Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 246–250, January 1992
Biochemistry

Determination of ligand-binding specificity by alternative splicing: Two distinct growth factor receptors encoded by a single gene

(fibroblast growth factor family/keratinocyte growth factor/expression cloning/tyrosine kinase/tissue-specific expression)

TORU MIKI*†, DONALD P. BOTTARO*, TIMOTHY P. FLEMING*‡, CHERYL L. SMITH*, WILSON H. BURGESS§, ANDREW M.-L. CHAN*, AND STUART A. AARONSON*

*Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892; and §Laboratory of Molecular Biology, Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, Rockville, MD 20855

Communicated by Hidesaburo Hanafusa, October 4, 1991 (received for review August 6, 1991)

ABSTRACT **Expression cDNA cloning and structural analysis of the human keratinocyte growth factor receptor (KGFR) revealed identity with one of the fibroblast growth factor (FGF) receptors encoded by the *bek* gene (FGFR-2), except for a divergent stretch of 49 amino acids in their extracellular domains. Binding assays demonstrated that the KGFR was a high-affinity receptor for both KGF and acidic FGF, while FGFR-2 showed high affinity for basic and acidic FGF but no detectable binding by KGF. Genomic analysis of the *bek* gene revealed two alternative exons responsible for the region of divergence between the two receptors. The KGFR transcript was specific to epithelial cells, and it appeared to be differentially regulated with respect to the alternative FGFR-2 transcript. Thus, two growth factor receptors with different ligand-binding specificities and expression patterns are encoded by alternative transcripts of the same gene.**

Growth factors and their receptors exhibit highly complex patterns of expression likely to be crucial to normal development processes as well as responses to injury and infection. Higher vertebrates contain multigene families of related ligands and their receptors, often with overlapping specificities. Presumably, this allows for greater flexibility in the timing and tissue distribution of these molecules. Another level of complexity has been appreciated only recently. Variants of the same growth factor or receptor can be encoded as alternative transcripts of the same gene. In the case of growth factor receptors, some insights have emerged with evidence that such variants may remain membrane associated or may be efficiently secreted (1). However, the functions of variants of membrane-spanning tyrosine kinase receptors remain to be elucidated. The number of such variants of fibroblast growth factor receptors (FGFRs) is particularly striking with at least 6 and potentially 12 isoforms (2).

In a search for epithelial cell-specific mitogens, we have isolated (3) and molecularly cloned (4) keratinocyte growth factor (KGF), a member of the FGF family. This factor differs from other characterized FGF-related molecules, which are active on a broad range of cell types, in that its mitogenic activity is tightly restricted to epithelial cells (3, 4). We recently isolated the KGF receptor (KGFR) cDNA by means of an expression cloning strategy, in which a cDNA library from mouse keratinocytes, which express the KGFR, was used to transfect NIH 3T3 cells. Since NIH 3T3 cells synthesize KGF, it was possible to identify KGFR cDNA transfectants as transformed foci, in which introduction of the KGFR cDNA created an autocrine KGF transforming loop (5).

The mouse KGFR is related to but distinct from the basic FGFR (*flg*/FGFR-1) (6–9). Its catalytic domain was found to be identical to the partial cDNA sequence for the mouse bek protein, indicating that the KGFR was encoded by the *bek* gene. The bek protein was initially identified as a tyrosine kinase by an approach of prokaryotic expression cloning using anti-phosphotyrosine antibodies (10). Other cDNAs closely resembling mouse *bek* have been identified by screening human or avian cDNA libraries (9, 11). In one case, the human *BEK* gene was shown to encode a receptor (FGFR-2) for basic FGF (bFGF) and acidic FGF (aFGF) (9). Here we use the designations of FGFR-1 and FGFR-2 for the *flg*- and *bek*-encoded FGFRs, respectively, as suggested by Keegan *et al.* (12). A human cDNA closely related to FGFR-2 was also identified as an amplified gene, designated K-*SAM*, in a stomach carcinoma line (13). These variants exhibit differences in their external domains including the presence or absence of the first immunoglobulin-like loop (Ig loop) and an acidic stretch of amino acids between the first and second Ig loops. A region of striking divergence in the second half of the third Ig loop and stem has been observed as well (5). In the course of expression cloning human mammary epithelial cell cDNAs with transforming activity for NIH 3T3 cells, we identified and cloned the human KGFR cDNA.¶ These studies show that alternative transcripts of a growth factor receptor gene give rise to two receptor species exhibiting striking differences in their ligand-binding properties.

MATERIALS AND METHODS

RNAs. Human poly(A)$^+$ RNAs were extracted from B5/589 (mammary epithelial cells), M426 (lung embryonic fibroblasts), umbilical cord endothelial cells, melanocytes, and OM431 (eye melanoma). Other human poly(A)$^+$ RNAs from monocytes (from peripheral blood), A1623 (anaplastic tumor groin node), A172 (glioblastoma), and Jurkat (lymphoma) were generous gifts of J. S. Gutkind, M. H. Kraus, G. D. Kruh, and S. Katamine (National Institutes of Health, Bethesda, MD), respectively.

Expression cDNA Cloning. A B5/589 cDNA library was constructed in λpCEV27 (14) by the automatic directional cloning method (15). Transfection of the library DNA, focus identification, and plasmid rescue were performed as described (14).

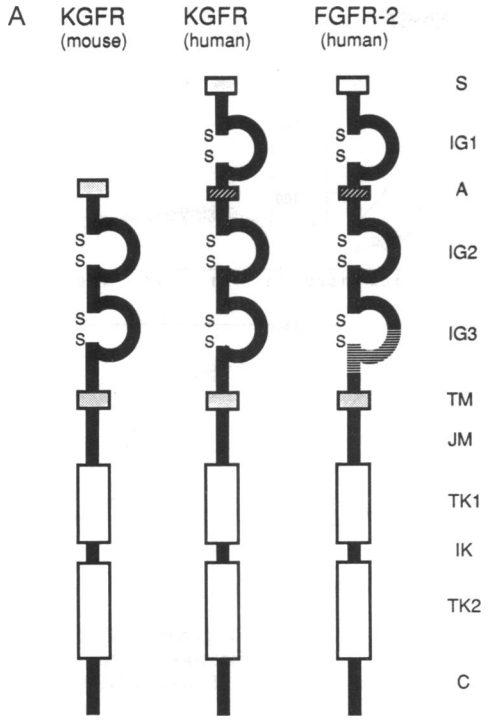
PCR Analysis. PCR was performed as described (16). The reaction was cycled 30 times at 94°C for 1 min, at 60°C for 3 min, and at 72°C for 3 min.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FGF, fibroblast growth factor; aFGF, acidic FGF; bFGF, basic FGF; FGFR, FGF receptor; KGF, keratinocyte growth factor; KGFR, KGF receptor.

†To whom reprint requests should be addressed.
‡Present address: Department of Ophthalmology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110.

¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M80634 and M80638).



B

```
Mouse KGFR   (MK)    1 ...........L.....................---------
Human KGFR   (HK)    1 MVSWGRFICLVVVTMATLSLARPSFSLVEDTTLEPEEPPTKYQIS
Human K-sam  (KS)    1 .....................................---------
Human FGFR-2 (F2)    1 .....................................

MK  37 ---------------------------------------------------------
HK  46 QPEVYVAAPGESLEVRCLLKDAAVISWTKDGVHLGPNNRTVLIGEYLQIKGATPRDSGLYA
KS  37 ---------------------------------------------------------
F2  46 .....................................................

MK  37 ----------------------------------------------G.........
HK 107 CTASRTVDSETWYFMVNVTDAISSGDDEDDTDGAEDFVSENSNNKRAPYWTNTEKMEKRLH
KS  37 ----------.....................................
F2 107 .....................................................

MK  53 ................T....................................
HK 168 AVPAANTVKFRCPAGGNPMPTMRWLKNGKEFKQEHRIGGYKVRNQHWSLIMESVVPSDKGN
KS  79 .....................................................
F2 168 .....................................................

MK 114 ...L.................................................
HK 229 YTCVVENEYGSINHTYHLDVVERSPHRPILQAGLPANASTVVGGDVEFVCKVYSDAQPHIQ
KS 140 .....................................................
F2 229 .....................................................

MK 175 .....................--.................M.............
HK 290 WIKHVEKNGSKYGPDGLPYLKVLK--HSGINSSNAEVLALFNVTEADAGEYICKVSNYIGQ
KS 201 .....................--...............................
F2 290 ....................AAGVNTTDKEI...YIR...FE.....T.LAG.S..I

MK 234 ..............V.....................................F....T......
HK 349 ANQSAWLTVLPKQQAPGREKEITASPDYLEIAIYCIGVFLIACMVVTVILCRMKNTTKKPD
KS 260 .....................................................
F2 351 SFH.....---.....................................

MK 295 .....................................................
HK 410 FSSQPAVHKLTKRIPLRRQVTVSAESSSSMNSNTPLVRITTRLSSTADTPMLAGVSEYELP
KS 321 ..................--...............................
F2 409 .....................................................

MK 356 .....................................................
HK 471 EDPKWEFPRDKLTLGKPLGEGCFGQVVMAEAVGIDKDKPKEAVTVAVKMLKDDATEKDLSD
KS 380 .....................................................
F2 470 .....................................................

MK 417 .....................................................
HK 532 LVSEMEMMKMIGKHKNIINLLGACTQDGPLYVIVEYASKGNLREYLRARRPPGMEYSYDIN
KS 441 .....................................................
F2 531 .....................................................

MK 478 ....................G....................................
HK 593 RVPEEQMTFKDLVSCTYQLARRMEYLASQKCIHRDLAARNVLVTENNVMKIADFGLARDIN
KS 502 .....................................................
F2 592 ....................G....................................

MK 539 .....................................................
HK 654 NIDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLMWEIFTLGGSPYPGIPVEELF
KS 563 .....................................................
F2 653 .....................................................

MK 600 .............T.....................................T.
HK 715 KLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRILTLTTNEEYLDLSQ
KS 624 ..........................................PPNPSLMSIFRK*
F2 714 .....................................................

MK 661 ..............................................*
HK 776 PLEQYSPSYPDTRSSCSSGDDSVFSPDPMPYEPCLPQYPHINGSVKT*
KS
F2 775 ..............................................*
```

Growth Factor Binding Assays and Crosslinking Experiments. Recombinant KGF and bovine brain aFGF were purified and labeled with $Na^{125}I$ as described (8, 17). Bovine brain bFGF was obtained from R & D Systems, Minneapolis. Bovine brain ^{125}I-labeled b-FGF (^{125}I-bFGF) was obtained from Amersham. Specific activities of all three tracers were $\approx 0.1\ \mu Ci/ng$ (1 Ci = 37 GBq). The NIH 3T3/FGFR-2 (human *BEK* transfectant) and NIH 3T3/NEO (vector transfectant) cells used have been described (9). Binding assays and covalent affinity crosslinking experiments were performed as described (5, 17).

RESULTS

Expression cDNA Cloning of a Human Transforming cDNA Related to FGFR-2. A cDNA expression library was constructed from human mammary epithelial cell line B5/589 by using the λpCEV27 vector system (14). Among 50 plates of NIH 3T3 cells transfected with B5/589 library cDNA, we identified and isolated several morphologically transformed foci. To screen for known cDNA sequences, PCRs were performed on samples of genomic DNA from independent foci by using primers derived from the sequences of human oncogenes, growth factors, and receptors. This screening procedure led to our identification of DNAs from three independent transformants that generated PCR fragments of the predicted size with primers from the intracellular domain of FGFR-2. Further PCR analysis suggested that the proteins encoded by the cDNA sequences in two foci contained two Ig loops and one contained three Ig loops. Integrated plasmid sequences were rescued from cellular DNA of the transformed focus expected to express the three-loop receptor. Transfection analysis revealed that a rescued plasmid, which scored positive by PCR for the FGFR-2 tyrosine kinase domain, exhibited a high-titered transforming activity of 10^4 focus-forming units/pmol.

Predicted Transforming Protein Is Identical to FGFR-2 Except in the Third Ig Loop Region. Sequence analysis of the 4.5-kilobase cDNA insert revealed an open reading frame encoding a membrane-spanning tyrosine kinase structurally related to the FGFR family. As shown in Fig. 1, it contained three Ig loops in its extracellular domain with an acidic amino acid stretch between the first and second loops. The predicted protein was most closely related to FGFR-2 and K-SAM, as well as mouse KGFR (Fig. 1). Its similarity to human FGFR-1 (9), FGFR-3 (12), or FGFR-4 (18) was significantly lower. Comparison of the predicted transforming protein with FGFR-2 revealed essentially complete identity with the exception of a strikingly divergent 49-amino acid stretch spanning the second half of the third Ig loop into the stem region (Fig. 1).

The predicted protein was also very similar to human K-SAM and mouse KGFR. However, each of these proteins

Fig. 1. Structure of the human KGFR and comparison with related molecules. (*A*) Schematic representation of structures of the human KGFR and related molecules. S, signal peptide; IG1, IG2, and IG3, immunoglobulin-like domains; A, acidic region; TM, transmembrane domain; JM, juxtamembrane domain; TK1 and TK2, tyrosine kinase domains; IK, interkinase domain; C, C terminus domain. (*B*) Predicted amino acid sequence of the human KGFR and its comparison with closely related proteins. The sequence of the human KGFR is shown in the second line. In the case of other molecules, only the amino acid residues different from the KGFR are shown. The residues identical to the human KGFR are indicated by dots. Dashes indicate that the residues are not present in the molecule. Potential signal peptide and transmembrane domains are underlined. The interkinase domain is shown by italic letters. Cysteine residues, which delimit three Ig loops in the extracellular portion of the molecule, are shown by the outline font. Termination codons are shown by asterisks.

contained only two Ig loops, and the mouse KGFR lacked the acidic amino acid stretch as well (Fig. 1*B*). K-SAM contained a C-terminal deletion and substitution and a small deletion of two amino acids between positions 340 and 341 (Fig. 1*B*). Both K-SAM and the mouse KGFR showed much higher predicted amino acid sequence similarity in their second half of the third Ig loop and stem regions than did human FGFR-2. In fact, the degree of relatedness to mouse KGFR was 98% (one amino acid difference) compared to only 47% with FGFR-2 in this region. These results were consistent with the possibility that we had isolated a human homologue of the mouse KGFR.

Third Ig Loop Region Determines KGF-Binding Properties. The mouse KGFR has been shown to bind KGF and aFGF at similar high affinity and bFGF at 20-fold lower affinity (5). In contrast, FGFR-2 has been reported to bind both aFGF and bFGF at high affinity (9), but there is no available evidence concerning its ability to interact with KGF. The isolation of a human cDNA whose product closely resembled the mouse KGFR, and yet differed only by a stretch of 49 amino acids in the second half of its third Ig loop from FGFR-2, allowed us to characterize its binding properties and compare them to those of FGFR-2. For those studies, we used NIH 3T3 transfectants overexpressing either protein and marker-selected NIH 3T3 cells transfected with the vector alone as a control.

As shown in Table 1, the transfectant containing our newly isolated cDNA demonstrated substantial ^{125}I-KGF binding, while neither FGFR-2 nor the vector transfectant showed detectable binding. These findings established that the cDNA encoded a human KGFR and suggested further that KGF lacked high affinity for FGFR-2. Evidence that FGFR-2 was indeed functional was derived from binding analyses with ^{125}I-aFGF and ^{125}I-bFGF. Both demonstrated a substantially greater number of binding sites on the FGFR-2 transfectant than on NIH 3T3 cells, which are known to express FGFR-1 (8) and to show mitogenic response upon stimulation by either growth factor (unpublished results). Whereas the human KGFR transfectant also bound increased amounts of ^{125}I-aFGF, we observed no increase in ^{125}I-bFGF binding over the level observed with control NIH 3T3 cells. All of these results demonstrated striking differences in the patterns of FGF and KGF binding by these two closely related human receptors.

Further analysis of the aFGF, bFGF, and KGF binding properties of human KGFR and FGFR-2 transfectants is shown in Fig. 2. ^{125}I-KGF binding to the KGFR transfectant was blocked to a similar degree by competition with unlabeled KGF and aFGF [50% displacement at 4 ng/ml (180 pM) and 10 ng/ml (600 pM), respectively] but bFGF was a relatively poor competitor [50% displacement at 50 ng/ml (3 nM)]. The pattern of FGF competition and 50% displacement values were nearly identical to those reported for the mouse KGFR (15, 17). In the same experiments, no specific ^{125}I-KGF binding to the FGFR-2 transfectant was observed (Fig. 2). ^{125}I-bFGF binding to the FGFR-2 transfectant was blocked equally well by competition with unlabeled bFGF and aFGF [50% displacement at 10 ng/ml (600 pM)], but only

Table 1. Specific binding of radiolabeled growth factors to cells transfected with human KGFR, FGFR-2, or vector alone

Ligand	Transfectant		
	NIH 3T3/KGFR	NIH 3T3/FGFR-2	NIH 3T3/NEO
^{125}I-KGF	211 ± 8	3.3 ± 0.4	3.0 ± 0.3
^{125}I-aFGF	188 ± 13	153 ± 8	34 ± 5
^{125}I-bFGF	30 ± 2	165 ± 5	41 ± 3

Values are expressed as mean cpm bound per μg of total cell protein (± SD) as determined from triplicate samples and are representative of results from three separate experiments. Specific binding is defined as cpm that are displaced with 100-fold excess unlabeled ligand. Tracer concentration is ≈6 ng/ml for each ligand.

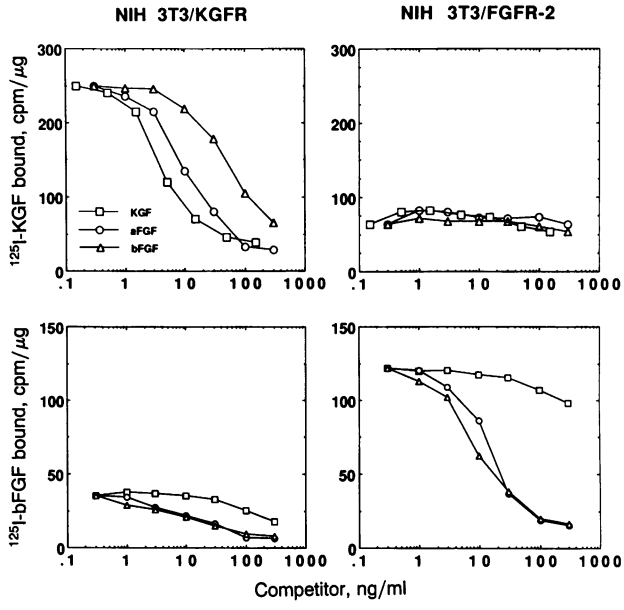


FIG. 2. Specific binding of radiolabeled ligands to NIH 3T3 cells transfected with KGFR or FGFR-2 expression vector. Specific binding of ^{125}I-KGF (*Upper*) and ^{125}I-bFGF (*Lower*) to NIH 3T3 cells transfected with human KGFR (*Left*) or FGFR-2 (*Right*) are shown. Displacement of tracers (6 ng/ml) by increasing concentrations of unlabeled KGF (□), aFGF (○), or bFGF (△) is expressed as cpm bound per μg of cell protein. Values shown are the means of triplicate samples and are representative of three or more experiments. A combination of added heparin (1–3 μg/ml) and brief salt extraction was used to block low-affinity tracer binding in all competition studies shown.

marginally by KGF. The displacement pattern of the low level ^{125}I-bFGF bound to the KGFR transfectant probably reflects the relatively low level of endogenous NIH 3T3 high-affinity FGFR, since similar binding levels and patterns of competition were observed with NIH 3T3 cells (data not shown). Since we used a combination of heparin addition and brief salt extraction in our binding assays, ^{125}I-bFGF binding to the KGFR with low affinity was not observed. This treatment eliminates low-affinity FGF binding to heparin-like cell-surface proteoglycans and probably low-affinity bFGF binding to the KGFR as well. As the only structural difference between the extracellular domains of FGFR-2 and KGFR resides in the 49-amino acid stretch within the third loop and stem, this region must confer the striking reciprocal differences in KGF and bFGF binding by the two receptors.

Absence of First Ig Loop Does Not Affect KGF Binding. The mouse KGFR contained two Ig loops, while the human clone contained three Ig loops (Fig. 1). However, this structural difference may not represent a species-specific difference, because we found several mouse KGFR isoforms, including three loop forms similar to the human clone (unpublished results). Among transformants detected by expression cloning with the B5/589 library, we identified a human FGFR-2-related cDNA possessing the second and third Ig loops of the KGFR (two-loop isoform; data not shown). Covalent affinity crosslinking of ^{125}I-KGF to the two- and three-loop KGFR isoforms revealed that both could bind KGF and confirmed the anticipated difference in receptor size (Fig. 3*A*). ^{125}I-KGF crosslinked to the three-loop KGFR had an apparent molecular mass of 160 kDa (corrected receptor size, 138 kDa), while the size of the two-loop KGFR–^{125}I-KGF complex was 131 kDa (corrected receptor size, 109 kDa). To determine the influence of the first Ig loop on KGF affinity, a Scatchard analysis of ^{125}I-KGF binding to NIH 3T3 transfectants expressing either receptor isoform was performed. Fig. 3*B* shows that both the number of KGF binding sites and binding

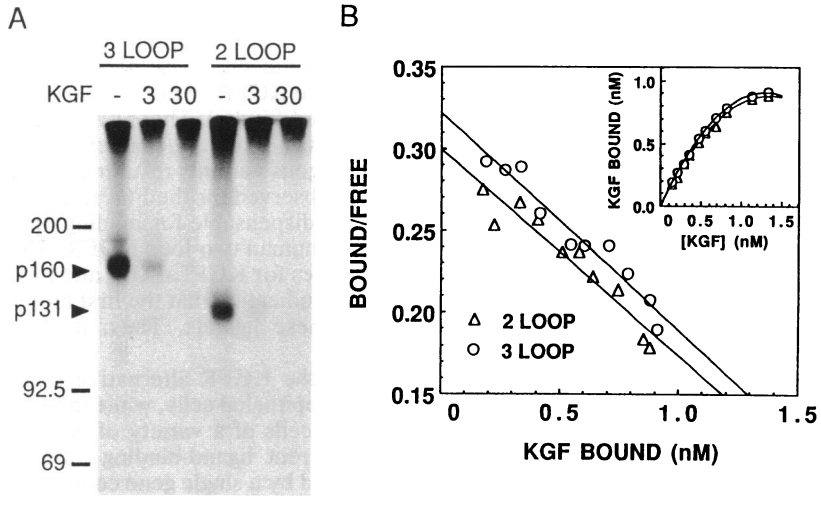


FIG. 3. Binding and crosslinking analysis of KGFR isoforms. (*A*) Covalent affinity crosslinking of ^{125}I-KGF to NIH 3T3 cells transfected with three-loop and two-loop human KGFR isoforms. With each isoform, ^{125}I-KGF binding was performed in the absence (−) or presence of 3- or 30-fold excess unlabeled KGF (as indicated). Arrowheads indicate the major ^{125}I-KGF-crosslinked complexes in the three-loop and two-loop KGFR transfectants. The migration of molecular size standards (kDa) is shown on the left. (*B*) Scatchard analysis of ^{125}I-KGF binding to NIH 3T3 cell transfectant expressing two-loop (△) or three-loop (○) KGFR isoforms. (*Inset*) Corresponding saturation binding of ^{125}I-KGF to two-loop (△) or three-loop (○) KGFR transfectants.

affinities (dissociation constant, ≈200 pM) were very similar. Thus, while the third loop divergence profoundly affected ligand-binding properties, the presence or absence of the first loop appeared to have little if any effect on KGF binding.

Determination of Ligand-Binding Specificity by Alternative Splicing. The high degree of sequence identity of the FGFR-2 and KGFR strongly suggested that both the receptor species were encoded by the same gene. If so, we reasoned that their divergent regions were likely to be encoded by different exons (exons K and B for KGF and bFGF, respectively) located between common upstream and downstream exons (U and D, respectively). To map these putative exons within the genomic sequence, we first compared nucleotide sequences of the divergent region. Two possible alternative locations of such exons could be postulated, and PCR primers were synthesized from the sequences of these hypothetical exons (Fig. 4*A*).

PCR analysis was performed to investigate the locations of these exons. As shown in Fig. 4*B*, all of the bands expected from the configuration of the exon/intron structure in Fig. 4*A*(*a*) could be detected (see Fig. 4*B*, lanes a, b, d, and g). In contrast, the respective PCR products, which should be synthesized if the exon/intron structure shown in Fig. 4*A*(*b*) were the case, could not be observed (Fig. 4*B*, lanes e and h). Together with the estimated sizes of the PCR products, our mapping data were consistent with the order of the exons as U-K-B-D [Fig. 4*A*(*a*)]. These results were further confirmed by the PCR analysis shown in Fig. 4*B* (lanes i–k). The intron/exon map of this region was determined as shown in Fig. 4*C*. Some of the PCR products (Fig. 4*B*, lanes i, j, and k) were cloned and sequenced, and consensus sequences for intron/exon junctions were found in the expected positions (Fig. 4*D*). All of these results established that two receptors with very different ligand-binding affinities were encoded by the same human gene (*BEK*) and messages for the two receptors were generated by alternative splicing.

Cell Type-Specific Regulation of KGFR and FGFR-2 Alternative Transcripts. To examine the level at which expression of the two receptor species might be regulated in various cell types, we used the PCR primers specific for the unique alternative exons of the KGFR and FGFR-2. As shown in Fig. 5, these exon-specific primers generated bands of 162 and 153 base pairs, respectively, using normal human genomic DNA as a template. These results were consistent with PCR analysis of intron/exon structure of the gene (Fig. 4*B*). We reverse-transcribed RNAs of different human cell types

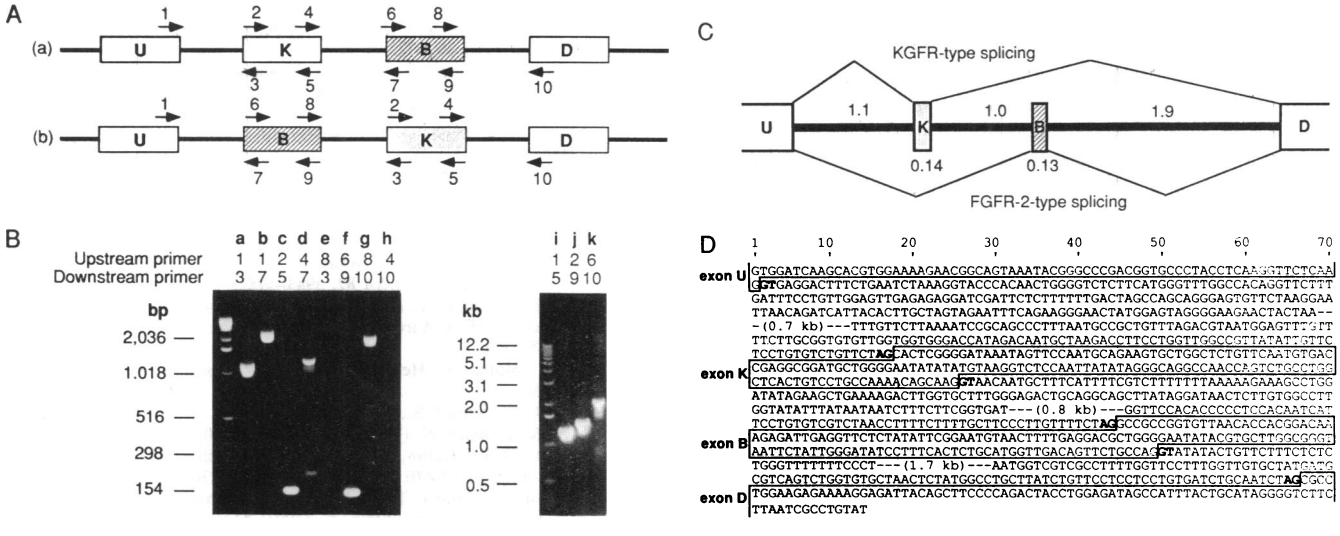


FIG. 4. Genomic analysis of the human *BEK* gene. (*A*) Postulated configuration of two alternative exons and location of the PCR primers used for analysis. Arrows indicate position and direction of the primers. (*B*) Analysis of the PCR products by agarose gel electrophoresis. Primers used are shown on the top. bp, Base pairs; kb, kilobase(s). (*C*) Intron/exon structure of a part of the human *BEK* gene, which gives rise to the divergence. Exons and introns are shown by boxes and thick lines, respectively, with the approximate sizes in kb. The splicing events specific to generate the two receptors are also shown. (*D*) Nucleotide sequence of the *BEK* gene surrounding the alternative exons. The exons are boxed and labeled by tentative designations (U, K, B, and D). The consensus sequences of the exon/intron borders are shown in boldface letters.

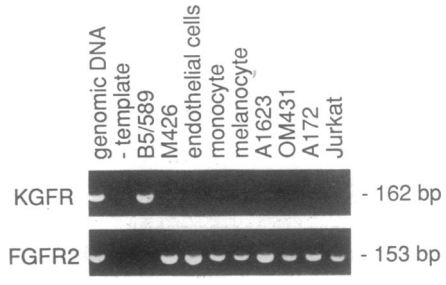


FIG. 5. Differential expression of the messages for KGFR and FGFR-2 in various cell lines. Poly(A)$^+$ RNAs extracted from the indicated cells were reverse-transcribed by primer 10, which can hybridize with the messages for either of the receptor species (see Fig. 4). Segments specific to KGFR and FGFR-2 are amplified from aliquots of the synthesized cDNAs by PCR using the primers specific to KGFR (primers 2 and 5) or FGFR-2 (primers 6 and 9). Human placental DNA was used as a positive control for PCR to show equal amplification of the segments of KGFR and FGFR-2 cDNAs (first lane). bp, Base pairs.

from a primer specific to both the alternative transcripts. The source of the RNAs includes mammary epithelial cells (B5/589), fibroblasts (M426), endothelial cells, melanocytes, and monocytes, as well as several tumor cell lines. The synthesized cDNA was then used to amplify KGFR- or FGFR-2-specific sequences from the exon-specific primers. Fig. 5 shows the striking contrast in patterns observed with only one of the alternative transcripts demonstrated in each of the cells analyzed. While the epithelial cells expressed transcripts containing only the KGFR-specific exon sequence, each of the other cell types expressed transcripts corresponding to FGFR-2-specific exon sequence. These findings are in complete accordance with the tightly restricted specificity of KGF for cells of epithelial derivation (3).

DISCUSSION

An expression cloning strategy aimed at identifying growth control genes in mitogenic signaling led to our isolation of a transforming cDNA from a human breast epithelial cell cDNA library. This cDNA encoded a predicted tyrosine kinase closely related to human FGFR-2. In fact, its sequence was essentially identical, except for a consecutive stretch of 49 amino acid residues that corresponded to the second half of the third Ig loop region of the extracellular ligand-binding domain. Like the mouse KGFR, the human protein bound both KGF and aFGF with high affinity and bFGF at lower affinity. In contrast, FGFR-2 failed to detectably bind KGF, although it showed high affinity for both aFGF and bFGF. Thus, the striking differences in the ligand-binding specificity of these receptors could be localized to this 49-amino acid stretch. PCR analysis of human genomic DNA further demonstrated that the region of divergence between the receptors was determined by single alternative exons, which encoded the domains responsible for their very different ligand-binding properties.

The transforming activity of human KGFR cDNA resulted from creation of an autocrine transforming loop in NIH 3T3 cells, which normally synthesize KGF. Recently, a gene designated K-*SAM* was identified as an amplified sequence in a human stomach carcinoma cell line (13). Its cDNA predicts a two-loop form of the KGFR, although it is truncated at its C terminus and exhibits a deletion of two consecutive amino acids at position 340 as well. Based on knowledge of the involvement of a number of growth factor receptors in malignant progression, it will be important to further investigate the role of the KGFR in human epithelial tumors as well as mechanisms that may lead to its oncogenic activation.

The human KGFR contained three Ig loops, while the first Ig loop and an acidic region are missing in the mouse KGFR isolated by an analogous strategy from a mouse keratinocyte expression cDNA library (5). These structural differences are not species specific, since we identified human KGFRs with two Ig loops as well as mouse KGFRs with three Ig loops and an acidic region (unpublished results). These two- and three-loop variants are presumably products of alternative transcripts as well, since analogous variants resulting from alternative splicing have been observed for the FGFR-1 (19). The first Ig loop of FGFR-1 is dispensable for binding of bFGF and Kaposi FGF (20). The human two-loop KGFR exhibited very similar relative affinities for KGF, aFGF, and bFGF as did the three-loop variant, indicating that the first loop is not required for binding of these ligands. Thus, its function remains to be elucidated.

In our present studies, the KGFR alternative transcript was found to be specific to epithelial cells, while the FGFR-2 transcript was detected in cells of a variety of other tissue types. The strikingly different ligand-binding affinities of these two receptors encoded by a single gene combined with their different patterns of expression provides a new dimension to growth factor receptor diversity and may reflect a general mechanism for increasing the repertoire of these important cell-surface molecules.

After submission of this manuscript for review, Johnson *et al.* (21) reported the partial genomic structure of the human *BEK* gene and identified exons corresponding to the K and B exons described by us.

We thank J. S. Rubin and D. Ron for purified KGF and helpful discussions; M. Moore and E. Fortney for excellent technical assistance; M. H. Kraus, G. D. Kruh, J. S. Gutkind, and S. Katamine for materials; and S. R. Tronick for computer analysis.

1. Mosley, B. M., Beckmann, P., March, C. J., Idzerda, R. L., Gimpel, S. D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J. M., Smith, C., Gallis, B., Urdal, D., Widmer, M. B., Cosman, D. & Park, L. S. (1989) *Cell* **59**, 335–348.
2. Hou, J., Kan, M., McKeehan, K., McBride, G., Adams, P. & McKeehan, W. L. (1991) *Science* **251**, 665–668.
3. Rubin, J. S., Osada, H., Finch, P. W., Taylor, W. G., Rudikoff, S. & Aaronson, S. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 802–806.
4. Finch, P. W., Rubin, J. S., Miki, T., Ron, D. & Aaronson, S. A. (1989) *Science* **245**, 752–755.
5. Miki, T., Fleming, T. P., Bottaro, D. P., Rubin, J. S., Ron, D. & Aaronson, S. A. (1991) *Science* **251**, 72–75.
6. Lee, P. L., Johnson, D. E., Cousens, L. S., Fried, V. A. & Williams, L. T. (1989) *Science* **245**, 57–60.
7. Ruta, M., Howk, R., Ricca, G., Drohan, W., Zabelshansky, M., Laureys, C., Barton, D. E., Francke, U., Schlessinger, J. & Givol, D. (1988) *Oncogene* **3**, 9–15.
8. Ruta, M., Burgess, W., Givol, D., Epstein, J., Neiger, N., Kaplow, J., Crumley, G., Dionne, C., Jaye, M. & Schlessinger, J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8722–8726.
9. Dionne, C. A., Crumley, G., Bellot, F., Kaplow, J. M., Searfoss, G., Ruta, M., Burgess, W. H., Jaye, M. & Schlessinger, J. (1990) *EMBO J.* **9**, 2685–2692.
10. Kornbluth, S., Paulson, K. E. & Hanafusa, H. (1988) *Mol. Cell. Biol.* **8**, 5541–5544.
11. Pasquale, E. B. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5812–5816.
12. Keegan, K., Johnson, D. E., Williams, L. T. & Hyman, M. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1095–1099.
13. Hattori, Y., Odagiri, H., Nakatani, H., Miyagawa, K., Naito, K., Sakamoto, H., Katoh, O., Yoshida, T., Sugimura, T. & Terada, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5983–5987.
14. Miki, T., Fleming, T. P., Crescenzi, M., Molloy, C. J., Blam, S. B., Reynolds, S. H. & Aaronson, S. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5167–5171.
15. Miki, T., Matsui, T., Heidaran, M. A. & Aaronson, S. A. (1989) *Gene* **83**, 137–146.
16. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
17. Bottaro, D. P., Rubin, J. S., Ron, D., Finch, P. W., Florio, C. & Aaronson, S. A. (1990) *J. Biol. Chem.* **265**, 12767–12770.
18. Partanen, J., Makela, T. P., Eerola, E., Korhonen, J., Hirvonen, H., Claesson-Welsh, L. & Alitalo, K. (1991) *EMBO J.* **10**, 1347–1354.
19. Johnson, D. E., Lee, P. L., Lu, J. & Williams, L. T. (1990) *Mol. Cell. Biol.* **10**, 4728–4736.
20. Mansukhani, A., Moscatelli, D., Talarico, D., Levytska, V. & Basilico, C. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4378–4382.
21. Johnson, D. E., Lu, J., Chen, H., Werner, S. & Williams, L. T. (1991) *Mol. Cell. Biol.* **11**, 4627–4634.