Cell Type-specific Inhibition of Keratinocyte Collagenase-1 Expression by Basic Fibroblast Growth Factor and Keratinocyte Growth Factor

A COMMON RECEPTOR PATHWAY*  
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Collagenase-1 is invariably expressed by migrating basal keratinocytes in all forms of human skin wounds, and its expression is induced by contact with native type I collagen. However, net differences in enzyme production between acute and chronic wounds may be modulated by soluble factors present within the tissue environment. Basic fibroblast growth factor (bFGF, FGF-2) and keratinocyte growth factor (KGF, FGF-9), which are produced during wounding healing, inhibited collagenase-1 expression by keratinocytes in a dose-dependent manner. However, KGF was >100-fold more effective than bFGF at inhibiting collagenase-1 expression, suggesting that this differential signaling is transduced via an FGF receptor that binds these ligands with different affinities. Reverse transcriptase-polymerase chain reaction analysis of human keratinocyte mRNA for fibroblast growth factor receptors (FGFRs) revealed expression of only FGFR-2 IIIb, the KGF-specific receptor, which also binds bFGF with low affinity, and FGFR-3 IIIb, which does not bind bFGF or KGF. FGFRs that bind bFGF with high affinity were not detected. Our results suggest that bFGF and KGF inhibit collagenase-1 expression through the KGF cell-surface receptor (FGFR-2 IIIb). Because bFGF induces collagenase-1 in most cell types, cell-specific expression of FGFR family members may dictate the regulation of matrix metalloproteinases in a tissue-specific manner.

Wound repair is a highly organized process that requires a series of spatially and temporally regulated events to heal a tissue defect. Among these, effective proteolytic degradation of extracellular matrix (ECM)1 macromolecules by various proteases is necessary to remodel the damaged tissue, promote neovascularization, and facilitate efficient migration of cells during re-epithelialization (1). Yet, in chronic ulcers, the overproduction of matrix-degrading proteases and/or the lack of production of their natural inhibitors probably contributes to the underlying pathogenesis of the non-healing state by interfering with normal repair processes and by perpetuating matrix destruction.

Matrix metalloproteinases (MMPs) constitute a family of zinc-dependent enzymes that collectively have the capacity to degrade virtually all components of the ECM (2). While most members of this family possess overlapping substrate specificities, the metallocollagenases, a subgroup of the MMP gene family, have the unique ability to initiate cleavage of fibrillar collagens I, II, and III at a specific locus in their triple helical domain. At physiologic temperature, cleaved collagen molecules denature into gelatin and become susceptible to further digestion by other proteases. Of the three known human collagens, collagenase-1 (MMP-1) is the enzyme principally responsible for collagen turnover in most tissues and, in particular, the skin.

Previous studies from our laboratories and others have shown that basal keratinocytes at the leading edge of migration in both normally healing wounds and chronic ulcers invari-antly express collagenase-1 (3–5). Signal for collagenase-1 is confined to the basal layer of epidermis, diminishes progressively away from the wound edge, and is absent in intact skin. Furthermore, collagenase-1 expression is rapidly induced in wound edge keratinocytes after injury, persists during the healing phase, and ceases following wound closure (6). In chronic, non-healing wounds expression of this MMP is prominent and excessive, whereas in normally healing wounds its expression is transient and localized precisely to areas of active re-epithelialization (3, 7). We have demonstrated that collagenase-1 expression by basal keratinocytes is induced following contact with native type I collagen,2 and the activity of this enzyme is required for cell migration (9). Thus, expression of matrix-degrading enzymes by keratinocytes during cutaneous wound repair is a normal and programmed response to injury, and altered cell-matrix interactions may play a critical role in regulating this response.

In addition to cell-matrix interactions, soluble mediators present in the ECM during wound repair may influence collagenase-1 expression. Keratinocyte collagenase-1 production is stimulated by several growth factors including transforming growth factor-α (TGF-α); epidermal growth factor (10), hepatocyte growth factor/scatter factor (11), transforming growth factor β1 (TGF-β1) (12, 13), and interferon-λ (14). Furthermore, several of these growth factors (e.g. epidermal growth factor

1 The abbreviations used are: ECM, extracellular matrix; FGF, fibroblast growth factor; bFGF, basic FGF; MMP, matrix metalloproteinase; TGF, transforming growth factor; KGF, keratinocyte growth factor; FGFR, FGF receptor; ELISA, enzyme-linked immunosorbent assay; bp, base pairs.

2 B. D. Sudbeck, B. K. Pilcher, H. G. Welgus, and W. C. Parks, (1997) J. Biol. Chem. 272, in press.
and hepatocyte growth factor/scatter factor) can augment ECM-directed collagenase-1 expression by keratinocytes (11, 15). In effect, while cell contact with specific matrices establishes the primary "on and off" signals, soluble mediators may finely control the net output of collagenase-1 by keratinocytes.

Basic fibroblast growth factor (bFGF, FGF-2) and keratinocyte growth factor (KGF, FGF-9) belong to a family of heparin-binding growth factors that exert a variety of effects on multiple cell types (16). bFGF is widely expressed in vivo, is a potent angiogenic factor, and induces collagenase-1 production by cultured fibroblasts (17, 18), endothelial cells (19, 20), and osteoblasts (21). In addition, bFGF stimulates growth and proliferation of human keratinocytes (22, 23). In contrast, KGF is expressed exclusively by cells of mesenchymal origin, such as fibroblasts (24) and microvascular endothelial cells (25), yet it specifically influences epithelial cells by a paracrine signaling mechanism (24, 26, 27). Both bFGF and KGF are expressed during epidermal wound repair (28, 29), and topical application of bFGF to wounds accelerates healing (30). Likewise, inhibition of KGF signaling in basal keratinocytes of epithermis following injury impairs re-epithelialization, presumably by inhibiting keratinocyte proliferation (31).

In this report, we demonstrate that bFGF and KGF down-regulate collagenase-1 expression by keratinocytes in a cell type-specific manner. Additionally, we show that KGF is >100-fold more potent than bFGF in suppressing collagenase-1 production and that keratinocytes express only two fibroblast growth factor receptors (FGFRs): FGFR-3 IIIb, which does not bind bFGF or KGF, and FGFR-2 IIIb, which binds KGF with high affinity, but poorly to bFGF. Thus, bFGF and KGF inhibition of keratinocyte collagenase-1 expression probably occurs exclusively through the KGF (FGFR-2 IIIb) receptor.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human bFGF, recombinant human KGF, and a polyclonal neutralizing antiserum to bFGF were obtained from R & D Systems (Minneapolis, MN). Bovine type I collagen (Vitrogen-100) was purchased from Celtix Laboratories (Palo Alto, CA).

Isolation and Culture of Human Keratinocytes—Human keratinocytes were harvested from healthy adult skin from reduction mammoplasties or abdominoplasties as described previously (15, 32). Briefly, the subcutaneous fat and deep dermis were removed, and the remaining tissue was incubated in 0.25% trypsin in phosphate-buffered saline. After 16 h, the epidermis was separated from the dermis with forceps, and tissue was rinsed with Dulbecco's modified Eagle's medium. The keratinocyte suspension was added to fresh Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum and 0.1% penicillin/streptomycin. A specified amount of keratinocyte suspension was then plated onto tissue culture dishes coated with 1 mg/ml type I collagen and the keratinocytes were scraped into Dulbecco's modified Eagle's medium. The keratinocyte suspension was then plated onto tissue culture dishes coated with 1 mg/ml type I collagen and the keratinocytes were scraped into Dulbecco's modified Eagle's medium.

Enzyme-linked Immunosorbent Assay (ELISA)—The amount of collagenase-1 accumulated in keratinocyte-conditioned medium was measured by indirect competitive ELISA (33). This ELISA is completely specific for collagenase-1, has nanogram sensitivity, and detects both active and zymogen enzyme forms, as well as collagenase-1 bound to tissue inhibitor of metalloproteinases (TIMP) or bound to substrate. Results were expressed in picograms of collagenase-1 and normalized to total cell protein as described by the BCA protein assay (Pierce) using bovine serum albumin as a standard.

Metabolic Labeling—Postconfluent keratinocytes plated on type I collagen were cultured for 24 h in the presence of Dulbecco's modified Eagle's medium/fetal calf serum containing control or experimental solutions. The wells were then washed with methionine-free Dulbecco's modified Eagle's medium containing 5% dialyzed fetal calf serum (to remove free amino acids), 1 mM sodium pyruvate, and the identical concentrations of experimental reagents. Conditioned medium was collected after 24 h and stored at -70 °C for analysis by immunoprecipitation.

Immunoprecipitation and Total Protein Synthesis—Specific polyclonal antisera to collagenase-1 (11), stromelysin-1 (34), 92-kDa gelatinase (35), or TIMP-1 (36) were used to immunoprecipitate the ~35S-labeled metalloproteinases from keratinocyte-conditioned medium as described (37). Samples were precleared with protein A-Sepharose (Zymed, San Francisco, CA) and supernatants were incubated with antibody for 1 h at 37 °C and then overnight at 4 °C. Immune complexes were precipitated with protein A-Sepharose and washed extensively. Radiolabeled proteins were resolved by polycrylamide gel electrophoresis and visualized by fluorography. Total incorporated radioactivity was determined from the same conditioned medium by trichloroacetic acid precipitation.

RNA Isolation and Northern Hybridization—Total RNA was isolated from cultured keratinocytes by phenol-chloroform extraction (38). RNA (5 µg) was denatured and resolved by electrophoresis through a 1% formaldehyde-agarose gel, transferred overnight to Hybond N+ (Amer sham Corp.), and hybridized with radiolabeled collagenase-1 (39) and GAPDH cDNA probes. The cDNA probes were labeled by random priming (Boehringer Mannheim, Mannheim, Germany) with [α-32P]dCTP (NEN Life Science Products). Following hybridization, the membranes were washed and exposed to x-ray film for an appropriate duration.

Reverse Transcriptase-PCR Analysis—To determine which FGFRs were expressed by both human keratinocytes and fibroblasts, total RNA was harvested as above. RNA was treated with RQ1 RNase-free DNase (Promega, Madison, WI) to remove any contaminating DNA. RT reactions was from nucleotides 2093–2112 of the cDNA coding for human keratinocytes and fibroblasts, we amplified random primed cDNA with specific primers as described (40). Thus, bFGF and KGF inhibition of keratinocyte collagenase-1 expression probably occurs exclusively through the KGF (FGFR-2 IIIb) receptor.

B: 5'-TCNAGATGGGAGRTGAA-3' and DO158 (5'-CCAAGTCGGC- DATCCTCTCAT-3') were used to amplify conserved sequences in the tyrosine kinase domain of all FGFRs (41). The primer sequences used for PCR were DO156 (5'-TCNAGATGGGAGRTGAA-3') and DO158 (5'-CCAAGTCGGC-DATCCTCTCAT-3'), which produce a 341-bp product. PCR was for 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 7 min. To determine which members of the FGFR family were expressed, PCR products were analyzed by restriction digestion analysis with PstI, BalI, ScaI, or NarI. Digestion fragments were separated by nondenaturing polycrylamide gel electrophoresis and visualized by silver staining.

To determine the expression of FGFR-2 isoforms (IIb and IIIc) by human keratinocytes and fibroblasts, we amplified random primed cDNA with specific primers as described (40). bFGF was amplified for FGFR-2 IIIb using the 5'S primer corresponding to a region within the FGFR-2 IIb-specific exon K. 5'-CAATGCAAGAGT- GTGGCTCCTGTCCA-3'. FGFR-2 IIc was amplified using the 5'S primer corresponding to a region within the FGFR-2 IIc-specific exon B. 5'-GGTTAACACCGGACCAC-3'. The 3' primer used in both PCR reactions was from nucleotides 2093–2112 of the cDNA coding for FGFR-2. The same 3' primer was used for all FGFRs, since the nucleotide sequence is identical for both isoforms in this region (27, 43, 44). PCR was for 40 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 7 min. The predicted fragment size was 822 bp for the FGFR-2 IIb and 830 for FGFR-2 IIc. The products were separated through a 2% agarose gel and visualized by ethidium bromide staining. Further specificity was determined by transfer to Hybond N+ followed by Southern hybridization with a radiolabeled product-specific oligonucleotide probe. The probe was labeled by terminal transferase (Boehringer Mannheim) with [α-32P]dCTP. Following hybridization, the membranes were washed and exposed to x-ray film for an appropriate duration.

To determine the expression of FGFR-3 isoforms (IIb and IIIc) by human keratinocytes and fibroblasts, we amplified random primed cDNA with specific primers as described (45). The 5'S primer used (5'-GGACGGCGCCCCCATCTGAGCCGCGC-3') corresponds to nucleotides 789–811 of the human FGFR-3 gene, and the 3'AS primer used (5'-TACACAGGGGCGGCGTGTCAGC-3') corresponds to nucleotides 1135–1158 of the FGFR-3 gene, generating a product with a predicted fragment size of 341 bp. PCR was for 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 7 min. The predicted fragment size was 822 bp for the FGFR-3 IIb and 830 for FGFR-3 IIc. The products were separated through a 2% agarose gel and visualized by ethidium bromide staining. Further specificity was determined by transfer to Hybond N+ followed by Southern hybridization with a radiolabeled product-specific oligonucleotide probe. The probe was labeled by terminal transferase (Boehringer Mannheim) with [α-32P]dCTP. Following hybridization, the membranes were washed and exposed to x-ray film for an appropriate duration.

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RESULTS

bFGF Inhibits Collagenase-1 Production by Keratinocytes in a Cell Type-specific Manner—Previous reports have documented the capacity of bFGF to stimulate collagenase-1 production in cells of mesenchymal origin (18, 19, 46, 47). Consistent with these studies, we found that bFGF increased collagenase-1 production by human dermal fibroblasts in a dose-dependent manner (Fig. 1A). At 1.0 ng/ml bFGF, collagenase-1 production was augmented 5-fold over control levels. To assess if bFGF modulates keratinocyte collagenase-1 production, cells were exposed to increasing concentrations of growth factor for 72 h, and collagenase-1 accumulation in the medium was quantified by ELISA. In contrast to other cell types, bFGF potently inhibited keratinocyte collagenase-1 expression, with an ED₅₀ of ~1.0 ng/ml (Fig. 1B). Preincubation with anti-bFGF neutralizing antiserum abolished collagenase-1 down-regulation (Fig. 1C), thus demonstrating that the effect was due to the growth factor itself and not to a contaminant.

Metabolic labeling and immunoprecipitation experiments confirmed that bFGF inhibited keratinocyte collagenase-1 production at the level of new enzyme synthesis (Fig. 2A). Immunosuppression of the same conditioned media for stromelysin-1 showed similarly reduced expression of this MMP (Fig. 2B), whereas the synthesis of 92-kDa gelatinase and TIMP-1 was unchanged (data not shown). Inhibition of collagenase-1 and stromelysin-1 expression was specific, since synthesis of total secreted proteins by keratinocytes increased slightly following bFGF treatment (Table I). The disparity in bFGF concentrations required to effectively inhibit keratinocyte collagenase-1 production in Figs. 1 and 2 reflect the individual skin

FIG. 1. bFGF inhibits keratinocyte collagenase-1 production in a cell type-specific and dose-dependent manner. Human dermal fibroblasts (A) were cultured on tissue culture plastic and keratinocytes (B and C) on type I collagen until confluent. Increasing concentrations of bFGF were added to the cell cultures, and collagenase-1 protein accumulated in the conditioned media after 72 h of incubation was quantified by ELISA. In C, cells were cultured on type I collagen alone (control), or in the presence of bFGF neutralizing antiserum (10 μg/ml). bFGF (25 ng/ml) was preincubated with antibody 2 h prior to addition to cultures. Cell layers were analyzed for total protein content as described under “Experimental Procedures.” Data shown are the means of triplicate observations from the same cell preparation.

FIG. 2. bFGF inhibits biosynthesis of keratinocyte collagenase-1 and stromelysin-1. Human keratinocytes were cultured on type I collagen until confluent. Cellular proteins were metabolically labeled as described under “Experimental Procedures,” and conditioned medium was analyzed for the presence of collagenase-1 (A) or stromelysin-1 (B) with specific antisera. Cells were treated with 1.0 or 25 ng/ml of bFGF as indicated. Results from one representative experiment of two different cell preparations analyzed are shown.
donors examined, whom we have found to exhibit variable sensitivities to the growth factor.

bFGF Inhibits Collagenase-1 Production in Keratinocytes Pretranslationally—Total RNA was isolated from keratinocytes that had been treated for 24 h in the absence or presence of bFGF (25 ng/ml) and was analyzed by Northern hybridization. bFGF inhibited steady-state collagenase-1 mRNA levels, causing a 68% reduction when compared with untreated controls (Fig. 3A). Identically treated cells were cultured for 48 h, and collagenase-1 protein was quantified by ELISA (Fig. 3B). bFGF inhibited collagenase-1 protein expression (57%) proportionally to the drop in mRNA levels (68%), indicating pretranslational regulation.

KGF Inhibits Keratinocyte Collagenase-1 Production—Although bFGF consistently inhibited keratinocyte collagenase-1 expression, we often had to use relatively high concentrations (≥10 ng/ml) of the growth factor to observe this activity (Fig. 2 and other data not shown). Because multiple FGFs bind to more than one FGFR with different affinities (48–51), we postulated that other members of the FGF family might be more potent inhibitors of collagenase-1 production. KGF, a mesenchymal cell-derived cytokine that acts specifically on epithelial cells (24), was chosen as a candidate because of its relevance to epidermal wound repair. Paralleling the effects of bFGF, treatment of cultured keratinocytes with increasing concentrations of KGF resulted in a dose-dependent inhibition of collagenase-1 expression (Fig. 4A). Futhermore, KGF was more potent, consistently demonstrating an ED50 of 0.01 ng/ml, at least 10-fold lower than bFGF.

As demonstrated by metabolic labeling and immunoprecipitation, KGF inhibited collagenase-1 production (Fig. 4B). Again, KGF was effective at lower concentrations than bFGF (Fig. 4A versus Fig. 1B). As observed for bFGF, stromelysin-1 biosynthesis was also inhibited by KGF treatment, whereas 92-kDa gelatinase and TIMP-1 were unaffected (data not shown). Total protein synthesis was mildly increased by KGF (Table I), indicating the specificity of its collagenase-related activity.

KGF Inhibits Keratinocyte Collagenase-1 Expression Pre-
mRNA levels (Fig. 5) secreted collagenase-1 protein closely paralleled decreased by ELISA. Quantitation demonstrated that inhibition of secretion media samples from the same skin donor were analyzed by Northern hybridization. The upper panel represents hybridization with a radiolabeled collagenase-1 cDNA probe. The lower panel represents hybridization with a radiolabeled GAPDH cDNA probe, demonstrating loading equivalence of total RNA. A, keratinocytes from the same skin preparation were treated identically, and collagenase-1 content in the conditioned medium following 48 h of culture was quantified by ELISA. Each bar represents the mean generated from triplicate determinations.

Northern hybridization was performed to determine if KGF inhibited collagenase-1 production in a manner similar to bFGF. Keratinocytes treated with KGF (1.0 ng/ml) displayed a dramatic reduction in collagenase-1 mRNA compared with untreated cells (Fig. 5A). To compare KGF inhibition of collagenase-1 mRNA with collagenase-1 protein, conditioned media samples from the same skin donor were analyzed by ELISA. Quantitation demonstrated that inhibition of secreted collagenase-1 protein closely paralleled decreased mRNA levels (Fig. 5B; 69 versus 70%, respectively).

Inhibition of Keratinocyte Collagenase-1 Expression by bFGF and KGF Is Transduced through the KGF Receptor—FGFs activate a family of four receptor tyrosine kinases, which bind each member with different affinities (48, 49, 51, 52). Further specialization of these receptors occurs through alternative mRNA splicing, leading to unique ligand binding properties (27, 52, 53). We examined FGFRs present on keratinocytes to determine whether cell surface receptor expression could explain the differences in ED50 between bFGF and KGF required to obtain an equivalent inhibition of collagenase-1 expression by keratinocytes.

We used established reverse transcriptase-PCR methods to determine which members of the FGFR family are expressed by human keratinocytes (41, 42, 45). Total RNA was isolated from keratinocytes of two separate skin donors, and a random-primed cDNA library was generated by reverse transcription. Amplification of the cDNA using a single primer pair to generate all FGFRs yielded the expected 341-bp fragment (Fig. 6A). As seen by differences in band intensities using EtBr staining, levels of FGFR expression varied among the two populations of keratinocytes. To distinguish among keratinocyte FGFRs 1–4, PCR products were analyzed by restriction digestion analysis. Cultured keratinocytes expressed similar levels of FGFRs 2 and 3 but did not express FGFRs 1 and 4 (Fig. 6B). Extraneous bands of sizes different from that predicted were seen following silver staining. These bands probably resulted from low level contamination by genomic DNA. Our assignment of receptor isotypes remains unchanged, however, because nonspaced regions produce fragments larger than those predicted by restriction digestion. The expression of only FGFRs 2 and 3 was a consistent finding among several skin donors (n = 5), but, as previously stated, expression levels varied among samples.

Alternative splicing of primary transcripts of FGFRs 1–3 generates cell surface receptors having unique sequences within the ligand-binding Ig-like domain III (52–54). These isoforms, designated IIb and IIc, have distinct ligand affinities that regulate FGF signaling (51). Because cultured keratinocytes expressed only FGFRs 2 and 3 (Fig. 6), we determined which isoform(s) (IIb or IIc) of each receptor were expressed.

Alternative splicing of FGFR-2 produces two distinct isoforms: FGFR-2 IIb and FGFR-2 IIc (27, 44). The IIb isoform binds KGF with high affinity but does not efficiently bind to bFGF. In contrast, FGFR-2 IIc affinity for bFGF is high, whereas KGF does not bind (51). FGFR-2 IIb, but not FGFR-2 IIc, was expressed by primary keratinocytes as demonstrated by EtBr staining (Fig. 7, A and C). In contrast, human foreskin fibroblasts expressed only FGFR-2 IIc (Fig. 7, A and C). Specificity was verified by Southern hybridization with a product-specific oligonucleotide probe (Fig. 7, B and D). These data agree with our findings that equivalent inhibition of keratino-
cyte collagenase-1 expression required much higher concentrations of bFGF than KGF and that keratinocytes and fibroblasts exhibited different responses to bFGF.

Similar to FGFR-2, alternative splicing of FGFR-3 primary transcripts results in two distinct isoforms, FGFR-3 IIIb and FGFR-3 IIIc (53, 55, 56). FGFR-3 IIIb does not bind bFGF or KGF, whereas FGFR-3 IIIc binds bFGF with high affinity but does not bind to KGF (51). Keratinocytes expressed only FGFR-3 IIIb, as determined by restriction digestion analysis and EtBr staining of PCR products (Fig. 8A). Specificity was verified by Southern hybridization with a product-specific oligonucleotide probe (Fig. 8B).

**DISCUSSION**

The precise regulation of MMP expression is critical for normal wound repair and for maintaining tissue homeostasis. Aberrant expression following tissue injury may lead to a failure of healing. Indeed, we have demonstrated increased expression of collagenase-1 and stromelysin-1 in certain ulcerative skin lesions when compared with normally healing wounds (54, 57, 58). Furthermore, inflammatory/proliferative diseases, such as rheumatoid arthritis (59), are associated with unregulated production of MMPs, leading to widespread matrix destruction. Therefore, precise control of MMP expression in multiple cell types is necessary to maintain proper tissue organization and to promote events essential to postinjury repair.

Previous reports from our laboratories and others have shown that expression of collagenase-1 during cutaneous wound repair is restricted to basal keratinocytes at the leading edge of re-epithelialization (3, 4, 57, 60). These cells are in contact with dermal ECM (5, 7), and collagenase-1 production by keratinocytes in vitro is primarily induced by contact with native type I collagen (7, 8), facilitating cell migration on this matrix (9). Three human interstitial collagenases have been reported to date. In a variety of normal and disease-associated tissue remodeling events, collagenase-1 may be expressed by epithelial cells, fibroblasts, endothelial cells, chondrocytes, and macrophages (3, 4, 57, 61, 62). In contrast, expression of collagenase-2 (MMP-8) is limited to neutrophils and chondrocytes (63, 64), and collagenase-3 (MMP-13), originally cloned from a breast carcinoma cell line (65), is expressed in cartilage (66, 67) and developing bone (68). Recent studies by Johansson et al. (69) have reported expression of collagenase-3 by HaCaT keratinocytes following treatment with TGF-α and TGF-β. In contrast, however, primary human epidermal keratinocytes fail to express both collagenase-2 and -3, and our results confirm these observations (data not shown), thereby suggesting that collagenase-1 is the principal collagen-degrading enzyme produced by keratinocytes during repair.

In addition to cell-matrix interactions, soluble factors present within the extracellular environment may also play an important role in regulating the expression of MMPs by keratinocytes (10, 11, 13, 14, 70). Indeed, in this report we demonstrate that members of the FGF family inhibit the production of collagenase-1 by keratinocytes. Perhaps more interesting, however, are the findings that inhibition by these growth factors is cell type-specific and that ligand signaling most likely occurs through the KGF binding isoform (IIIb) of FGFR-2.

The molecular mechanisms responsible for cell type-specific regulation of MMP expression may be numerous and distinct. For example, intranuclear events mediate TGF-β inhibition of collagenase-1 production in fibroblasts and its induction in keratinocytes (13). Mauviel et al. (13) demonstrated that distinct *jun* trans-activating factors result in the differential regulation of collagenase-1 transcription in these two cell types. Although not reported to date, other cell-specific post-receptor signal transduction pathways could also mediate the different responses of cell types to a soluble factor. Furthermore, responses of distinct cell types to extracellular cation concentrations also regulate MMP production. Indeed, increased intracellular Ca$^{2+}$ induces collagenase-1 in fibroblasts (71), whereas its secretion is inhibited in keratinocytes (72). Finally, the binding of a single cytokine or growth factor to distinct cell-surface receptors provides yet another potential pathway for cell-specific MMP regulation. Many studies had previously shown bFGF to induce the expression of MMPs in various cell...
bFGF/KGF Inhibition of Keratinocyte Collagenase-1

The expression of IIIb and IIIc exons of the FGFR-3 in human keratinocytes. A and B, cDNA fragments of FGFR-3 IIIb and IIIc were amplified from human keratinocytes. The resulting product of 369 bp was visualized by EtBr staining (uncut). PCR products were analyzed by restriction digestion analysis with TaqI or HaeII to determine which isoforms were expressed (IIIb or IIIc, respectively). EtBr staining (A) and Southern hybridization with a radiolabeled oligonucleotide probe (B) revealed a fragment of the predicted size for FGFR-3 IIIb (264 bp) following digestion with TaqI. No digestion fragments were evident for FGFR-3 IIIc following digestion with HaeII. Thus, human keratinocytes express only the IIIb isoform of FGFR-3.

Recent studies have begun to delineate a role for KGF production during wound repair. Following tissue injury, the expression of KGF is markedly up-regulated by fibroblasts within the damaged dermis and acts in a paracrine manner to stimulate the overlying epithelium (29, 75). Additionally, KGF applied to full-thickness wounds results in increased epithelialization associated with epidermal thickening (76), and the targeted overexpression of this growth factor to keratinocytes leads to marked acanthosis (8). In contrast, expression of a dominant negative FGFR-2 IIIb driven by the K-14 promoter in transgenic mice resulted in epidermal atrophy, abnormal hair follicles, and impaired epidermalization (31). Taken together, these data suggest that the primary influence of KGF following injury is to promote proliferation and differentiation of basal keratinocytes.

Our data suggest that KGF may restrict keratinocyte MMP expression after wounding, thereby preventing the excessive degradation of the ECM. Interestingly, KGF receptors are expressed throughout the full thickness of intact skin. Upon wounding, receptor expression is dramatically decreased in migrating keratinocytes, and this pattern persists throughout the healing phase. However, KGF receptors are still prominently expressed by proliferating basal cells just behind the migrating front and in noninvolved areas of epidermis (75). When injury results in the production of KGF by underlying dermal fibroblasts, keratinocytes at the edge of tissue damage augment their basal proliferating phenotype, supplying new cells for the migrating front. Inhibition of MMP expression by KGF in these proliferating wound edge keratinocytes may be needed to prevent the degradation of reforming basement membrane or the aberrant destruction of underlying ECM. Because migrating wound keratinocytes have markedly downregulated KGF receptor (i.e. FGFR-2 IIIb) expression, KGF would not affect these cells, allowing collagenase-1 production to facilitate migration. Thus, KGF may play a dual role in wound repair, as a factor that stimulates cell proliferation and differentiation at the wound edge but also restricts MMP production to just the actively migrating cells.

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