylation of tyrosines at the C-terminal tail, inducing downstream signaling. (**C, D**) Therapeutic anti-EGFR antibodies bind the extracellular domain of EGFR and inhibit ligand binding (**C**), whereas tyrosine kinase inhibitors compete for ATP binding at the tyrosine kinase domain, thereby inhibiting kinase activity (**D**).

additionally consists of ErbB2/neu, ErbB3, and ErbB4. Upon binding of EGFR-specific ligands such as epidermal growth factor (EGF), amphiregulin (AREG), transforming growth factor α (TGFα), epigen, or ligands shared with ErbB4, such as epiregulin (EREG), betacellulin, or heparin-binding epidermal growth factor (HB-EGF) a conformational change of the EGFR is induced that allows homo- or hetero-dimerization with other family members (**Fig. 1A, B**).[6]

EGFR ligands are generated as membrane-bound pro-forms that require cleavage by proteases to induce autocrine and paracrine EGFR signaling. Ectodomain shedding of EGFR ligands is mainly performed by a disintegrin and metalloproteinase (ADAM) proteins 10 and 17.[7] However, juxtacrine signaling by membrane-bound EGFR ligands has also been reported and it is not yet clear whether these different modes of action have distinct biological consequences.[8] Dependent on ligand and dimerization partners, EFGR activation may result in signaling via MAPK, STATs, PI3K, or PLCγ.[9] Analysis of mice lacking EGFR revealed that EGFR plays an essential role during fetal development and also in tissue homeostasis during adult life.[10-14] Mutant mice develop neurodegeneration shortly after birth and display defects in several epithelial compartments depending on the genetic background.[10,13-15] The skin is particularly affected in EGFR-deficient mice, showing impaired hair follicle development and hair growth and strong inflammation.[16-18] Recently, a child carrying an inherited loss-of-function mutation of the EGFR was reported who showed lifelong inflammation in the skin, gut, and lung that caused early death of the infant, highlighting the importance of EGFR signaling for establishment and maintenance of tissue homeostasis.[19]

EGFR Inhibitors

Overexpression of EGFR or its ligands and activating mutations in the EGFR signaling pathway may lead to epithelial neoplasms and can be found in a large number of cancers in various tissues.[20-22] EGFR activation promotes multiple tumorigenic processes by regulating proliferation, cell survival, angiogenesis, and metastasis.[23] Knowing this, strategies aimed at inhibiting EGFR signaling by targeted therapies were developed. Currently, 2 strategies to inhibit EGFR signaling—monoclonal antibodies and tyrosine kinase inhibitors (TKI)—have been approved for the treatment of cancer either alone or in combination with cytotoxic therapies such as standard chemotherapy or radiation therapy. Starting in the 1980s, several monoclonal antibodies against the ligand binding domain of EGFR have been developed.[24] Anti-EGFR antibodies currently used in the clinic are cetuximab and panitumumab. These agents are highly specific for EGFR and, by blocking ligand binding, prevent the conformational change in EGFR necessary for dimerization (**Fig. 1C**).[25] Cetuximab and panitumumab are approved for the treatment of patients with squamous cell carcinoma and colorectal cancer (CRC). However, the efficacy of cetuximab against CRC is restricted to defined patient collectives expressing characteristic biomarker patterns. Mutation at codon 12 of *KRAS* is negatively predictive for the response to cetuximab whereas wild-type (wt) *KRAS* or the *KRAS* G13D mutation has no prognostic effect.[26,27] Not all patients with wt *KRAS* respond to cetuximab or panitumumab; other prognostic parameters in patients with wt *KRAS* codon12 include *BRAF* mutation, *PI3K* mutation, localization in the left colon (reduced likelihood of *KRAS* or *BRAF* mutation), and AREG expression in the tumor tissue, whereas EREG expression is a positive prognostic factor for the response to cetuximab in metastatic CRC that may be independent of the *KRAS* mutation status.[28-31] Additionally, *AREG* polymorphisms have been shown to affect the efficiency of cetuximab in CRC.[32]

As an alternative strategy to inhibit EGFR, signaling inhibitors of the tyrosine kinase domain have been developed (**Fig. 1D**). These small molecules bind to the intracellular tyrosine kinase domain and inhibit receptor autophosphorylation by

competing with ATP. TKIs have varying affinities for the ATP binding sites of other tyrosine kinases, for example ErbB2 and VEGR, and are therefore not as specific for EGFR as anti-EGFR antibodies. However, TKIs like erlotinib, gefitinib, or afatinib bind certain mutated forms of EGFR that frequently occur in lung cancer with higher affinity and are therefore recommended for treatment of these mutated types of non-small cell lung cancer.[33,34] Irrespective of the treatment modality, use of EGFR inhibitors (EGFR-I) is associated with 2 common problems, adverse side effects and acquired resistance.[5]

Side Effects of EGFR-I Treatment

The most common side effects of EGFR-targeted therapies are dermatologic toxicities and diarrhea. Other events, such as nausea, emesis, neurological, or hematologic side effects, are rare compared to cytotoxic chemotherapy.[34] Among the cutaneous toxicities observed in cancer patients treated with EGFR-I the most common are papulopustular rash of the upper trunk and face skin (60–90%), dry and itchy skin (12–16%), and microbial infections (38–70%).[35,36] Less frequently, pruritus, hair modifications, and paronychial inflammation can occur. The cutaneous side effects seem to be largely independent of the type of EGFR-I used and combined treatment with the monoclonal antibody cetuximab and the TKI erlotinib lead to side effects similar to those induced by treatment with the individual drugs.[37] Although side effects induced by EGFR-I are generally classified as moderate, they are usually chronic and may significantly impact the patient's quality of life and thus necessitate dose reduction or even interruption of treatment.

Most importantly, the severity and the timing of the onset of the skin rash significantly correlate with the effectiveness of the treatment.[38,39] Correlation was reported when either TKIs or monoclonal antibodies were used as monotherapy or in combination with chemotherapy.[39-41] Importantly, rapid onset of moderate to severe skin rash is the best biomarker currently available to predict the effectiveness of EGFR-I treatment, resulting in improved tumor progression parameters such as time to tumor progression and overall survival.[42] Meta-analysis revealed that there are minor differences in the response efficiency between ethnic groups and between the individual drugs.[40] At present, it is believed that the cutaneous side effects induced by EGFR-I are due to inhibition of EGFR in basal keratinocytes and hair follicles, which express high levels of EGFR similar to tumor cells. Indeed, we and others have recently discovered that lack of EGFR in murine keratinocytes is sufficient to induce skin alterations similar to those observed in the skin of EGFR-I–treated patients, supporting this hypothesis.[16,17] However, given that EGFR plays a central role in skin biology, it is currently unclear why the skin rash does not develop in every patient treated with EGFR-I. It has been shown that the severity of the rash is dependent on the dose of EGFR-I used[43]. Increasing the dose of EGFR-I in patients who do not develop the skin rash at the standard dose was shown to increase skin toxicity along with therapy response rates.[38]

Recent findings in animal models have shed light on a previously underestimated role of EGFR in immune cells that might be targeted by systemic EGFR-I.[44-46] Based on these findings, in the following sections we will discuss the large body of evidence for a central role of EGFR signaling in keratinocytes to maintain homeostasis in the skin and other potential mechanisms underlying the side effects of EGFR-I treatment.

Mechanisms Underlying EGFR-I Induced Cutaneous Side Effects

Skin inflammation, or rash and folliculitis

The skin contains a network of immune cell populations summarized as skin-associated lymphoid tissue (SALT) residing in both the epidermis and the dermis. The cells of this system face the challenge of fulfilling 2 apparently contradictory tasks: on the one hand, to maintain homeostasis and tolerance although confronted with the vast number of microbes that are part of the normal flora, and on the other hand to be prepared to fight against pathogens. The epidermis contains specialized dendritic cells, the Langerhans cells, as well as αβ T-cells and γδ T-cells (also called dendritic epidermal T cells or DETCs), which are rare in human skin but the main T-cell population in mouse epidermis.[47] The dermis contains various subpopulations of dendritic cells, macrophages, mast cells, and innate lymphoid cells as well as αβ T cells and γδ T cells. Some of these cell types, like Langerhans cells, constantly migrate to skin-draining lymph nodes under homeostatic conditions whereas other cell types, like neutrophil granulocytes or monocytes, are recruited to the skin to contribute to specific immune responses.[48]

Skin rash is the most frequently observed side effect in EGFR-I–treated patients, macroscopically appearing with inflammatory papulopustular eruptions that usually start in the face and upper trunk. The rash typically develops within 1 or 2 weeks after therapy initiation, peaks after 2–4 weeks, and slowly regresses with continuation of therapy.[49] Histological analysis of skin rash showed mainly CD4-positive T cells and CD1a-positive Langerhans cells throughout the dermis and epidermis, whereas the lesional dermis was dominated by mononuclear myeloid cells like macrophages and activated dendritic cells. Of note, neutrophils were predominantly located at distorted hair follicles.[50-54] In mice deficient for Egfr in basal epidermal keratinocytes ($Egfr^{dEP}$) the resident immune cell populations of the epidermis, Langerhans cells and γδ T-cells, progressively disappeared from the epidermis and were replaced by inflammatory DC and αβ T-cell populations, whereas in the dermis mainly macrophages and mast cells accumulated.[16,17] Increased expression of the death receptor ligand TRAIL in cells infiltrating into the dermis that might contribute to rash development has been reported.[52] The inflammatory infiltrate is likely triggered by primary changes in epidermal epithelial cells. It may, however, be amplified and maintained by secondary infections and barrier defects, as discussed later in the text. To date, the exact cell type triggering inflammation remains elusive. Application of EGFR-I inhibits EGFR activation in basal cells of the interfollicular epidermis,

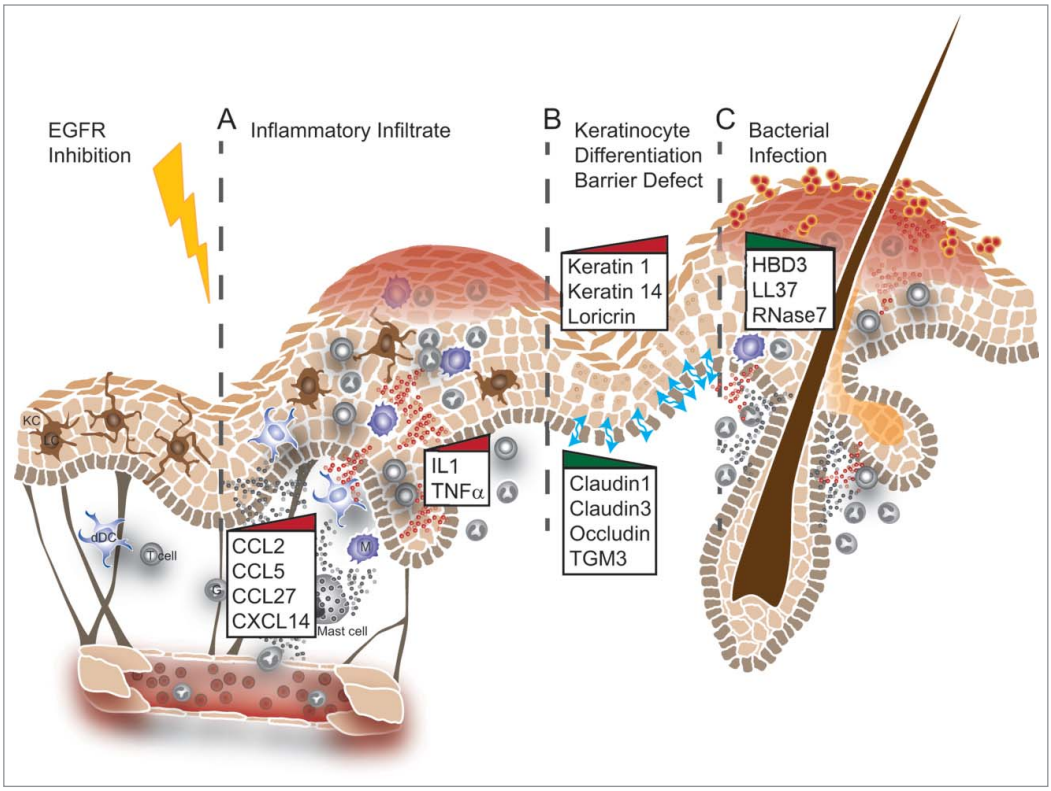

Figure 2. Schematic representation of potential defects observed in the skin of EGFR-I–treated patients. (**A**) Alterations in chemokine and cytokine production in keratinocytes may result in attraction of inflammatory cells. (**B**) Disturbed keratinocyte differentiation impairs proper formation of tight junctions and barrier function. (**C**) The barrier defect and reduced expression of antimicrobial peptides result in bacterial infections accompanied by massive infiltration of neutrophil granulocytes and macrophages. These events often appear sequentially but may also occur independently. The graphic representation is modified from Lacouture M., Rodeck U., (DOI: 10.1126/scitranslmed.3006993)

hair follicle, and eccrine glands.[55] Indication for a central role of hair follicle cells in the pathogenesis of skin rash comes from the observation that patients treated with EGFR-I after radiation therapy failed to develop skin rash in irradiated skin areas.[56-58] Indeed, hair follicle cells have been reported to respond to radiation by apoptosis whereas epidermal cells responded with cell cycle arrest and temporal depletion of basal layer stem cells.[59,60] An alternative hypothesis suggests that depletion of immune cells such as DCs from the skin may be the cause of the transient absence of lesions in previously irradiated skin.[61] On the other hand, simultaneous administration of cetuximab in combination with radiation therapy may lead to very severe skin toxicity because of the important role of EGFR in the repair of radiation-induced DNA damage.[62-64]

Increased expression of CCL-2, CCL-5, and CXCL-10 has been described in EGFR-I–treated cultured human keratinocytes and lesional epidermis.[65] Moreover, in interfollicular epidermis, massive production of CCL-27 and CXCL-14 was found that might be responsible for recruitment of T cells and monocyte-derived inflammatory cells, respectively, into the skin (**Fig. 2A**).[16,65] However, high levels of CCL27 detectable in patient serum did not correlate with rash severity.

Levels of other chemokines that are under the control of EGFR ligands in inflamed skin, for example CXCL-1, CXCL-2, CCL-20, CXCL-8, and GM-CSF, are reduced in EGFR-I–treated epidermis.[16,65,66] GM-CSF has pleiotropic effects and is not only responsible for differentiation and recruitment of myeloid cells but is also capable of regulating keratinocyte proliferation and apoptosis.[67] Similar to EGFR ligands, GM-CSF produced by keratinocytes has been shown to enhance wound healing.[68] Little is currently known about the regulatory mechanisms that lead to these changes in cytokine and chemokine expression. GM-CSF seems to be expressed in an ERK/AP1-dependent manner *in vitro* and *in vivo* and this regulation is amplified under inflammatory conditions.[66] However, in mice some chemokines such as CCL-2 are already upregulated during late embryogenesis, under sterile conditions, and in the absence of detectable inflammatory infiltrate, pointing to a role as transcriptional targets of EGFR. Recent findings indicate another mechanism that might be responsible for the recruitment of neutrophils in particular: treatment of human, but not mouse, keratinocytes with EGFR-I induced expression of complement components and complement activation, resulting in increased deposition of the complement component C3 that might also act as a chemotactic factor.[69]

Although T cells are abundantly present in the immune infiltrate in lesional skin of patients treated with EGFR-I, experiments in mice lacking Egfr in the epidermis showed that lack of B cells and T cells did not ameliorate the skin inflammation and also did not reduce the immune cell infiltrate, suggesting that the rash might be driven by an innate immune response.[16,17] Therefore, therapeutic strategies targeting innate immune cells might be more effective for the management of skin rash in EGFR-I–treated patients. In human skin, blocking IL-8 reduced the side effects induced by locally injected anti-EGFR antibody by reducing infiltration of neutrophils.[70] In $Egfr^{dEP}$ mice, local deletion of macrophages or inhibition of mast cell activation reduced skin inflammation and altered the composition of the skin infiltrate, respectively.[16,17] Interleukin-1 (IL-1) and its superfamily

members IL-18 and IL-33 are candidate cytokines involved in the activation of innate immune responses and rash development. Indeed, IL-1R/MyD88-dependent signaling has been implicated in the induction of sterile inflammation (inflammation occurring in the absence of pathogens) in response to cell death.[71] IL-1α is also important for the initiation of the wound healing response that might be responsible for generating an immune cell infiltrate.[72,73] Increased expression of IL1α and IL-1β is indeed found in epidermal lysates of *Egfr*^{dEP} mice, but is probably derived from immune cells since keratinocytes lacking EGFR expressed lower mRNA levels of both cytokines.[16] Interestingly, patients with low serum levels of IL-18 before the start of treatment were more likely to develop a higher grade (grade 2 or above) rash than those with high serum levels;[17] however, during treatment IL-18 serum levels increased in both patient groups. IL-33, another IL-1 family cytokine, is induced in mouse or human skin after irradiation with UV-B, contributing to the development of inflammation.[74] Application of sunscreen can reduce the side effects of EGFR-I in patients, suggesting that sun exposure might potentiate cytokine production in conjunction with reduced EGFR signaling.[75]

The increased levels of IL1 in *Egfr*^{dEP} mouse skin, together with the increased IL18 in EGFR-I treated patient serum, suggest that strategies targeting this pathway might be of therapeutic benefit. In a mouse model of neutrophil-rich hair follicle inflammation induced by injection of an anti-mouse Egfr antibody, skin inflammation could be prevented when the IL-1 antagonist kineret was co-injected with the anti-EGFR antibody. However, kineret injection had no effect when injected during ongoing inflammation.[76] IL-1 expression seems to be regulated downstream of TNFα since in the same study treatment with etanercept had comparable effects to kineret and reduced IL-1 expression. Discouraging results concerning the inhibition of these proinflammatory pathways come from the analysis of *Egfr*^{dEP} mice lacking either IL-1 or TNF signaling components. Genetic deletion of MyD88, an adaptor protein necessary for signaling via IL1R and IL18R, did not rescue the inflammatory skin phenotype in mice lacking EGFR in the epidermis and neither did combined deletion of both TNF receptors TNFR1 and TNFR 2.[16,17]

Barrier defect/xerosis

A major function of the skin is to protect the organism from the environment and from excessive water loss. To fulfill this task, proper differentiation of keratinocytes to form the stratified epithelium and cornification of the outmost layers is necessary. The human interfollicular epidermis consists of 4 layers, the basal layer, spinous layer, granular layer and cornified layer, each of which is characterized by a specific expression pattern of structural and matrix proteins like keratins.[77] During terminal differentiation cells become enucleated, involucrin is degraded, the content of lamellar bodies is extruded, and transglutaminases crosslink keratins and other proteins.[78] Strong intercellular interactions are formed by tight junction proteins and the lipids in the intercorneocyte space.

Patients receiving EGFR-I can develop xerosis within weeks after treatment start, manifesting as cutaneous dryness, itching, and scaling that are typically found on the limbs and in areas affected by rash but may affect all areas of the skin.[53] Mice lacking EGFR signaling in the epidermis develop progressive transepidermal water loss starting around day 10 after birth.[16,79] The barrier function of the skin may be perturbed by EGFR-I treatment at multiple levels (**Fig. 2B**). EGFR ligands like EGF or TGFα induce proliferation of cultured keratinocytes and epidermal hyperplasia and hyperkeratosis,[80,81] whereas in benign papilloma EGFR has been shown to provide a survival signal to keratinocytes.[82]

Furthermore, EGFR signaling regulates differentiation of keratinocytes and the activity of transglutaminases (TGM) that are critical for crosslinking structural proteins like involucrin, loricrin, and small proline-rich proteins by forming 3-(γ-glutamyl) lysine isopeptide bonds. In *Egfr*^{dEP} mice and in *Adam17*$^{\Delta KC}$ mice the expression of *tgm3* is strongly reduced accompanied by altered epidermal differentiation, mirrored by retained expression of loricrin in basal keratinocytes and a derangement of keratin expression.[16,79] TGFα produced by keratinocytes is the main EGFR ligand responsible for barrier integrity since mice lacking *tgfα* develop a similar phenotype.[83,84] This might in part be mediated by Ca^{2+} signaling. The calcium channel transient receptor potential 3 (trpv3) protein has recently been shown to be an essential part of the EGFR signaling cascade. Lack of *trpv3* in the epidermis affects hair development and skin barrier function and results in a phenotype similar to that of *waved (wa)1* and *wa2* mice, which are either deficient for TGFα (wa1) or harbor a hypomorphic EGFR variant (wa2).[83,84,109] Calcium influx via trpv3 induces further transactivation of EGFR and is necessary for transglutaminase activity in the superficial epidermis. Consequently, changes in stratification, parakeratosis (the retention of nuclei in stratum corneum), vacuolar degeneration, and apoptotic keratinocytes in the basal layer can be observed in EGFR-I–treated patients.[55]

EGFR is also implicated in regulating cell–cell contacts, including those in cancer cells. Treatment of the A431 tumor cell line with EGFR-I decreased expression of claudin-4, and increased claudin-2 expression in the human lung adenocarcinoma cell line A549 was dependent on EGFR activation.[85,86] Expression of claudin-3 was reduced in *Egfr*^{dEP} epidermis, as was expression of claudin-1 in human lesional skin (**Fig. 2B**).[16] Therefore, skin barrier defects can lead to compensatory hyperproliferation, as indicated by increased proliferation and keratin 6 expression, and might be responsible for some of the proinflammatory factors secreted by lesional skin as well as the production of "danger" cytokines like IL-36.[79,87]

Antimicrobial defense/infections

The skin not only constitutes a physical barrier protecting from infection but is also an environmental niche hosting a plethora of commensal organisms such as bacteria and fungi. These microorganisms are specifically adapted for this niche and protect the body by preventing colonization and invasion of opportunistic organisms.[88] Defects in epidermal structural proteins may

disturb this barrier function. For example, mutations of filaggrin are associated with atopic dermatitis, and a shift in skin microbiota has been observed in a related mouse model with abnormal filaggrin processing.[89] Combined with the physical epidermal barrier, antimicrobial peptides (AMPs) constitute a major component of the active innate immune defense against invading microbes in the skin. Under homeostatic conditions AMPs in the skin are produced mainly by keratinocytes, but also by mast cells and eccrine sweat glands.[90] Only recently it has become understood that a substantial contribution also comes from commensal bacteria, which produce AMPs and also TLR ligands.[91,92] Under inflammatory conditions, a large number of "inducible" AMPs are produced by infiltrating immune cells like neutrophils or dendritic cells. The basic functions of all AMPs are activation of the host innate immune response and direct killing of pathogens.[93] The major groups of AMPs found in the skin are defensins, cathelicidin, dermcidin, and a group of other proteins/peptides including RNAse7 and S100 proteins.

Defensins have a broad spectrum of antimicrobial activity and their expression can be induced by bacterial infection or proinflammatory cytokines like IL-1, but may be inhibited by pretreatment with retinoic acid.[93] Cathelicidin (CAMP, LL-37) has broad-spectrum activity and has been shown to bind to lipopolysaccharide (LPS) on gram-negative bacteria but also activates keratinocytes. Skin inflammation and 1,25-dehydroxy vitamin D3 are potent inducers of cathelicidin expression.[93] Expression of some AMPs is also under the control of EGFR. Bacterial products like the *Helicobacter pylori* virulence effector CagA or LPS function via EGFR signaling to either suppress or upregulate expression of human β-defensin 3, respectively.[94,95] Treatment of human keratinocytes with erlotinib reduced expression of hBD3, RNase7, and CAMP; similarly, expression of murine β-defensin 14, the mouse homolog of hBD3, was reduced in *egfr*-deficient keratinocytes (**Fig. 2C**).[16] However, expression of β-defensin 1 and 2 was increased in these cells, which is consistent with reports of differential regulation of expression of hBD1-4 in humans.[93] EGFR also closely interacts with S100 proteins and regulates the transcription of S100A2 and S100A7 (Psoriasin).[96,97] S100A7 protein has been shown to interfere with EGFR signaling and increase survival of cancer cell lines, whereas S100A4 protein binds to EGFR ligands to enhance proliferation.[98,99] In normal human keratinocytes, induction of CCL20, hBD4, and S100A7 RNA expression requires the synergistic action of integrins, EGFR, and IL-1 to promote antimicrobial defense.[100]

The question of whether treatment with EGFR-I affects antimicrobial defense has already been addressed in early clinical trials. The folliculitis observed in EGFR-I–treated patients typically begins as a sterile inflammatory process associated with neutrophil-rich infiltrates, which is in contrast to classic acne.[25,54] Acne is believed to be caused by infection with *Propionibacterium acnes* and colonization of lesions with this bacterium can be found at day 1 in 70% of patients.[101] Interestingly, EGFR-I–treated patients showed no significant changes in the cutaneous microflora at early stages, although *Staphylococcus aureus* was cultured from some persistent lesions.[51,52] More recent studies revealed that up to 40% of patients developed infections after EGFR-I treatment, mainly at sites of toxic lesions, with the vast majority of infections being caused by *S. aureus* and fewer by infection with enterobacteriaceae and herpes viridae.[16,102] That the increased rate of infections might indeed be the result of reduced AMP production by keratinocytes is indicated by the finding that supernatants from erlotinib-treated human keratinocytes were less potent in killing *S. aureus* than those from mock-treated control keratinocytes.[16] Infection with pathogenic strains like *S. aureus* may worsen the course of EGFR-I–induced cutaneous disease and thus further compromise the patient's quality of life, creating the need for effective management of complicating infections. Antibiotics of the tetracycline family, namely doxycycline and minocycline, have recently been evaluated for their potential to alleviate EGFR-I–induced skin disease. In one study, combined treatment with moisture, topical sun screen, prednisolone, and doxycycline from day 1 before the start of EGFR-I and continuing over a 6-week period resulted in a significant reduction in the number of patients with rash of grade >2.[75] Another study that evaluated the use of doxycycline alone observed a lower incidence of grade 2–3 folliculitis in the tetracycline arm.[49] Thus, preventive application of tetracyclines seems to be a promising approach to improve the quality of life that is affected by the impaired antimicrobial defense caused by EGFR-I treatment. Tetracyclines are also applied in a variety of dermatologic diseases because of their anti-inflammatory activity through inhibiting cytokine and chemokine secretion.[49,103] Doxycycline has already been shown to be effective in rosacea therapy at sub-antimicrobial doses.[104] Furthermore, high rates of tetracycline-resistant *S. aureus* are found in lesions of EGFR-I patients, suggesting that the ameliorative effects of preemptive tetracycline therapy on EGFR-I induced skin rash are due to its anti-inflammatory properties.[102]

Hair alterations

Hair modifications in EGFR-I–treated patients develop at later time points than folliculitis, usually after 2–3 months, and differentially affect distinct hair types. Although scalp hair grows more slowly and some patients eventually develop androgen-like alopecia, facial hair and eyelashes may grow progressively.[35,36]

Hair follicles are constantly self-renewing throughout life, a process called the hair cycle. The hair cycle consists of 3 stages: anagen (hair growth), catagen (phase of involution), and telogen (the resting phase). The EGFR ligands EGF and TGFα play a critical role during the hair cycle because they are involved in triggering anagen and catagen phases and in the differentiation of sebocytes.[105,106] The threshold of EGFR activation during hair growth and development seems to be very important, as hyper- and hypoactivation of the EGFR can lead to a similar outcome. Overexpression of EGF in mice results in thinner hair, and EGF treatment of newborn mice delays hair development.[107,108] Moreover, mice harboring a hypomorphic EGFR (wa2) develop wavy hair and curly whiskers, as do mice harboring a mutated form (wa1) or homozygous deletion of TGFα.[83,84,109] Studies in different EGFR mutant mouse models showed that, after the first hair cycle, EGFR-deficient hair follicles fail to enter catagen and

remain in aberrant anagen, which results in hair follicle degradation and hair loss.[18,110] These changes are accompanied by fibrosis and an inflammatory infiltrate. The complex functions of EGFR in the epidermis and hair follicles are also highlighted by transplantation experiments with EGFR-deficient skin. A block in hair cycle progression was observed when EGFR-deficient skin was grafted onto wild-type mice, suggesting a cell-autonomous requirement for EGFR.[111] However, progressive hair follicle loss in these transplants was always accompanied by a dense dermal immune infiltrate. Whether similar mechanisms cause hair alterations in EGFR-I–treated patients remains to be determined. Since immune cell infiltration and hair loss are also always associated in EGFR-I–treated patients it is likely that hair loss is a result of immune-mediated damage, suggesting that EGFR signaling might be protective against immunological reactions.[18,111]

Some studies also point toward an involvement of hormone signaling in EGFR-I–induced alopecia. Indeed, data from breast cancer studies indicate interactions between hormone receptors and EGFR.[112,113] Additionally, crosstalk between EGFR and the estrogen receptor pathway has been reported in squamous cell carcinoma.[114] Some data indicate that trichomegaly preferentially occurs in female patients.[115] However, careful analyses of the hormone pathways in treated patients are still lacking.

Conclusion

Our understanding of the pathogenesis underlying EGFR-I–induced skin toxicities has substantially increased during the last years due in part to the generation of novel animal models addressing the specific role of EGFR and its ligands in keratinocytes. Clinical studies have evaluated new treatment regimens that led to novel guidelines for the treatment of patients receiving EGFR-I, involving pre-emptive treatment with antibiotics and intensive skin care.[75] However, more prospective clinical studies are needed to optimize these treatment strategies. Additionally, evidence-based approaches must be established. One example would be treatment with inhibitors of IL-1, based on its high expression in the epidermis. However, with the increasing number of clinical and preclinical studies it has become evident that systemic treatment strategies might also reduce the effectiveness of EGFR-I against tumor growth that seems to be mediated in part by an antitumor immune response.[116-119] Therefore, novel topical strategies to reduce side effects in the skin are needed. Case reports of topical recombinant human EGF or topical vitamin K cream resulting in a reduction of rash grade within a few weeks are very promising.[120,121] Menadione, a synthetic pro-vitamin K3, inhibits phosphatases and thereby increases baseline EGFR phosphorylation.[122] However, topical pretreatment with vitamin K1 (phytomenadione) did not profoundly affect rash severity.[123] With EGFR-I treatment being applied more and more in early stages of antitumor therapy, the need for strategies to reduce its side effects will further increase to avoid dose reductions and maintain patients on therapy at an effective dose. Future preselection strategies for patients should consider testing skin biopsies for the positive development of skin rash *in vitro* in organ cultures to predict the patient's response to anti-EGFR therapies. This would certainly increase the treatment success rate and avoid unnecessary treatments and related costs. Only then we will be able to exploit the full potential of anti-EGFR therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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