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# Altered Expression of Keratinocyte Growth Factor and Its Receptor in Psoriasis

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**One of the biological characteristics of psoriasis is excessive flaking of the skin. This is directly related to the marked hyperplasia of epidermal keratinocytes and to incomplete epidermal differentiation. Keratinocyte growth factor (KGF), a potent mitogen for human keratinocytes, is expressed by stromal cells. Alterations in the KGF signaling pathway might account for the epidermal hyperplasia associated with psoriasis. To test this hypothesis, we investigated the expression of KGF and its receptor (KGFR) in psoriasis tissue. KGF and KGFR mRNA levels were found to be frequently elevated in psoriatic skin specimens as compared with normal skin. Increased KGF transcript expression was localized to the dermal layer of the involved skin specimen using *in situ* hybridization. In contrast, KGFR transcript and protein expression was localized to the basal layer of keratinocytes in normal skin and to the basal and suprabasal layers of the psoriatic epidermis, coincident with the expanded proliferative keratinocyte pool. To identify molecules that might regulate KGFR expression we investigated the effects of various pharmacological agents and cytokines on KGFR synthesis by keratinocytes. Phorbol ester, interleukin-6, interferon-γ, and ultraviolet B (UVB) treatment all led to substantial down-regulation of KGFR expression. The down-regulation of KGFR synthesis by UVB suggests a possible mechanism for the antiproliferative action of this agent in the treatment of psoriasis. Taken together, these results suggest that increased KGFR-mediated signaling in keratinocytes in the lesional epidermis might account in part for the epidermal hyperplasia in psoriasis. *(Am J Pathol 1997, 151:1619–1628)***

Psoriasis is a chronic skin disease that is characterized histopathologically by epidermal hyperplasia, an abnormal differentiation sequence of keratinocytes in the affected epidermis, and the presence of inflammatory cells. Recent evidence suggests a primary immunological basis for this disorder linked to T lymphocyte infiltration of defined regions of the skin.[1–3] A conditional state of tissue activation may result from chronic activation of a physiologically appropriate wound-repair program that is initially triggered by the actions of effector T lymphocytes. These observations provide a basis for the immune activation in psoriasis but do not explain the profound epidermal hyperplasia, which accounts in large part for the clinical appearance of the disease. Rather, this may be related to lymphocyte-derived cytokines acting directly or indirectly on keratinocytes[4–6] or increased expression of growth factors and/or their receptors that might directly regulate epidermal hyperproliferation.[4]

Keratinocyte growth factor (KGF) is a fibroblast-derived member of the fibroblast growth factor (FGF) family[7] that has potent mitogenic activity on a wide variety of epithelial cells, and keratinocytes in particular, but no corresponding activity on fibroblasts, endothelial cells, or other nonepithelial targets of FGF action.[8] The KGF receptor (KGFR) is a transmembrane tyrosine kinase[9] that is a splice variant of the FGFR-2/*bek* gene.[10] The KGFR binds KGF and acidic FGF with high affinity and basic FGF at a lower affinity[11] and is expressed only by epithelial cells whereas FGFR-2 is present in a variety of different cell types.[10]

KGF and KGFR mRNA are expressed in the dermis and epidermis, respectively, of developing and adult mammalian skin.[12–15] After cutaneous wounding, there is a large induction of KGF expression in fibroblasts below and at the wound edge.[12,16] Furthermore, expression of a dominant-negative KGFR transgene in basal keratinocytes leads to epidermal atrophy, dermal thickening, and a substantially delayed re-epithelialization of wounded skin.[17] All of these data point to a critical role for KGFR signaling in the morphogenesis of the epidermis and in wound re-epithelialization. In addition, it has recently been reported that expression of the KGF gene can be induced by a number of cytokines and growth factors.[18–20] Given that expression of many of these factors are increased in psoriasis[4] and that the epidermis appears to be one of the major targets of endogenous KGF

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action *in vivo*, we examined psoriatic tissue for evidence of alterations in KGF and KGFR expression associated with this disease.

## Materials and Methods

### Skin Biopsies

Skin biopsies were obtained from 20 patients with plaque-type psoriasis vulgaris. Tissue was taken from representative lesional tissue as well as from adjacent uninvolved skin using a 6-mm punch. Routine histopathology and immunohistochemistry was performed on fresh-frozen skin biopsies. *In situ* hybridization (ISH) analysis was performed on formalin-fixed tissues that were subsequently dehydrated through graded alcohols, cleared with toluene, and embedded in paraffin. Additional biopsy specimens were snap-frozen in liquid nitrogen and stored at −70°C for subsequent RNA extraction. Total cellular RNA was isolated using the TRIZOL reagent (Life Technologies, Gaithersburg, MD) according to the manufacturer's instructions.

### Generation of KGF and KGFR cDNA Probes

A 145-bp *Bam*HI/*Eco*RI fragment of the human cDNA sequence (nucleotides 1 to 145) served as a probe for ribonuclease protection whereas a 324-bp *Eco*RI/*Bam*HI fragment (nucleotides 145 to 469)[7] was used for ISH. A 148-bp PCR fragment specific for exon K of the human *bek* gene[10] served as a KGFR probe for ribonuclease protection and ISH. These fragments were cloned into pGEM3Zf(−) (Promega, Madison, WI). Transcription with T7 RNA polymerase generated antisense transcripts from the 145-bp KGF and 148-bp KGFR constructs, and SP6 RNA polymerase generated the antisense strand transcript from the 324-bp KGF probe. The human vimentin probe was a 396-bp *Bam*HI/*Pst*I fragment (nucleotides 331 to 727) from cHuVim1[21] (purchased from the American Type Culture Collection, Rockville, MD) subcloned into pGEM3Zf(−) such that transcription with SP6 polymerase generated the antisense transcript. For the ribonuclease protection assay (RPA), transcription reactions were performed using $^{32}$P-labeled UTP (800 Ci/mmol; New England Nuclear, Boston, MA) as the labeled nucleotide, whereas for ISH experiments, transcripts were labeled using [$^{33}$P]UTP (1000 to 3000 Ci/mmol; New England Nuclear). Labeled transcripts were prepared as described.[22]

### Ribonuclease Protection Assay

Ten micrograms of total cell RNA was hybridized overnight at 43°C with $1 \times 10^5$ cpm of gel-purified probe. Hybrids were digested with 0.1 U of RNAse A and 20 U of RNAse T1 (Ambion, Austin, TX) for 60 minutes at 35°C. Protected fragments were resolved on 6% polyacrylamide, 8 mol/L urea gels and visualized by autoradiography.

### In Situ *Hybridization*

ISH was performed on deparaffinized tissue sections as previously described.[14] After hybridization, sections were dipped in NTB-2 emulsion (Eastman Kodak, Rochester, NY). After appropriate exposure times, slides were developed in Kodak D-19 developer and counterstained with hematoxylin.

### Immunohistochemistry of Skin Specimens

Frozen sections, 6 μm in thickness, were stained using the avidin-biotin-peroxidase complex staining system (Vector Laboratories, Burlingame, CA) and 3-amino-9-ethylcarbozole as the chromogenic substrate. An affinity-purified rabbit polyclonal antibody (C-17) raised against a peptide corresponding to the amino acids 805 to 821 mapping within the carboxy terminus of the human FGFR2/KGFR (Santa Cruz Biotechnology, Santa Cruz, CA) was used to detect KGFRs in samples from normal and pathological skin. Monoclonal antibody to Ki67 protein (Amac, Westbrook, ME) was used to identify cycling cells.[23] Monoclonal antibodies to α3-integrin, which react only with basal keratinocytes in normal skin,[24] were obtained from A. Albino (Memorial Sloan-Kettering Cancer Center, New York, NY). Negative controls for these immunoperoxidase studies included matched isotype monoclonal or polyclonal antibodies with irrelevant antigenic specificities or the omission of the primary antibody.

### In Vitro *Cell Culture*

Normal human keratinocytes were cultured as previously described[25] in low calcium (<0.1 mmol/L) keratinocyte growth medium (Clonetics Corp., San Diego, CA) or a similar medium (keratinocyte SFM, Life Technologies) supplemented with recombinant epidermal growth factor (EGF; 0.1 ng/ml) and 0.4% bovine pituitary extract, 0.1% insulin, and 0.5% hydrocortisone. Cells were used after the first or second subculture.

### KGFR Protein Analysis

Protein lysates were prepared in *Staphylococcus* A buffer (10 mmol/L sodium phosphate, pH 7.5, 100 mmol/L NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), and 0.5% deoxycholate) containing the protease inhibitors phenylmethylsulfonyl fluoride (1 mmol/L) and aprotinin (10 μg/ml), and clarified by centrifugation at 10,000 × *g* for 30 minutes. Cell lysates (1 mg of protein) were immunoprecipitated using 1 μg of the C-17 rabbit polyclonal antibody. Immunocomplexes were precipitated with Gammabind G Sepharose (Pharmacia, Piscataway, NJ) and washed four times in *Staphylococcus* A wash buffer containing 0.1% bovine serum albumin (BSA). The pellets were boiled for 5 minutes in Laemmli buffer and proteins resolved by electrophoresis through 8% SDS-polyacrylamide gels. After transfer to polyvinylidine difluoride (Immobilon P) membranes (Millipore, Bedford, MA), the

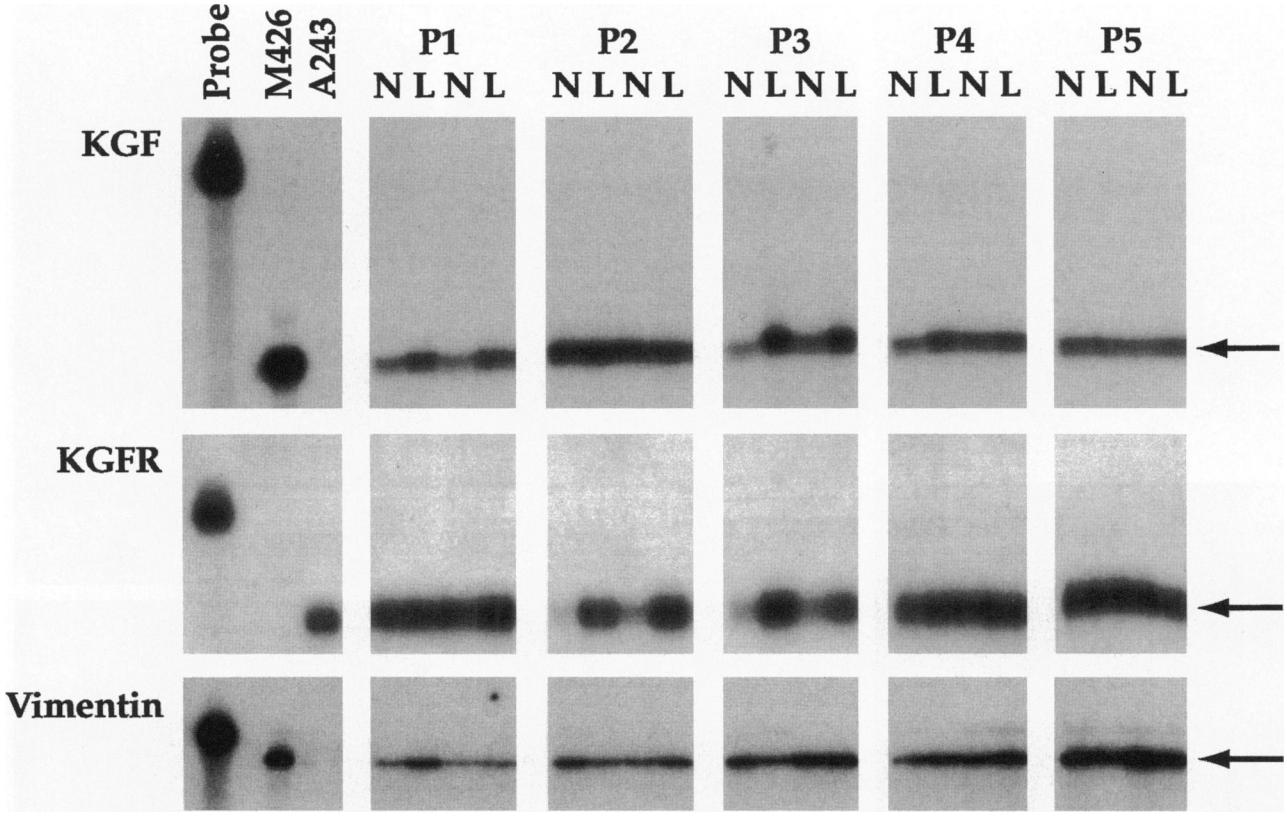


**Figure 1.** Analysis of KGF and KGFR mRNA in psoriasis. Total cellular RNA was extracted from two separate regions of normal-appearing (N) or lesional (L) skin from patients with plaque-type psoriasis vulgaris. RNA (5 $\mu$g) was hybridized to $^{32}$P-labeled antisense KGF, KGFR, and vimentin probes. After digestion with RNAse, protected hybrids were resolved by electrophoresis through denaturing polyacrylamide gels. RNAs extracted from the human embryonic lung fibroblast cell line, M426, and a squamous epithelial carcinoma cell line, A253, were included as positive controls for KGF and KGFR expression, respectively. Exposure times were as follows: KGF, 48 hours; KGFR, 48 hours; and vimentin, 12 hours.

membranes were blocked with 5% BSA in PBS for 2 hours and then incubated for 1 hour with 0.5 $\mu$g/ml C-17 antibody in incubation buffer (1% BSA/PBS). After extensive washing in 0.05% Tween 20/PBS, the blots were incubated with $^{125}$I-labeled protein A ($2 \times 10^5$ cpm/ml) for 30 minutes in incubation buffer and subsequently exposed to Kodak X-Omat film at −70°C.

## Results

### Analysis of KGF and KGFR Transcript Expression in Normal and Psoriatic Skin Specimens

RNAs extracted from regions of psoriatic plaque and corresponding normal-appearing tissue were analyzed for KGF and KGFR transcript expression by RPA. Results of studies with tissue taken from five patients with psoriasis vulgaris are shown in Figure 1. For each patient, samples were taken from two separate regions of lesional and normal tissue. Control samples included RNA extracted from M426, an embryonic lung fibroblast cell line, and a squamous cell carcinoma cell line, A253. M426 was used to collect the conditioned medium from which KGF was originally purified and contains high levels of KGF mRNA and protein,[7,8] whereas A253 had previously been identified to contain relatively high KGFR transcript levels (P. W. Finch, unpublished data). Increased levels of the KGF transcript were detected in RNA extracted from psoriatic skin in comparisons with the normal skin specimens for patients 1, 3, and 4. No increase in KGF transcript levels was detected in RNA isolated from patients 2 and 5. Increased KGFR signal was apparent in RNA isolated from psoriatic tissue of patients 1 through 4 (although elevated signal KGFR was detected for only one of the two sets of tissue collected from patient 1), whereas there was no change in the KGFR levels in RNAs isolated from patient 5. RNA from an additional two patients was also analyzed, and in each case increased levels of KGF and KGFR transcripts were observed in psoriatic samples in comparison with normal skin (results not shown). As a positive control, analysis of vimentin gene expression demonstrated comparable transcript levels between the normal and psoriatic RNA samples (Figure 1). These results therefore demonstrate that increased expression of KGF and KGFR transcripts occurs frequently in psoriasis.

### ISH Analysis of KGF/KGFR Expression in Psoriatic Skin Specimens

To further evaluate the pathological implications of increased KGF and KGFR expression in psoriasis, we ex-

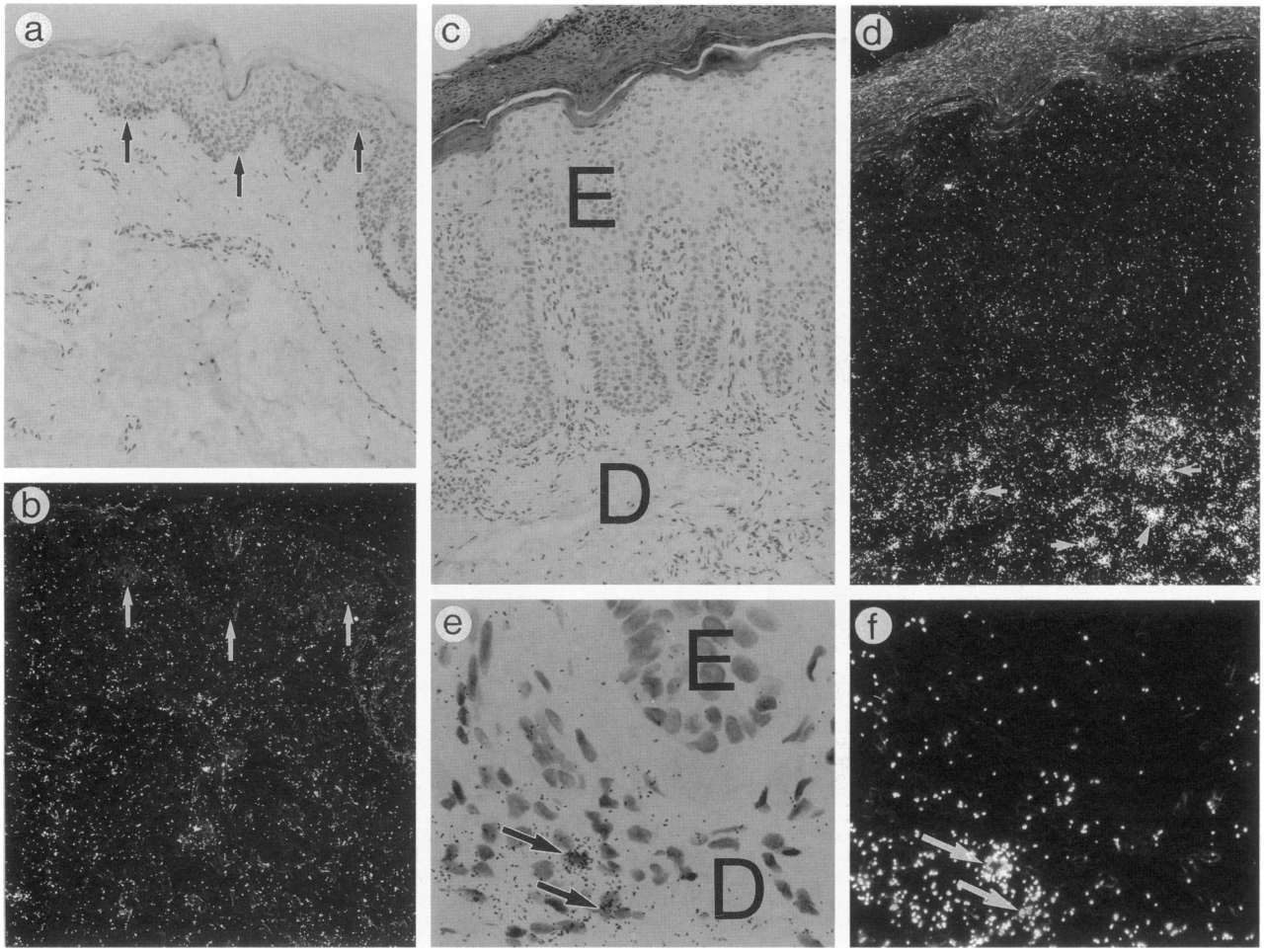


**Figure 2.** ISH of KGF mRNA expression in uninvolved and lesional psoriatic epidermis, shown in bright-field (**a**, **c**, and **e**) and corresponding dark-field (**b**, **d**, and **f**) micrographs of human skin tissue sections. In normal-appearing skin (**a** and **b**), diffuse KGF expression is present throughout the papillary layer of the epidermis. **Arrows** mark the position of the dermal-epidermal junction. In the psoriatic specimen (**c** and **d**), increased KGF hybridization signal is apparent in the subepithelial papillary layer of the dermis (D) but not epidermis (E). Representative KGF-expressing cells are indicated by **arrows** in **d**. Hematoxylin; original magnification, ×31.2). Higher magnification (**e** and **f**) of the dermal-epidermal junction in the psoriatic plaque demonstrates the increased KGF signal in the dermal (D) mesenchymal cells but not the epidermal (E) keratinocytes. Representative KGF-expressing cells are indicated by **arrows**. Hematoxylin; original magnification, ×156.

amined the spatial distribution of their transcripts in normal and lesional tissue using ISH. Six-micron-thick paraffin-embedded tissue sections were hybridized to $^{33}$P-labeled antisense cRNA hybridization probes complementary to the primary KGF and KGFR transcripts, and the resulting hybrids detected by nuclear track emulsion autoradiography. The localization of silver grains over the tissue section demonstrated that in normal-appearing skin specimens diffuse KGF signal was present in mesenchymal cells throughout the subepithelial papillary layer of the dermis (Figure 2, a and b). In contrast, increased KGF mRNA expression was observed in the involved psoriatic specimens (Figure 2, c and d), with signal located over mesenchymal cells in the papillary layer of the dermis, and in particular the cells subjacent to the epidermis (Figure 2, e and f).

In normal skin sections the KGFR ISH signal was localized predominantly to the basal layer of the epidermis (stratum basale, Figure 3, a and b). Increased KGFR mRNA was apparent in the basal as well as the suprabasal layers of the psoriatic epidermis in comparison with uninvolved epidermis (Figure 3, c and d). To quantify the differences in KGFR expression both between different cell layers in the normal epidermis and between corresponding layers in the normal and psoriatic epidermis, silver grains were counted from representative cells within the specific cell layers of five matched specimens of lesional *versus* nonlesional tissue. As shown in Table 1, basal keratinocytes in the normal epidermis express significantly more KGFR mRNA compared with spinous keratinocytes ($P = 0.027$). There was a 2.7-fold increase ($P = 0.001$) in KGFR RNA expression in basal keratinocytes in psoriatic tissue compared with the corresponding cells in normal-appearing skin. Suprabasal keratinocytes in psoriatic skin express KGFR RNA levels that are somewhat lower than in psoriatic basal cells, but at levels that are comparable to basal keratinocytes in normal skin, and significantly different from that found in normal spinous keratinocytes ($P = 0.018$). Furthermore, as psoriatic keratinocytes differentiate beyond the suprabasal region and into the spinous layers, there is also a significant reduction in KGFR expression ($P = 0.006$). There-

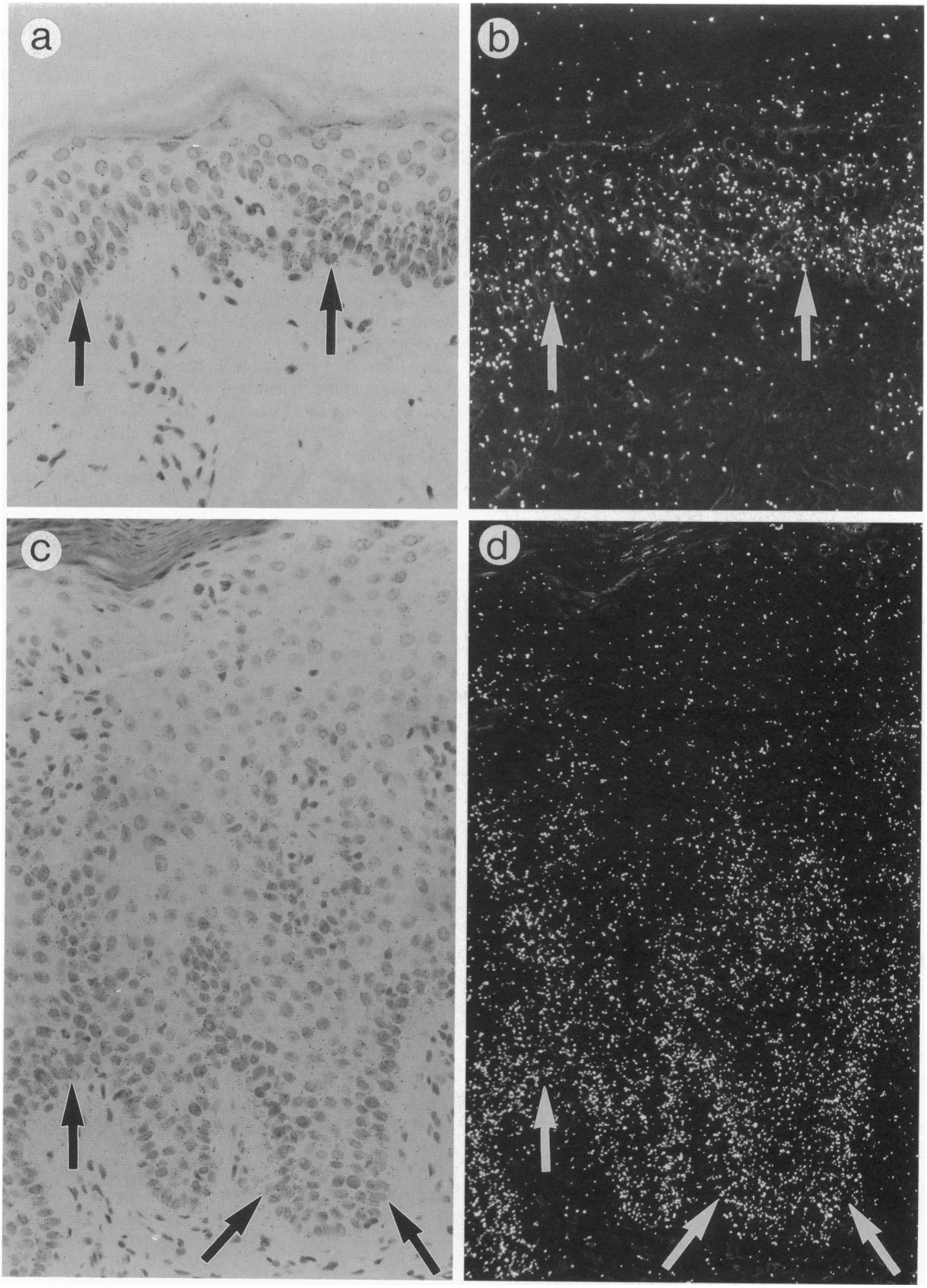


**Figure 3.** ISH of KGFR mRNA expression in uninvolved and lesional psoriatic epidermis, shown in bright-field (**a** and **c**) and corresponding dark-field (**b** and **d**) micrographs of human skin tissue sections. **a** and **b**: Weak KGFR mRNA expression is present over the basal layer (stratum basale) of the epidermis of normal-appearing skin. **c** and **d**: Increased KGFR signal is evident over the basal and suprabasal layers of keratinocytes in psoriatic plaques. In each figure, **arrows** indicate the position of the basal epidermal layer expressing the highest levels of KGFR mRNA. Hematoxylin; original magnification, ×62.5.

**Table 1.** Increased KGFR Gene Expression in Lesional *versus* Nonlesional Psoriatic Tissue

| Epidermal cell layer | Grains/cell (mean ± SD) | | |
|---|---|---|---|
| | Nonlesional | Lesional | *P** |
| Proliferative | | | |
| Basal | 5.2 ± 2.8 | 13.9 ± 2.8 | 0.001 |
| Suprabasal | Not present | 8.2 ± 2.1 | |
| Differentiating | | | |
| Spinous | 1.4 ± 0.4 | 3.7 ± 1.3 | 0.018 |
| Granular | 0.7 ± 0.5 | 0.7 ± 0.1 | 0.866 |

Results are shown as the average grain count from four representative cells within specific cell layers of five matched (lesional *versus* nonlesional) psoriatic epidermis samples after ISH using an antisense riboprobe specific for KGFR gene expression. Matched tissues from the same patient were cut onto the same slide and hybridized using the same probe to ensure they received equal treatment. Quantitative measures of the numbers of grains/cell were obtained using the public domain IMAGE software developed at the National Institutes of Health (Bethesda, MD).

*Paired Student's *t*-test; $P < 0.05$ was considered statistically significant.

fore, the increased signal is due to both increased KGFR transcript expression per cell and an increase in the numbers of KGFR-expressing keratinocytes in the psoriatic epidermis. Hybridization of serial sections with the $^{33}$P-labeled sense strand cRNA KGF or KGFR probes resulted in sparse background labeling of cells (results not shown). ISH of equivalent areas with an antisense probe for vimentin, which served as a positive control, resulted in a strong signal throughout the papillary layer of the dermis, consistent with expression from primarily mesenchymal cells (results not shown). Epidermal keratinocytes were not positive for vimentin signal.

## *Analysis of KGFR Expression by Immunohistochemistry*

Biopsy samples of active psoriatic plaques and normal-appearing nonlesional skin were sectioned on a cryostat microtome and incubated with antibodies to KGFR/FGFR-2 (C-17), α3-integrin, or Ki67. The specificity of the C-17 antisera for the KGFR is described in Figure 6. Representative results are shown in Figure 4. In normal human epidermis, plasma membrane staining by C-17 is confined to basal keratinocytes (Figure 4A). There is weak staining of the remainder of the epidermis, but as it is not membrane associated and does not correspond to regions in which KGFR transcripts were detected (Figure 3b), it most likely represents cross-reaction with an unrelated protein species. In contrast to the normal speci-

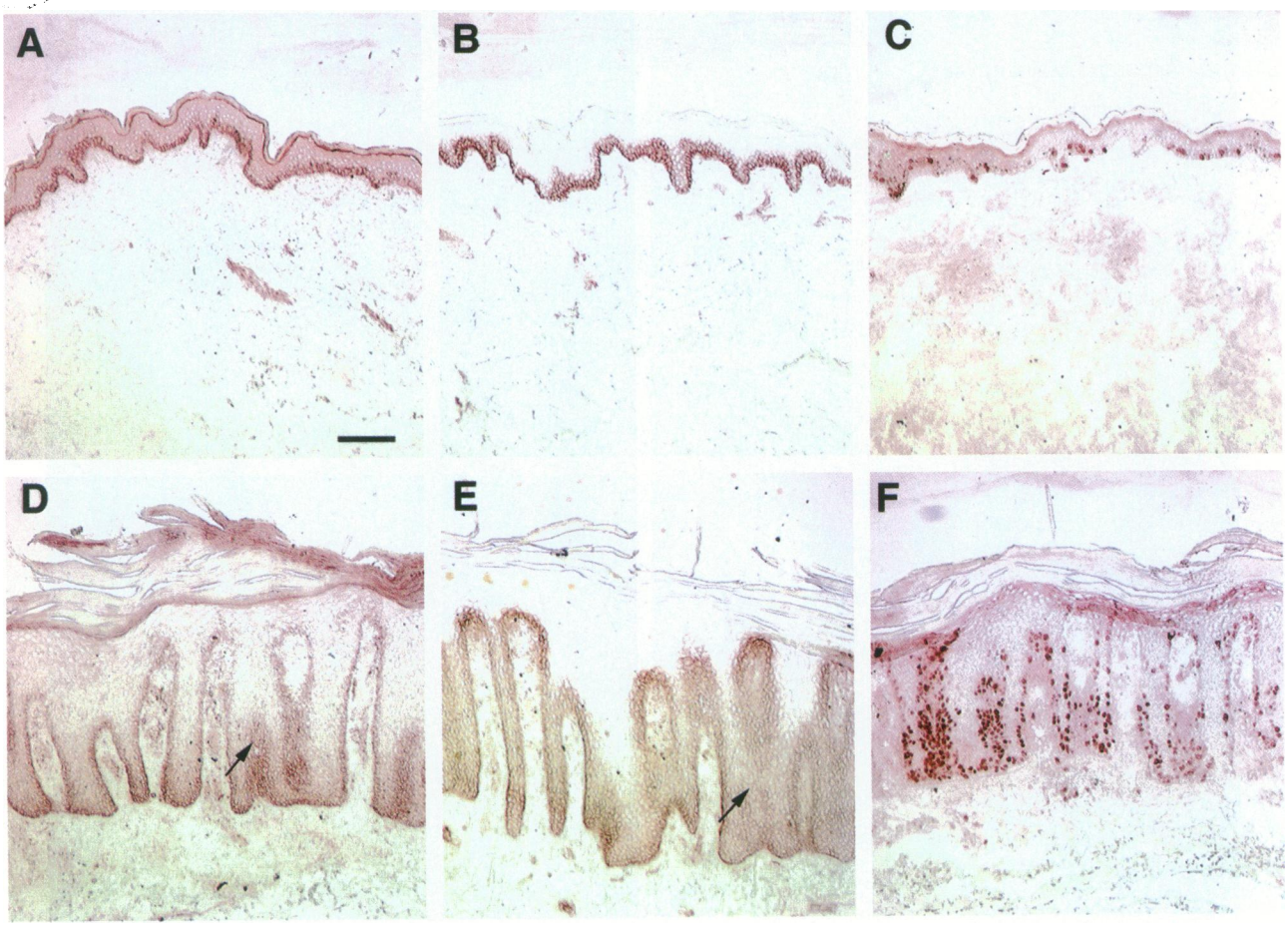


**Figure 4.** Immunohistochemical staining of KGFRs in normal skin and lesional psoriasis. Six-micron cryostat sections of normal skin (**A** to **C**) or lesional psoriasis (**D** and **E**) were stained with antibodies to KGFR (**A** and **D**), α3 integrin (**B** and **E**), or Ki67 (**C** and **F**), as described in Materials and Methods. In normal skin, plasma membrane staining of KGFRs and α3 integrin and nuclear Ki67 staining is localized to the basal layer of keratinocytes. In psoriasis, these antibodies also stain cells in the suprabasal layers (indicated by **arrows** in **D** and **E**). Scale bar in **A**, 100 μm.

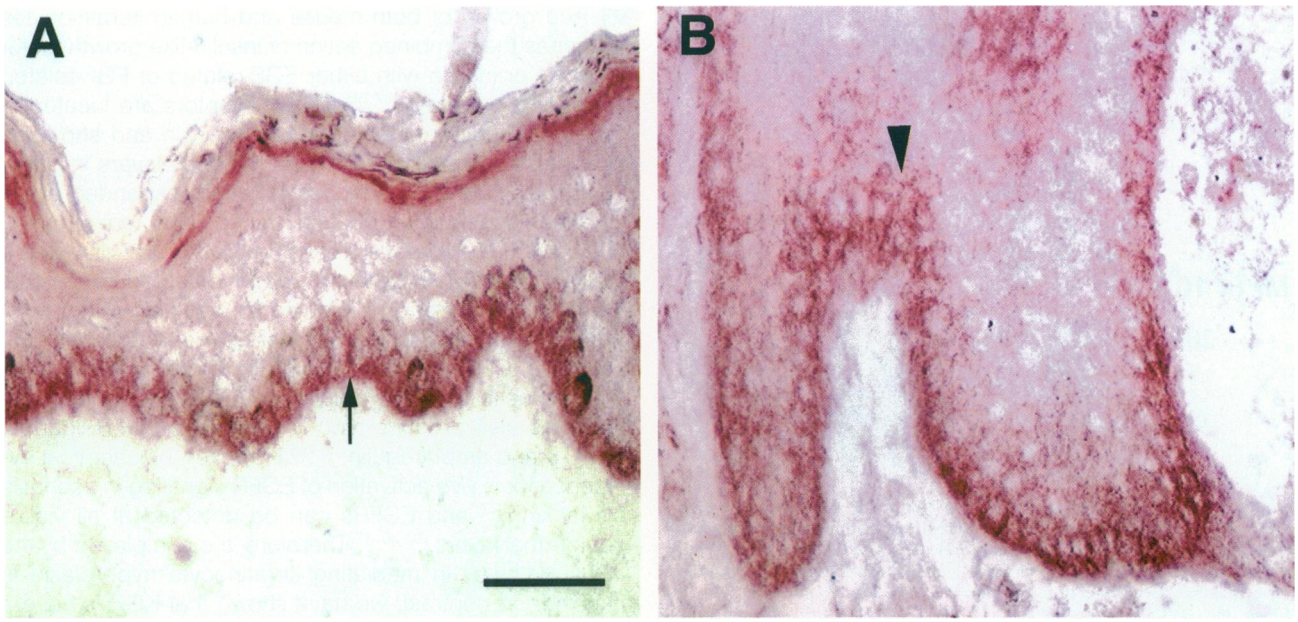


**Figure 5.** Immunohistochemical staining of KGFRs in normal skin and lesional psoriasis. Six-micron cryostat sections of normal skin (**A**) or lesional psoriasis (**B**) were stained with a polyclonal KGFR antisera as described in Materials and Methods. In normal skin, the basal and lateral membranes are stained to a greater degree than the apical membrane. The **arrow** indicates the staining of a lateral membrane region between two basal keratinocytes. In psoriasis, KGFR staining is present in suprabasal cells (indicated by an **arrowhead**). Cytoplasmic staining (indicated by the presence of granular nonmembrane signal) is also apparent. Scale bar in **A**, 40 $\mu$m.

mens, epidermis from lesional psoriatic plaques shows plasma membrane staining with C-17 in both basal and suprabasal keratinocytes (Figure 4D). These patterns of expression were similar to those detected by staining with an anti-$\alpha$3-integrin antibody (Figure 4, B and E). The $\alpha$3-integrin is expressed in basal keratinocytes in normal skin but is also found in the suprabasal layers in hyperproliferative skin.[24] In normal epidermis, proliferating keratinocytes, as identified by nuclear staining with the Ki67 antibody, were sparse and were restricted to the basal epidermal layer (Figure 4C). However, in psoriatic plaques, increased numbers of Ki67-positive keratinocyte nuclei were present in both the basal and suprabasal compartments (Figure 4F), therefore defining the increased proliferative compartment in psoriasis.

When examined at higher magnification, the KGFR staining in normal skin was predominantly detected in the basal and lateral membranes of the basal keratinocytes, with noticeably less staining apparent on the apical membrane (Figure 5A). In lesional skin, suprabasal staining is also present. Furthermore, upon examination of multiple specimens, the intensity of KGFR staining in lesional skin was consistently less than that seen in normal skin. Given the increased transcript expression in the psoriatic epidermis, this may indicate receptor down-regulation.

We also examined KGFR staining in a group of eight patients after treatment of the disease with 6-thioguanine, a nucleoside analogue that selectively depletes proliferating T lymphocytes. Before treatment, the altered pattern of KGFR staining described above correlated with clinical and histopathological disease expression. However, in each case after 1 to 6 months of 6-thioguanine treatment, reductions in epidermal acanthosis and inflammatory infiltrates correlated with restoration of the normal, basal-cell-restricted pattern of KGFR staining (data not shown).

## *Analysis of KGFR Expression in Cultured Keratinocytes*

Our results have demonstrated the presence of increased KGF and KGFR gene expression in psoriatic plaques compared with uninvolved tissue. KGF gene expression in fibroblasts can be induced by a number of cytokines and growth factors, including interleukin (IL)-6, transforming growth factor (TGF)-$\alpha$, and platelet-derived growth factor (PDGF).[18–20] As increased bioactivity of many of these factors is present in psoriatic tissue,[4] this provides a number of potential mechanisms for the elevated levels of KGF expression present in lesional tissue. However, the factors that modulate KGFR gene expression have yet to be identified. To address this question, we incubated primary cultures of keratinocytes with a variety of pharmacological agents and cytokines that have known effects on keratinocyte growth and differentiation and looked for effects on KGFR synthesis. The agents tested included phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C and a major regulator of keratinocyte differentiation[26]; granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-6, potent keratinocyte mitogens that might serve to link epidermal proliferation with local tissue inflammation[4]; interferon (IFN)-$\gamma$, an inducer of certain features of the keratinocyte psoriatic phenotype[4]; and ultraviolet B (UVB), an antiproliferative agent for keratinocytes that is used in the treatment of psoriasis. After these treatments, KGFR protein was examined by immunoprecipitation and

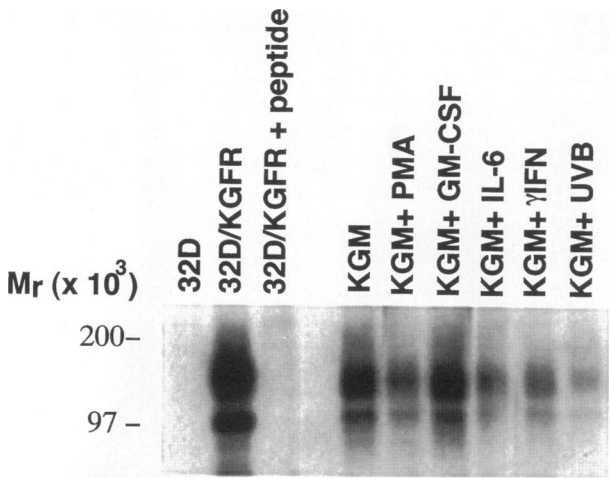


**Figure 6.** Analysis of KGFR expression in cultured keratinocytes. Primary cultures of foreskin keratinocytes were grown in keratinocyte growth medium (KGM) until 80 to 90% confluence. Cultures were then treated with various growth- and differentiation-modulating agents for 24 hours. The various agents used were 10 ng/ml phorbyl 12-myristate 13-acetate (PMA), 10 ng/ml; GM-CSF, 10 ng/ml; IL-6, 10 ng/ml; IFN-γ, 10 ng/ml; UVB, 32 mJ/cm². Cell lysates were prepared and 1-mg samples were immunoprecipitated using the C-17 polyclonal KGFR antisera. Immunoprecipitates were separated by electrophoresis through 8% SDS-PAGE, transferred to polyvinylidine membranes, and then blotted with C-17. As controls for the specificity of this antibody, 32D hematopoietic cells transfected with the human KGFR were used. An approximately 130-kd protein, corresponding to the KGFR was immunoprecipitated from the lysate prepared from 32D/KGFR transfectants but not from control untransfected 32D cells. Furthermore, immunoprecipitation of this protein species was blocked when the antibody was preincubated with the antigenic peptide (32D/KGFR plus peptide) prior to immunoprecipitation.

subsequent immunoblot analysis using the C-17 antisera (Figure 6). Treatment with either PMA, IL-6, IFN-γ, or UVB all led to a substantial decrease in KGFR protein expression relative to untreated keratinocytes.

## *Discussion*

In this study, we observed increased KGF and KGFR expression associated with lesional psoriatic tissue. KGF is perhaps the most potent mitogen for human keratinocytes[8,27] and, therefore, might also be expected to play a role in promoting epidermal hyperproliferation in this disease. Indirect evidence for such a role can be inferred from studies in transgenic mice in which KGF[28] or a dominant-negative KGFR[17] was targeted for overexpression in basal keratinocytes by the keratin K14 promoter. KGF overexpression was associated with epidermal hyperplasia, whereas inhibition of functional KGF signaling by the dominant-negative KGFR resulted in epidermal atrophy, a reduced steady-state proliferation of the basal epithelial layer, and reduced re-epithelialization after wounding. Furthermore, *in vitro* studies have shown that KGF is able to promote an increase in cell number in confluent monolayers of keratinocytes relative to untreated or EGF-treated controls.[29] These results point to the importance of KGF in maintaining the normal morphogenesis of the epithelium and the potential of increased KGF signaling to result in the accumulation of keratinocytes that have retained proliferative potential.

The growth of both mouse and human keratinocytes requires the combined action of insulin-like growth factor (IGF)-1 or insulin with either EGF-related or FGF-related signaling pathways.[27,30] IGF-1 receptors are located in the basal epidermal layer in normal skin and show expanded expression to the lower spinous layers in psoriasis, coincident with the location of the expanded proliferative keratinocyte pool in the lesional epidermis.[31] Furthermore, increased tyrosine kinase activity of the IGF-1 receptor β-chain has been detected in lesional *versus* nonlesional skin, arguing for active involvement of IGF-1 receptor mitogenic signaling in the psoriatic epidermis.[31] The number of EGFRs is also increased in hyperplastic psoriatic epidermis,[32] and psoriatic keratinocytes also produce high levels of two EGFR ligands, TGF-α and amphiregulin.[33–35] However, biochemical evidence for *in vivo* activation of EGFR signaling in psoriasis is lacking,[27] and EGFRs can be detected at all viable epidermal layers.[32,36,37] Therefore, the role played by the EGF pathway in mediating keratinocyte hyperplasia is unclear. In contrast, we have shown that KGFRs have a similar distribution to IGF-1 receptors in that they are restricted to the basal epidermal compartment in normal skin and to both the basal and lower spinous layers in lesional psoriatic epidermis. Furthermore, we have shown that altered expression of KGFR in keratinocytes of psoriatic lesions is not fixed and is modulated during clinical disease expression. Based upon these data, we hypothesize that IGF-1 and KGF represent the main mediators of normal epidermal proliferation as well as hyperproliferation in psoriasis.

We have used a combination of immunohistochemistry, performed using a well characterized polyclonal KGFR antiserum, and ISH to localize KGFRs to basal keratinocytes in normal skin and the basal and suprabasal layers in psoriatic epidermis. A KGF immunoglobulin fusion protein has been reported to recognize KGF-binding sites predominantly in the spinous layer and, to a lesser extent, the basal layer in normal skin.[15,16] Under standard conditions used for immunohistochemistry, the KGF immunoglobulin fusion protein can recognize some low-affinity binding sites in tissue sections (unpublished observations), which may account for the differences in staining patterns observed in the present study and those using the chimeric KGF fusion protein.

The observation of increased KGFR transcript expression in the psoriatic epidermis is consistent with the situation in re-epithelializing human skin where KGFR transcript levels increased several-fold over basal levels in control skin during the intermediate period of wound repair.[16] However, the mechanisms responsible for modulating KGFR expression have yet to be identified. To address this question, we determined the effects of various cytokines and growth-modulating agents on KGFR protein expression *in vitro*. Of the agents used, PMA, IL-6, IFN-γ, and UVB all led to decreased KGFR protein expression (Figure 5). PMA, an activator of protein kinase C, elicits changes that occur during the spinous to granular cell transition in the epidermis.[26] Therefore, the decrease in KGFR expression after PMA treatment would be consistent with these results as KGFRs are present only in

undifferentiated keratinocytes with proliferative potential (Figures 3 and 4). Also of note was the fact that UVB treatment resulted in a several-fold reduction in KGFR protein expression. Similar results have also recently been reported by Zhou et al.[38] UVB is an antiproliferative agent for keratinocytes that is used in the treatment of psoriasis. It is tempting to speculate that a major mechanism through which UVB exerts its clinical effect is by down-regulation of KGFR synthesis in psoriatic plaques, thereby decreasing the ability of the epidermis to respond to increased dermal synthesis of KGF.

The implications of KGFR down-regulation by IFN-$\gamma$ and IL-6, both of which are found at increased levels in psoriasis, remain to be determined. It should be recognized, however, that we have not performed an exhaustive analysis of the many cytokines that are known to be present in lesional psoriatic tissue, and of these there are likely to be some that up-regulate KGFR expression. Furthermore, it might be the case that up-regulation of KGFR expression is mediated by the action of multiple cytokines, including those such as IL-6 and IFN-$\gamma$ that have a negative effect on KGFR expression under the experimental conditions used. Finally, we have evidence that regulation of KGFR expression in cultured keratinocytes is complex, with the level of expression dependent on the growth maintenance media used and the supplements added. Additional work aimed at identifying the factors that positively regulate KGFR expression in psoriatic tissue should take these variables into account as well as the interaction of keratinocytes with extracellular matrix components and underlying dermal cells.

Recently, a KGF null mutant mouse has been described[39] that demonstrated no major abnormalities in epidermal growth or wound healing. This surprising result probably reflects overlapping specificities among the various members of the FGF gene family. However, it seems clear that disruption of the KGFR results in profound alterations to those organs that have been targeted.[17,40] Therefore, the signaling pathway mediated by the KGFR appears to be critical for the normal development of at least certain organ systems, including the skin. We would therefore argue, based upon various *in vitro* and *in vivo* data, that despite the fact that other FGF activities can compensate for the lack of KGF, increased expression of KGF could result in profound effects on epidermal proliferation in physiological and pathological settings.

Two novel KGF-regulated genes (KRGs) have recently been isolated from a human keratinocyte cell line by using differential display. KRG-1 encodes a novel nonselenium glutathione peroxidase,[41] suggesting a role in epidermal protection mediated by the detoxification of reactive oxygen species. KRG-2 corresponds to a human homologue of the yeast *CHL1* gene, a putative helicase that is involved in correct chromosome transmission and cell cycle progression.[42] KRG-1 was shown to be expressed at high levels in two specimens of psoriatic skin in comparison with the corresponding normal skin specimens.[41] We have also examined KRG-2 transcript expression in normal and psoriatic skin specimens (results not shown). KRG-2 was expressed at very low levels in normal skin and was generally elevated two- to threefold in the psoriatic counterpart. However, this may be in part be due to the increased number of epidermal cells present in the lesional tissue compared with the corresponding normal specimen. Additional studies will be necessary to fully determine the level of overexpression, if any, of these genes in psoriatic tissue and, if so, how they may modulate the keratinocyte phenotype in an inflammatory environment.

In summary, we have demonstrated altered KGF and KGFR expression in lesional psoriatic tissue compared with normal skin. Given the known importance of the KGFR signaling pathway in the normal morphogenesis of the epidermis,[17] and the demonstration that KGF can promote excessive epidermal proliferation,[28,29] our results suggest that increased KGF signaling may play a role in mediating the epidermal hyperplasia that accounts in large part for the clinical appearance of psoriasis.

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