## K-sam gene encodes secreted as well as transmembrane receptor tvrosine kinase

(heparin-binding growth factor receptor/fibroblast growth factor receptor/secreted receptor)

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Contributed by Takashi Sugimura, December 20, 1991

ABSTRACT K-sam was first identified as a gene amplified in the stomach cancer cell line KATO-III. The size of the major transcript of the K-sam gene was 3.5 kilobases in KATO-III cells, and we have previously shown that K-sam encodes a receptor tyrosine kinase that belongs to the heparin-binding growth factor receptor, or fibroblast growth factor receptor, gene family. The K-sam gene expresses multiple sizes of mRNAs in brain tissue, the immature teratoma cell line NCC-IT, and KATO-III. RNA blot analyses with a variety of K-sam probes indicate that there are at least four classes of K-sam mRNAs. Three types of K-sam cDNAs in addition to the previously reported type of K-sam cDNA were isolated, and their nucleotide sequences encode a full-length transmembrane receptor, a secreted receptor with a tyrosine kinase domain, and a secreted receptor without a tyrosine kinase domain.

The K-sam gene (1) was first identified as amplified DNA fragments in the stomach cancer cell line KATO-III (2) by the in-gel DNA renaturation method (3-5). K-sam cDNA, corresponding to a 3.5-kilobase (kb) mRNA, encodes a receptor tyrosine kinase. The K-sam cDNA is homologous to mouse bek (6), mouse keratinocyte growth factor (KGF) receptor (7), chicken Cek3 (8), human bek (9, 10), and human TK14 (11) cDNAs. N-sam is a K-sam-related gene. N-sam cDNAs (1) are highly homologous to cDNAs for chicken basic fibroblast growth factor (FGF) receptor (12)/Cek1 (13) and mouse basic FGF receptor (14, 15), and N-sam is identical with flg (10, 16, 17). Heparin-binding growth factors, or the FGF family, include acidic and basic FGFs, INT2 protein (18), HST1 protein (19-21), FGF5 (22), HST2 protein/FGF6 (23-25), and KGF (26). The K-sam/bek and N-sam/flg genes establish a gene family of heparin-binding growth factor receptors or FGF receptors.

The K-sam gene is amplified preferentially in poorly differentiated types of stomach cancer (5). K-sam mRNA of 3.5 kb is overexpressed in stomach cancer cells with K-sam amplification (1). During studies of K-sam gene expression, multiple sizes of K-sam mRNA were found. We describe here molecular cloning and characterization of three additional K-sam cDNAs. K-sam cDNA type I (K-sam-I) was obtained from a cDNA library of human brain, K-sam cDNA type III (K-sam-III) was obtained from a cDNA library of the immature teratoma cell line NCC-IT (27), and K-sam cDNA type IV (K-sam-IV) was obtained from a cDNA library of KATO-III cells. The previously published K-sam cDNA derived from KATO-III cells was designated K-sam cDNA type II (K-sam-II). K-sam-I, corresponding to a 4.5-kb K-sam mRNA, encoded a transmembrane receptor with a tyrosine kinase domain, identical with the previously reported human Bek protein. Sequence analyses predicted that K-sam-III encoded a secreted receptor with a tyrosine kinase domain and that K-sam-IV encoded a secreted receptor without a tyrosine kinase domain.<sup>‡</sup>

## MATERIALS AND METHODS

RNA Blot Analysis. RNA blot analyses were performed under high-stringency conditions (19, 20). Poly(A)<sup>+</sup> RNA of postmortem human brain (28) was kindly provided by H. Kobayashi and S. Tsuji of Niigata University. Poly(A)<sup>+</sup> RNAs of NCC-IT cells and KATO-III cells were prepared as described (1). RA0.7 is a specific probe for the K-sam gene (1) and corresponds to the 5' noncoding region and a small (1)portion of the coding region of K-sam-II (nucleotides 1-719 of K-sam-II). The EC probe was made by polymerase chain reaction (PCR) and corresponds to the region of K-sam-I encoding the extracellular domain (nucleotides -46 to 1071). The ATM probe was made by PCR and corresponds to the region of K-sam-II encoding the transmembrane domain (nucleotides 1259-1595). The SR0.5 probe corresponds to the region encoding the second part of the K-sam-II tyrosine kinase domain (nucleotides 2071-2604) and cross-hybridizes with N-sam cDNAs (1). The DD0.4 probe was made by Dra I restriction endonuclease digestion and corresponds to the 3' noncoding region of K-sam-I (nucleotides 2927-3275). The SKT probe was made by PCR and corresponds to the 3'-terminal region of K-sam-IV (nucleotides 751-1110).

Isolation of K-sam cDNA Clones. A human brain cDNA library was constructed from poly(A)<sup>+</sup> RNA of postmortem human brain (28) and cloned into  $\lambda gt10$ . Size fractionation of cDNA inserts (>0.5 kb) was added to the procedure described previously (20). An NCC-IT cDNA library was constructed and cloned into  $\lambda gt10$ . A KATO-III cDNA library was constructed and cloned into  $\lambda$ ZAPII (Stratagene). These cDNA libraries were screened with appropriate probes under high-stringency conditions (20).

DNA Sequencing. cDNA sequences were determined by the dideoxy chain-termination method as described (1).

cDNA PCR. cDNA PCR was carried out as described (1). The DNA sequences of primers were as follows: primer U1 (sense), 5'-CTGACAAGGGAAATTATACC-3' (corresponding to nucleotides 671-690 of K-sam-I); primer U2 (sense), 5'-GACTGCCGGCAAATGCCTCCA-3' (corresponding to nucleotides 782-802 of K-sam-I) with a Sal I site added to the 5' terminus; primer D1 (antisense), 5'-TTTGCACAGAGGAAATAGATGCC-3' (corresponding to nucleotides 1110-1088 of K-sam-IV); primer D2 (antisense),

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Abbreviations: FGF, fibroblast growth factor; KGF, keratinocyte

growth factor. <sup>‡</sup>The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M87770 (K-sam-I), M87771 (K-sam-III), and M87772 (K-sam-IV)].

5'-ACCAGCGGGGTGTTGGAG-3' (corresponding to nucleotides 1334–1317 of K-sam-I) with a Sal I site added to the 5' end; primer D3 (antisense), 5'-ATCATCTTCATCATCTC-CAT-3' (corresponding to nucleotides 1622–1603 of K-sam-I).

## RESULTS

K-sam Gene Expression. RNA blot hybridization with a specific probe for the K-sam gene, RA0.7, showed bands of various sizes (Fig. 1, lanes 1–3). RNA from postmortem human brain showed bands of 4.5, 4.0, and 1.6 kb. RNA from NCC-IT cells gave bands of 4.5, 4.0, 3.2, and 1.6 kb. The intensity of the 4.0-kb band was weak in human brain RNA, whereas it was as strong as that of the 4.5-kb band in NCC-IT RNA. In RNA from KATO-III cells, the major bands were at  $\approx$ 3.5 kb, ranging from 4.2 to 3.2 kb, and less intense bands of 4.5 kb and  $\approx$ 1.6 kb (1.8 to 1.4 kb) were also detected.

Further analyses were performed with other K-sam probes to predict the structure of the multiple mRNAs derived from the K-sam gene. The EC probe, corresponding to the region of K-sam-I encoding the extracellular domain, showed the same pattern in RNA blot hybridization as RA0.7 with RNA from KATO-III (data not shown). The ATM probe, corresponding to the region of K-sam-II encoding the membranespanning domain, hybridized to 4.5- and 4.0-kb bands in NCC-IT RNA and to 4.5- and 3.5-kb bands in KATO-III RNA (Fig. 1, lanes 4 and 5). SR0.5, corresponding to the region of K-sam-II encoding the second part of the tyrosine kinase domain, hybridized to 4.5-, 4.0-, and 3.2-kb bands in NCC-IT RNA and to 4.5- and 3.5-kb bands in KATO-III RNA (Fig. 1, lanes 6 and 7).

**Isolation and Characterization of K**-sam-I. To isolate K-sam cDNAs corresponding to the 4.5-kb mRNA in normal tissue, a human brain cDNA library was screened with RA0.7. A clone that contained a 4.2-kb insert was isolated out of  $2 \times 10^5$  clones and designated K-sam-I.

K-sam-I contains a large open reading frame that encodes a protein of 821 amino acids with calculated  $M_r$  of 92,024 (Fig. 2A). The N-terminal 21 amino acid residues of the K-sam-I product correspond to the signal peptide, while the following 354, 23, and 423 residues constitute the extracellular, transmembrane, and cytoplasmic domains, respectively. The extracellular domain of the K-sam-I product contains three immunoglobulin-like (Ig-like) domains (29). An uninterrupted stretch of acidic residues (12) exists between the first and second Ig-like domains of the K-sam-I product. The cytoplasmic domain contains a tyrosine kinase domain with a 14-amino acid kinase insert region (30). The 3' noncoding region of K-sam-I has an internal polyadenylylation signal (31) in addition to the three terminal polyadenylylation signals.



FIG. 1. K-sam gene expression. Poly(A)<sup>+</sup> RNA (2  $\mu$ g per lane) was fractionated in agarose gels, transferred to Nitroplus membranes (MSI), and probed with <sup>32</sup>P-labeled RA0.7 (lanes 1–3), ATM (lanes 4 and 5), SR0.5 (lanes 6 and 7), DD0.4 (lane 8), or SKT (lane 9). Lane 1, human brain; lanes 2, 4, and 6, NCC-IT; lanes 3, 5, and 7–9, KATO-III. Films of lanes 1, 2, 4, 6, and 9 were exposed for 24 hr at -70°C, and those of lanes 3, 5, 7, and 8 for 9 hr at -70°C.

The nucleotide sequence of K-sam-I is almost identical with that of human bek. The reported nucleotide sequence of human bek terminates at nucleotide 3236 of K-sam-I. Although the nucleotide sequence of K-sam-I and that of human bek differ at nucleotides -179, -129, -126, -103, 159, and 2724 of K-sam-I, the deduced amino acid sequence of K-sam-I is identical with that of human bek.

K-sam-I differs from K-sam-II in four regions. The first Ig-like domain and 6 base pairs (bp) in the juxtamembrane domain of K-sam-I (nucleotides 110-376 and 1282-1287, respectively) are absent from K-sam-II (Fig. 3). The region encoding the second half of the third Ig-like domain of K-sam-I (nucleotides 940-1083) is replaced by a nucleotide sequence without significant homology in K-sam-II. The C-terminal coding region and the 3' noncoding region of K-sam-I (nucleotides 2277-3995) is also replaced by a different sequence in K-sam-II. The reason for the change in the C-terminal tail and the 3' noncoding region remains unknown.

The DD0.4 probe hybridized to a 4.5-kb band, but not to a 3.5-kb band, in KATO-III RNA (Fig. 1, lane 8). DD0.4 hybridized to a 4.5-kb band, but not to a 4.0-kb band, in NCC-IT RNA (data not shown). K-sam-I most likely corresponds to a 4.5-kb mRNA.

**Isolation and Characterization of K-sam-III.** An NCC-IT cDNA library was screened with SR0.5 and RA0.7, and a clone that hybridized to both probes was isolated (1). The nucleotide sequence of the cDNA was almost identical with that of K-sam-I, except that the membrane-spanning region of K-sam-I (nucleotides 940–1287, Fig. 2A) was deleted. The cDNA was designated K-sam-III and was analyzed.

The nucleotide sequence of K-sam-III terminates 19 bp downstream of the internal polyadenylylation signal of K-sam-I (nucleotide 3105), and adenine at position -235 of K-sam-I is replaced by cytosine in K-sam-III. K-sam-III encodes a secreted receptor with a tyrosine kinase domain and consisting of 705 amino acids with calculated  $M_r$  of 79,211.

cDNA PCR with primers U2 and D3 revealed 846-bp bands from poly(A)<sup>+</sup> RNA of human brain, NCC-IT, and KATO-III (Fig. 4). The 846-bp bands are likely to be derived from K-sam mRNA encoding a receptor with a membranespanning region. A 498-bp band was also amplified from poly(A)<sup>+</sup> RNA of NCC-IT, but not from RNA of human brain and KATO-III (Fig. 4). Both RA0.7 and SR0.5 hybridized to a 3.2-kb K-sam mRNA, but ATM did not (Fig. 1). These results show the existence of a K-sam mRNA that encodes a secreted receptor with a tyrosine kinase domain in NCC-IT.

Isolation and Characterization of K-sam-IV. A 1.6-kb band hybridized to RA0.7 and EC, but not to ATM or SR0.5 (Fig. 1), suggesting the existence of a class of K-sam mRNA that encodes a secreted receptor without a tyrosine kinase domain. A KATO-III cDNA library in  $\lambda$ ZAPII was screened to isolate clones that hybridized to EC but not to ATM. Fourteen clones were isolated out of 2 × 10<sup>5</sup> clones. Restriction enzyme mapping revealed that two clones, with 1.5-kb and 2.2-kb inserts, had different 3'-terminal sequences. Neither of the 3'-terminal sequences of these two inserts was contained in K-sam-II, K-sam-III, or K-sam-III. The 1.5-kb cDNA was designated K-sam-IV and was analyzed.

The nucleotide sequence of K-sam-IV is identical with that of K-sam-I amino acid codons 1-249, but further 3' the two sequences differed (Fig. 2B). From residue 250, the open reading frame of K-sam-IV continues downstream for another 5 amino acids and is then followed by a stop codon, TGA. The 3' noncoding region of K-sam-IV, 504 bp, includes a polyadenylylation signal 30 bp upstream from the 3' end. The deduced amino acid sequence of K-sam-IV contains the signal peptide, the first Ig-like domain, the acidic region, the second Ig-like domain, and the additional 5 amino acids but Ά

	CCCAAGGACCACTCTTCFGCGTTTGCAGTTGCT
-239	CCCCACAACCCCGGGCTCGTTGCCGTTTCTCCATCCCGACCCACGCGGGGGCGCGGGGGACAACACAGGTCGCGGAGGACGCTTGCCATTCAAGTGACTGCAGCGCAGCGCAGCGCGCGC
-119	TCCTGAGCCCACCGCAGGCTGAAGGCATTGCGCGTAGTCCATGCCCGTAGAGGAAGTGCGCAGATGGGATTAACGTCCACATGGAGATATGGAAGAGGACCGGGGATTGGTACCGTACCGTAGCG
1	ATGGTCAGCTGGGGTCGTTTCATCTGCCTGGTCGTGGTCACCATGGCAACCTTGTCCCTGGCCCGGCCCTCCTTCAGTTTAGTGAGCAACAATTAGAGCCAGAAGAGCCACCAACC
1	<u>M V S W G R F I C L V V V T M A T L S L A</u> R P S F S L V E D T T L E P E E P P T
121	AAATACCAAATCTCTCAACCAGAAGTGTACGTGCCGCCAGGGGAGTCGCTAGAGGTGCGCTCCTGTTGAAGATGCCGCCGTGATCAGTTGGACTAGGAGTGGCGCGCCAGGGGAGTCGCTAGAGGTGCGCTGCGCCGCGCGCG
41	KYQISQPEVYVAAPGESLEVR <b>O</b> LLKDAAVISWTKDGVHLG
241	CCCAACAATAGGACAGTGCTTATTGGGGAGTACTTGCAGATAAAGGGCGCCACGCCTAGAGACTCCGGCCTCTATGCTTGTACTGCCAGTAGGACTGTAGACAGTGAAACTTGGTACTTC
81	PNNRTVLIGEYLQIKGATPRDSGLYACOTASRTVDSETWYF
361	ATGGTGAATGTCACAGATGCCATCTCATCCGGAGATGATGAGGATGACACCGATGGTGCGGAAGATTTTGTCAGTGAGAACAGTAACAAGAGAGCACCATACTGGACCAACAACAAGAA
121	M V N V T D A I S S G <u>D D E D D T</u> D G A E D F V S E N S N N K R A P Y W T N T E
481	ANGATGGAAAAAGCGGCTCCATGCTGTGCCTGCGGCCAACACTGTCAAGTTTCGCTGCCCAGCCGGGGGAACCCAATGCCAACCATGCGGTGGCTGAAAAAACGGGAAGGAGTTTAAGCAG
161	KMEKRLHAVPAANTVKFR 🜍 PAGGNPMPTMRWLKNGKEFKQ
601	GAGCATCGCATTGGAGGCTACAAGGTACGAAACCAGCACTGGAGCCTCATTATGGAAAGTGTGGTCCCATCTGACAAGGGAAATTATACCCTTGTGGTGGAGAAATGAATACGGGTCCATC
201	E H R I G G Y K V R N Q H W S L I M E S V V P S D K G N Y T 🖸 V V E N E Y G S I
	$\nabla$
721	AATCACACGTACCACCTGGATGTTGTGGAGCGATCGCCTCACCGGCCCATCCTCCAAGCCGGACTGCCGGCAAATGCCTCCACAGTGGTCGGAGGAGACGTAGAGTTTGTCTCCAAGGTT
241	N H T Y H L D V V E R S P H R P I L Q A G L P A N A S T V V G G D V E F V 💟 K V
841	TACAGTGATGCCCAGCCCCACATCCAGTGGATCAAGCACGTGGAAAAGAACGGCAGTAAATACGGGCCCGACGGGCTGCCCTACCTCAAGGTTCTCAAGGCCGCCGGTGTTAACACCACG
281	Y S D A Q P H I Q W I K H V E K N G S K Y G P D G L P Y L K V L K <b>b</b> A G V N T T -
	-
961	GACAAAGAGATTGAGGTTCTCTATATTCGGAATGTAACTTTTGAGGACGCTGGGGAATATACGTGCTGGCGGGTAATTCTATTGGGATATCCTTTCACTCTGCATGGTTGACAGTTCTG
321	D K E I E V L Y I R N V T F E D A G E Y T 🖸 L A G N S I G I S F H S A W L T V L
	<b>U</b>
1081	CCAGCGCCTGGAAGAGAAAAGGAGATACAGCTTCCCCAGACTACCTGGAGATAGCCATTTACTGCATAGGGGTCTTCTTAATCGCCTGTATGGTGGTGAACAGTCATCCTGTGCCGAATG
361	PAPGREKEITASPDYLEIAIYCIGVFLIACMVVTVILCRM
1201	DOTOGATORADOTOCONCOLOGICA DE CONCOLORIZADA DE CONCOLORIZADOS DE CONCOLORIZ
401	when the kind of the second and the second
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1321	A CACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
441	N TOT V D T T T D T S S T A D T D M T. A C V S F Y F T. P F. D P K W F F P R D K
1441	
401	
401	FILGKELGEGCEGVVVMKEKVVGIDKDKEKEKVVVVKHDK
1561	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
521	$\alpha_1$ and $\alpha_2$ and $\alpha_3$ and $\alpha_4$ and $\alpha_3$ and $\alpha_4$ and $\alpha_3$ and $\alpha_4$
521	
1691	
561	
501	
1801	AAGGACTTGGTGTCATGCACCTACCAGCTGGCCAGAGGCATGGAGTACTTGGCTTCCCAAAAATGTATTCATCGAGATTTAGCAGCCAGAAATGTTTTGGTAACAGAAAACAATGTGATG
601	K D L V S C T Y O L A R G M E Y L A S O K C I H R D L A A R N V L V T E N N V M
1921	ANAATAGCAGACTTTGGACTCGCCAGAGATATCAACAATATAGACTATTACAAAAAGACCACCAATGGGCGGCTTCCAGTCAAGTGGATGGCTCCAGAAGCCCTGTTTGATAGAGTATAG
641	KTADFGIARDTNING
011	
2041	
691	
001	
21 61	
2101	
/21	K M D K F A N C I N E D I M M M K D C W H A V F S Y K F I F K Y E V E D E D K I
2281	CTCACTCTCACAACCAATGAGGAATACTTGGACCTCAGCCAACCATCTCCGAACAGTATTCACCTAGTTACCCTGACAACAAGAAGTTCTTGTTCTTCAGGAGAATGATTCTGTTTTTTCTCCCA
761	LT LTT NEEYLDLS QPLE QYSPSYPDT KSSCSSGDDSVFSP
2401	GACCCCATGCCTTACGAACCATGCCTTCCTCAGTATCCACACATAAACGGCAGTGTTAAAACATGAATGA
801	D P M P Y E P C L P Q Y P H I N G S V K T
2521	GAGCAGGGAGACCATGCCTCCCCAGAGCTTGTTGTGTCTCCCACTTGTATATATGGATCAGAGGAGTAAATAATTGGAAAAGTAATCAGCATATGTGTAAAGATTTATACAGTTGAAAAACTTGT
2641	AATCTTCCCCAGGAGGAGAAGAAGGTTTCTGGAGCAGTGGACTGCCACAAGCCACCATGTAACCCCTCTCACCTGCCGTGCGTTCTGGACCAGTAGGACTCAAGGTGGACGTAC
2761	GTTCTGCCTTCCTTGTTAATTTTGTAATAATTGGAGAAGATTTATGTCAGCACAACATTACAGAGCACAAATGCAGTATATAGGTGCTGGATGTATGT
2881	ANTATATATATATATATATATATATAAAGGAGTTATTTTTTGTATTGATTGATTTAAAAGGATGTCCCAATGCACCTAGAAAATTGGTC <u>TCTT</u> TTTTTAAAAGCTATTTGGTAAATGCTGTTCTTAA
3001	ACATAATTTCTTAATTTTCACCGAGCAGAGGTGGAAAAAACCTTTTGCTTTCAGGGAAAATGGTATAACGTTAATTTAAT <mark>AATAAA</mark> TTGGTAATATACAAAAAAAAAAAATTAAACAATTAAACAATTAAACAATTAAA
3121	ttttttgtaatttaagtggcatttctatgcaggcaggacaggcagactagttaatctattgcttggacttaactagttatcagatcctttgaaaagaaaaaagagaatatt
3241	TTTGGGGAAAATGAAGTTTTGATTTAATTTGTGTTTAAATGCTGCTGTCAGACGATTGTTCTTAGACCTCCTAAATGCCCCATATTAAAAGAACTCATTCAT
3361	GTGTGCAACCCTGTCATTACGTCAACGCAACGTCTAACTGGACTTCCCAAGATAAATGGTACCAGCGTCCTCTTAAAAGATGCCTTAATCCATTCCTTGAGGACAGACCTTAGTTGAAAAT
3481	GATAGCAGAATGTGCTTCTCTCGGCAGCTGGCCTTCTGGCTTCTGAGTTGCACATTAATCAGATTAGCCTGATTCTCTTCAGTGAATTTTGATAATGGCTTCCAGACTCTTTGCGTTGGA
3601	GACGCCTGTTAGGATCTTCAAGTCCCATCATAGAAAATTGAAACACAGAGTTGTTCTGCTGATAGTTTTGGGGATACGTCCATCTTTTTAAGGGATTGCTTTCATCTAATTCTGGCAGGA
3721	CCTCACCAAAAGATCCAGCCTCATACCTACATCAGACAAAATATCGCCGTTGTTCCTTCTGTACTAAAGTATTGTGTTTTGGTATGGAAACACCCCACTCACT
3841	ATGAATGCAGATTACACTGATCTTATGTGTTACAAAAATTGGAGAAAGTATTTAATAAAACCTGTTAATTTTTATACTGACAAAAATGTTTCTACAGATATTAATGTTAACAAGACAA
3961	ATTAA TIGTCACGCAACTTAAAAAAAAAAAAAAAA

В

**B** 721 AATCACACGTACCACCTGGATGTTGTGGGCAGCCAGGGTTTATGAGCTTTGCATGATCCTCATGGTTCCCAAGCGTCATCTGTGTAAAGTGGACGTGGTATGAAATGTCTGACATTTTGG 241 N H T Y H L D V V G S Q G L

841 AAGCTGAGATTACTCTGAAAATGTTAATTGGGCAGGTGAAAAGGGTACAGATGTGCTGTAGCAGACCTTTGGTTTTAAAAGAGAAGCATCATTTCCCCCAACAGGGCAACTGTAGAAGGCC 961 AGCTGAAGAGTAAAGGAAAAAGGTCTGAGGACTGAGGCTGGGCTGGGCTGGAAAAGTGTGAGGGGCCCTTCACTTCCATACAAAAAAAGTAAAGCAGTAACCAATTCAGTGGCC

FIG. 2. Nucleotide sequence and deduced amino acid sequence of K-sam cDNAs. (A) K-sam-I. Nucleotides are numbered at left, as are the deduced amino acids, which are shown below the nucleotide sequence. Heavy underlines indicate the putative hydrophobic signal peptide and the transmembrane domain. Underline indicates the kinase insert region in the tyrosine kinase domain. Double underline indicates an uninterrupted stretch of acidic residues (DDEDD). Cysteine residues that define Ig-like domains are circled. The region deleted in K-sam-III is bracketed. Open arrowhead indicates the point at which sequence identity between K-sam-I and K-sam-IV diminishes. Polyadenylylation signals are boxed. (B) K-sam-IV. Nucleotide sequence of K-sam-IV is identical with that of K-sam-I at positions -272 to 747. Nucleotide sequence downstream from nucleotide position 720 is shown.

lacks the transmembrane domain and tyrosine kinase domain. Thus, K-sam-IV seems to encode a secreted receptor that lacks a tyrosine kinase domain and consists of 254 amino acids with calculated  $M_r$  of 28,298.

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K-sam-l

K-sam-II



FIG. 3. K-sam cDNAs and probes. Noncoding region is indicated by bar. The 3' noncoding regions specific for K-sam-II and K-sam-IV are indicated by thicker bars. Shown for each cDNA are the hydrophobic signal peptide (filled box), Ig-like domains (numbered open boxes), acidic region (checked box), transmembrane domain (striped box), tyrosine kinase domain (stippled box), divergent coding region of K-sam-II and K-sam-IV (hatched box), and polyadenylylation signal(s) [small circle(s)].

cDNA PCR with primers U1 and D1 amplified 440-bp products from  $poly(A)^+$  RNA of human brain and KATO-III (data not shown). These PCR products showed the expected sizes and contained the expected internal *Pvu* II sites. PCR with primers U1 and D1 amplified 650-bp bands from genomic DNA of human placenta and KATO-III (data not shown), which also contained the internal *Pvu* II sites.

RNA blot hybridization with the SKT probe showed a 1.8-kb band in KATO-III (Fig. 1, lane 9) but not in human brain and NCC-IT (data not shown).

## DISCUSSION

K-sam mRNAs of different sizes were present in various types of cells. K-sam mRNAs of 4.5, 4.0, and 1.6 kb were detected in normal tissue, human brain. K-sam mRNAs of 3.5 and 1.8 kb were found in KATO-III stomach cancer cells. K-sam mRNA of 3.2 kb was detected in NCC-IT teratoma cells. RNA blot analyses with a variety of K-sam probes, coupled with analyses of different types of K-sam cDNAs, indicate that there are at least four classes of mRNAs. The K-sam mRNA of 4.5 kb encodes the full-length receptor, which is identical with the human bek product. The K-sam mRNAs of 4.0 and 3.5 kb, which are truncated at least in the 3' noncoding region, encode membrane-spanning receptors with a tyrosine kinase domain. The K-sam mRNA of 3.2 kb probably encodes a secreted receptor with a tyrosine kinase domain, and the K-sam mRNAs of 1.6 and 1.8 kb are likely



FIG. 4. cDNA PCR with primers U2 and D3. cDNA PCR products with primers U2 and D3 were fractionated in a 3% agarose gel, and transferred to Nitroplus membrane. The filter was hybridized with a  $^{32}$ P-labeled probe made by PCR with primers U2 and D2 using K-sam-III as the template. Lane 1, human brain; lane 2, NCC-IT; lane 3, KATO-III.

to encode secreted receptors without a tyrosine kinase domain.

The structures of K-sam-I and K-sam-II are different in four regions. The first Ig-like domain of K-sam-I is absent from K-sam-II, probably due to alternative splicing or cassette splicing (31). Such a variant receptor with two Ig-like domains was also reported for human bek (10). The second half of the third Ig-like domain of K-sam-I is replaced by another sequence in K-sam-II. The structures of bek and TK14 in this region have the K-sam-I pattern, whereas that of the KGF receptor has the K-sam-II pattern. According to the recently published partial genomic sequences around the third Ig-like domain of K-sam/human bek (32, 33), the difference in this region may be due to alternative splicing of a mutually exclusive exon (31). The human bek product exhibits high affinity for both acidic and basic FGFs (9), whereas the KGF receptor exhibits high affinity for both acidic FGF and KGF (7). Thus, the second half of the third Ig-like domain is important for ligand recognition. Six base pairs of K-sam-I that encode valine and threonine in the juxtamembrane domain are absent from K-sam-II, and this region is also absent from TK14. The 3'-terminal region of K-sam-I is replaced by another sequence in K-sam-II. The reason for the replacement remains to be determined. These changes in the extracellular domain and in the cytoplasmic domain may lead to altered ligand binding affinity and signal transduction.

The boundary of the region deleted in K-sam-III is also the boundary of structural difference between K-sam-I and K-sam-II (Fig. 3). The boundary is located at nucleotides 939/940 and 1287/1288 of K-sam-I. The nucleotide sequence of K-sam-I around the upstream boundary is  $A\underline{AG}/\underline{G}CC$ , which satisfied the consensus sequence of the 5' and 3' splice sites of an exon (34). The nucleotide sequence around the downstream boundary is  $C\underline{AG}/\underline{G}TAACA/\underline{G}TT$ . As 6 bp, GTAACA, are absent from K-sam-II, the G at position 1288 may be the 3' splice site. K-sam mRNA corresponding to K-sam-III lacks the membrane-spanning region, probably due to alternative splicing or cassette splicing.

K-sam-IV was isolated during studies on secreted receptors without a tyrosine kinase domain. K-sam-IV corresponded not to the 1.6-kb K-sam mRNA, but to the 1.8-kb K-sam mRNA in KATO-III. K-sam mRNA corresponding to K-sam-IV probably results from alternative splicing using alternative 3'-terminal exons (31).

Among many receptor tyrosine kinases, epidermal growth factor receptor (35) and basic FGF receptor (36) were reported to have secreted forms. The K-sam gene also is likely to generate secreted receptors and may generate a secreted receptor with a tyrosine kinase domain.

The biological significance of secreted receptors encoded by K-sam remains to be determined. It also remains unknown whether the secreted receptor with a tyrosine kinase domain and that without a tyrosine kinase domain have different biological functions. Secreted receptors may act as carrier proteins, as described for a secreted form of the growth hormone receptor that is also generated by alternative splicing (37, 38). Another possibility is that secreted receptors could modulate the response of target cells to ligands, heparin-binding growth factors, by acting as a trap for those growth factors or by competing with transmembrane receptors for their ligands. Similar mechanisms were postulated for cytokine receptors (39). Alternatively, a secreted receptor may transduce signals, as reported for the secreted interleukin 6 receptor, which may be able to transduce signals in association with the interleukin 6 signal transducer, gp130 (40, 41). Although the physiological role of secreted receptors encoded by K-sam remains to be elucidated, serological examination to detect such products could be a diagnostic 2964 Medical Sciences: Katoh et al.

clue for certain types of stomach cancer with K-sam amplification.

We thank Drs. H. Kobayashi and S. Tsuji for providing  $poly(A)^+$ RNA of postmortem human brain and Dr. S. Teshima for NCC-IT cells. This work was supported in part by a Grant-in-Aid for a Ten-Year Strategy for Cancer Control from the Ministry of Health and Welfare and from the Ministry of Education, Science, and Culture.

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