Characterization of a Soluble KGF Receptor cDNA from Human Corneal and Breast Epithelial Cells

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PURPOSE. Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor (FGF) family FGF-7. It exhibits potent mitogenic activity for epithelial cells, including corneal and mammary epithelial cells. A messenger RNA has been reported that is generated by alternative splicing of bek that putatively codes only for the extracellular ligand-binding domain of KGF receptor (soluble KGF receptor). In the present study, the expression of the mRNA coding for this alternative bek transcript was examined and the corresponding protein characterized.

METHODS. Alternative messenger RNA transcripts were detected in various cell lines or tissues using reverse transcription-polymerase chain reaction (RT-PCR) and RNase protection assay. NIH/3T3 fibroblast cells and 293 kidney embryonic epithelial cells were stably transfected with soluble KGF receptor cDNA and transmembrane KGF receptor cDNA. Soluble KGF receptor protein was produced using a baculovirus-insect expression system. Soluble KGF receptor protein was detected using western and dot blot analyses. Binding assays and cross-linking labeling were used to determine the affinity and specificity of soluble KGF receptor. A mitogenic assay was performed to examine the function of the soluble KGF receptor.

RESULTS. The soluble KGF receptor mRNA was primarily expressed in epithelial cells, including cells from the cornea and breast. Cross-linking labeling and affinity-binding assays with 125I-KGF showed that the soluble KGF receptor bound KGF (FGF-7) but not FGF-1 or FGF-2. Soluble KGF receptor was detected in the culture medium of cells stably transfected with soluble KGF receptor cDNA but not with transmembrane KGF receptor cDNA, suggesting that the soluble receptor was generated by mRNA splicing and probably not by proteolysis or posttranslational processing. Soluble KGF receptor inhibited KGF binding to transmembrane KGF receptor and DNA synthesis in BALB/MK epidermal keratinocytes in response to KGF, suggesting that soluble KGF receptor expression could provide a mechanism for the cell to downregulate responses to KGF.

CONCLUSIONS. A truncated soluble KGF receptor expressed in corneal and other epithelial cells probably functions to downregulate the response of the cell to KGF. (Invest Ophthalmol Vis Sci. 1998;39:2584-2593)

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family (FGF-7) that is best characterized as a mediator of stromal–epithelial interactions.1-3 KGF is expressed by cells of mesenchymal origin and is thought to regulate some of the functions of epithelial cells.1 For example, KGF stimulates proliferation of corneal epithelial cells,2 breast epithelial cells,3 and skin keratinocytes.4

The cognate KGF receptor is a transmembrane tyrosine kinase that is a product of the bek gene.5,6 FGF receptor-2 is also a product of the bek gene that is generated by alternative mRNA splicing.5 bek contains alternative exons (Fig. 1) that contribute to the extracellular ligand-binding domains of KGF receptor and FGF receptor-2 (often designated as the K and B exons, respectively).5 These alternative exons determine the ligand-binding specificity of each of the transmembrane receptors. The protein-coding sequences, including the entire intracellular domain, of the mRNA transcripts are otherwise identical.5-7 The transmembrane KGF receptor has a high affinity for KGF and FGF-1, but binds FGF-2 with significantly lower affinity.5,8 Conversely, FGF receptor-2 has high affinity for FGF-1 and FGF-2, but does not bind KGF.5

While studying the expression and function of KGF and KGF receptor in the breast and cornea, we discovered an alternative reverse transcription-polymerase chain reaction (RT-PCR) amplification product in which the K and B exons were linked together (Fig. 1).3,5 Based on the sequence of this cDNA, we hypothesized that the alternative bek-derived mRNA containing both exons would code for a soluble protein consisting of the extracellular domain of KGF receptor, because a translation termination codon was generated at bp 13 in the B exon when the K and B exons were spliced (Fig. 1). In the
MATERIALS AND METHODS

pOPRSVI vector was obtained from Stratagene (La Jolla, CA); pcDNA 3.1 vector, transfection kit, *Spodoptera frugiperda* (SF9; Bac-N-Blue), insect cells (High Five), and insect cell medium (Grace's) from Invitrogen (San Diego, CA); Earle's minimum essential medium with nonessential amino acids (EMEM/NEAA), sodium pyruvate, MEM vitamins, and insect cell medium (EXCELL-400) from JRH Biosciences (Lenexa, KS); Dulbecco's modified Eagle's medium (DMEM); Dulbecco's modified Eagle's medium/Nutrient mixture F-12 (DMEM-F12), fetal bovine serum (FBS), calf serum, nonessential and essential amino acids, L-glutamine, insulin, cholaera toxin, human epithelial growth factor (EGF), ampicillin, gentamicin, lipofectamine, DNase, RNAase, RNase protected sequence. The translation product is a soluble protein without transmembrane and cytoplasmic domains of the full-length KGFR with KGF binding specificity. The RNA probe constructed for use in Rnase protection assay. Each of the alternatively spliced bek mRNA will yield a different-sized RNase protected sequence.

present study, we examined the expression of the mRNA coding for this alternative bek transcript and characterized the corresponding protein.

Materials and Methods

Protein coding sequence for the soluble KGF receptor was cloned from HCE cells by 5' rapid amplification of cDNA ends (RACE) using a commercial kit (Amplifinder RACE Kit; Clon...
A DNA probe spanning the 3' end of exon U (nucleotides 66-71), all exon K and the 5' end of exon B (nucleotides 1-130) of the beel gene (Fig. 1) was cloned by RT-PCR from HME cells into pcCR2.1 TA clone vector (Invitrogen). The plasmid construct was linearized with the NdeI restriction enzyme. RNA probe was synthesized with this probe using T7 RNA polymerase (RNA Transcription Kit; Stratagene) and [α-32P]cytidine triphosphate (800 Ci/mmol; Amersham, Arlington Heights, IL). The RNA probe was purified by ethanol precipitation to remove free nucleoside triphosphates. Fifty micrograms total cellular RNA was incubated in hybridization buffer containing 2 × 10^6 cpm probe RNA at 45°C overnight and digested with 40 μg/ml ribonuclease A and 1000 U/ml ribonuclease T1 (Boehringer Mannheim, Mannheim, Germany) at 30°C for 1 hour using a published method. The protected mRNA were analyzed on a 6% denaturing polyacrylamide-urea (sequencing) gel and exposed with film (BioMax; Kodak, Rochester, NY).

**Constructs and Transfection**

All constructs were generated using PCR amplification to introduce restriction sites for insertion into appropriate vectors and were verified by nucleic acid sequencing (Sequenase 2.0; USB). Soluble KGF receptor and transmembrane KGF receptor cDNA were cloned into a Rous sarcoma virus (RSV)-LTR promoter-driven pCR3-1 expression vector, respectively. The construct containing soluble KGF receptor cDNA sequence was cotransfected with the pMelBacA baculovirus transfer vector containing a honeybee melittin secretion signal residues at the N-terminus and the six histidine residues at the C-terminus. The transfer vector containing soluble KGF receptor cDNA sequence was cotransfected with the kit DNA (Bac-N-Blue; Invitrogen) into Sf9 insect cells by cationic liposome-mediated transfection, according to the manufacturer’s instructions. Sf9 cells were used to identify recombinant plaques. Insect cells were used to express the soluble KGF receptor protein.

**Iodination of KGF**

Human recombinant KGF was radiolabeled with 125I-sodium using the chloramine-T method. Three micrograms KGF in 50 μl 20 mM phosphate-buffered saline (PBS; pH 7.4) containing 1 M NaCl were mixed with 1 mCi 125I-sodium and 1.2 μl 1 μg/ml chloramine-T at room temperature for 1 minute, and 10 μl 1 mg/ml sodium metabisulfite was added to stop the reaction. The mixture was added to 200 μl PBS containing 0.1% bovine serum albumin (PBS-BSA) and applied to a 300-μl heparin Sepharose CL-6B column equilibrated with PBS-BSA. The column was washed with 25 ml PBS-BSA and eluted with PBS-BSA containing 0.85 M NaCl. The specific activity of the 125I-KGF was approximately 200 mCi/mg.

**Receptor-Binding Assays and Cross-Linking Labeling**

**Binding Assay 1.** The soluble KGF receptor binding assay was performed by mixing 1.35 ml sKGF6His medium, 0.15 ml 10X binding buffer (25 mM Hepes [pH 7.4] 1 μg/ml heparin, 1 mg/ml BSA; HBB) containing 0.1 nM 125I-KGF and a dilutional series containing unlabeled KGF, FGF-1, or FGF-2 and incubating at room temperature for 1.5 hours. The reaction mixtures were added to 70 μl protein G agarose–6xhis monoclonal antibody and incubated with rotational shaking for an additional 1.5 hours. The protein G agarose beads were washed three times with cold PBS and the ligand-soluble KGF receptor complex recovered by microcentrifugation at 10,000g for 15 seconds (Eppendorf; Brinkmann, Westbury, NY). Radioactivity bound to the soluble KGF receptor was measured with a
KGF receptor were grown to confluence in 24-well plates and HCN, human corneal endothelial cells; T-HCE, human cornea with the defective human papilloma virus E6 and E7 genes; epithelial cells transformed with simian virus 40; HCE, human gamma counter (Cobra auto-gamma; Packard Instruments, New York, NY). Curve-fitting for the binding data and median effective concentrations (EC50) were determined with commercial software (GraphPad Prism 2.0; Prism, San Diego, CA).

This alternative band indicates the PCR product amplified with these primers that also contains the K exon (arrow). This alternative band originally led to the discovery of the alternative mRNA coding for soluble KGF receptor. Note that this mRNA is primarily expressed in epithelial cells (HCE, T-HCE, and HME). This band was cloned and used as the probe in the RNase protection assay in Figure 4. Amplification product of expected 172-bp size for transmembrane KGF receptor was detected in each of the cell lines. Amplification product of expected 229-bp size for soluble KGF receptor was detected in all the cells except HCN-E6/E7, although the band was very faint in HSF, HT1080, and SKF cells. SKF, skin fibroblasts; HT1080, fibrosarcoma cells; 293, embryonic kidney epithelial cells; HepG2, hepatocarcinoma cells; A549, lung cancer-derived cells; LYM, lymphocytes; A382, astrocytoma cells; HABEC, human aortic endothelial cells; HVEC, human umbilical endothelial cells; HME, human mammary stromal cells; HMEC, human mammary epithelial cells; HSF, human corneal stromal fibroblasts; HCN-E6/E7, human corneal endothelial cells transduced with the defective human papilloma virus E6 and E7 genes; HCN, human corneal endothelial cells; T-HCE, human corneal epithelial cells transformed with simian virus 40; HCE, human corneal epithelial cells; EX-HCE, ex vivo human corneal epithelial cells.

Figure 3. Reverse transcription-polymerase chain reaction (RT-PCR) detection of fibroblast growth factor (FGF) receptor-2, transmembrane keratinocyte growth factor (KGF) receptor, and soluble KGF receptor mRNA in different cells and cell lines. (A) Amplification product of expected 199-bp size for FGF receptor-2 was detected in most cell lines. The arrow indicates the PCR product amplified with these primers that also contains the K exon (arrow). This alternative band originally led to the discovery of the alternative mRNA coding for soluble KGF receptor. Note that this mRNA is primarily expressed in epithelial cells (HCE, T-HCE, and HME). This band was cloned and used as the probe in the RNase protection assay in Figure 4. (B) Amplification product of expected 172-bp size for transmembrane KGF receptor was detected in each of the cell lines. (C) Amplification product of expected 229-bp size for soluble KGF receptor was detected in all the cells except HCN-E6/E7, although the band was very faint in HSF, HT1080, and SKF cells. SKF, skin fibroblasts; HT1080, fibrosarcoma cells; 293, embryonic kidney epithelial cells; HepG2, hepatocarcinoma cells; A549, lung cancer-derived cells; LYM, lymphocytes; A382, astrocytoma cells; HABEC, human aortic endothelial cells; HVEC, human umbilical endothelial cells; HME, human mammary stromal cells; HMEC, human mammary epithelial cells; HSF, human corneal stromal fibroblasts; HCN-E6/E7, human corneal endothelial cells transduced with the defective human papilloma virus E6 and E7 genes; HCN, human corneal endothelial cells; T-HCE, human corneal epithelial cells transformed with simian virus 40; HCE, human corneal epithelial cells; EX-HCE, ex vivo human corneal epithelial cells.

Western and Dot Blot Analyses

The KGF-KGF receptor-protein G agarose beads or 30 µl sKGFR6His medium was added to SDS-PAGE loading buffer without reducing, boiled for 5 minutes, and centrifuged for 5 minutes at 10,000g. The samples were resolved by electrophoresis on a 16% polyacrylamide SDS gel and electrotransferred (Bio-Rad) to a nitrocellulose membrane (0.2 µm, Bio-Rad). Soluble KGF receptor protein in the media from NIH/3T3 or 293 transfectants was detected by dot blot analysis using a KGF-lgG1 Fc domain chimera that has high affinity and specificity for the KGF receptor. The medium was concentrated 10X in a vacuum concentrator system (Speedvac; Savant Instruments, Farmingdale, NY), and 10 µl medium was loaded on the nitrocellulose membrane. Nitrocellulose membranes used in western or dot blot analyses were blocked with 5% nonfat milk (Bio-Rad) and 0.1% Tween-20 in 10 mM phosphate buffer containing 0.1 M NaCl (PBS-NaCl; pH 7.4) at 4°C overnight. The membranes were incubated with 1:1000 6xhis antibody or 1:25 KGF-Fc chimera in PBS-NaCl containing 0.01% Tween-20 (PBS-NaCl-Tween) at room temperature for 1.5 hours and washed four times for 5 minutes with PBS-NaCl-Tween and incubated in 1:2000 anti-mouse IgG (from sheep) linked with horseradish peroxidase (Amer sham) for 1.5 hours. Finally, the membranes were washed four times in PBS-NaCl-Tween. Soluble KGF receptor protein was detected with a western blot detection system (ECL; Amersham).

Mitogenic Assay. Mouse BALB/MK epidermal keratinocytes were grown to confluence in 96-well plates precoated with 50 µl of 8 mg/ml human fibronectin at 37°C for 1 hour. The medium was then replaced with serum-free low-calcium EMEM containing 1 µg/ml of transferrin and 3 X 10^-8 sodium selenite. After 48 hours in the sodium selenite medium, the medium was replaced with sodium selenite medium containing 1 ng/ml KGF. Different volumes of sKGFR6His medium that had been concentrated and dialyzed against PBS at 4°C overnight were added to a final volume of 100 µl. After 16 hours incubation at 37°C, 20 µl 50 mCi/ml [3H]thymidine was added per well, and incubation was continued for 6 additional hours. The cells were washed once with 250 µl cold PBS, washed twice with cold 3% trichloroacetic acid, and solubilized in 150 µl 0.25 M NaOH for 30 minutes. Then, 125 µl of each sample was added to 10 ml Scinti Vese (Fisher Scientific, Fair Lawn, NJ), and counting was performed in a liquid scin-
FIGURE 4. Expression detected by RNase protection assay. An RNA probe (Fig. 1A) was designed to distinguish fibroblast growth factor (FGF) receptor-2, transmembrane keratinocyte growth factor (KGF) receptor, and soluble KGF receptor mRNA. The protected RNA for FGF receptor-2, KGF receptor, and soluble KGF receptor were the expected size of 131, 154, and 285 bp, respectively. (A, B) Two experiments with different samples, although both included 293 embryonic kidney epithelial and simian virus 40 large T-antigen-transfected human corneal epithelial cells (T-HCE) for comparison. Soluble KGF receptor mRNA was detected in T-HCE, 293, hepatocarcinoma (HepG2), cells, and lung tissue (arrowheads). There was some carryover of the T-HCE sample into the adjacent empty well (B). Note that soluble KGF receptor mRNA was not detected in five independent ex vivo human corneal epithelial cell samples (EX-HCE), although transmembrane KGF receptor mRNA and FGF receptor-2 mRNA were detected. Soluble KGF receptor mRNA was not detected by RNase protection assay in some cell lines in which it had been detected by reverse transcription-polymerase chain reaction (RT-PCR; Fig. 3), suggesting that expression was at low levels in these cells. FGF receptor-2 and transmembrane KGF receptor mRNA expression also varied from cell to cell and in the different tissues. The cause of the doublet or triplet bands for each of the receptor mRNA differing by only one or two nucleotides is probably related to one or two nucleotide variations in the length of the RNA probe. Sequencing reaction products are provided as markers (M). A549, King cancer-derived cells; SKF, skin fibroblasts; HCN, human corneal endothelial cells; HCN-E6/E7, human corneal endothelial cells transduced with the defective human papilloma virus E6 and E7 genes; HSF, human corneal stromal fibroblasts; HMS, human mammary stromal cells; HME, human mammary epithelial cells; HAEC, human aortic endothelial cells.

RESULTS

5' RACE was performed using cDNA from HCE cells with a 3' primer specific for the bek-coded cDNA in which the K and B exons were spliced. This yielded a cDNA coding for the extracellular domain of the KGF receptor (Fig. 1) that was identical in nucleic acid sequence to a cDNA we had isolated from HME cells by RT-PCR, except for additional sequence 5' to the translation initiation site that was identical with that reported for the transmembrane KGF receptor sequence. The protein coding cDNA sequence for the alternative KGF receptor had been reported (accession number U11814).

Alternative Transcripts of bek Cell Lines and Tissues

Alternative mRNA coding for FGF receptor-2 (Fig. 3A), transmembrane KGF receptor (Fig. 3B), and soluble KGF receptor (Fig. 3C) were detected by RT-PCR in several human cell lines and in lymphocytes. These PCR products were confirmed by verifying that representative bands of the expected sizes had the correct sequence by nucleic acid sequencing. Transmem-
TABLE 1. RNase Protection Assay in Various Human Cell Lines and Tissues

<table>
<thead>
<tr>
<th>Cells or tissues</th>
<th>FGFR-2</th>
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<th>Soluble KGFR</th>
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<tr>
<td>293</td>
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A DNA fragment spanning partial exon U, exon K, and the 5' end of exon B of the bok gene was cloned into the pCR2.1 TA cloning vector from human mammary epithelial cells. The plasmid construct was linearized with the NdeI restriction enzyme. RNA probe was synthesized using T7 RNA polymerase.

Soluble Keratinocyte Growth Factor Receptor

Soluble KGFR receptor mRNA was detected in each of the cell lines, including epithelial, fibroblast, and endothelial cell lines. Soluble KGFR receptor mRNA was detected in most of the cell lines. The cell lines in which KGFR receptor could not be detected or in which it was detected at low levels by RT-PCR tended to be fibroblast cell lines (SKF, HT1080, and HSF), although the soluble KGFR receptor transcript was detected in HMS cells. FGF receptor-2 mRNA was not detected in lymphocytes, HME, or HCE cells, but was detected in the other cell lines. Of note, although FGF receptor-2 mRNA was not detected in HCE cells in primary culture, it was detected in ex vivo human corneal endothelial tissue (Fig. 3A).

An RNase protection assay (Fig. 4) was also performed with a probe (Fig. 1) that detected the three alternative bok-

coded receptor transcripts. The results are summarized in Table 1. The soluble KGFR receptor mRNA was detected with the less sensitive (compared with the sensitivity of RT-PCR) RNase protection assay in epithelial cell lines and lung tissue.

Specificity and Affinity of Soluble KGFR Receptor

Western blot analysis and immunoprecipitation-western blot with antibody to 6xhis showed (Fig. 5) that a 26-kDa protein was secreted by baculovirus containing the soluble KGFR receptor cDNA with a fused honeybee melittin secretion signal. Competitive binding analysis indicated that soluble KGFR receptor expressed by baculovirus-bound KGFR (EC50 4.7 nM; Fig. 6A), but not FGF-1 or FGF-2. Transmembrane KGFR receptor expressed by 293 cells transfected with plasmid coding for the transmembrane KGFR receptor-bound KGFR (EC50 2.6 nM) and FGF-1 (EC50 2.7 nM). FGF-2 bound with much lower affinity (EC50 52 nM; Fig. 6B).

Cross-linking labeling also indicated that the soluble KGFR receptor expressed by baculovirus had affinity (Fig. 7A) and high specificity for KGFR (Fig. 7B), because binding of [125I] KGFR to the soluble KGFR receptor was competitively inhibited by KGFR, but not FGF-1 or FGF-2. Heparin is required for KGFR binding to the soluble KGFR receptor, because binding did not occur when heparin was removed from HBB binding buffer (data not shown).

Soluble KGFR Receptor Protein Generated by mRNA Splicing

Dot blot analysis was performed with conditioned medium from cells transfected with pOPRSVI vector coding for soluble KGFR receptor or transfected with pcDNA 3.1 vector coding for transmembrane KGFR receptor and with vectors alone, using the KGFR-Fc chimera protein, which is capable of detecting KGFR receptor under native conditions. The KGFR portion of the chimera binds KGFR receptor, and the Fc portion binds the second antibody linked with horseradish peroxidase. Results are shown in Figure 8 and summarized in Table 2. Soluble KGFR receptor was detected in medium from the NIH/3T3 cells transfected with soluble

![Figure 5](https://example.com/figure5.png)

**FIGURE 5.** Western blot analysis of baculovirus expression of soluble keratinocyte growth factor (KGFR) receptor. In lane A, 0.03 ml (1 mg/ml) sKGFR6His medium was directly loaded on the gel used for western blot analysis. In lane B, the protein was first immunoprecipitated from the medium with antibody to 6xhis to concentrate the protein, and bound complex was dissolved in the sodium dodecyl sulfate (SDS) gel loading buffer without reductant and subjected to the SDS-polyacrylamide gel electrophoresis gel. The 6xhis antibody to six histidines on the carboxyl-termini of soluble KGFR receptor was used to detect soluble KGFR receptor protein (26 kDa) expressed in the baculovirus system.
Concentration of KGF (M)

**Figure 6.** Specific binding of $^{125}$I-keratinocyte growth factor (KGF), $^{125}$I-fibroblast growth factor (FGF)-1, and $^{125}$I-FGF-2 to soluble KGF receptor and transmembrane KGF receptor. (A) Specific binding of $^{125}$I-KGF to soluble KGF receptor protein (median effective concentration [EC$_{50}$], 4.7 nM). Neither FGF-1 nor FGF-2 showed affinity for the soluble KGF receptor protein. (B) Specific binding of $^{125}$I-KGF (EC$_{50}$, 2.5 nM) and $^{125}$I-FGF-1 (EC$_{50}$, 2.7 nM) to transmembrane KGF receptor. $^{125}$I-FGF-2 bound with much lower affinity (EC$_{50}$, 52 nM) to the transmembrane KGF receptor. Soluble KGF receptor produced in baculovirus and 293 embryonic kidney epithelial cells expressing transmembrane KGF receptor were incubated in duplicate with 0.1 nM $^{125}$I-KGF in the presence of increasing concentrations of unlabeled KGF. Each point represents the average of duplicate measurements. The data are representative of three experiments.

KGF receptor (Fig. 8A), but not in medium conditioned by 293 cells transfected with vector coding for transmembrane KGF receptor (Fig. 8B). Conversely, using the competitive binding assay, KGF receptor was detected on 293 cells transfected with vector coding for the transmembrane KGF receptor, but not on NIH/3T3 cells transfected with vector coding for the soluble KGF receptor (Table 2).

Together, the dot blot and the competitive binding analyses indicate that only cells transfected with vector coding for transmembrane KGF receptor have receptor associated with the cell membrane, whereas only cells transfected with vector coding for soluble KGF receptor secrete soluble receptor into the medium. Thus, in these cell types, soluble KGF receptor is not generated by proteolysis or posttranslational processing of the transmembrane KGF receptor protein.

**Inhibition of KGF Binding to Transmembrane KGF Receptor and DNA Synthesis in BALB/MK by Soluble KGF Receptor**

Soluble KGF receptor produced by baculovirus competitively inhibited $^{125}$I-KGF binding to human embryonic kidney 293 cells stably transfected with vector coding for the transmembrane KGF receptor (Fig. 9A). It also inhibited KGF-induced DNA synthesis in mouse BALB/MK epidermal keratinocytes (Fig. 9B).

**Figure 7.** Cross-linking labeling of $^{125}$I-keratinocyte growth factor (KGF) to soluble KGF receptor. (A) sKGFR6His media were incubated with 0.1 nM $^{125}$I-KGF and unlabeled KGF (100, 10, 1, 0.1, 0.01, and 0.001 nM). The resultant bound complexes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. (B) To sKGFR6His media were added 0.1 nM $^{125}$I-KGF without KGF and with 500x unlabeled KGF, FGF-1, or FGF-2. The resultant bound complexes were subjected to SDS-PAGE and phoshoimaging. bFGF, basic fibroblast growth factor.

**Figure 8.** Dot blot analysis of NIH/3T3 cells transfected with soluble keratinocyte growth factor (KGF) receptor cDNA and 293 embryonic kidney epithelial cells transfected with transmembrane KGF receptor cDNA. (A) NIH/3T3 cells transfected with vector alone (lane 1), and three colonies (lanes 2 through 4) transfected with soluble KGF receptor cDNA with different levels of expression. (B) Positive control NIH/3T3 cells expressing soluble KGF receptor (lane 1). 293 embryonic kidney epithelial cells transfected with vector alone (lane 2), and 293 transfected with transmembrane KGF receptor cDNA (lane 3).
Table 2. Comparison of Mouse NIH/3T3 Cells Transfected with Soluble KGFR cDNA and Embryonic Kidney Epithelial 293 Cells Transfected with Transmembrane KGFR cDNA

<table>
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<td>Binding assay</td>
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The table above shows the results of dot blot analysis and binding assay for NIH 3T3 cells and 293 cells transfected with transmembrane or soluble KGFR cDNA. ND indicates none detected.

Discussion

KGF, in addition to several other growth factors, is thought to have an important role in regulating corneal epithelial wound healing. Receptor expression and regulation is an important determinant of the function of growth factors in all organs. Thus, it is important to characterize KGF receptor expression and function in the cornea to understand the specific functions regulated by KGF in corneal wound healing.

In this study an alternative mRNA transcript derived from the bek gene coded for a soluble KGF receptor. The soluble KGF receptor seemed to be expressed primarily in epithelial cells, although the transcript could be detected by RT-PCR in other cell types. To rule out the possibility that soluble KGF receptor was generated by posttranslational processing of the transmembrane receptor, we attempted to detect soluble KGF receptor in the medium of 293 cells transfected to overexpress the transmembrane KGF receptor. The result showed that soluble KGF receptor was not derived from transmembrane KGF receptor by proteolysis, although we cannot exclude the possibility that soluble receptor could be generated by this mechanism in some cell types.

The transcript coding for soluble KGF receptor is derived from an alternative splicing event in which the K and B exons of the bek gene that contribute residues determining ligand-binding specificity of KGF receptor and FGF receptor-2, respectively, are linked together. This linkage directs translation of a truncated protein that contains each of the amino acid residues specified by the K exon in the transmembrane KGF receptor, but which is terminated near the carboxyl-terminal end of the extracellular domain.

KGF receptor is preferentially expressed in epithelial cells. The mechanisms regulating mRNA splicing that determine whether the transmembrane FGF receptor-2 or transmembrane KGF receptor is preferentially expressed in a particular cell type remain unknown. Similarly, the mechanisms that control the relative expression of alternative mRNA coding for transmembrane or soluble KGF receptor are unknown.

The soluble KGF receptor mRNA was consistently detected in ex vivo corneal epithelium using RT-PCR (Fig. 3). However, the mRNA was not detected by RNase protection assay in five ex vivo corneal epithelium samples removed by scraping (Fig. 4). Soluble KGF receptor mRNA can be detected by RNase protection assay in cultured simian virus 40 large, T-antigen–transformed corneal epithelial cells (Fig. 4). Thus, the soluble KGF receptor mRNA seems to be present at low levels in ex vivo corneal epithelium. There could be several explanations for this finding. Firstly, the soluble KGF receptor mRNA could have degraded in the ex vivo epithelium samples, because 1 to 2 minutes were required to scrape the epithelium from the human eye during corneal surgery and transfer the tissue into RNA extraction solution. During this time, RNase released from the disrupted epithelial tissue could have degraded the RNA. Secondly, rapid downregulation of the expression of the soluble KGF receptor may have occurred after
wounding of the corneal epithelium. This would make functional sense, because the soluble KGF receptor would inhibit the mitogenic effect of KGF that promotes corneal epithelial healing. Our data suggest this could have been the case, because transmembrane KGF receptor mRNA, but not soluble KGF receptor mRNA, was detected in each of the ex vivo corneal epithelium samples. Finally, it could be that the soluble KGF receptor we have detected has a minor role in the corneal epithelium in vivo and is therefore not expressed at high levels in this tissue.

Many truncated receptors (soluble and membrane spanning) have been shown to be generated by proteolytic processing. These include the p130 MET and p140 MET hepatocyte growth factor receptors, 32 insulin-like growth factor II, 24 p185-Neu receptor, 25 nerve growth factor receptor II, 26 interleukin (IL)-2 receptor, 27 IL-6 receptor, 28 tumor necrosis factor receptor, 29,30 and transferrin. 31 However, human epidermal growth factor receptor, 23 and truncated receptor encoded by fr&4, 34 and soluble Fas 35,36 are generated by translation of alternatively spliced transcripts, as we found for soluble KGF receptor.

It is surprising that the soluble KGF receptor binds KGF, but not FGF-1 or FGF-2. These results are different from the transmembrane KGF receptor, which also binds FGF-1 and FGF-2 (Fig. 6). We hypothesize that the tertiary structure of the ligand-binding domain of the soluble KGF receptor is somehow different from that of the membrane-spanning KGF receptor.

The soluble KGF receptor and transmembrane KGF receptor had moderate affinities for KGF (EC50 4.7 nM and 2.6 nM, respectively), which were close to that reported by Zimmer et al. (1.9 nM). 37 Another group, however, found an EC50 for KGF receptor in the range of 180 PM. 5 We cannot explain the difference in this affinity between our observations and the latter study.

Many soluble cytokine receptors have been described that have high affinity for the same ligand as the corresponding transmembrane receptor. 35,36-43 The functions of these soluble cytokine receptors have not been well characterized in most cases. Some viruses also direct synthesis of soluble receptors binding cytokines that modulate immune response. 44 In this case, it seems likely that the viruses direct synthesis of the soluble cytokine receptors to modulate the immune response to their advantage. Competitive inhibition of ligand binding to transmembrane receptor and inhibition of KGF-induced DNA synthesis in mouse BALB/MK epidermal keratinocytes by the soluble KGF receptor, suggest that the soluble receptor may be expressed by the cell to modulate its response to available ligand. Further study may provide insights into the physiologic functions of the soluble KGF receptor and the regulatory mechanisms that control its expression.

References


