Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5983–5987, August 1990
Medical Sciences

K-*sam*, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes

(fibroblast growth factor/amplification/gastric cancer/in-gel DNA renaturation method)

YUTAKA HATTORI, HIROKI ODAGIRI, HIROSHI NAKATANI, KIYOSHI MIYAGAWA, KENICHIRO NAITO, HIROMI SAKAMOTO, OSAMU KATOH, TERUHIKO YOSHIDA, TAKASHI SUGIMURA, AND MASAAKI TERADA

Genetics Division, National Cancer Center Research Institute, Tsukiji 5-Chome, Chuo-ku, Tokyo 104, Japan

Contributed by Takashi Sugimura, May 10, 1990

ABSTRACT **DNA fragments amplified in a stomach cancer-derived cell line, KATO-III, were previously identified by the in-gel DNA renaturation method, and a 0.2-kilobase-pair fragment of the amplified sequence was subsequently cloned. By genomic walking, a portion of the exon of the gene flanking this 0.2-kilobase-pair fragment was cloned, and the gene was designated as K-*sam* (KATO-III cell-derived stomach cancer amplified gene). The K-*sam* cDNAs, corresponding to the 3.5-kilobase K-*sam* mRNA, were cloned from the KATO-III cells. Sequence analysis revealed that this gene coded for 682 amino acid residues that satisfied the characteristics of the receptor tyrosine kinase. The K-*sam* gene had significant homologies with *bek*, *FLG*, and chicken basic fibroblast growth factor receptor gene. The K-*sam* gene was amplified in KATO-III cells with the major transcript of 3.5-kilobases in size. This gene was also expressed in some other stomach cancer cells, a small cell lung cancer, and germ cell tumors.**

Many cancer cells are characterized by aneuploidy. Increased cellular DNA content is often associated with advanced malignancy (1, 2). Yet, only a limited amount of information is available on genetic changes involved in stomach cancers, which have the highest incidence among malignant diseases worldwide (3–14). Amplification of as-yet-unrecognized genes was expected to occur in stomach cancer, especially in the late stage of carcinogenesis. We have previously demonstrated the presence of amplified DNA sequences in several cancers (15–17), including a KATO-III cell line, established from a signet-ring cell carcinoma of the stomach (18), by the in-gel DNA renaturation method (19, 20). We also reported cloning of a 0.2-kilobase-pair (kbp) amplified DNA fragment from KATO-III cells. This 0.2-kbp fragment was designated as $SAM_{0.2}$ (16, 17). We have shown that DNA sequences containing $SAM_{0.2}$ were amplified preferentially in poorly differentiated types of stomach cancer (17).

Here we report isolation of the exon of a gene flanking $SAM_{0.2}$, designated as the K-*sam* gene (KATO-III cell-derived stomach cancer amplified gene), and characterization of the K-*sam* cDNA.* Sequence analysis of the K-*sam* cDNA showed that the gene encoded a receptor tyrosine kinase. The K-*sam* gene had significant homologies with mouse tyrosine kinase gene, *bek* (21), chicken basic fibroblast growth factor (bFGF) receptor gene (22), and human *fms*-like gene, *FLG* (23).

MATERIALS AND METHODS

Cells and Tumors. The gastric cancer cell lines KATO-III (signet-ring cell carcinoma), OKAJIMA (poorly differentiated adenocarcinoma), SCH (choriocarcinoma), TMK1 (poorly differentiated adenocarcinoma), MKN1 (adenosquamous carcinoma), MKN7 (well-differentiated tubular adenocarcinoma), MKN28 (moderately differentiated tubular adenocarcinoma), MKN45 (poorly differentiated adenocarcinoma), and MKN74 (moderately differentiated tubular adenocarcinoma) were maintained as described (16, 18, 24, 25). NSC3, NSC4, NSC8, and NSC10, the human stomach cancer cells transplanted to nude mice, are all poorly differentiated adenocarcinomas. Culture conditions and characteristics of the following cell lines have been mentioned elsewhere: small cell lung cancer, Lu135 (26); immature teratoma, NCC-IT (27); teratocarcinoma, NCC-EC-1 (27).

Identification of the Exon. The $SAM_{0.2}$ fragment was used to identify clones containing the exon flanking $SAM_{0.2}$ from the bacteriophage charon 4A genomic library constructed from human placental DNA.

cDNA Library Construction. A cDNA library was constructed from the poly(A)$^+$ RNA of KATO-III cells and cloned to the bacteriophage vector λgt10 (28, 29). Phage DNA screening was carried out under the same conditions as described (30).

cDNA Sequence Analysis. The K-*sam* cDNAs were inserted into the *Eco*RI site of M13mp18 phage. The overlapping subclones were generated by the stepwise deletion method (31, 32). Both strands of cDNA were sequenced in full by the dideoxy chain-termination method using deoxy-7-deazaguanosine triphosphate (33, 34). Nucleotide and amino acid sequences were analyzed by the GENETYX programs (Software Development Co. Ltd., Tokyo, Japan) for a microcomputer and the IDEAS programs (35) for the VAX/VMS computer. The GenBank nucleic acid data base (release 60.0) and the Protein Identification Resource National Biomedical Research Foundation protein data base (release 20.0) were used for the homology search.

Nucleic Acid Hybridization. Southern blot hybridization analyses and blot hybridization analyses of RNA were performed under the high-stringency condition described previously (30, 36).

Polymerase Chain Reaction (PCR). The method of cDNA PCR was essentially as described (37). Briefly, cDNAs were synthesized from 1 μg of total cellular RNA or 0.5 μg of poly(A)$^+$ RNA by murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) using oligo(dT) or random hexamer as a primer. The RNA·cDNA hybrid was then denatured and amplified for 30 cycles using a DNA thermal cycler and a GeneAmp kit (Perkin–Elmer/Cetus). The DNA sequences of primers were as follows: primer 1 (sense), 5′-GGCTGTGCACAAGCTGACCAA-3′ (corresponding to nucleotide positions 1569–1589 of K-*sam*7);

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; bFGF, basic FGF; PCR, polymerase chain reaction.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M35718).

primer 2 (antisense), 5'-ATCATCTTCATCATCTCCAT-3' (1946–1927); primer 3 (sense), 5'-ATGGAGATGATGAA-GATGAT-3' (1927–1946); primer 4 (antisense), 5'-TCCCT-CATCATCATGTACAG-3' (2537–2518); primer 5 (sense), 5'-AACTGCAGTGCTGGCTCTGTTCAATG-3' (1296–1315) with a *Pst* I site created at the 5' terminus; primer 6 (antisense), 5'-TTAAGCTTCCGACCACTGTGGAGGCAT-3' (1140–1121) with a *Hin*dIII site added to the 5' end. The PCR product with primer 1 and primer 2 and the product with primer 3 and primer 4 were expected to contain an internal *Sau*3AI site and *Bal* I site, respectively. Combination of primers 5 and 6 will generate a 184-bp fragment, only if the region spanning nucleotides 1073–1411 is duplicated on the K-*sam* transcripts. Thermal cycle parameters for primers 1 to 4 were denaturation at 94°C for 1 min, annealing at 60°C for 2 min, and extension at 72°C for 3 min; parameters for primers 5 and 6 were denaturation at 94°C for 20 sec, annealing at 62°C for 20 sec, and extension at 72°C for 30 sec.

RESULTS

Isolation of the K-*sam* cDNA. Since the $SAM_{0.2}$ fragment did not contain an exon portion, this fragment was used to screen the normal human placental genomic library for identification of exons flanking $SAM_{0.2}$. Among 200,000 plaques, a clone containing a 1.6-kbp fragment (K-*sam*HP-1.6) was isolated. This K-*sam*HP-1.6 fragment hybridized to 3.5-kb mRNA in KATO-III cells, indicating that this fragment contained the K-*sam* exon. The fragment of K-*sam*HP-1.6 was used for screening of the cDNA library constructed from poly(A)$^+$ RNA of KATO-III cells. Among the 300 positive clones obtained by screening of 400,000 cDNA clones, 17 were selected at random and purified. Based on the restriction enzyme maps, the 17 clones were divided into two groups: 12 clones represented by the clone K-*sam*7 possessed one *Pvu* I site and one *Ava* I site in the middle of their sequences (Fig. 1), whereas the remaining 5 clones were represented by the clone K-*sam*17, which contained two *Pvu* I sites and two *Ava* I sites (Fig. 1). K-*sam*7, K-*sam*17, and K-*sam*22 bore the inserts of 3.0 kbp, 2.2 kbp, and 2.6 kbp, respectively. By blot analysis of RNA from KATO-III cells, the inserts of these three clones hybridized to the 3.5-kb mRNA (data not shown).

cDNA Sequence. K-*sam*7, K-*sam*17, and K-*sam*22 were examined by nucleotide sequence analyses. The nucleotide sequence of K-*sam*7 is shown in Fig. 2 and consists of 3025 nucleotides. The translation was probably initiated by the ATG codon at positions 595–597 (*i*) because this codon largely satisfied Kozak's translation initiation rule (38) and

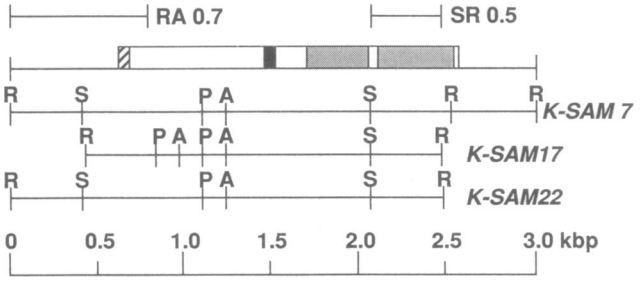

FIG. 1. Schematic diagram of the cDNA clone K-*sam*7. Represented are untranslated (—) and translated (boxed) regions, signal peptide (hatched box), transmembrane domain (black box), and tyrosine kinase domain (stippled boxes). RA0.7 and SR0.5 represent the *Eco*RI/*Acc* III 0.7-kbp fragment and the *Sma* I/*Eco*RI 0.5-kbp fragment, respectively. RA0.7 and SR0.5 were used as the probes for cDNA cloning, Southern blot analysis, and RNA blot analysis. Major restriction enzyme recognition sites are designated: R, *Eco*RI; P, *Pvu* I; A, *Ava* I; S, *Sma* I.

(*ii*) because there were three in-frame stop codons upstream of this ATG codon. The longest open reading frame was composed of 2046 nucleotides from positions 595 to 2640, and the translated amino acid sequence of 682 residues is also shown in Fig. 2. The calculated molecular size was 76,704 Da. At the 3' end of the cDNA sequence, there was neither the classical poly(A) signal AATAAA nor the poly(A) stretch. Compared with K-*sam*17 and K-*sam*22, K-*sam*7 revealed two discrepancies in sequence. In K-*sam*7 and K-*sam*22, nucleotide positions 1075–1413 were single stretch, whereas in the K-*sam*17 this region was tandemly doubled. Thus, the difference of the two types of K-*sam* cDNAs was due to the presence or absence of the in-frame 339-bp tandemly repeated sequence of positions 1075–1413 of K-*sam*7. cDNA PCR with primers 5 and 6 amplified a 184-bp fragment, which is specific to the 339-bp duplication, from the mRNA of KATO-III but not from mRNA of OKAJIMA, MKN7, MKN28, MKN45, MKN74, SCH, or NCC-IT (data not shown). The initiation codon of K-*sam* mRNA containing this repeated sequence remains to be determined. The second sequence difference was that K-*sam*17 and K-*sam*22 contained a six-base insertion, TAACAG, between nucleotide positions 1612 and 1613 of K-*sam*7.

Deduced Amino Acid Sequence. The amino acid sequence deduced from the nucleotide sequence data of cDNA showed that the presence of 15 hydrophobic amino-terminal residues preceded by several basic residues exhibited the features of the signal peptide. The putative extracellular domain extended from 21 to 287 amino acid residues. The consensus of N-linked glycosylation sites (NXS/T, where X = unspecified amino acid) was observed in six places. Four cysteine residues were distributed in the extracellular domain. These cysteine residues and adjoining amino acids residues satisfied the immunoglobulin-like domain conserved sequences of $VX_3CX_{11}W$ and DXGXYXC (39); the first set is $VKFRC^{90}PAGGNPMPTMRW$ and $DKGNYTC^{142}$; the second is $VEFVC^{189}KVYSDAQPHIQW$ and $DAGEYIC^{251}$. The 339-bp repeated sequences lead to a 113-amino acid insertion in the extracellular domain of the product. Next to the extracellular domain existed the highly hydrophobic 23 amino acids (residues 288–310) characteristic of a transmembrane domain. The remaining region was thought to be the cytoplasmic domain, which contained the consensus tyrosine kinase (40): the putative nucleotide binding site $G^{398}XGX_2GXV$ and K^{427}, the sequences that distinguished protein tyrosine kinase from serine/threonine kinase $I^{533}HRDLAARNV$ and $K^{578}WMAPE$ (Fig. 2). Y^{567} residue was the likely target of autophosphorylation reactions (40). Compared with other tyrosine kinases (40), the tyrosine kinase domain was split by a 14-amino acid insertion, residues 494–507. The amino acid sequence suggested the product of the K-*sam* gene to be a receptor tyrosine kinase. In K-*sam*17 and K-*sam*22, the six-base insertion between nucleotide positions 1612 and 1613 resulted in addition of 2 amino acids in the cytoplasmic domain near the transmembrane.

Homology Search of Deduced Amino Acid Sequence. Although the sequence for the transmembrane and extracellular domains of the *bek* cDNA has not been reported, the tyrosine kinase domain of the K-*sam* gene shared 97% homology at the amino acid level with that of the *bek* cDNA. Tyrosine kinase domains of chicken bFGF receptor (22), the *FLG* protein (23), and the chicken *cek* 1 protein (48) were also 89%, 81%, and 89% homologous at the amino acid level, respectively. The chicken *cek* 1 protein was 99.8% homologous to chicken bFGF receptor.

We examined the genomic Southern blot hybridization patterns of different species with the K-*sam* cDNA probe. The K-*sam* cDNA probe hybridized in mouse, rat, and chicken under the high-stringency condition. Under the low-stringency condition (36), this probe also hybridized to *Xe-*

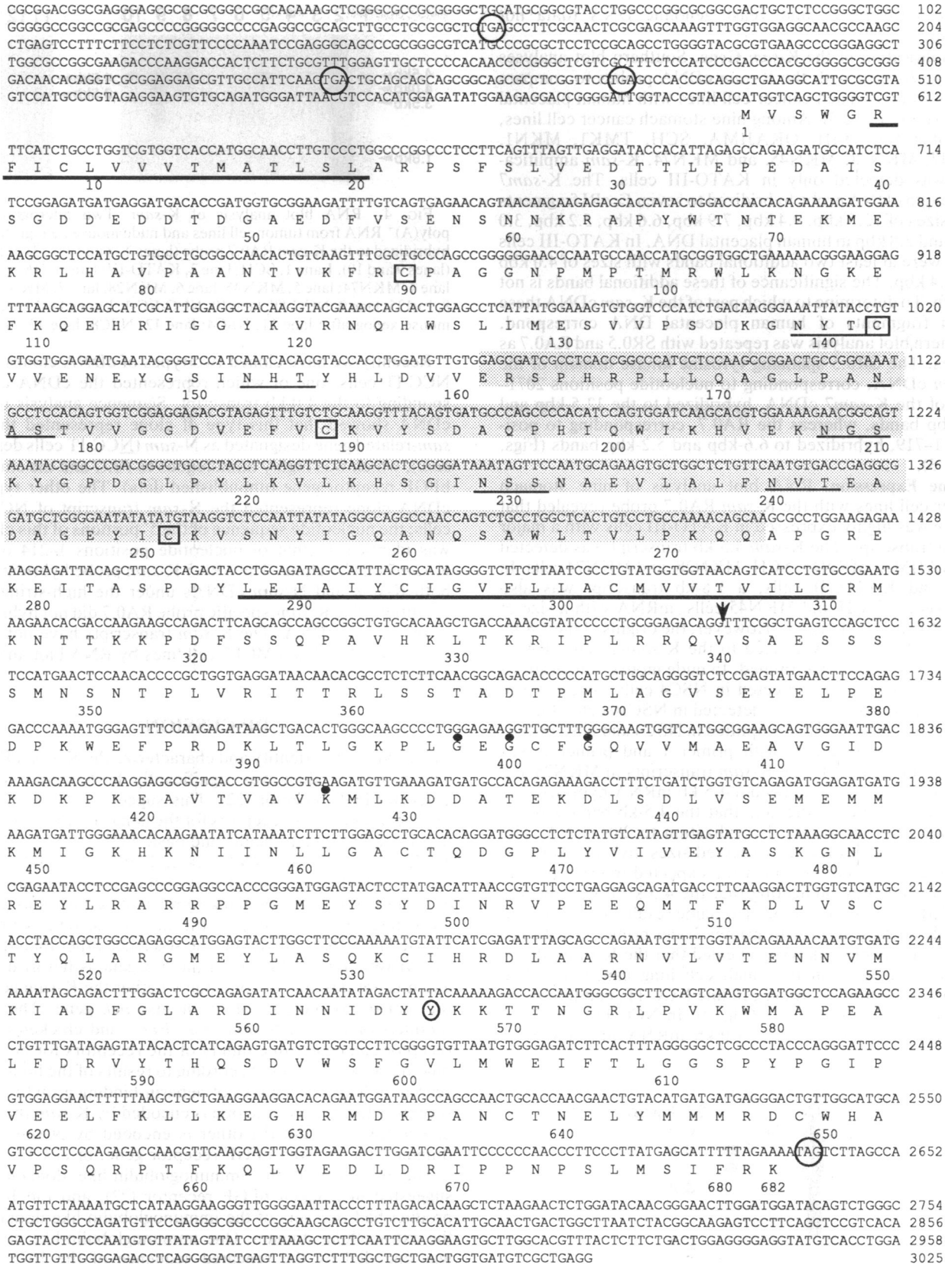

FIG. 2. Nucleotide and deduced amino acid sequences of the K-*sam*7 cDNA. Numbers at the right indicate positions of nucleotides, and numbers below the amino acids refer to the amino acid sequences. The open reading frame began at the ATG at nucleotide positions 595–597, followed by the 15-amino acid signal sequence (heavily underlined). The circled TGAs or TAG indicate in-frame stop codons. Cysteine residues in the extracellular domain are boxed. Potential N-glycosylation sites are underlined. The shaded region was tandemly doubled in the K-*sam*17 clone. The heavy black bar demarcates the putative transmembrane region. The arrow above the nucleotide sequence represents the insertion site of six bases, TAACAG, in K-*sam*17 and K-*sam*22. Dots over "G" at residues 398, 400, and 403 and over "K" at 427 indicate the potential ATP-binding sites. The circled tyrosine at the 567 amino acid residue identifies the putative site of autophosphorylation.

nopus, Drosophila, and yeast genomic DNA (data not shown).

Amplification of the K-*sam* Gene. Southern blot analyses were performed with the K-*sam* cDNA probes to compare DNAs from stomach cancer cell lines with human placental DNA (Figs. 1 and 3). Among nine stomach cancer cell lines, including KATO-III, OKAJIMA, SCH, TMK1, MKN1, MKN7, MKN28, MKN45, and MKN74, K-*sam* amplification was detected only in KATO-III cells. The K-*sam*7 full-length cDNA probe hybridized to seven *Eco*RI fragments with sizes of 12.5 kbp, 9.4 kbp, 7.9 kbp, 6.6 kbp, 5.2 kbp, 3.0 kbp, and 2.8 kbp in human placental DNA. In KATO-III cells there were at least two additional bands with sizes of 4.0 kbp and 3.4 kbp. The significance of these additional bands is not known. To determine to which part of the K-*sam* cDNA these seven fragments of human placental DNA correspond, Southern blot analysis was repeated with SR0.5 and RA0.7 as probes. The SR0.5 spanning tyrosine kinase domain of the K-*sam* cDNA, corresponding to nucleotide positions 2071–2604 of the K-*sam*7 cDNA, hybridized to the 12.5-kbp and 9.4-kbp bands, whereas the RA0.7, corresponding to positions 1–719, hybridized to 6.6-kbp and 5.2-kbp bands (Figs. 1 and 3).

Gene Expression. RNA blot analysis of nine stomach cancer cell lines with the K-*sam* RA0.7 probe revealed that K-*sam* was highly expressed in KATO-III cells with a major 3.5-kb transcript. The K-*sam* 4.5-kb transcript was detected in OKAJIMA, MKN45, SCH, MKN28, and MKN74 cells. In SCH and KATO-III cells, a 1.8-kb transcript was also observed. In SCH and MKN45 cells, mRNA with a size of about 4.0 kb was detected. However, the origins of the 1.8-kb and 4.0-kb signals hybridized to the K-*sam*-specific RA0.7 probe are yet to be determined. In nude mouse xenografts, a 3.5-kb transcript was detected in NSC4 cells, whereas 3.5- and 4.5-kb transcripts were detected in NSC10 cells (Fig. 4). The K-*sam* gene was also amplified in NSC4 and NSC10 cells (17). PCR amplification with primer 1 and primer 2 was carried out to identify the K-*sam* transcripts in MKN28 and MKN45 cells, which showed a 4.5-kb mRNA hybridized to the K-*sam* probes, indicating that the 4.5-kb band was not generated by nonspecific hybridization to 28S rRNA. The PCR products showed the expected sizes, hybridized to the K-*sam* cDNA, and contained the expected internal restriction enzyme sites, indicating that the 4.5-kb transcript was encoded by the K-*sam* gene. The same results were obtained by PCR amplification with primer 3 and primer 4 (data not shown). RNA blot analysis revealed that the K-*sam* mRNA was also detected in the small cell lung cancer cell line Lu-135, immature teratoma cell line NCC-IT, and teratocarcinoma cell line NCC-EC-1 (Fig. 4). In NCC-IT, the SR0.5 K-*sam* probe hybridized to 4.0-kb mRNA in addition to 4.5-kb mRNA.

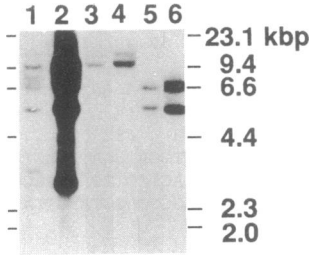

FIG. 3. Southern blot analysis with the K-*sam* cDNA probe. Ten micrograms of genomic DNAs was digested by *Eco*RI and hybridized to the ^{32}P-labeled 3.0-kbp full length of the K-*sam*7 cDNA probe (lanes 1 and 2), SR0.5 (lanes 3 and 4), or RA0.7 (lanes 5 and 6). Lanes 1, 3, and 5, human placenta; lanes 2, 4, and 6, KATO-III cells. Films of lanes 4 and 6 were exposed for 30 min at room temperature, and those of other lanes were exposed overnight at −70°C.

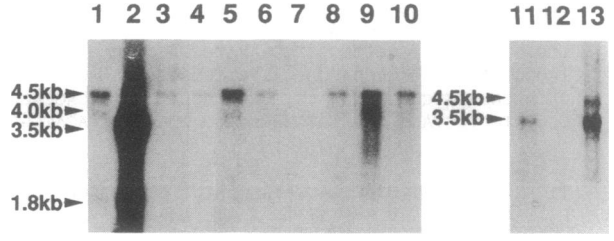

FIG. 4. RNA blot analysis of K-*sam*. Two micrograms of poly(A)$^+$ RNA from tumor cell lines and nude mouse xenografts was hybridized to the K-*sam* RA0.7 probe (lanes 1–8 and 11–13) or SR0.5 (lanes 9 and 10). Lane 1, SCH; lane 2, KATO-III; lane 3, OKAJIMA; lane 4, MKN74; lane 5, MKN45; lane 6, MKN28; lane 7, MKN7; lane 8, Lu135; lane 9, NCC-IT; lane 10, NCC-EC-1. Lanes 11–13 are nude mouse xenografts: lane 11, NSC4; lane 12, NSC8; lane 13, NSC10.

Recently we have cloned two types of cDNA clones from NCC-IT cells, one of which represented the cDNA corresponding to the 4.0-kb transcript. Sequence analysis of the cDNA showed that this type of clone represented the K-*sam*-related gene designated as N-*sam* (NCC-IT cells derived *sam*), which was highly homologous to *FLG* and the chicken bFGF receptor gene (unpublished data). The other type of cDNA clone represented the K-*sam* transcript of NCC-IT cells; the nucleotide sequence of the 5′ portions of this cDNA was identical to that of nucleotide positions 1–214 of the K-*sam*7 cDNA clone. The SR0.5 K-*sam* probe hybridized to both K-*sam* and N-*sam* cDNAs under the high-stringency condition. The K-*sam*-specific probe RA0.7 did not hybridize to the N-*sam* cDNA. The K-*sam* transcripts have not been detected in K562 and MCF7 cell lines by RNA blot analysis (data not shown).

DISCUSSION

In this paper, we identify and characterize the K-*sam* cDNA. The K-*sam* product was significantly homologous to the chicken bFGF receptor (22). This suggests that the K-*sam* product is one of the receptors for the heparin-binding growth factor family, such as acidic and basic FGFs, *HST*1 (30, 36), *HST*2/FGF6 (41, 42), *INT*-2 (43), FGF5 (44), and keratinocyte growth factor (45). The tyrosine kinase domain of the K-*sam* cDNA, corresponding to nucleotide positions 1750–2668, had 88% homology at the nucleotide level with the mouse *bek* cDNA. However, the sequence other than that of the tyrosine kinase domain of the *bek* gene is not available, and it is not possible to conclude whether the K-*sam* gene is the human counterpart of the mouse *bek* gene. The gene products of K-*sam*, N-*sam*, *bek*, *FLG*, and chicken bFGF receptor can establish a family of the receptors for heparin-binding growth factors. According to results of the homology search, at least two classes of heparin-binding growth factor receptors likely exist: the one is encoded by K-*sam* (human) or *bek* (mouse), and the other is encoded by N-*sam*/*FLG* (human), the chicken bFGF receptor gene, or *cek* 1.

The presence of the immunoglobulin-like domain was suggested in chicken bFGF receptor (22), and the K-*sam* product had two sets of the immunoglobulin-like domain in the extracellular domain. One immunoglobulin-like domain was encoded by the repeated sequence of the 1075–1413 nucleotides. Thus, this repeated sequence could have some important biological function such as ligand recognition.

The 14 amino acids inserted in the tyrosine kinase domain were as long as those of the *bek* protein, the chicken bFGF receptor, and the *FLG* protein and were much shorter than those of platelet-derived growth factor receptors, the colony-stimulating factor 1 receptor, and the c-*kit* protein (40, 46). We do not know whether the kinase insert was essential to

signal transduction as has been studied in detail in platelet-derived growth factor receptor (47).

Amplification of the K-*sam* gene was restricted to poorly differentiated types of stomach cancer (17). In contrast, amplification of c-*erbB-2*, the gene for another receptor tyrosine kinase, was confined to well-differentiated adenocarcinomas (12). The biological significance of amplification of these two different genes remains yet to be determined.

Thus far, the size of the predominant mRNA hybridized to the K-*sam* probe was 3.5 kb in the cells with the amplified K-*sam* gene, such as KATO-III cells and nude mice xenografts NSC4 and NSC10, whereas in the cells without the amplified K-*sam* gene it was 4.5 kb. It is likely that the 3.5-kb and 4.5-kb K-*sam* transcripts are generated by differential splicing of this gene.

Identification of the ligand for the K-*sam* protein will give us a crucial insight into the biological roles of the family of heparin-binding growth factors and their receptors in normal cells and in cancers, especially in gastric cancers.

We gratefully acknowledge the gift of the human placental genomic library from Dr. T. Maniatis. We are grateful to the following scientists for providing us with cell lines, xenografts, or DNAs used in the present studies: Drs. Y. Hayata, K. Nakatani, M. Sekiguchi, Y. Shimosato, E. Tahara, T. Terasaki, S. Teshima, H. Watanabe, I. Uno, T. Higashinakagawa, and F. Hishinuma. This work was supported in part by a Grant-in-Aid for the Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare and by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare and from the Ministry of Education, Science, and Culture. Y.H., H.O., K.M., O.K., and T.Y. were awardees of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research.

1. Merkel, D. E., Dressler, L. G. & McGuire, W. L. (1987) *J. Clin. Oncol.* **5**, 1690–1703.
2. Sandberg, A. A., Turc-Carel, C. & Gemmill, R. M. (1988) *Cancer Res.* **48**, 1049–1059.
3. Nakasato, F., Sakamoto, H., Mori, M., Hayashi, K., Shimosato, Y., Nishi, M., Takao, S., Nakatani, K., Terada, M. & Sugimura, T. (1984) *Jpn. J. Cancer Res. (Gann)* **75**, 737–742.
4. Shibuya, M., Yokota, J. & Ueyama, Y. (1985) *Mol. Cell. Biol.* **5**, 414–418.
5. Koda, T., Matsushima, S., Sasaki, A., Danjo, Y. & Kakinuma, M. (1985) *Jpn. J. Cancer Res. (Gann)* **76**, 551–554.
6. Seki, T., Fujii, G., Mori, S., Tamaoki, N. & Shibuya, M. (1985) *Jpn. J. Cancer Res. (Gann)* **76**, 907–910.
7. Shimizu, K., Nakatsu, Y., Sekiguchi, M., Hokamura, K., Tanaka, K., Terada, M. & Sugimura, T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5641–5645.
8. Yokota, J., Yamamoto, T., Toyoshima, K., Terada, M., Sugimura, T., Battifora, H. & Cline, M. J. (1986) *Lancet* **i**, 765–767.
9. Bos, J. L., Verlean-de Vries, M., Marshall, C. J., Veeneman, G. H., van Boom, J. H. & van der Eb, A. J. (1986) *Nucleic Acids Res.* **14**, 1209–1217.
10. Deng, G., Lu, Y., Chen, S., Miao, J., Lu, G., Li, H., Cai, H., Xu, X., E, Z. & Liu, P. (1987) *Cancer Res.* **47**, 3195–3198.
11. Staal, S. P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5034–5037.
12. Yokota, J., Yamamoto, T., Miyajima, N., Toyoshima, K., Nomura, N., Sakamoto, H., Yoshida, T., Terada, M. & Sugimura, T. (1988) *Oncogene* **2**, 283–287.
13. Wada, M., Yokota, J., Mizoguchi, H., Sugimura, T. & Terada, M. (1988) *Cancer Res.* **48**, 2988–2992.
14. Yoshida, M. C., Wada, M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, T., Kakinuma, M., Sugimura, T. & Terada, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4861–4864.
15. Nakatani, H., Tahara, E., Sakamoto, H., Terada, M. & Sugimura, T. (1985) *Biochem. Biophys. Res. Commun.* **130**, 508–514.
16. Nakatani, H., Tahara, E., Yoshida, T., Sakamoto, H., Suzuki, T., Watanabe, H., Sekiguchi, M., Kaneko, Y., Sakurai, M., Terada, M. & Sugimura, T. (1986) *Jpn. J. Cancer Res. (Gann)* **77**, 849–853.
17. Nakatani, H., Sakamoto, H., Yoshida, T., Yokota, J., Tahara, E., Sugimura, T. & Terada, M. (1990) *Jpn. J. Cancer Res.* **81**, 707–710.
18. Sekiguchi, M., Sakakibara, K. & Fujii, G. (1978) *Jpn. J. Exp. Med.* **48**, 61–68.
19. Roninson, I. B., Abelson, H. T., Housman, D. E., Howell, N. & Varshavsky, A. (1984) *Nature (London)* **309**, 626–628.
20. Roninson, I. B. (1987) *Methods Enzymol.* **151**, 332–371.
21. Kornbluth, S., Paulson, K. E. & Hanafusa, H. (1988) *Mol. Cell. Biol.* **8**, 5541–5544.
22. Lee, P. L., Johnson, D. E., Cousens, L. S., Fried, V. A. & Williams, L. T. (1989) *Science* **245**, 57–60.
23. Ruta, M., Howk, R., Ricca, G., Drohan, W., Zabelshansky, M., Laureys, G., Barton, D. E., Francke, U., Schlessinger, J. & Givol, D. (1988) *Oncogene* **3**, 9–15.
24. Motoyama, T., Hojo, H. & Watanabe, H. (1986) *Acta Pathol. Jpn.* **36**, 65–83.
25. Ochiai, A., Yasui, W. & Tahara, E. (1985) *Jpn. J. Cancer Res. (Gann)* **76**, 1064–1071.
26. Terasaki, T., Shimosato, Y., Nakajima, T., Tsumuraya, M., Morinaga, S., Hirohashi, S., Yamaguchi, K., Kato, K., Ichinose, H. & Nagatsu, T. (1986) *Jpn. J. Clin. Oncol.* **16**, 203–212.
27. Teshima, S., Shimosato, Y., Hirohashi, S., Tome, Y., Hayashi, I., Kanazawa, H. & Kakizoe, T. (1988) *Lab. Invest.* **25**, 328–336.
28. Huynh, T. V., Young, R. A. & Davis, R. W. (1984) *DNA Cloning: A Practical Approach* (IRL, Oxford), Vol. 1, pp. 49–78.
29. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263–269.
30. Taira, M., Yoshida, T., Miyagawa, K., Sakamoto, H., Terada, M. & Sugimura, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2980–2984.
31. Henikoff, S. (1984) *Gene* **28**, 351–359.
32. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33**, 103–119.
33. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
34. Mizusawa, S., Nishimura, S. & Seela, F. (1986) *Nucleic Acids Res.* **14**, 1319–1324.
35. Kanehisa, M. (1982) *Nucleic Acids Res.* **10**, 183–196.
36. Sakamoto, H., Mori, M., Taira, M., Yoshida, T., Matsukawa, S., Shimizu, K., Sekiguchi, M., Terada, M. & Sugimura, T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3997–4001.
37. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
38. Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872.
39. Williams, A. F. & Barcley, A. N. (1988) *Annu. Rev. Immunol.* **6**, 381–405.
40. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 42–52.
41. Sakamoto, H., Yoshida, T., Nakakuki, M., Odagiri, H., Miyagawa, K., Sugimura, T. & Terada, M. (1988) *Biochem. Biophys. Res. Commun.* **151**, 965–972.
42. Marics, I., Adelaide, J., Raybaud, F., Mattei, M.-G., Coulier, F., Planche, J., Lapeyriere, O. & Birnbaum, D. (1989) *Oncogene* **4**, 335–340.
43. Peters, G., Brookes, S., Smith, R. & Dickson, C. (1983) *Cell* **33**, 369–377.
44. Zhan, X. I., Bates, B., Hu, X. & Goldfarb, M. (1988) *Mol. Cell. Biol.* **8**, 3487–3495.
45. Finch, P. W., Rubin, J. S., Miki, T., Ron, D. & Aaronson, S. A. (1989) *Science* **245**, 752–755.
46. Matsui, T., Heidaran, M., Miki, T., Popescu, N., Rochelle, W. L., Kraus, M., Pierce, J. & Aaronson, S. A. (1989) *Science* **243**, 800–804.
47. Williams, L. T. (1989) *Science* **243**, 1564–1570.
48. Pasquale, E. B. & Singer, S. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5449–5453.