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Review

Beyond Wavy Hairs

The Epidermal Growth Factor Receptor and Its Ligands in Skin Biology and Pathology

Marlon R. Schneider,* Sabine Werner,† Ralf Paus,‡§ and Eckhard Wolf*

From the Institute of Molecular Animal Breeding and Biotechnology and Laboratory for Functional Genome Analysis, Gene Center, LMU Munich, Munich, Germany; the Department of Dermatology,‡ University Hospital Schleswig-Holstein, University of Lübeck, Lübeck, Germany; the Department of Biology,† Institute of Cell Biology, ETH Zurich, Zürich, Switzerland; and the School of Translational Medicine,§ University of Manchester, Manchester, United Kingdom*

The epidermal growth factor receptor (EGFR) network, including its seven ligands and four related receptors, represents one of the most complex signaling systems in biology. In many tissues, including the skin and its appendages (notoriously the hair follicles), its correct function is necessary for proper development and tissue homeostasis, and its deregulation rapidly results in defects in cellular proliferation and differentiation. The consequences are impaired wound healing, development of psoriasis-like lesions, structural and functional defects of the hair follicles, and tumorigenesis. In addition to *in vitro* experiments and data from clinical studies, several genetically modified mouse models displaying alterations in the interfollicular skin and hair follicles attributable to mutations in components of the EGFR system have been reported. These animals, in many cases representing *bona fide* models of known human diseases, have been seminal in the study of the role of EGFR and its ligands in the skin and its appendages. In this review, we take the multiple phenotypes of these animal models as a basis to summarize and discuss the effects elicited by members of the EGFR system in diverse aspects of skin biology and pathology, including cellular proliferation and differentiation, wound healing, hair follicle morphogenesis, and tumorigenesis. ***(Am J Pathol 2008, 173:14–24; DOI: 10.2353/ajpath.2008.070942)***

The epidermal growth factor receptor (EGFR) and its ligands represent one of the most powerful and complex signaling networks in higher vertebrates. In this pleiotropic system that exerts an unusually wide array of diverse bioregulatory functions, several peptide growth factors promote the homo- or heterodimerization and subsequent autophosphorylation of a family of tyrosine kinase receptors. Consequently, adaptor proteins and enzymes initiate signaling cascades, culminating in biological outcomes ranging from cell division to cell death, differentiation, or malignant transformation.

Retrospectively, the first indications that EGFR-mediated signaling plays a central role in skin biology and pathology, can be traced back to 1933, when Francis A.E. Crew published a report describing mice with "coats which looked exactly as though the animals had been to the hairdresser and had had a permanent wave treatment."[1] This pleasantly written account (considering the sobriety of today's scientific literature style) is probably the first description of the phenotypic consequences of a perturbation in the activity of the epidermal growth factor receptor. The mouse line described, carrying a point mutation in the gene encoding transforming growth factor-α (TGF-α), is known today as *waved-1* (*wa-1*). Only 2 years after Crew's description of the *wa-1* mutation, mice with a similar phenotype were described by Clyde Keeler[2] and named *waved-2*. Keeler[2] reported (without missing the obligatory reference to a hairdresser) that *wa-2* mice, as compared with *wa-1*, showed a more marked phenotype of "marcelled" hairs and curled vibrissae (Figure 1). Sixty years later, the identification of a point mutation in

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Address reprint requests to Dr. Marlon R. Schneider, Institute of Molecular Animal Breeding and Biotechnology, Gene Center, LMU Munich, Feodor-Lynen-Str. 25, D-81377 Munich, Germany. E-mail: schnder@lmb.uni-muenchen.de.

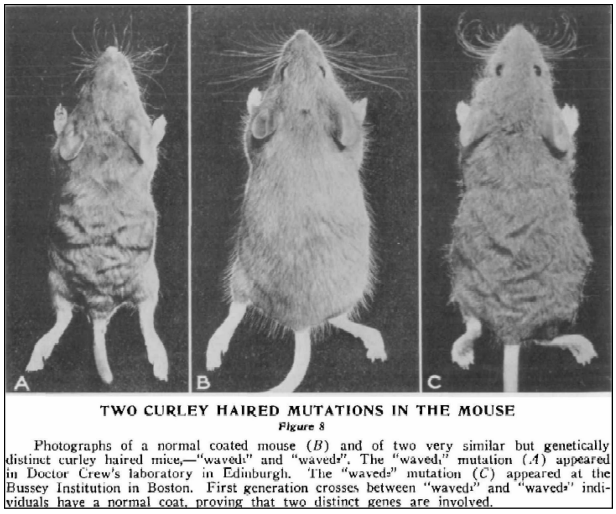

Figure 1. Photographs published by Clyde E. Keeler[2] in 1935 comparing a *wa-1* (**A**), a *wa-2* (**C**), and a normal coated mouse (**B**). Reproduced from *The Journal of Heredity*, 1935, Vol 26, pp 189–191, with permission from Oxford University Press.

the *egfr* gene as the genetic basis of the wa-2 phenotype[3,4] confirmed the correctness of his observations: a mutation in the receptor is expected to result in a more severe phenotype than a mutation affecting only one of its ligands. Both mouse lines are still being used by researchers for examining the effects of reduced levels of these proteins in different organs.

Since the initial description of *wa-1* mice, evidence has accumulated from numerous molecular, cellular, and whole-organism studies that, collectively, indicate a central role for the EGFR family and its ligands in cutaneous biology and pathology: 1) EGFR ligands are autocrine- and paracrine-acting growth factors for keratinocytes, playing a central role in controlling proliferation of these cells[5–10]; 2) EGFR ligands also activate mesenchymal cells and stimulate fibroblast proliferation and angiogenesis[11,12]; 3) diverse EGFR ligands were detected in wound fluid[13–15] and the expression of EGFRs transiently increases after wounding,[16] indicating a role for this network in healing of skin wounds; 4) overexpression of multiple EGFR ligands is a hallmark of psoriatic epidermis[9,17–19]; and 5) epithelial squamous cell carcinomas overexpress EGFR,[20,21] and substantial evidence implicates EGFR signaling as a major component in the pathogenesis of melanoma[22] and nonmelanoma skin cancer.[23–26]

Numerous genetically engineered mouse models displaying alterations in the skin and hair follicles attributable to changes in the activity of members of the EGFR family (Table 1) or their ligands (Table 2) have been reported during the last decades. Their phenotypes, ranging from alopecia and psoriasis-like lesions to skin tumors, highlight the exceptionally wide range of effects

Table 1. Summary of the Skin Phenotype of Genetically Modified Mouse Models of EGFR and ERBB2

Receptor	Mutation	Phenotype	References
EGFR (ERBB1)	KO	Open eyelids at birth, impaired epidermal as well as hair follicle differentiation; minor differences and severity depend strongly on the genetic background	27–29
		Delayed hair development and multiple hair shaft abnormalities; EGFR-deficient skin grafts respond aberrantly to the wound environment	30
		The growth of squamous papillomas produced by grafting EGFR-deficient, *v-ras*-transformed keratinocytes onto nude mice is strongly impaired	31
		EGFR is essential for the development of skin tumors in K5-SOS transgenic mice	23
		EGFR is essential for maintaining the proliferative population in the basal cell compartment of papillomas	24
	Humanized (KI)	Curly whiskers, altered morphology, and distribution of hair follicles; progressive degeneration with loss of most follicles over time	32
	TG (K5-EGFR-DN)	Waved hairs, curly whiskers, progressive hair degeneration, and alopecia	33
ERBB2	TG (K5-ERBB2*)	Thickened skin, patchy hair growth, severe follicular hyperplasia, and spontaneous papilloma; lethal	34
	Transgenic (K5-ERBB2)	Alopecia, follicular hyperplasia, and sebaceous gland enlargement as well as spontaneous skin tumor development	35
	Transgenic (K14-ERBB2*)	Extensive skin phenotype; epidermal hyperplasia, preneoplasia, papilloma, hyperkeratosis, and dyskeratosis; disruption in hair follicle morphogenesis; lethal	36
	Transgenic (K14-ERBB2*-I)	Conditional overexpression causes reversible hyperproliferation of epidermal basal cells and hyperplasia of the skin and other stratified epithelia	37

KO, knockout; KI, knockin; K5, keratin 5 promoter; K14, keratin 14 promoter; DN, dominant-negative; *, constitutively active form; I, doxycycline-inducible.

Table 2. Summary of the Skin Phenotypes of Genetically Modified Mouse Models of EGFR Ligands

Ligand	Mutation	Phenotype	References
AREG	TG (K14-AREG)	Psoriasis-like phenotype; erythematous skin with alopecia, occasional papillomatous growth; dermal and epidermal lymphocytic and neutrophilic infiltration	38
	TG (INV-AREG)	Similar to K14-AREG mice; reduced E-cadherin levels in psoriatic lesions	39, 40
BTC	TG (CBA-BTC)	Delayed hair cycle induction; increased angiogenesis at the wound site	41
EGF	TG (CMV-EGF)	Hyperproliferation of basal layer cells, arrest of hair follicle development	42
EREG	KO	Chronic dermatitis	43
HBEGF	KO	Impaired wound healing	44
TGF-α	KO	Wavy hair, curly whiskers, defective ORS, and altered hair follicle structure; impaired early wound epithelialization	45–47
	MT-TGF-α	Hyperplastic skin, papillomas, sebaceous adenomas, and more rarely, sebaceous and squamous cell carcinomas after DMBA treatment; synergy with TPA promotion	48, 49
	TG (K14-TGF-α)	Epidermal thickening and stunted hair growth; spontaneous papillomas; psoriasis-like lesions; tumor formation without an initiator agent	50, 51
	TG (K1-TGF-α)	Epidermal hyperproliferation and hyperkeratosis; spontaneous papilloma formation with high sensitivity to tumor promotion by TPA	52, 53
		TGF-α/*v-fos* double-transgenic mice: aberrant keratinocyte differentiation; accelerated papillomatogenesis and malignant conversion after TPA promotion	54, 55
		TGF-α/*v-Ha-ras* double-transgenic mice: increased epidermal hyperproliferation and tumorigenesis and malignant conversion	56, 57

TG, transgenic; KO, knockout; K14, keratin 14 promoter; K1, keratin 1 promoter; INV, involucrin promoter; CBA, chicken β-actin promoter; CMV, cytomegalovirus promoter; MT, metallothionein promoter.

elicited by the EGFR signaling system in the skin. Here, we summarize and critically discuss the most relevant genetic mouse models with altered activity of these molecules and, based on their phenotypes, examine the role of the EGFR system in the physiology and pathology of the skin and its appendages.

The EGFR Network

Together with their ligands, EGFR and its related receptors (see below) are essential for normal embryonic development and adult tissue homeostasis, and their deregulation has been associated with many human diseases, including cancer.[58] Because they are interesting therapeutic targets, the role of components of this system in tumorigenesis has attracted much attention during the past few years. In cancer cells, constitutive activation of EGFR is achieved by several mechanisms, including increased production of ligands, elevated levels of the receptor, mutations in EGFR extracellular or intracellular domains, defective down-regulation of EGFR or extensive cross talk with other systems like G-protein-coupled receptors, other tyrosine kinase receptors, or with cell adhesion molecules.[59] Overexpression of ligands and receptors appears to play a major role in many tumor entities, including non-small-cell lung cancers, ovarian, breast, gastric, and bladder cancer, and their co-expression is often associated with poor survival.[58] A useful way to describe the EGFR system is as a multilayered signal-computing network comprising an input layer, a signal-processing layer, and an output layer.[60,61]

Input Layer

The uppermost level comprises the seven ligands amphiregulin (AREG), betacellulin (BTC), heparin-binding EGF-like growth factor (HBEGF), TGF-α, epiregulin (EREG), epigen (EPGN), and EGF itself (Figure 2). The existence of additional ligands is unlikely because a genome-wide search using algorithms based on genomic and cDNA structures failed to identify further potential EGFR ligands.[62] EGFR ligands are initially synthesized as membrane-bound precursors consisting of an EGF motif flanked by an N-terminal extension and a C-terminal membrane-anchoring region. The EGF-like domain, characterized by a consensus sequence composed of six conserved cysteines forming three intramolecular disulfide bonds, can be cleaved (shedded) to release the mature, circulating form.[63] The released ligand can activate EGFRs on the cell of its origin (autocrine mode of action), on neighboring cells (paracrine mode, for example, the activation of epithelial cells by mesenchyme-derived factors), or on distant cells after systemic

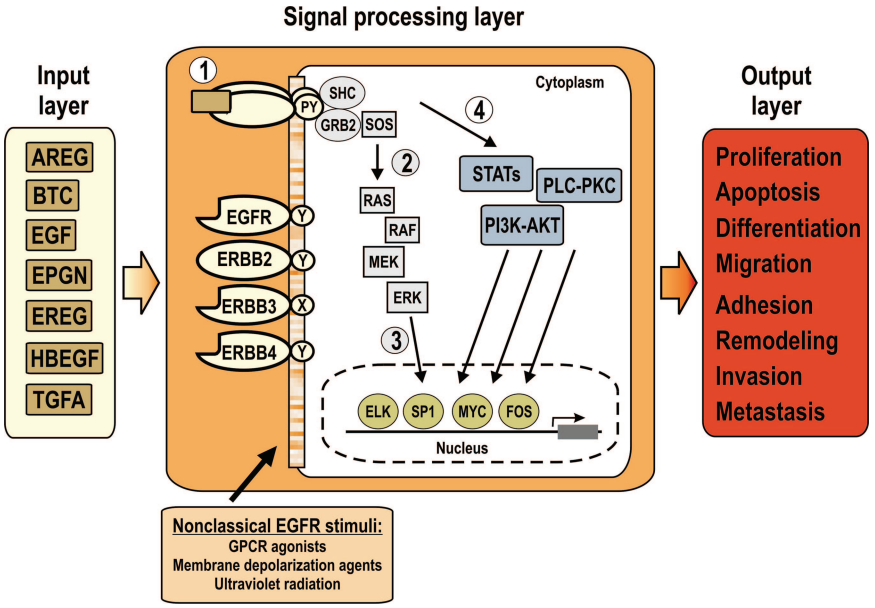

Figure 2. The EGFR network can be viewed as a multilayered system. The input layer comprises the seven EGFR ligands. In the signal processing layer, the four receptors form 10 possible dimeric combinations after ligand binding. Note that ERBB2 and ERBB3 homodimers are nonfunctional because of a lack of a ligand or to a dead kinase, respectively. Once activated by a ligand (**1**), molecules with adaptor or enzymatic function are recruited to the receptors and activate downstream signaling pathways including the RAS-MAPK cascade (**2**), which is shown in more detail because of its relevance for keratinocyte proliferation and survival. Finally, activated downstream components change the activity of multiple nuclear transcription factors, altering the cellular transcriptional program (**3**). Signaling via the STAT, PLC-PKC, and PI3K-AKT pathways are also important in mediating EGFR activity (**4**). EGFR signaling is also activated by EGFR-independent signaling pathways (nonclassical use) including ligand-free receptor activation. The output layer is the result of the altered cellular transcriptional program and represents the cellular effects elicited by the combined activity of the previous layers.

distribution (endocrine mode). In addition, a juxtacrine mechanism, in which the precursor form exerts biological activity by stimulating adjacent cells via cell-cell contacts, has been described for AREG, HBEGF, and TGF-α.[64] Interestingly, juxtacrine signaling can possibly induce different biological responses as compared with the effects of the shedded growth factor.

Signal-Processing Layer

This layer includes the ERBB receptors and a myriad of downstream signaling molecules including phosphotyrosine-binding proteins, adaptor molecules, and transcription factors (Figure 2). EGFR (ERBB1; HER1) is the prototype of a family of four tyrosine kinase receptors, which also includes ERBB2 (neu; HER2), ERBB3 (HER3), and ERBB4 (HER4). The four ERBBs share an overall structure with an extracellular ligand-binding domain, a single hydrophobic transmembrane domain and an intracellular kinase domain flanked by a carboxy-terminal tail with tyrosine autophosphorylation sites. Ligand binding induces the formation of homo- or heterodimers and subsequent activation of the kinase domains. Next, tyrosine phosphorylation on residues within the carboxy-terminal tail enables the recruitment and activation of effectors containing Src homology 2 and phosphotyrosine binding domains.[58,60,61] The sites that undergo autophosphorylation and the identity and relative strength of the activated signaling cascades are determined by the individual ligand as well as by the heterodimerization partner. On ligand binding, multiple signaling cascades are activated simultaneously, including the mitogen-activated protein kinase pathway (MAPK), the phosphoinositide-3-kinase pathway, protein kinase C, and signal transducers and activators of transcription (STATs). This leads to changes in the cellular transcriptional program, mediated by the proto-oncogene products c-Fos, c-Jun, and c-Myc, by zinc-finger-containing transcription factors such as Sp1 and Egr1, and others.[60] The signaling cascade RAS-MAPK is the major pathway mediating keratinocyte survival and proliferation[65] and it is shown in more detail in Figure 2. It is, however, beyond the scope of this review to summarize the knowledge on EGFR signaling in detail, and the authors recommend recently published, excellent overviews.[58,61,66,67] Signal attenuation is reached predominantly by the internalization of receptor-ligand units through clathrin-coated invaginations of the plasma membrane. Subsequent sorting steps direct the receptors either back to the cell surface or to lysosomes for degradation.[68]

Although all seven ligands can bind and activate EGFR, some of them (BTC, EREG, HBEGF, EPGN) can additionally bind to ERBB4. Because ERBB2 has no known ligand[69] and because ERBB3 carries a defective kinase activity because of substitutions in critical residues,[70] homodimers of these two receptors are believed to be inactive. However, both receptors are able to generate potent cellular signals by forming heterodimeric complexes with ERBB1, ERBB4, or with each other.[71] A further and important aspect concerns the integration of heterologous signals by the signal-processing layer (Figure 2). These nonclassical stimuli include ligands of other transmembrane receptors, membrane depolarization agents, and stress inducers.[72] The interconnection to other signaling modules supports the integration and coordination of cellular responses to extracellular stimuli.

Output Layer

This level embraces essentially every imaginable aspect of cellular and organismic biology, ranging from cell division, migration, to differentiation, and apoptosis (Figure 2). Reflecting its essential roles in mammalian development, complete loss of EGFR activity in knockout mice results in death either at an embryonic stage, in the perinatal period, or after a few weeks of postnatal life,

depending on the genetic background.[27–29] Knockout mice lacking ERBB2, -3, or -4 die inevitably during embryonic development.[73–75] The specific output depends on several factors. Ligands and receptors are present at different levels in various tissues, and, as impressively demonstrated by their overexpression in tumors,[58,59] quantitative changes are important. The type of ERBB dimer formed is obviously also decisive. Interestingly, homodimeric receptor combinations have been shown to be less mitogenic and transforming than the heterodimeric complexes, with heterodimers containing ERBB2 being most potent.[60] Biochemical properties of the ligands, such as heparin binding, their presence in a predominantly soluble or membrane-bound (precursor) form, or differential binding strength and trafficking of receptor-ligand complexes, can possibly also alter their activity. However, the exact mechanisms responsible for distinct biological activities after the activation of the same receptor dimer by different ligands are unknown.

EGFR and ERBB2: Gatekeepers of Skin Homeostasis

EGFR (ERBB1)

EGFR is most strongly expressed in the proliferation-competent basal cells of the rodent[30,76] and human[77] epidermis. The number of receptors decreases as keratinocytes enter the program of terminal differentiation and migrate to the suprabasal layers of the epidermis.[30,78,79] EGFR transcripts have been localized along the entire length of the outer root sheath of vibrissal and pelage hair follicles.[3] Although no obvious skin phenotype was noted in *egfr*$^{+/-}$ mice, further decreases in EGFR activity result in the appearance of the waved phenotype. This is seen in transgenic mice expressing a dominant-negative EGFR mutant in the epidermis,[33] in mice carrying a humanized (and hypomorphic) EGFR,[32] and in mice carrying the antimorphic alleles *wa5*[80] or *velvet*.[81] Surprisingly, constitutive activation of this receptor, as observed in the mutant mouse line *Dsk5*,[82] results in very similar phenotypic manifestations, including wavy hairs and curly whiskers. Although the reason for this contradictory observation is not clear, it indicates that the wavy coat is a general response to altered (in either direction) EGFR signaling. Surviving EGFR knockout mice display a severe phenotype of epidermal atrophy and extremely low rates of keratinocyte proliferation.[27–29] Further studies (including graft-based experiments) revealed that EGFR-deficient hair follicles are highly proliferative, but undergo premature differentiation and are unable to progress normally from anagen via catagen to telogen.[30] Instead, cell proliferation in the interfollicular epidermis (but not in the hair follicle epithelium) is strongly reduced in the absence of EGFR. Interestingly, multiple EGFR-null grafts were consumed by an inflammatory reaction.[30,83] This suggests that EGFR may play a role in protecting the hair follicle from immunological reactions.

Although apparently not essential for tumor initiation per se, EGFR was shown to facilitate tumor development. Squamous papillomas produced by grafting EGFR-null, v-ras^{Ha}-transformed keratinocytes onto nude mice were strongly reduced in size as compared with v-ras^{Ha}-transformed keratinocytes with intact EGFR.[31] Further studies revealed that EGFR is necessary to maintain the proliferative population in the basal cell compartment of papillomas.[24] EGFR deficiency results in the migration of proliferating cells into the suprabasal layer and premature differentiation in association with cell cycle arrest.[24] This concept is supported by experiments showing that the tumor formation seen in transgenic mice expressing a dominant form of the signaling protein son of sevenless (SOS) in the epidermis under the control of the keratin 5 promoter is impaired in the absence of a functional EGFR. This was attributable to increased apoptosis of EGFR-deficient tumor cells.[23] Finally, UV irradiation was shown to enhance EGFR signaling (via different mechanisms, including blocking phosphatase deactivation, altering receptor internalization and degradation, and increasing the expression of EGFR ligands), leading to keratinocyte proliferation, reduced apoptosis, and epidermal hyperplasia.[25] The phenotype of EGFR-deficient hair follicles and squamous papillomas collectively indicates that the main function of EGFR is to delay commitment to differentiation and to maintain keratinocytes in a proliferative state. Thus, EGFR can be considered as a survival factor for tumor cells, rendering this signaling system an interesting target for therapeutic intervention.

ERBB2

The expression pattern of ERBB2 is similar to that of EGFR. ERBB2 is predominantly expressed in the basal cell layer of the epidermis, in the epithelial cells of the sebaceous glands,[35,84,85] and in the outer root sheath of hair follicles.[86] ERBB2 was shown to be activated in EGF-treated epidermal keratinocytes, after treatment of the epidermis with the phorbol ester and tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and in the skin of transgenic mice expressing TGF-α in the epidermis,[87] raising the hypothesis that activation of this receptor plays an important role in tumor promotion.

To study the role of ERBB2 in epidermal homeostasis and skin carcinogenesis, diverse transgenic mouse lines were generated. Constitutive expression of the activated form of ERBB2 (neu*) in epidermal basal cells under the control of the keratin 5 (K5)[34] or K14[36] promoter resulted in a dramatic phenotype characterized by epithelial hyperplasia, which was particularly severe in the hair follicles. Early mortality because of the severity of the phenotype precluded further analysis and forced the development of additional lines. Doxycycline-inducible, conditional expression of activated ERBB2[37] in adult animals resulted in hyperplasia of the epidermis and hair follicles; prenatal expression caused perinatal death.

More informative results were obtained by the overexpression of wild-type ERBB2 under the control of the K5 promoter.[35] These animals show a milder skin phenotype (nevertheless including alopecia, follicular and interfollicular epidermal hyperplasia, and enlarged sebaceous

glands) and have a longer life span. Analysis of proliferation and differentiation markers indicated an increase in epidermal proliferation and a delay in differentiation. Importantly, spontaneous papillomas, which sometimes converted to squamous cell carcinomas, appeared in homozygous animals as early as 6 weeks of age. K5-ERBB2 transgenic mice were also more sensitive to TPA treatment and to two-stage carcinogenesis. The results of this study indicate that ERBB2 overexpression provides both an initiating and promoting stimulus and demonstrates an important role for this receptor in tumorigenesis of the skin. Finally, similar to EGFR, ERBB2 appears to play an important role in UV light-induced pathologies such as skin cancer.[88]

ERBB3

This receptor is expressed in all layers of the epidermis (with highest levels in the suprabasal and spinous layers), in hair follicles, but not in sebaceous glands.[35,89] Its localization to the upper strata of the epidermis, in contrast to the predominantly basal localization of EGFR and ERBB2, suggests a role in epidermal maturation and differentiation. Little is known, however, about its actions in the epidermis. In a study involving transfection of partial thickness porcine wounds with adenoviral particles containing an *ERBB3* expression cassette, a positive influence on wound repair was observed.[90]

ERBB4

Expression of this receptor is undetectable in the mouse epidermis[35,79,87] and its possible role in cutaneous biology is unknown.

Summary

In summary, EGFR and ERBB2 play central roles in many aspects of cutaneous biology and pathology. This signaling system is important for normal hair follicle morphogenesis and cycling, and may serve to protect the follicle from immunological reactions. Furthermore, it regulates proliferation and differentiation of follicular and interfollicular keratinocytes and strongly influences the outcome of oncogenic transformation. In the next section, we will discuss how this broad spectrum of potential actions is initiated and modulated by individual EGFR ligands.

The EGFR Ligands: Individual and Overlapping Effects

AREG

Although AREG is present at relatively low levels in the normal epidermis, its expression increases in cutaneous squamous cell carcinomas,[26] after topical retinoic acid treatment,[91] in psoriatic lesions,[92] and in several other hyperproliferative skin diseases.[17] Although no skin phenotype has been reported for knockout mice lacking AREG,[93] targeted expression of AREG in the basal[38] or suprabasal[39] layers of the epidermis of transgenic mice triggered the development of a severe, highly vascularized inflammatory-proliferative psoriasis-like cutaneous pathology. In the latter model, transgenic mice also showed synovitis, a precursor to psoriatic arthritis. Thus, AREG is remarkable in its ability to induce a complete psoriasis-like response, including, in addition to epidermal hyperproliferation, skin and joint inflammation. Recently, an anti-AREG antibody was shown to reduce the epidermal thickness of transplanted psoriatic skin,[94] suggesting that inhibiting AREG activity may be an efficient strategy for the treatment of psoriasis.

BTC

Although BTC is expressed in the skin, its role in skin biology is largely unknown. In normal human skin, BTC expression appears to be restricted to suprabasal keratinocytes, in particular to the granular cell layer.[89,91] BTC expression is down-regulated in psoriatic skin,[89] after retinoid-induced cell hyperplasia[91] and in basal and squamous cell carcinomas.[26] Interestingly, AREG and many other EGFR ligands are up-regulated under these situations (see below). Mice with targeted inactivation of the *Btc* gene are viable and show no obvious phenotype,[95] possibly because of functional redundancy among EGF family members. However, overexpression of BTC in transgenic mice resulted in increased keratinocyte proliferation, delayed hair follicle morphogenesis, and delayed hair cycle induction.[41] In addition, although wound closure was normal, angiogenesis at the wound site was significantly increased in BTC-overexpressing mice.

EGF

The founding member of the EGF family of ligands was soon recognized as an important factor for the normal development of the skin and hair follicles, because its administration to neonatal mice delayed the development of hair follicles and reduced hair diameter.[96] In sheep, EGF administration caused a weakness in the hair (wool), allowing the fleece to be sheared by hand.[97] Expression of this ligand has been reported in the upper layers of both adult mouse and fetal sheep epidermis as well as in the outer root sheath and the differentiating cells of the sebaceous glands in developing and mature sheep follicles.[42,98,99]

As for AREG and BTC, no skin abnormalities have been detected in mice lacking EGF.[93] Transgenic mice overexpressing EGF showed an increase in the proliferation rate of basal keratinocytes and a delay in epidermal differentiation, resulting in a thicker epidermis as compared with control animals.[42] Expression of endogenous EGF in hair follicles from control mice is normally turned off once the telogen phase is reached. In the transgenic mice, the continuous expression of EGF in hair follicles arrested follicular development at the final stage of morphogenesis.[42] According to a model proposed by these authors, EGF could function as a biological switch regulating the entry to and the exit from the anagen phase.

However, arrest of EGF overexpression in late-stage morphogenesis would rather suggest an inhibition of entry into hair follicle cycling (ie, first catagen induction around P17); this, however, is confusing, because EGF is a powerful catagen inducer in sheep and human hair follicles.[97,100] Therefore, the exact function of EGF in the control of murine and human hair follicle morphogenesis and cycling under physiological conditions remains to be clarified.

Epigen

The newest EGFR ligand was cloned from a mouse keratinocyte cDNA library.[101] EPGN is expressed in the inner and outer root sheath of hair follicles in newborn mouse skin, but not in the hair shaft.[62] Further characterization of potential actions of this EGFR ligand in skin biology will require the generation of knockout or transgenic mouse models.

Epiregulin

Levels of this protein in skin are relatively low.[91] EREG was shown to act as an autocrine growth factor for normal human keratinocytes *in vitro*.[10] Although the targeted elimination of EREG did not significantly affect keratinocyte growth or wound healing, the loss of this EGFR ligand was associated with a late-onset (5 months at the earliest) chronic dermatitis affecting ear and face with gradual involvement of the neck.[43] Histologically, the lesions were characterized by increased thickness of epithelial layers and fibrosis with infiltration of mast cells, eosinophils, and other inflammatory cells. This study also revealed distinct functions of the secreted and membrane-anchored forms of EREG: whereas the former is critical for the regulation of IL-18 in keratinocytes, the latter is essential for proper cytokine production by tissue resident macrophages. It remains to be determined why an independently generated EREG knockout mouse line did not show any overt abnormal phenotype.[102] Possibilities include the deletion of different gene regions, different genetic backgrounds, or maintenance conditions.

HBEGF

This EGFR ligand is predominantly found in the suprabasal layer of the epidermis and is also up-regulated in squamous cell carcinomas[26] and by retinoic acid treatment.[91] HBEGF is a major component of the mix of growth factors present in wound fluid[14] and its expression was detected in the advancing epithelial margin, in islands of regenerating epithelium within burn wounds, and in eccrine sweat glands,[103] suggesting a role in wound healing. This idea was further supported by the report of rapid and robust induction of *Hbegf* mRNA expression after scrape-wounding of epithelial cell monolayers.[44,104] HBEGF was also detected in hair follicle epithelial cells and keratinocytes at the wound edge, with particularly high levels during maximal, anagen-associated keratinocyte proliferation.[105] Retinoid-induced keratinocyte proliferation appears to depend on the induction of HBEGF and subsequent activation of EGFR,[19,91,106,107] indicating a major role for this EGFR ligand in keratinocyte biology.

The targeted deletion of the *hbegf* gene[108] or its replacement by an uncleavable form[109] results in early postnatal lethality because of defects in cardiac chamber dilation and valve malformations. Replacement of the *hbegf* gene by a transmembrane truncated mutant form, resulting in higher than normal levels of the shedded, soluble growth factor, was associated with epidermal hyperplasia and perturbed differentiation of keratinocytes.[109] Unfortunately, mutant mice were short-lived, precluding further studies. This problem was overcome by the generation of keratinocyte-specific HBEGF-deficient mice.[44] These mice, although otherwise apparently normal, showed a marked impairment in wound closure. Although keratinocyte proliferation was unaffected, cell migration was impaired at the wound site. *Hbegf* mRNA was up-regulated at the migrating epidermal wound edge. It should be considered, however, that (subtle) hair follicle abnormalities, which could contribute to the wound healing phenotype, were not rigorously excluded.[44] Taken together, these findings indicate that HBEGF is the major, and essential, EGFR ligand involved in re-epithelialization of skin wounds.

TGF-α

This ligand is expressed in the basal, spinous, and granular layers of the epidermis and in the inner root sheath of hair follicles, limited longitudinally to a specific region above the bulb.[45,110] *Tgfa* knockout mice show an obvious epithelial phenotype with wavy hairs, curly whiskers, and altered hair follicle structure, including septulation of the hair medulla and reduced dermal adipose tissue.[45,46] This phenotype is remarkably similar to the phenotype associated with the spontaneous mutant mouse *wa-1*, and the mutations are allelic. The wound healing process was shown to occur normally in *tgfa* knockout mice, although an increased variability in the rate of wound closure was reported by one group.[45] However, in an ear wound model, in which healing is mainly achieved by re-epithelialization, a delay of this process was seen in *tgfa* knockout mice.[47]

The most important actions of TGF-α, however, are related to its role in tumorigenesis. TGF-α expression is up-regulated by the oncogene *v-ras*^{Ha} in keratinocytes,[111] in papillomas elicited by chemical carcinogenesis,[112] and in human cutaneous squamous cell carcinomas.[26] TGF-α overexpression under the control of a ubiquitously active promoter did not result in spontaneous skin lesions, probably attributable to relatively low expression in this tissue. However, the increased TGF-α levels were sufficient to cause the development of hyperplasia, papillomas, adenomas, and even, although less frequent, squamous cell carcinomas after a single dose of the mutagenic agent 7,12-dimethylbenzanthracene.[48] Furthermore, arising tumors could be separated in two mutually exclusive genetic classes: tumors harboring *ha-ras* mutations displayed low transgene-derived TGF-α expression, whereas tumors harboring only wild-type *ha-ras* genes showed highly increased TGF-α expression. This indicates that TGF-α can act as an autonomous

tumor promoter and can functionally substitute for *ha-ras* mutational activation in skin tumorigenesis.

This concept is further supported by the epidermal hyperplasia and spontaneous papilloma formation seen on targeted overexpression of TGF-α in the epidermis via K14[50] or K1[52] promoters. In these transgenic mice, tumors arise without the need for an initiator (eg, 7,12-dimethylbenzanthracene), while the severity of the phenotype is enhanced by TPA treatment (tumors were negative for activating *ha-ras* mutations).[51,53] The interaction of TGF-α with other oncogene products in tumor initiation, promotion, and progression was further analyzed using K1-TGF-α transgenic mice.[54–57,113]

Conclusion and Perspectives

The existence of seven EGFR ligands with specific affinities to members of a family of four receptors allows numerous combinatorial possibilities of signaling. The complexity of this multifaceted, multipurpose signaling system appears to reflect the need for sophisticated pathways that regulate the intricate interactions between adjacent and spatially distant cell populations of different tissues, including the skin, in higher vertebrates. In addition to their ability to initiate distinct signaling cascades after receptor binding, the EGFR ligands exert specific effects because of distinct biochemical properties (like heparin binding), to differential expression of ligand precursor forms and their activating proteinases, and as a result of regulated shedding.

As reflected by the mostly mild phenotypes of knockout mice lacking single EGFR ligands, redundancy appears to be the evolutionary strategy that assures correct development and tissue homeostasis even in case expression of a single EGFR ligand is lost (with the notable exception of HBGEF, which is indispensable for correct heart development and normal re-epithelialization after wounding). Nevertheless, more subtle skin/hair phenotypes may have been overlooked in knockout mice lacking AREG, BTC, or EGF, and functional studies such as experimental tumorigenesis, wounding, or the application of other stressors, may be necessary to reveal them. Transgenic and knockout mice with altered expression of individual components of the EGFR network have been very useful tools for obtaining insight into the roles of these molecules in skin biology and pathology. For instance, although multiple EGFR ligands are overexpressed in psoriatic epidermis,[9,17–19] only AREG induced a corresponding disease when overexpressed in transgenic mice. Furthermore, multiple EGFR ligands are present in wound fluid, but a clear phenotype of impaired wound healing is only present in HBEGF-deficient mice.[44] Along the same lines, expression of multiple EGFR ligands is induced by *v-ras*^{Ha} in keratinocytes,[111] but neoplastic lesions arise only after TGF-α overexpression. Notably, TGF-α is dispensable for skin tumorigenesis,[111,114] demonstrating the fine balance between overlapping and specific actions of EGFR ligands.

There are several examples of EGFR ligands acting both individually and as a collective to orchestrate essential processes in tissues other than skin. In each situation, different ligands take on the major role. For instance, although primarily AREG and HBEGF prepare the uterine epithelium for interacting with the embryo during blastocyst implantation, at least three additional EGFR ligands are expressed in the uterus around implantation time.[115] Not unexpectedly, implantation defects have not been reported in mice lacking individual EGFR ligands. Further examples are the induction of AREG, BTC, and EREG by luteinizing hormone in ovary granulosa cells[116] or the induction of AREG, TGF-α, and HBEGF by parathyroid hormone in osteoblasts.[117]

An emerging aspect in the biology of the EGFR network is the importance of feedback inhibition. Loss of the negative feedback regulator of ERBBs, RALT/MIG6 adaptor protein, resulted in hyperactivation of EGFR signaling, increased proliferation, and reduced differentiation of keratinocytes, as well as higher susceptibility to neoplastic transformation.[118] In contrast, skin-targeted expression of the molecule caused a waved-like phenotype typical for situations of reduced EGFR activity.[119] Because of its remarkable sensitivity to reduced or increased EGFR-mediated activity, the skin will certainly continue to serve as an excellent readout system for the dissection of basic mechanisms of EGFR signaling.

In addition, the clinical relevance of mammalian skin's exquisite sensitivity to altered EGFR activity has become acutely evident in recent years with the increasing clinical use of different EGFR tyrosine kinase inhibitors and anti-EGFR monoclonal antibodies for treating patients with various types of cancer. A common, severe, and often therapy-limiting undesired treatment effect of these, oncologically very attractive, new agents is the development of extensive inflammatory rashes, often in association with prominent, disseminated (sterile) folliculitis. In many cases, there is a consistent positive correlation between the presence of cutaneous drug eruptions and tumor regression or even survival.[120] This suggests that the skin offers an excellent clinical read-out system for evaluating the efficacy of EGFR-antagonistic agents, and raises the question whether the cutaneous adverse effects even award (as yet undefined) clinical anti-tumor benefits.[120]

Future research aimed at elucidating the pleiotropic functions of the EGFR network in the skin and its multiple layers of regulation should place more emphasis on as yet insufficiently explored aspects like nonclassical activation of EGFR, possible EGFR-independent actions of EGFR ligands, and the regulation of their shedding (as well as its consequences for ligand binding). A better understanding of this powerful signaling system should allow the development of innovative therapeutic strategies for diseases of the skin and its appendages.

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