The American Journal of Pathology, Vol. 170, No. 6, June 2007

DOI: 10.2353/ajpath.2007.060537

Tumorigenesis and Neoplastic Progression

Differential ErbB1 Signaling in Squamous Cell versus Basal Cell Carcinoma of the Skin

Laure Rittié,* Sanjay Kansra,* Stefan W. Stoll,* Yong Li,* Johann E. Gudjonsson,* Yuan Shao,* Lowell E. Michael,* Gary J. Fisher,* Timothy M. Johnson,*†‡ and James T. Elder*§¶

From the Departments of Dermatology, Otolaryngology,† Surgery,‡ and Radiation Oncology,§ University of Michigan Medical Center, Ann Arbor; and the Ann Arbor Veterans Affairs Health System,¶ Ann Arbor, Michigan*

In this study, we examined ErbB1 signaling in human basal and squamous cell carcinomas (BCC and SCC) of the skin *in vivo*. We used enzyme-linked immunosorbent assay, laser capture microdissection-coupled real-time reverse transcriptase-polymerase chain reaction, and immunohistochemistry to assess expression and activation levels of ErbB1 protein, ligands, and potential downstream effectors, in BCC and SCC tumors, stroma, and adjacent epidermis. Although total ErbB1 protein and mRNA were similar in cancerous and normal skin, we found that ErbB1 activation (phospho-Tyr^{1068}) was greater in bulk SCC versus BCC or normal skin. In addition, three ErbB1 ligand transcripts (amphiregulin, heparin-binding epidermal growth factor-like growth factor, and transforming growth factor-α) were up-regulated in tumor cells of SCC but not BCC. Expression of these ligands was also increased in asymptomatic epidermis adjacent to both SCC and BCC, relative to normal skin. Interestingly, betacellulin transcript levels were inversely regulated compared with the other ligands. Consistently, downstream ErbB1 effectors (Erk1/2 and Akt) were activated in tumor cells of SCC but not of BCC and in adjacent epidermis of both BCC and SCC. These results demonstrate that ErbB1 signaling is hyperactive in tumor cells of SCC but not of BCC and in nearby asymptomatic epidermis of both tumor types. Our results suggest that targeting ErbB1 signaling might be of benefit in the treatment of SCC. *(Am J Pathol 2007, 170:2089–2099; DOI: 10.2353/ajpath.2007.060537)*

Basal cell carcinoma (BCC) is the most common cancer diagnosed in the United States, representing ~30% of all new US cancers, with ~1 million cases diagnosed annually.[1] The incidence of BCC appears to be increasing.[2,3] Although less common than BCC, squamous cell carcinoma (SCC) of the skin carries a >10-fold higher risk of metastasis and mortality.[4] Surgical treatment of BCC and SCC is frequently disfiguring, and local recurrence is not uncommon. Clearly, BCC and SCC constitute serious health problems.

Early histological studies suggested that BCC often arise from the hair follicle epithelium.[5,6] This link has been strengthened by the observation that the Patched signaling pathway, which is constitutively activated by mutation in BCC,[7–10] plays a critical role in hair follicle development[11] and cycling.[12] Recent studies have strongly implicated ErbB1 (also known as epidermal growth factor receptor, or EGFR) in the initiation of hair growth,[13] and a variety of natural and engineered mutants in ErbB receptors and their ligands have very significant effects on hair follicle morphogenesis.[14–21] Moreover, signaling through ErbB1 has also been implicated in chemical and oncogene-induced carcinogenesis in mouse skin, leading to papillomas that progress to SCC.[22–26] ErbB signaling also plays an important role in controlling the proliferation of normal skin in the context of wound re-epithelialization and in hyperproliferative disorders such as psoriasis.[27] Together with substantial data implicating overexpression and/or mutation of ErbB family members in human cancer,[28–30] and of a role for ErbB inhibitors in cancer

Supported in part by the National Institute for Arthritis, Musculoskeletal, and Skin Diseases, National Institutes of Health (award R21 AR48405 and award T32-AR07197 to S.K.); the Babcock Memorial Trust; Chesebrough Pond's Lever Brothers Dermatology Foundation (research career development award and a dermatology foundation research grant to S.W.S.); and the Ann Arbor Veterans Affairs Hospital (to J.T.E.).

Accepted for publication February 22, 2007.

Supplemental material for this article can be found on *http://ajp.amjpathol.org*.

Current address of S.K.: Division of Endocrinology, Department of Pharmacology, Medical College of Wisconsin, Milwaukee, WI.

Address reprint requests to James T. Elder, 3312 CCGC, Box 0932, 1500 East Medical Center Dr., University of Michigan, Ann Arbor, MI 48109-0932. E-mail: jelder@umich.edu.

therapy,[31] these observations suggested to us that ErbB signaling could be an important effector of malignant behavior in BCC and/or SCC, providing opportunities for improved therapy.

The mammalian c-ErbB receptor family consists of four closely related receptor tyrosine kinases (RTKs; ErbB1 to 4) that interact with multiple ErbB receptor ligands.[32,33] Ligand binding promotes receptor dimerization, with subsequent auto- and transphosphorylation of specific tyrosine residues, resulting in activation of multiple signal transduction pathways. Receptor activation ultimately affects many cellular functions, including cell migration, proliferation, differentiation, and survival.[34] Normal human skin has been shown to express ErbB1, ErbB2, and ErbB3, but not ErbB4.[35–38]

Previous studies of ErbB protein expression in BCC and SCC have yielded inconsistent results.[39–47] The overall impression gleaned from most of those studies is that both ErbB1 and ErbB2 are expressed in BCC at the same or lower levels than in normal skin. Recently, BCC, SCC, and normal skin were found to express ErbB1, ErbB2, and ErbB3, but not ErbB4 mRNA, reminiscent of normal skin.[48] Relative expression of these receptors in tumor cells versus adjacent epidermis was not addressed in that study, which used bulk tumor specimens. In cutaneous SCC, studies have reported variable levels of ErbB1 and ErbB2 in primary tumors but increased expression of ErbB1[47] and ErbB2[46] proteins in metastatic lesions.

In the current study, we examined the ErbB1 signaling in BCC and SCC, differentiating tumor cells, and adjacent epidermis. Although total ErbB1 expression was not altered in BCC or SCC versus normal skin, we showed that ErbB1 activation (phospho-Tyr^{1068}) was greater in bulk SCC versus BCC or normal skin. ErbB1 activation in SCC tumor was accompanied by increased expression of three ErbB1 ligands [amphiregulin (AREG), heparin-binding epidermal growth factor-like growth factor (HB-EGF), and transforming growth factor-α (TGF-α)] and increased activation of potential ErbB1 downstream effectors. Taken together, these results demonstrate that the ErbB1 pathway is hyperactivated in SCC tumors versus BCC and normal skin.

Materials and Methods

Reagents

Mouse monoclonal antibodies to ErbB1 were purchased from Transduction Laboratories (clone 13) (Lexington, KY), Labvision (Ab-13 and Ab-14) (Freemont, CA), or Invitrogen (Zymed no. 28-005; Carlsbad, CA). Antibodies against Akt (CST no. 2966), phospho-Akt (CST no. 9277), Erk1/2 (p42/44 mitogen-activated protein kinase, CST no. 9102), and phospho-Erk1/2 (CST no. 9101) were from Cell Signaling Technologies (Beverly, MA). Horseradish peroxidase-conjugated secondary antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). EGFR (full length) and phospho-EGFR (pY1068) enzyme-linked immunosorbent assay (ELISA) kits were from Invitrogen. All other chemicals were purchased from Sigma (St. Louis, MO).

Procurement of Human Tissue Samples

Tumor tissue samples were obtained from the University of Michigan Cutaneous Oncology Unit. OCT-embedded tumor blocks were also obtained from the Cutaneous Oncology Unit in the course of Mohs micrographic surgery. Mohs stages are convex tissue fragments that are sequentially removed in a radial manner after tumor debulking, to establish the tumor limits. Because these specimens are laid flat before sectioning, they also contain epithelium that resides in the immediate vicinity of the tumor (or as much as 2 to 3 mm away from the tumor margin). BCC and SCC samples were ascertained without regard to histological subtype. Normal skin was obtained from sun-protected skin, either from excess skin removed during abdominoplasty or mammoplasty or by punch biopsy. All procedures involving human patients were approved by the Institutional Review Board of the University of Michigan Medical School, and all patients provided written informed consent.

Skin Sample Processing for ELISA

Tumor specimens were removed by shave debulking so as to contain a minimum amount of grossly normal skin, although admixture with noncancerous follicular structures and epidermis could not be excluded. Samples were snap-frozen in liquid nitrogen on removal. Tissue lysates were prepared as follows: samples were homogenized on ice in RIPA buffer [50 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, 1% Igepal (Nonidet P-40), 0.25% sodium deoxycholate, 1 mmol/L ethylenediaminetetraacetic acid, 1 mmol/L phenylmethyl sulfonyl fluoride, protease inhibitors (Complete Mini; Roche, Indianapolis, IN), 1 mmol/L sodium orthovanadate, and 1 mmol/L sodium fluoride] and vortexed in the presence of glass beads (Biospec, Bartlesville, OK). After 10 minutes of centrifugation at 10,000 $\times$ *g* and 4°C, supernatants were assayed for total protein using a protein assay reagent (Bio-Rad, Hercules, CA) and bovine serum albumin as a standard. Total and phospho-EGFR ELISAs were performed according to the manufacturer's instructions.

Immunohistochemistry

Six-μm frozen sections were prepared from OCT-embedded blocks containing Mohs surgical stages and immediately fixed for 20 minutes in 4% paraformaldehyde containing phosphatase inhibitors (10 mmol/L sodium orthovanadate and 50 mmol/L sodium fluoride). After three washes in TBST (50 mmol/L Tris-HCl, 0.15 mol/L NaCl, and 0.1% Triton X-100, pH 7.5), tissue sections were blocked in blocking buffer (TBST containing 1% bovine serum albumin) for 1 hour at room temperature. After three washes in TBST, sections were incubated with the primary antibody in blocking buffer overnight at 4°C at the following dilutions: anti-ErbB1 (1:800), anti-Erk1/2 (1:100), anti-phospho-Erk1/2 (1:400), and anti-phospho-Akt (1:200). After three washes in TBST, samples were

Table 1. Sequences of Primers and Probes for QRT-PCR

Gene	Sense primer (5′ → 3′)	Anti-sense primer (5′ → 3′)	Probe
Amphiregulin	5′-AGGCCATTATGCTGCTG GAT-3′	5′-TGTGGTCCCCAGAAAAT GGT-3′	5′-CCTCAATGACACCTACTCT GGGAAGCGTGA-3′
Betacellulin	5′-GGGAGATGCCGCTTCGT-3′	5′-TGCTCCAATGTAGCCTTC ATCA-3′	5′-CCGAGCAGACGCCCTCC TGTG-3′
EGF	5′-AATACCGTTAAGATACAGT GTAGGCACTTTA-3′	5′-ATCACAACTCATTTTGG CAAAATC-3′	5′-CTCCTCATTGGCGTGGTC CATGCTGAT-3′
Epiregulin	5′-GGCTCCTTCATCGAATGC TAA-3′	5′-CAGGATAAACGTAGAGGA AGAACAGA-3′	5′-CCTTTGAGTAGAGTCTCC CTGGATCACATACCA-3′
HB-EGF	5′-TGGCCCTCCACTCCTC ATC-3′	5′-GGGTCACAGAACCATCCTA GCT-3′	5′-CACCCACCTTTGCCACA-3′
TGF-α	5′-AGGACAGCACTGCCAGAGA TG-3′	5′-CGACGGAGTTCTTGACAGA GTTT-3′	5′-CTGTGCAGCCTTTTGTGGG CCTTC-3′
36B4	5′-ATGCAGCAGATCCGCA TGT-3′	5′-TTGCGCATCATGGTGT TCTT-3′	5′-CGCGGGAAGGCTGTGG TGCT-3′

incubated with biotin-conjugated secondary antibodies 1 hour at room temperature followed by the addition of ABC reagent (Vectastain Elite; Vector Laboratories, Burlingame, CA). Color was developed using AEC (3-amino-9-ethyl-carbazole) (no. A-5754; Sigma), according to the manufacturer's instructions. Slides were counterstained with Mayer's hematoxylin. Sections were examined independently by two observers (J.T.E. and J.E.G.), who were blinded to the antibody used for staining and tumor type. The staining intensities were scored on a 0 to 4 semiquantitative scale (from 0 = absent to 4 = very strong). In assigning scores, the observers took into account both the intensity and the extent of staining.

RNA Extraction and Quantitative Reverse Transcriptase-Polymerase Chain Reaction (QRT-PCR)

Total RNA from frozen samples or laser-captured material were extracted using a commercial kit (RNeasy; Qiagen, Chatsworth, CA) and quantified using a RiboGreen RNA quantification kit (Invitrogen) according to the manufacturer's instructions. QRT-PCR was performed on 100 ng of total RNA as described previously.[49] Labeled PCR primers and probes for ErbB1 ligands[50] were produced by the custom oligonucleotide synthesis service (Applied Biosystems, Foster City, CA) and are shown in Table 1. The primers and probe for ErbB1 (predesigned TaqMan gene expression assay no. Hs00193306_m1) were obtained from Applied Biosystems. Efficiency of QRT-PCR reactions was determined using cDNA standards for 36B4, AREG, HB-EGF, and TGF-α. Data on figures are presented relative to 36B4 (fold versus 36B4 = $2^{-(\Delta CTtarget\ -\ \Delta CT36B4)}$).

Laser Capture Microdissection (LCM)

LCM was performed as recently described.[50] Tumor specimens were microdissected to obtain either tumor cells, adjacent asymptomatic interfollicular epidermis (without appendages or hair follicle infundibulum), or peritumoral stroma. Normal skin was microdissected to obtain epidermis, dermis (equal portions of papillary and reticular dermis in a defined length of section), or appendages (whole hair follicles, sebaceous glands, and eccrine glands in a defined length of section).

Statistical Analyses

Data are expressed as mean ± SEM. Comparisons among groups were made using a two-sample *t*-test under a two-tailed hypothesis. For QRT-PCR experiments, exponential transformations were used to depict the data on figures. However, statistical analyses of QRT-PCR were made on the untransformed scale. All *P* values are considered significant when lower than 0.05. In the figures, error bars indicate SEM, and significance is depicted with asterisks.

Results

ErbB1 Levels Are Not Altered in BCC and SCC, Relative to Normal Skin

To evaluate the role of ErbB1 signaling in BCC and SCC, we first assessed total ErbB1 protein expression in BCC and SCC tumor samples by ELISA. As shown in Figure 1A, ErbB1 protein expression was not significantly different between normal skin, BCC, and SCC. In parallel, ErbB1 expression pattern was studied by immunohistochemistry (Figure 1B). ErbB1 staining intensity in SCC and BCC was variable in tumor cells of both BCC and SCC (examples of weaker and stronger staining are given in Figure 1B) but in general were similar to that observed in adjacent normal-appearing skin. ErbB1 staining intensity was evaluated by two blinded investigators in tumor cells, adjacent epidermis, and noncancerous appendages, using a semiquantitative 0 to 4 scale (Table 2). No significant differences in ErbB1 staining intensity could be detected, either between tumor and asymptomatic adjacent epidermis or between BCC and SCC.

ErbB1 mRNA levels were quantified in various cellular compartments of BCC, SCC, and normal skin, using QRT-PCR. LCM was applied to each sample to isolate RNA from tumor cells, adjacent/overlying epidermis, and

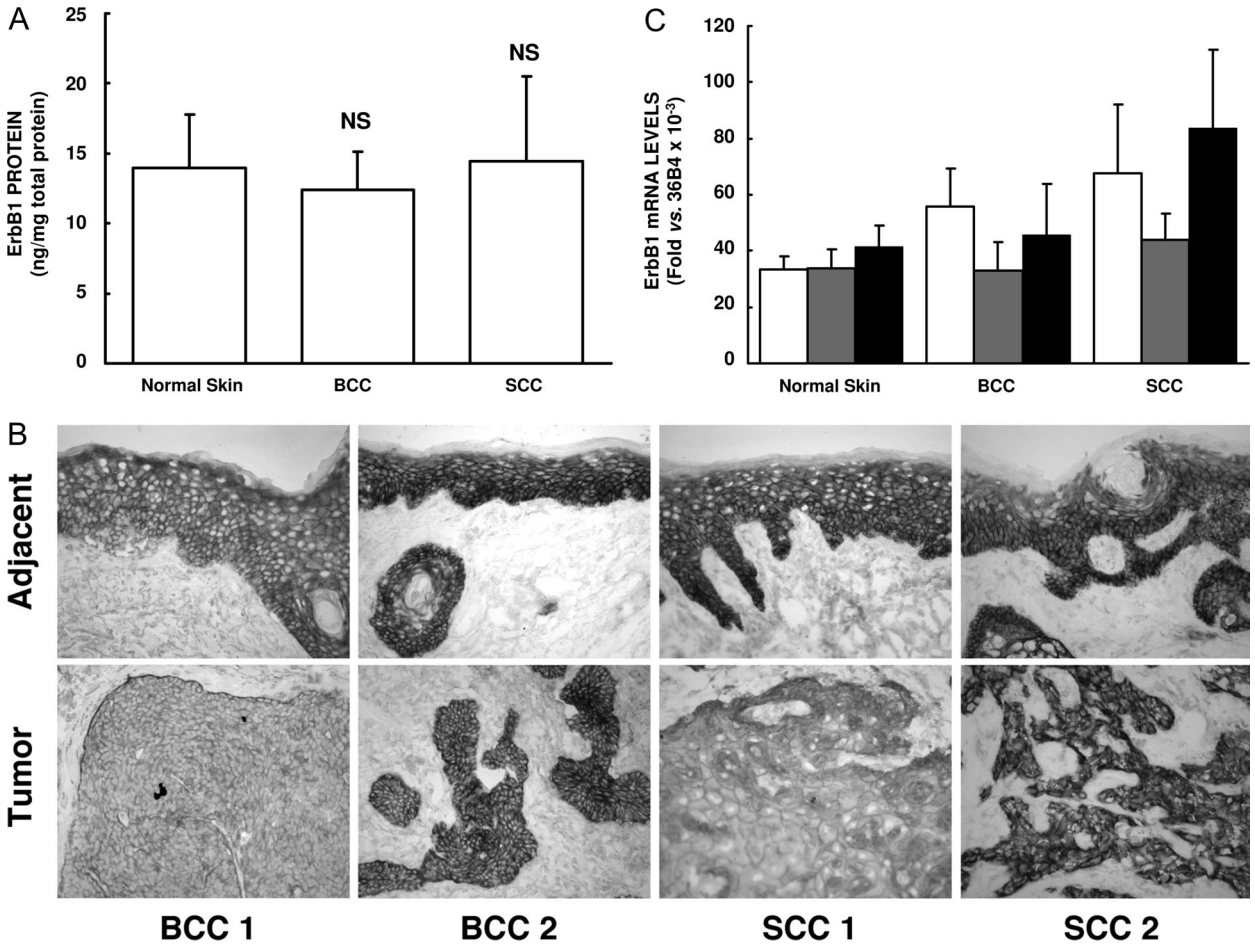

Figure 1. ErbB1 expression is not altered in BCC and SCC, relative to normal skin. **A:** ELISA quantification of total ErbB1 protein in normal skin ($n = 5$), bulk BCC ($n = 9$), and bulk SCC ($n = 7$). NS, nonsignificant versus normal skin. **B:** ErbB1 immunostaining in BCC and SCC. For each tumor type, one example of relatively weak (BCC1 and SCC1) and one example of relatively strong (BCC2 and SCC2) ErbB1 staining of tumor cells are shown to illustrate the variability in staining that was observed. Adjacent indicates clinically normal-appearing skin adjacent to and/or overlying the tumor. Original magnifications, ×200. **C:** ErbB1 mRNA expression was quantified in microdissected normal skin ($n = 4$), BCC ($n = 5$), and SCC ($n = 5$) samples by QRT-PCR. White bars, normal epidermis (for normal skin) or epidermis adjacent to tumor (for tumor specimens); gray bars, normal dermis (for normal skin) or tumor stroma (for tumor specimens); black bars, normal epidermal appendages (for normal skin) or tumor cells (for tumor specimens). None of the differences was statistically significant.

stroma. For comparison, sun-protected buttock skin of healthy individuals was dissected to isolate epidermis, dermis, and appendages. As shown in Figure 1C, ErbB1 mRNA levels were similar in all studied compartments. These results are in agreement with the data presented in Figure 1, A and B, and Table 2, ie, alteration of ErbB1 expression is not a consistent feature of either SCC or BCC.

Table 2. Semiquantitative Immunohistochemical Evaluation of ErbB1 in BCC and SCC

Evaluator	Tumor type		ErbB1 Tumor	ErbB1 Epid*	ErbB1 App†
1	BCC	Avg	2.80	3.00	2.83
		SEM	0.14	0.10	0.12
		n =	15	15	15
	SCC	Avg	2.50	2.86	2.94
		SEM	0.25	0.10	0.05
		n =	11	11	9
2	BCC	Avg	3.25	3.53	3.38
		SEM	0.16	0.08	0.07
		n =	16	17	16
	SCC	Avg	3.16	3.50	3.44
		SEM	0.20	0.13	0.15
		n =	11	11	9

*Asymptomatic overlying/adjacent epidermis.
†Noncancerous appendages (follicular structures).

ErbB1 Is Activated in SCC versus BCC and Normal Skin

ErbB1 receptor activation is characterized by increased tyrosine phosphorylation of the receptor, as well as several other proteins that act as signaling intermediates.[32,51] To estimate the activity of the ErbB1 receptor, phosphorylation of tyrosine residue 1068 (Tyr^{1068}) was quantified by ELISA in shave debulkings of BCC and SCC (consisting mainly, but not totally, of tumor cells) and compared with that measured in sun-protected skin from healthy controls. As shown in Figure 2, ErbB1 activation levels were similar between BCC and normal skin. Interestingly, activated ErbB1 levels were ~2.5-fold higher in SCC, compared with BCC or normal skin ($P < 0.05$). In

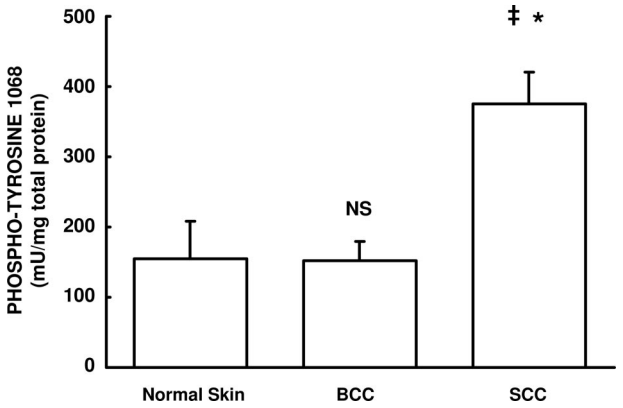

Figure 2. ErbB1 is activated in SCC versus BCC and normal skin. ELISA quantification of ErbB1 phospho-Tyr^{1068} in normal skin ($n = 5$), bulk BCC ($n = 9$), and bulk SCC ($n = 7$). *$P < 0.05$ versus normal skin; ‡$P < 0.05$ versus BCC. NS, nonsignificant versus normal skin.

parallel, we attempted to localize activated ErbB1 in SCC tissue sections by immunohistochemistry. Unfortunately, we could not detect any signal using two different phospho-ErbB1-specific antibodies either in tumor cells or in adjacent/overlying epidermis, whereas EGF-stimulated normal keratinocytes were positive (data not shown).

Expression of ErbB1 Ligand mRNAs in Microdissected BCC and SCC

Because ErbB1 requires ligand binding for its activation,[52–54] we quantified the expression of six ErbB1 ligands [AREG, betacellulin (BTC), EGF, epiregulin (EREG), HB-EGF, and TGF-α] in BCC and SCC by QRT-PCR. To this end, LCM was used to separate tumor cells, adjacent/overlying interfollicular epidermis, and stromal cells for each tumor specimen. Figure 3A shows ErbB1 ligand mRNA levels in BCC samples. Strikingly, we found that AREG, EREG, HB-EGF, and TGF-α transcript levels were markedly lower in tumor cells than in adjacent epidermis. In parallel, BTC transcript levels were similar in tumor cells versus adjacent epidermis, whereas EGF mRNA levels were just above limit of detection in some samples (depicted), and not detectable in others (not shown). Interestingly, stromal cells were found to produce relatively large amounts of AREG and TGF-α mRNAs along with lesser amounts of BTC and HB-EGF mRNAs, despite the fact that this compartment lacks epithelial elements.

As shown in Figure 3B, SCC tumors yielded a much different outcome than did BCC tumors. All ErbB1 ligands tested showed similar expression levels in SCC tumor cells and adjacent epidermis. AREG, BTC, HB-EGF, and TGF-α mRNAs were expressed at slightly higher levels in SCC tumor cells than in adjacent epidermis, although these differences were not statistically significant. Direct comparison of Figure 3, A and B, shows that AREG, EREG, HB-EGF, and TGF-α transcript levels were higher in SCC tumors than in BCC tumors (26.2-, 11.2-, 4.1-, and 22.5-fold, respectively). These differences were markedly and statistically significant (Supplemental Figure 1, see *http://ajp.amjpathol.org).*

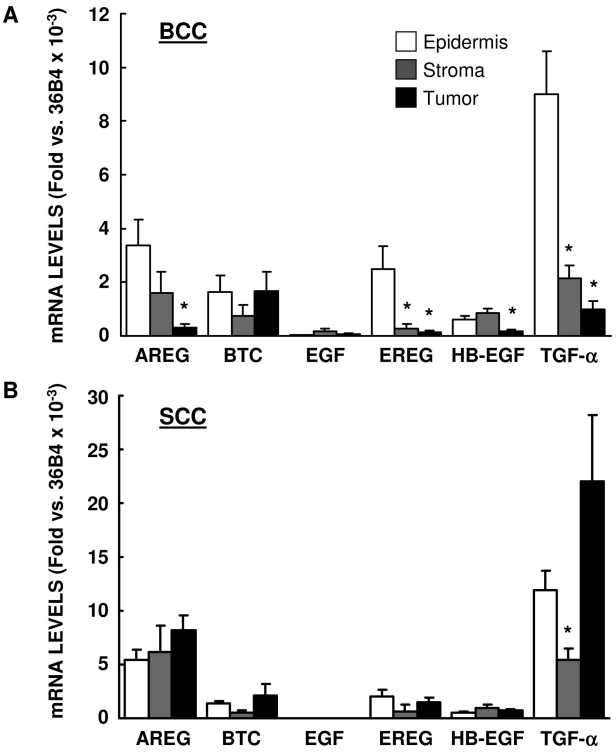

Figure 3. Expression of ErbB1 ligands in microdissected BCC and SCC. ErbB1 ligand mRNA levels were quantified by QRT-PCR in microdissected adjacent epidermis (white bars), stroma (gray bars), and tumor cells (black bars) for BCC (**A**) ($n = 5$) and SCC (**B**) ($n = 4$ to 5, except for EGF stroma in which values were unreliable because of being below limits of detection in two samples). *$P < 0.05$ versus adjacent epidermis.

Expression of AREG, HB-EGF, TGF-α, and BTC in Epithelial Compartment of Normal and Cancerous Skin

Transcript levels of AREG, HB-EGF, TGF-α, and BTC were then compared with those of normal skin epithelia, ie, epidermis and appendages (hair follicles, eccrine, and sebaceous glands) (Figure 4). It is interesting to note that, in normal skin, AREG mRNA levels were 11-fold higher in appendages as compared with epidermis (Figure 4A). HB-EGF and TGF-α levels were also significantly higher in appendages relative to normal epidermis (Figure 4, B and C), whereas expression of BTC was significantly decreased in this context (Figure 4D).

AREG, HB-EGF, and TGF-α mRNA levels were higher in asymptomatic epidermis adjacent to both BCC and SCC, when compared with normal interfollicular epidermis. AREG mRNA levels were 9.1- and 14.6-fold higher in epidermis adjacent to BCC and SCC, respectively, than in normal epidermis (Figure 4A). In addition, AREG mRNA levels were markedly increased in SCC tumors (22-fold the levels of normal epidermis), whereas BCC tumor cells expressed similar amounts of AREG mRNA (0.8-fold) relative to normal epidermis. The HB-EGF mRNA expression pattern was similar to that of AREG (Figure 4B). HB-EGF transcript levels were 3.0- and 2.7-times higher in adjacent epidermis of BCC and SCC, respectively, relative to normal epidermis. SCC tumor cells expressed 3.4-fold higher levels of HB-EGF mRNA

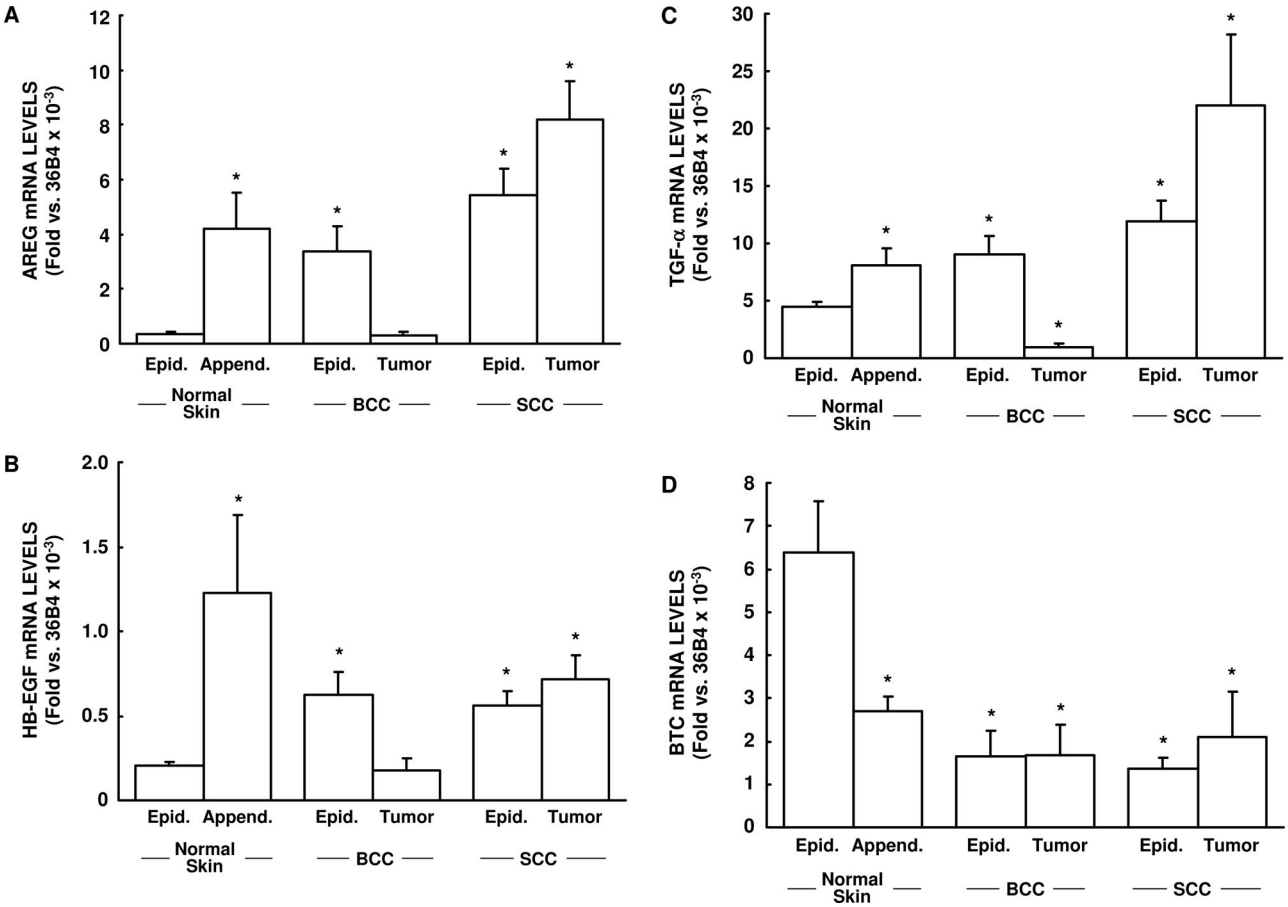

Figure 4. AREG, HB-EGF, TGF-α, and BTC mRNA levels in epithelial compartments of normal and cancerous skin. mRNA levels for AREG (**A**), HB-EGF (**B**), TGF-α (**C**), and BTC (**D**) were quantified by QRT-PCR in microdissected epithelial compartments of normal versus cancerous skin as indicated below the bars. $^*P < 0.05$ versus epidermis from normal skin; $n = 3$ to 5.

versus normal skin, whereas BCC tumor cells expressed similar amounts of HB-EGF mRNA (0.8-fold) relative to normal epidermis.

Figure 4C depicts TGF-α mRNA levels in epithelial cells of BCC, SCC, and normal skin. TGF-α transcript levels were, respectively, 2.0 and 2.7 times more abundant in adjacent epidermis of BCC and SCC than in normal epidermis. In addition, TGF-α mRNA levels were overexpressed (4.9-fold) in SCC tumors versus epidermis of normal skin. Expression of TGF-α in BCC tumor cells was significantly reduced (78% reduction) relative to normal epidermis, which had a relatively high level of TGF-α expression. Unlike AREG, HB-EGF, and TGF-α, BTC mRNA levels were markedly and significantly lower in adjacent epidermis of both BCC and SCC (74 and 79% reduction, respectively), when compared with epidermis of normal skin (Figure 4D). A similar reduction was noted in BCC tumors relative to normal epidermis (−74%) and in SCC tumors versus normal epidermis (−67%).

Erk1/2 and Akt Are Activated in SCC but Not BCC Tumors

As additional surrogates for evaluation of ErbB1 activation in SCC and BCC, we assessed Erk1/2 and Akt phosphorylation by immunohistochemistry. Erk1/2 and Akt are phosphorylated upon ligand activation of ErbB1, as it is the case in normal human keratinocytes *in vitro*[55,56] and in UV-treated human skin *in vivo*.[57,58] Figure 5A presents phospho-Erk1/2 and phospho-Akt staining in representative samples of BCC and normal skin. Normal skin revealed faint nuclear staining for phospho-Erk1/2 in the upper epidermal layers, whereas phospho-Akt was uniformly stained in the basal epidermal layer. In contrast, staining for both phospho-Erk1/2 and phospho-Akt was stronger in asymptomatic epidermis adjacent to BCC tumors, whereas tumor cells were primarily negative for both phospho-Erk1/2 and phospho-Akt. Control stainings (isotype-matched IgG for phospho-Erk1/2 and peptide preabsorption for phospho-Akt) were negative.

Figure 5B presents phospho-Erk1/2 and phospho-Akt staining in a representative SCC. In this tumor, both tumor cells and adjacent epidermis showed a very strong staining for phospho-Erk1/2 and phospho-Akt. In both cases, the intensity of the staining was much greater for SCC than for BCC. Phospho-Akt staining of adjacent epidermis was more discontinuous than was seen in normal skin and extended to the lower suprabasal layers as well as the basal layer.

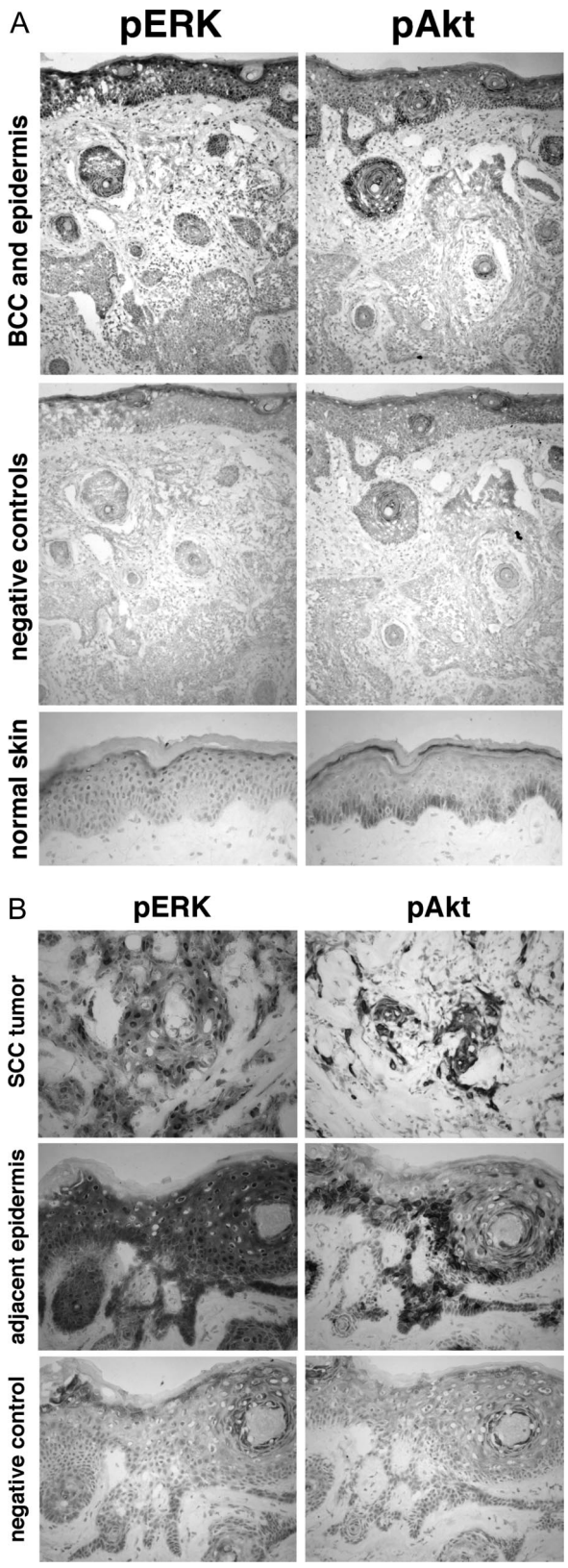

Figure 5. Phospho-Erk1/2 and phospho-Akt immunostaining in BCC, SCC, and normal skin. Phospho-Erk (left) and phospho-Akt (right) were revealed by immunohistochemistry in BCC (**A**) and SCC (**B**) samples. In **A** and **B**, negative control indicates isotype control antibody for phospho-Erk1/2 and peptide preabsorption for phospho-Akt. In **A**, normal skin refers to sun-protected buttocks skin from a different individual. In **B**, adjacent epidermis appears irregular because of tangential sectioning.

Semiquantitative evaluation of phospho-Erk1/2 and phospho-Akt were conducted by two blinded observers, as described in Materials and Methods. Results, presented in Table 3, show that phospho-Erk1/2 and phospho-Akt were similarly expressed in tumor versus adjacent epidermis of SCC, whereas staining was significantly more intense in adjacent epidermis versus tumor cells of BCC. In addition, staining for phospho-Erk1/2 and phospho-Akt was significantly higher in SCC epidermis and tumor relative to BCC epidermis. Interestingly, phospho-Akt was virtually absent in BCC tumor.

Discussion

In the present study, we examined ErbB1 signaling in cutaneous BCC and SCC. We used ELISA, LCM-coupled QRT-PCR, and immunohistochemistry to assess ErbB1 receptor protein and activation levels, ErbB1 ligand expression, and activation of potential downstream effectors in BCC and SCC tumors, including adjacent stroma and epithelium. We show that although ErbB1 protein and mRNA expressions were not consistently altered in either primary BCC or SCC, ErbB1 activation, as measured by Tyr^{1068} phosphorylation levels, was significantly higher in bulk SCC relative to BCC and normal skin.

Our multiple attempts to localize phosphorylated (ie, activated) ErbB1 by immunohistochemistry were unsuccessful. These results are consistent with previous reports indicating that it is much more difficult to document ErbB1 tyrosine phosphorylation in skin than in EGF-stimulated cultured keratinocytes.[55–57] Alternatively, we measured ErbB1 ligand transcript levels by LCM-coupled QRT-PCR and potential ErbB1 downstream effectors (phospho-Erk1/2 and phospho-Akt) by immunohistochemistry. Both strategies lead to similar results, ie, ErbB1 signaling is different in BCC and SCC: although both tumor types are characterized by increased ErbB1 signaling in asymptomatic adjacent epidermis, ErbB1 signaling is activated in SCC tumor cells but not in BCC tumor cells.

During revision of this article, Fogarty and colleagues[59] published a study in which they used Western blotting using the LiCor immunofluorescence detection system to quantify the expression and activation of ErbB1 in SCC of the skin. Results show that even using a relatively sensitive Western blot detection technique, ErbB1 protein was detectable in only 43% of studied tumors, and activated ErbB1 (as measured by phosphorylation of Tyr^{1068}), in only 24% of studied tumors. The use of ELISA in our study has proven to be a much more sensitive technique to quantify ErbB1 expression and activation, because both were detected in all tested samples, including normal healthy human skin.

ErbB1 is commonly overexpressed in a number of epithelial malignancies and is often associated with an aggressive phenotype.[28–30] However, previous studies of ErbB1 protein expression in BCC and cutaneous SCC have yielded inconsistent results,[39–47] which is conso-

Table 3. Semiquantitative Immunohistochemical Evaluation of Erk, Phospho-Erk, and Phospho-Akt in BCC and SCC

Evaluator	Tumor type		Total Erk1/2			Phospho-Erk1/2			Phospho-Akt		
			Tumor	Epid*	App†	Tumor	Epid*	App†	Tumor	Epid*	App†
1	BCC	Avg	0.83‖**	1.53	1.50	0.63‖**	1.60	1.37	0.03‖**	1.47	1.50
		SEM	0.09	0.13	0.15	0.12	0.20	0.16	0.03	0.20	0.21
		n =	15	15	15	15	15	15	15	15	15
	SCC	Avg	1.45	1.50	1.39	1.82	2.00	1.72	1.32	1.60	1.25
		SEM	0.14	0.13	0.13	0.10	0.21	0.17	0.32	0.28	0.20
		n =	111	11	9	11	11	9	11	10	8
2	BCC	Avg	2.18‡	2.79	2.53	1.44§¶	2.41	1.93	0.07‖**	2.74	2.67
		SEM	0.19	0.16	0.14	0.26	0.19	0.15	0.05	0.22	0.27
		n =	117	17	16	17	17	15	14	17	15
	SCC	Avg	2.48	2.75	2.42	2.41	2.64	2.17	1.73	2.00	1.85
		SEM	0.22	0.20	0.19	0.28	0.28	0.30	0.41	0.29	0.37
		n =	111	11	9	11	11	9	11	11	10

*Asymptomatic overlying/adjacent epidermis.
†Noncancerous appendages (follicular structures).
‡$P < 0.05$, tumor versus overlying/adjacent epidermis.
§$P < 0.05$, BCC versus SCC.
¶$P < 0.05$, tumor versus overlying/adjacent epidermis.
‖$P < 0.0005$, tumor versus overlying/adjacent epidermis.
**$P < 0.0005$, BCC versus SCC.

nant with our findings of variable levels of RNA and immunoreactive protein in both tumor types. Other recent studies using quantitative techniques have also reported an unaltered, albeit variable, expression of ErbB1 in BCC and SCC of the skin.[48,60] We could not find any evidence for increased ErbB1 protein and mRNA expression in either BCC or SCC samples ($n = 9$ to 16). Taken together, these data suggest that overexpression of ErbB1 per se is not a cardinal feature of either BCC or SCC.

Recently, Haider and colleagues[60] performed microarray analysis on cutaneous SCC tumors. These authors reported an increased expression of EREG and TGF-α mRNA in SCC when compared with normal skin, but no change in AREG, HB-EGF, or BTC transcript levels. Several points could explain this apparent discrepancy between this study and our results. First, the origin of the samples is different: Haider and colleagues[60] used bulk SCC whereas we purified tumor cells by LCM. Second, Haider and colleagues[60] used "uninvolved skin at the time of repair, after clear margins were achieved" as normal skin. Our results demonstrate that epidermis adjacent to tumor cells is different from normal epidermis, at least in terms of ErbB1 signaling, and shows that asymptomatic epidermis adjacent to SCC tumors expresses amounts of AREG, HB-EGF, and BTC similar to those expressed by the tumor cells themselves.

LCM-coupled QRT-PCR proved to be a powerful tool for assessing ErbB1 ligand expression in skin samples. The collected data allowed us to make several interesting observations. First, EGF was expressed at very low levels by all settings, as previously reported of Northern blotting for keratome biopsies of normal epidermis[61] and by QRT-PCR of full punch skin biopsies of normal skin.[50] Here, we report that EGF expression is similarly low in BCC and SCC samples. Second, we showed that mRNA expression levels of three ErbB1 ligands (AREG, HB-EGF, and TGF-α) were significantly higher in normal appendages compared with normal epidermis. Because all appendages found under a given length of epidermis were microdissected in this study, additional work will be necessary to localize the source(s) of appendageal expression of these ligands. Third, BTC was clearly different in its expression pattern, compared with the remaining ErbB1 ligands. Its expression was the highest in normal skin and, relative to this standard, was significantly decreased in normal epidermal appendages, asymptomatic adjacent epidermis, and tumor cells of both BCC and SCC. Expression of BTC was found to be reduced in psoriatic lesions[62] and in retinoid-treated skin,[50] suggestive of an inverse relationship between BTC expression and epidermal proliferation. Consistent with this interpretation, BTC protein has been localized primarily to the differentiated, nonproliferative spinous, and granular layers of normal and psoriatic epidermis.[62]

This study did not undertake immunostaining of ErbB1 ligands themselves. However, abundant immunohistochemical evidence exists to indicate that the mRNA patterns observed here have valid correlates at the protein level. For instance, overexpression of AREG has been detected by immunohistochemistry in tumor cells of SCC but not BCC.[63] Moreover, overexpression of TGF-α has been detected by immunohistochemistry in 86% of Bowen's disease cases (carcinoma *in situ*),[64] with intense focal staining in 40% of SCCs.[65] Strong expression of TGF-α has also been detected by immunohistochemistry in appendages and upper layers of normal skin,[66] with higher expression in the upper epidermal layers confirmed by *in situ* hybridization.[67] TGF-α has also been observed in a range of appendageal tumors, with the lowest expression being observed in primordial epitheliomas (ie, BCC).[68] In addition, marked overexpression of immunoreactive AREG and TGF-α proteins have been reported in the hyperproliferative epidermis overlying both BCC[63] and SCC.[69] HB-EGF protein has also been detected by immunofluorescence in nonmelanoma skin cancer such as BCC and SCC, although with no indication of its level of expression relative to normal skin.[70] The present study reveals, for the first time, quantification of

the relative expression of these ligands in isolated cellular compartments of skin tumors as well as of normal skin and provides strong evidence that the growth factors previously detected by immunohistochemistry are expressed *in situ*, rather than taken up from the circulation.

Activation of Erk1/2 and Akt signal transduction pathways, albeit being characteristic of ErbB1 activation, can also result from activation of upstream signals other than ErbB1. However, the intimate relationship of ErbB1 to Erk1/2 signaling[71] and the strong correlation of the localization of ligands and phospho-Erk1/2 and phospho-Akt provide further evidence for activation of ErbB1 in the implicated tissue compartments. The near-total absence of Akt phosphorylation in BCC tumor cells is remarkable and may help to shed further light on signaling events in BCC.

The marked activation of ErbB1 signaling in the asymptomatic adjacent epidermis of both BCC and SCC is of interest in the context of the role of the microenvironment in the maintenance and progression of tumors. The cellular environment of the tumor consists of microvasculature,[72] stroma,[73] inflammatory cells,[74] and adjacent epidermal elements. The roles of the first three of these in cancer biology have received greater attention than the fourth one. We and others have shown that the hyperplastic epidermis overlying BCC expresses high levels of the hyperproliferative keratins *KRT6* and *KRT16*.[75,76] These keratins have long been considered to be markers for a regenerative maturation program of epidermal differentiation in interfollicular human skin.[77,78] Both the *KRT6* and *KRT16* genes contain EGF-responsive elements in their promoters.[79] Although several other growth factors and cytokines also regulate these genes,[80,81] our results raise the possibility that hyperactive ErbB1 signaling in the nearby epidermis may make a significant contribution to the tumor microenvironment, either by encouraging microvascular proliferation via expression of vascular endothelial growth factor,[82] influx of inflammatory cells via chemokines such as interleukin-8,[83] or proliferation of stromal cells by ErbB1 ligands themselves. It is also possible that the asymptomatic adjacent epidermis may be altered by chronic sun exposure and could contain actinic keratoses (AKs). However, AKs are rarely seen in Mohs sections of BCC, and even in these cases, the proportion of the adjacent epithelium that is occupied by AKs is small. Although the incidence of AKs in SCC Mohs sections is higher, again the percentage of the adjacent epithelium that is involved by AK is small. Therefore, the possible presence of AKs in our specimens is unlikely to explain our results.

Our results demonstrate that ErbB1 signaling is activated in SCC tumor cells, but not in BCC tumor cells. This suggests that SCC might be more responsive to inhibition of ErbB1 signaling than BCC. Whether such an approach would be a useful adjuvant or alternative to surgical intervention is unclear and would need further investigation.

Acknowledgments

We thank Jessica L. Johnson for expert technical assistance and Dr. Andrzej Dlugosz of the Department of Dermatology and the University of Michigan Comprehensive Cancer Center for helpful discussions throughout the course of this study.

References

1. Miller DL, Weinstock MA: Nonmelanoma skin cancer in the United States: incidence. J Am Acad Dermatol 1994, 30:774–778
2. Plesko I, Severi G, Obsitnikova A, Boyle P, Cook J, Zitelli JA: Trends in the incidence of non-melanoma skin cancer in Slovakia. 1978–1995. Neoplasma 2000, 47:137–142
3. Karagas MR, Greenberg ER, Spencer SK, Stukel TA, Mott LA: Increase in incidence rates of basal cell and squamous cell skin cancer in New Hampshire, USA. New Hampshire Skin Cancer Study Group. Int J Cancer 1999, 81:555–559
4. Lewis KG, Weinstock MA: Nonmelanoma skin cancer mortality (1988–2000): the Rhode Island follow-back study. Arch Dermatol 2004, 140:837–842
5. Miller SJ: Etiology and pathogenesis of basal cell carcinoma. Clin Dermatol 1995, 13:527–536
6. Zackheim HS: Origin of the human basal cell epithelioma. J Invest Dermatol 1963, 40:283–297
7. Daya-Grosjean L, Sarasin A: UV-specific mutations of the human patched gene in basal cell carcinomas from normal individuals and xeroderma pigmentosum patients. Mutat Res 2000, 450:193–199
8. Evans T, Boonchai W, Shanley S, Smyth I, Gillies S, Georgas K, Wainwright B, Chenevix-Trench G, Wicking C: The spectrum of patched mutations in a collection of Australian basal cell carcinomas. Hum Mutat 2000, 16:43–48
9. Gailani MR, Bale AE: Acquired and inherited basal cell carcinomas and the patched gene. Adv Dermatol 1999, 14:261–284
10. Wolter M, Reifenberger J, Sommer C, Ruzicka T, Reifenberger G: Mutations in the human homologue of the Drosophila segment polarity gene patched (PTCH) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 1997, 57:2581–2585
11. Chuong CM, Patel N, Lin J, Jung HS, Widelitz RB: Sonic hedgehog signaling pathway in vertebrate epithelial appendage morphogenesis: perspectives in development and evolution. Cell Mol Life Sci 2000, 57:1672–1681
12. Sato N, Leopold PL, Crystal RG: Induction of the hair growth phase in postnatal mice by localized transient expression of Sonic hedgehog. J Clin Invest 1999, 104:855–864
13. Mak KK, Chan SY: Epidermal growth factor as a biologic switch in hair growth cycle. J Biol Chem 2003, 278:26120–26126
14. Bol D, Kiguchi K, Beltran L, Rupp T, Moats S, Gimenez-Conti I, Jorcano J, DiGiovanni J: Severe follicular hyperplasia and spontaneous papilloma formation in transgenic mice expressing the neu oncogene under the control of the bovine keratin 5 promoter. Mol Carcinog 1998, 21:2–12
15. Threadgill DW, Dlugosz AA, Hansen LA, Tennenbaum T, Lichti U, Yee D, LaMantia C, Mourton T, Herrup K, Harris RC, Barnard JA, Yuspa SH, Coffey RJ, Magnuson T: Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. Science 1995, 269:230–234
16. Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, Derynck R: Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature 1995, 376:337–341
17. Sibilia M, Wagner EF: Strain-dependent epithelial defects in mice lacking the EGF receptor [published erratum appears in Science 1995 Aug 18;269(5226):909]. Science 1995, 269:234–238
18. Murillas R, Larcher F, Conti CJ, Santos M, Ullrich A, Jorcano JL: Expression of a dominant negative mutant of epidermal growth factor receptor in the epidermis of transgenic mice elicits striking alterations in hair follicle development and skin structure. EMBO J 1995, 14:5216–5223
19. Luetteke NC, Phillips HK, Qiu TH, Copeland NG, Earp HS, Jenkins

NA, Lee DC: The mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase. Genes Dev 1994, 8:399–413
20. Xie W, Chow LT, Paterson AJ, Chin E, Kudlow JE: Conditional expression of the ErbB2 oncogene elicits reversible hyperplasia in stratified epithelia and up-regulation of TGFalpha expression in transgenic mice. Oncogene 1999, 18:3593–3607
21. Brakebusch C, Grose R, Quondamatteo F, Ramirez A, Jorcano JL, Pirro A, Svensson M, Herken R, Sasaki T, Timpl R, Werner S, Fassler R: Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes. EMBO J 2000, 19:3990–4003
22. Xian W, Rosenberg MP, DiGiovanni J: Activation of erbB2 and c-src in phorbol ester-treated mouse epidermis: possible role in mouse skin tumor promotion. Oncogene 1997, 14:1435–1444
23. Chan KS, Carbajal S, Kiguchi K, Clifford J, Sano S, DiGiovanni J: Epidermal growth factor receptor-mediated activation of Stat3 during multistage skin carcinogenesis. Cancer Res 2004, 64:2382–2389
24. Dlugosz AA, Hansen L, Cheng C, Alexander N, Denning MF, Threadgill DW, Magnuson T, Coffey Jr RJ, Yuspa SH: Targeted disruption of the epidermal growth factor receptor impairs growth of squamous papillomas expressing the v-ras(Ha) oncogene but does not block in vitro keratinocyte responses to oncogenic ras. Cancer Res 1997, 57:3180–3188
25. Kiguchi K, Bol D, Carbajal S, Beltran L, Moats S, Chan K, Jorcano J, DiGiovanni J: Constitutive expression of erbB2 in epidermis of transgenic mice results in epidermal hyperproliferation and spontaneous skin tumor development. Oncogene 2000, 19:4243–4254
26. Sibilia M, Fleischmann A, Behrens A, Stingl L, Carroll J, Watt FM, Schlessinger J, Wagner EF: The EGF receptor provides an essential survival signal for SOS-dependent skin tumor development. Cell 2000, 102:211–220
27. Elder JT, Kansra S, Stoll SW: Autocrine regulation of keratinocyte proliferation. J Clin Ligand Assay 2004, 27:137–142
28. Yarden Y: The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. Eur J Cancer 2001, 37(Suppl 4):S3–S8
29. Bell DW, Lynch TJ, Haserlat SM, Harris PL, Okimoto RA, Brannigan BW, Sgroi DC, Muir B, Riemenschneider MJ, Iacona RB, Krebs AD, Johnson DH, Giaccone G, Herbst RS, Manegold C, Fukuoka M, Kris MG, Baselga J, Ochs JS, Haber DA: Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol 2005, 23:8081–8092
30. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, Lu KV, Yoshimoto K, Huang JH, Chute DJ, Riggs BL, Horvath S, Liau LM, Cavenee WK, Rao PN, Beroukhim R, Peck TC, Lee JC, Sellers WR, Stokoe D, Prados M, Cloughesy TF, Sawyers CL, Mischel PS: Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2005, 353:2012–2024
31. Herbst RS, Onn A, Mendelsohn J: The role of growth factor signaling in malignancy. Cancer Treat Res 2003, 115:19–72
32. Klapper LN, Kirschbaum MH, Sela M, Yarden Y: Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. Adv Cancer Res 2000, 77:25–79
33. Olayioye MA, Neve RM, Lane HA, Hynes NE: The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 2000, 19:3159–3167
34. Hackel PO, Zwick E, Prenzel N, Ullrich A: Epidermal growth factor receptors: critical mediators of multiple receptor pathways. Curr Opin Cell Biol 1999, 11:184–189
35. Press MF, Cordon-Cardo C, Slamon DJ: Expression of the HER-2/neu proto-oncogene in normal human adult and fetal tissues. Oncogene 1990, 5:953–962
36. Prigent SA, Lemoine NR, Hughes CM, Plowman GD, Selden C, Gullick WJ: Expression of the c-erbB-3 protein in normal human adult and fetal tissues. Oncogene 1992, 7:1273–1278
37. Stoll SW, Kansra S, Peshick S, Fry DW, Leopold WR, Wiesen JF, Sibilia M, Zhang T, Werb Z, Derynck R, Wagner EF, Elder JT: Differential utilization and localization of ErbB receptor tyrosine kinase activities in intact skin compared to normal and malignant keratinocytes. Neoplasia 2001, 3:339–350
38. De Potter IY, Poumay Y, Squillace KA, Pittelkow MR: Human EGF receptor (HER) family and heregulin members are differentially expressed in epidermal keratinocytes and modulate differentiation. Exp Cell Res 2001, 271:315–328
39. Maguire Jr HC, Jaworsky C, Cohen JA, Hellman M, Weiner DB, Greene MI: Distribution of neu (c-erbB-2) protein in human skin. J Invest Dermatol 1989, 92:786–790
40. Liu B, Zhang H, Li S, Chen W, Li R: The expression of c-erbB-1 and c-erbB-2 oncogenes in basal cell carcinoma and squamous cell carcinoma of skin. Chin Med Sci J 1996, 11:106–109
41. Ogiso Y, Oikawa T, Kondo N, Kuzumaki N, Sugihara T, Ohura T: Expression of proto-oncogenes in normal and tumor tissues of human skin. J Invest Dermatol 1988, 90:841–844
42. Groves RW, Allen MH, MacDonald DM: Abnormal expression of epidermal growth factor receptor in cutaneous epithelial tumours. J Cutan Pathol 1992, 19:66–72
43. Springer EA, Robinson JK: Patterns of epidermal growth factor receptors in basal and squamous cell carcinoma. J Dermatol Surg Oncol 1991, 17:20–24
44. Nazmi MN, Dykes PJ, Marks R, Nanni P, Pupa SM, Nicoletti G, De Giovanni C, Landuzzi L, Rossi I, Astolfi A, Ricci C, De Vecchi R, Invernizzi AM, Di Carlo E, Musiani P, Forni G, Menard S, Lollini PL: Epidermal growth factor receptors in human epidermal tumours. Br J Dermatol 1990, 123:153–161
45. Kikuchi A, Amagai M, Hayakawa K, Ueda M, Hirohashi S, Shimizu N, Nishikawa T: Association of EGF receptor expression with proliferating cells and of ras p21 expression with differentiating cells in various skin tumours. Br J Dermatol 1990, 123:49–58
46. Ahmed NU, Ueda M, Ichihashi M: Increased level of c-erbB-2/neu/HER-2 protein in cutaneous squamous cell carcinoma. Br J Dermatol 1997, 136:908–912
47. Shimizu T, Izumi H, Oga A, Furumoto H, Murakami T, Ofuji R, Muto M, Sasaki K: Epidermal growth factor receptor overexpression and genetic aberrations in metastatic squamous-cell carcinoma of the skin. Dermatology 2001, 202:203–206
48. Krähn G, Leiter U, Kaskel P, Udart M, Utikal J, Bezold G, Peter RU, Nanni P, Pupa SM, Nicoletti G, De Giovanni C, Landuzzi L, Rossi I, Astolfi A, Ricci C, De Vecchi R, Invernizzi AM, Di Carlo E, Musiani P, Forni G, Menard S, Lollini PL: Coexpression patterns of EGFR, HER2, HER3 and HER4 in non-melanoma skin cancer. Eur J Cancer 2001, 37:251–259
49. Quan T, He T, Voorhees JJ, Fisher GJ: Ultraviolet irradiation blocks cellular responses to transforming growth factor-beta by down-regulating its type-II receptor and inducing Smad7. J Biol Chem 2001, 276:26349–26356
50. Rittié L, Varani J, Kang S, Voorhees JJ, Fisher GJ: Retinoid-induced epidermal hyperplasia is mediated by epidermal growth factor receptor activation via specific induction of its ligands heparin-binding EGF and amphiregulin in human skin in vivo. J Invest Dermatol 2006, 126:732–739
51. Pawson T, Nash P: Assembly of cell regulatory systems through protein interaction domains. Science 2003, 300:445–452
52. Ferguson KM, Berger MB, Mendrola JM, Cho HS, Leahy DJ, Lemmon MA: EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. Mol Cell 2003, 11:507–517
53. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Kofler M, Jorissen RN, Nice EC, Burgess AW, Ward CW: The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. Mol Cell 2003, 11:495–505
54. Cho HS, Mason K, Ramyar KX, Stanley AM, Gabelli SB, Denney Jr DW, Leahy DJ: Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature 2003, 421:756–760
55. Stoll SW, Kansra S, Elder JT: Metalloproteinases stimulate ErbB-dependent ERK signaling in human skin organ culture. J Biol Chem 2002, 277:26839–26845
56. Kansra S, Stoll SW, Johnson JL, Elder JT: Autocrine extracellular signal-regulated kinase (ERK) activation in normal human keratinocytes: metalloproteinase-mediated release of amphiregulin triggers signaling from ErbB1 to ERK. Mol Biol Cell 2004, 15:4299–4309
57. Fisher GJ, Talwar HS, Lin J, Lin P, McPhillips F, Wang Z, Li X, Wan Y, Kang S, Voorhees JJ: Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. J Clin Invest 1998, 101:1432–1440

58. Wan YS, Wang ZQ, Shao Y, Voorhees JJ, Fisher GJ: Ultraviolet irradiation activates PI 3-kinase/AKT survival pathway via EGF receptors in human skin in vivo. Int J Oncol 2001, 18:461–466
59. Fogarty GB, Conus NM, Chu J, McArthur G: Characterization of the expression and activation of the epidermal growth factor receptor in squamous cell carcinoma of the skin. Br J Dermatol 2007, 156:92–98
60. Haider AS, Peters SB, Kaporis H, Cardinale I, Fei J, Ott J, Blumenberg M, Bowcock AM, Krueger JG, Carucci JA: Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. J Invest Dermatol 2006, 126:869–881
61. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey Jr R, Ellingsworth L, Derynck R, Voorhees JJ: Overexpression of transforming growth factor alpha in psoriatic epidermis. Science 1989, 243:811–814
62. Piepkorn M, Predd H, Underwood R, Cook P: Proliferation-differentiation relationships in the expression of heparin-binding epidermal growth factor-related factors and erbB receptors by normal and psoriatic human keratinocytes. Arch Dermatol Res 2003, 295:93–101
63. Piepkorn M: Overexpression of amphiregulin, a major autocrine growth factor for cultured human keratinocytes, in hyperproliferative skin diseases. Am J Dermatopathol 1996, 18:165–171
64. Grant JJ, Howes G, McKee PH: Transforming growth factor-alpha expression in in situ epidermal neoplasia. Clin Exp Dermatol 1995, 20:208–212
65. Ho T, Horn T, Finzi E: Transforming growth factor alpha expression helps to distinguish keratoacanthomas from squamous cell carcinomas. Arch Dermatol 1991, 127:1167–1171
66. Finzi E, Harkins R, Horn T: TGF-alpha is widely expressed in differentiated as well as hyperproliferative skin epithelium. J Invest Dermatol 1991, 96:328–332
67. Turbitt ML, Akhurst RJ, White SI, MacKie RM: Localization of elevated transforming growth factor-alpha in psoriatic epidermis. J Invest Dermatol 1990, 95:229–232
68. Finzi E, Ho T, Anhalt G, Hawkins W, Harkins R, Horn T: Localization of transforming growth factor-alpha in human appendageal tumors. Am J Pathol 1992, 141:643–653
69. Kikuchi A, Sakuraoka K, Shimizu H, Nishikawa T: Immunohistochemical evaluation of epidermis overlying basal cell carcinomas. Br J Dermatol 1993, 128:644–649
70. Downing MT, Brigstock DR, Luquette MH, Crissman-Combs M, Besner GE: Immunohistochemical localization of heparin-binding epidermal growth factor-like growth factor in normal skin and skin cancers. Histochem J 1997, 29:735–744
71. Marmor MD, Skaria KB, Yarden Y: Signal transduction and oncogenesis by ErbB/HER receptors. Int J Radiat Oncol Biol Phys 2004, 58:903–913
72. Folkman J: Fundamental concepts of the angiogenic process. Curr Mol Med 2003, 3:643–651
73. Beacham DA, Cukierman E: Stromagenesis: the changing face of fibroblastic microenvironments during tumor progression. Semin Cancer Biol 2005, 15:329–341
74. Karin M: Inflammation and cancer: the long reach of Ras. Nat Med 2005, 11:20–21
75. Xia L, Stoll SW, Liebert M, Ethier SP, Carey T, Esclamado R, Carroll W, Johnson TM, Elder JT: CaN19 expression in benign and malignant hyperplasias of the skin and oral mucosa: evidence for a role in regenerative differentiation. Cancer Res 1997, 57:3055–3062
76. Markey AC, Lane EB, Macdonald DM, Leigh IM: Keratin expression in basal cell carcinomas. Br J Dermatol 1992, 126:154–160
77. Mansbridge JN, Knapp AM, Strefling AM: Evidence for an alternative pathway of keratinocyte maturation in psoriasis from an antigen found in psoriatic but not normal epidermis. J Invest Dermatol 1984, 83:296–301
78. Vallat VP, Gilleaudeau P, Battat L, Wolfe J, Nabeya R, Heftler N, Hodak E, Gottlieb AB, Krueger JG: PUVA bath therapy strongly suppresses immunological and epidermal activation in psoriasis: a possible cellular basis for remittive therapy. J Exp Med 1994, 180:283–296
79. Jiang CK, Magnaldo T, Ohtsuki M, Freedberg IM, Bernerd F, Blumenberg M: Epidermal growth factor and transforming growth factor alpha specifically induce the activation- and hyperproliferation-associated keratins 6 and 16. Proc Natl Acad Sci USA 1993, 90:6786–6790
80. Komine M, Rao LS, Kaneko T, Tomic-Canic M, Tamaki K, Freedberg IM, Blumenberg M: Inflammatory versus proliferative processes in epidermis. Tumor necrosis factor alpha induces K6b keratin synthesis trough a transcriptional complex containing NFkappaB and C/EBP beta. J Biol Chem 2000, 275:32077–32088
81. Komine M, Rao LS, Freedberg IM, Simon M, Milisavljevic V, Blumenberg M: Interleukin-1 induces transcription of keratin K6 in human epidermal keratinocytes. J Invest Dermatol 2001, 116:330–338
82. Detmar M, Yeo K-T, Nagy J, Van De Water L, Brown L, Berse BR, Ledbetter S, Dvorak H: Keratinocyte-derived vascular permeability factor (vascular endothelial growth factor) is a potent mitogen for dermal microvascular endothelial cells. J Invest Dermatol 1995, 105:44–50
83. Braff MH, Hawkins MA, Di Nardo A, Lopez-Garcia B, Howell MD, Wong C, Lin K, Streib JE, Dorschner R, Leung DY, Gallo RL: Structure-function relationships among human cathelicidin peptides: dissociation of antimicrobial properties from host immunostimulatory activities. J Immunol 2005, 174:4271–4278