Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4861–4864, July 1988
Medical Sciences

Human *HST1* (*HSTF1*) gene maps to chromosome band 11q13 and coamplifies with the *INT2* gene in human cancer

(transforming gene/growth factor/gene mapping/amplification)

MICHIHIRO C. YOSHIDA*, MAKIO WADA†, HITOSHI SATOH*, TERUHIKO YOSHIDA†, HIROMI SAKAMOTO†, KIYOSHI MIYAGAWA†, JUN YOKOTA†, TOSHIAKI KODA‡, MITSUAKI KAKINUMA‡, TAKASHI SUGIMURA†, AND MASAAKI TERADA†§

*Chromosome Research Unit, Faculty of Science, Hokkaido University, Kita-10, Nishi-8, Kita-ku, Sapporo 060, Japan; †National Cancer Center Research Institute, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan; and ‡Institute of Immunological Science, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo 060, Japan

Contributed by Takashi Sugimura, February 18, 1988

ABSTRACT **The human *HST1* gene, previously designated the *hst* gene, and now assigned the name *HSTF1* for heparin-binding secretory transforming factor in human gene nomenclature, was originally identified as a transforming gene in DNAs from human stomach cancers by transfection assay with mouse NIH 3T3 cells. The amino acid sequence of the product deduced from DNA sequences of the *HST1* cDNA and genomic clones had approximately 40% homology to human basic and acidic fibroblast growth factors and mouse *Int-2*-encoded protein. We have mapped the human *HST1* gene to chromosome 11 at band q13.3 by Southern blot hybridization analysis of a panel of human and mouse somatic cell hybrids and *in situ* hybridization with an *HST1* cDNA probe. The *HST1* gene was found to be amplified in DNAs obtained from a stomach cancer and a vulvar carcinoma cell line, A431. In all of these samples of DNA, the *INT2* gene, previously mapped to human chromosome 11q13, was also amplified to the same degree as the *HST1* gene.**

The *HST1* gene, now assigned the name *HSTF1* for heparin-binding secretory transforming factor in human gene nomenclature, was originally identified as a transforming gene (*hst*) in DNAs from human stomach cancers and a noncancerous portion of human stomach mucosa by transfection assay using mouse NIH 3T3 cells (1). The *HST* gene was subsequently identified as a transforming gene in stomach cancers from the other patients (2) and also in other types of cancers (2–4). The *KS* oncogene, recently identified as a transforming gene in DNA from a Kaposi's sarcoma, has been shown to be the *HST* gene (5, 6). The *HST* gene is at present the most frequently found transforming gene next to the *RAS* gene family. Cosmid clones containing the genomic *HST* from leukocyte DNA of a patient with leukemia (7) and a normal person (27) were shown to have transforming activity with NIH 3T3 cells upon transfection.

In addition to the previously described *HST* gene, the human genome contains another DNA sequence that hybridized to the *HST* cDNA. In this paper, we designate the previously described *HST* gene that has transforming activity the *HST1* gene. The other DNA sequence that hybridized to the *HST* cDNA probe is designated the *HST2* gene. The *HST2* gene may represent a pseudogene or a gene belonging to the *HST* gene family. Clones containing either the *HST1* gene or the *HST2* gene have been obtained (9, 27). The 206-amino acid sequence of the *HST* encoded protein deduced from DNA sequences of the HST1 cDNA (8) and the genomic *HST1* has significant homology to selected regions of human basic fibroblast growth factor (FGF), human acidic FGF, and mouse *Int-2*-encoded protein, respectively (9). FGFs are potent mitogens, and the mouse *Int-2* gene is considered to be involved in development of mouse mammary cancers induced by murine mammary tumor virus (10–12).

Here, we report that the *HST1* gene maps to human chromosome 11 at band q13.3 and that this gene is coamplified with *INT2* in some human cancers. The human *INT2* gene was previously mapped to chromosome 11 at band q13 (10). The results indicated that at least two of the genes encoding the FGF family are located closely on chromosome 11 at band q13.

MATERIALS AND METHODS

DNA Probes. The isolation, characterization, and DNA sequencing of the HST1 cDNA from T361-2nd-1 cells, a secondary transformant obtained by transfection of a stomach cancer DNA, and those of the *HST1* gene have been described (1, 8). A 0.59-kilobase-pair (kbp) *Ava* II–*Ava* II fragment, corresponding to nucleotides 281 to 872 of the HST1 cDNA (8), encompasses most of open reading frame 1 and is designated probe AA. This probe hybridized to DNA sequences derived from the *HST1* gene and to those from the *HST2* gene. A 0.78-kbp *Eco*RI–*Sst* I fragment of the genomic *HST1* is designated probe b (1). Probe b hybridized to the *HST1*-specific fragments but not to the *HST2* sequences. SS6 is a 0.9-kbp *Sac* I–*Sac* I fragment of the human *INT2* gene, and BB4 is a 2.3-kbp *Bam*HI–*Bam*HI fragment of the same gene (13).

Somatic Cell Hybrids. A panel of somatic cell hybrids was established by fusion of human diploid fibroblasts and mouse mutant B82 or FM3A cells, which are deficient in thymidine kinase or hypoxanthine phosphoribosyltransferase, respectively. These murine mutant cells permitted hybrid selection with hypoxanthine/aminopterin/thymidine (HAT) medium containing ouabain (14). Cytogenetic analyses by Q-banding were used to determine the human chromosome complement. High molecular weight DNA for Southern blot and cytogenetic analyses was prepared from hybrid cells at the same stage of passaging as described (1, 14, 15).

***In Situ* Hybridization.** Metaphase spreads were obtained from phytohemagglutinin-stimulated peripheral blood lymphocytes from a normal male as described (14). Before hybridization, the chromosome preparations were pretreated with RNase and denatured in 70% formamide/0.30 M sodium chloride/0.030 M sodium citrate at 70°C. The probe was ^{3}H-labeled by nick translation to a specific activity of 1–5 × 10^7 dpm/μg and used for hybridization at a concentration of

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Abbreviation: FGF, fibroblast growth factor.

§To whom reprint requests should be addressed.

3 μg/ml. Hybridization, washing, and autoradiography were carried out essentially as described (16), and the slides were subsequently Q-banded by double staining with quinacrine mustard and Hoechst 33258 (17). Silver grains on or touching chromosomes were scored and plotted on an idiogram established by the International System for Human Cytogenetic Nomenclature (18).

Southern Blot Hybridization. DNAs were isolated from a total of 43 stomach cancers, including 27 primary tumors and 16 lymph node metastases, and 32 noncancerous portions of stomach mucosae obtained at the time of surgery from 32 patients with stomach cancers (1, 15). DNAs were also isolated from 22 cell lines including a vulvar carcinoma cell line, A431 (19), and a colon carcinoma cell line, COLO 205 (20). Southern blot hybridization analyses of these DNA samples were performed as described (1, 21). Washing of the filters to remove the probe and to allow reutilization for hybridization with several different probes was performed as described (21). To ascertain that equal amounts of DNA were loaded in each lane, the filter was hybridized with several probes unrelated to these genes.

RESULTS

Chromosomal Localization of the *HST1* Gene. Hybridization with the HST1 cDNA probe AA to Southern blots of *Eco*RI-digested human DNA revealed four DNA fragments of 8.0, 5.8, 2.8, and 0.8 kbp (Fig. 1). Three of these four fragments, 5.8, 2.8, and 0.8 kbp, correspond to the sequences contained in the *HST1* gene, while the 8.0-kbp fragment corresponds to a sequence in the *HST2* gene. When *Eco*RI-digests of mouse DNA were hybridized with the HST1 cDNA probe AA, three bands of 6.7, 4.4, and 1.8 kbp were detected. Therefore, it was possible to identify the human *HST1* sequence in the DNA from mouse–human hybrid cell lines containing different human chromosomes. An analysis of the segregation of *Eco*RI-digested DNA fragments of the human *HST1* gene in a total number of 27 hybrid cell clones showed concordance between the presence of the human *HST1* gene and human chromosome 11, whereas discordance was found with all other human chromosomes (Fig. 1).

The genomic *HST1* probe b hybridized only to the 5.8-kbp *Eco*RI–*Eco*RI fragment of the *HST1* gene; it did not hybridize to the 2.8-kbp and 0.8-kbp *Eco*RI–*Eco*RI fragments of the *HST1* gene or the 8.0-kbp *Eco*RI–*Eco*RI fragment of the *HST2* gene. This probe b was used to identify the sublocalization of the *HST1* gene by *in situ* hybridization on normal human early metaphase or prophase chromosomes at the 500–700 band stage. On examination of 58 cells, 28 (35.9%) of 78 silver grains were found on chromosome 11. Of these, 22 (78.6%) were clustered on chromosome 11 at band q13. A significant distribution of silver grains (63.6%, 14 of 22) were found on subband q13.3 ($P < 0.001$) as shown in Fig. 2.

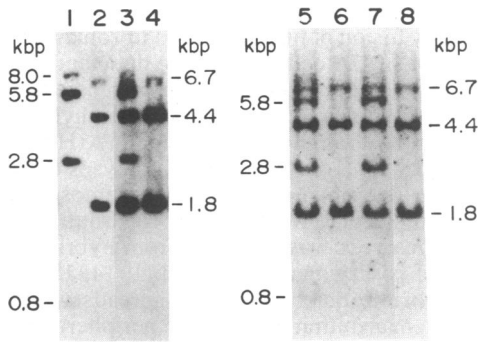

FIG. 1. Southern blot hybridization of DNAs from human and mouse cells and their cell hybrids. DNA was digested with *Eco*RI, and analyzed by Southern blot hybridization with human HST1 cDNA probe AA. Representative patterns are shown. Lanes: 1, human; 2, mouse; 3, hybrid 1-6; 4, hybrid 2-2; 5, hybrid H/B 6-3; 6, hybrid H/B 2A-4; 7, hybrid H/B 6-1; 8, hybrid H/B 5C3. Mouse–human cell hybrids 1-6, H/B 6-3, and H/B 6-1 contained human chromosome 11, whereas hybrids 2-2, H/B 2A-4, and H/B 5C3 cells did not.

Coamplification of the *HST1* and *INT2* Genes. A total of 65 tumor DNAs, including those from 43 human stomach cancers and 22 cell lines were digested with *Eco*RI and analyzed by Southern blot hybridization with the HST1 cDNA probe AA. In all of these DNA samples, the probe detected 8.0-, 5.8-, 2.8-, and 0.8-kbp *Eco*RI–*Eco*RI fragments, indicating that there was no gross rearrangement of the *HST1* and *HST2* genes. However, in DNAs from a lymph node metastasis of a stomach cancer of patient SC6 and from A431 cells, the signals corresponding to the *HST1*-specific 5.8-, 2.8-, and 0.8-kbp fragments were much more intense than those in DNAs from normal tissues or other cultured cell lines, which include COLO 205, a colon carcinoma cell line (Fig. 3). The primary tumor from the patient SC6 also contained the amplified *HST1* gene (data not shown). It should be noted that the intensity of the band corresponding to the 8.0-kbp fragment of the *HST2* gene was the same in these samples of DNAs as that in DNAs from normal tissues and COLO 205 cells. The degree of amplification of the *HST1* gene was approximately 6-fold for DNAs from both primary tumor and the lymph node metastasis of a stomach cancer patient, SC6, and 3-fold for DNA from A431 cells when they were determined by quantitative comparison of the *HST1* fragments between serially diluted amounts of DNAs of the tumors or the cell line and those of normal tissues. This stomach cancer with the amplified *HST1* gene was 1 of the 19 poorly differentiated stomach adenocarcinomas examined.

These samples of DNA were analyzed with an *INT2* probe, BB4 or SS6, after digestion with *Bcl* I or *Eco*RI, respectively. Upon *Bcl* I digestion, human genomic DNA exhibited one band of 9.0 kbp with BB4 as a probe, whereas *Eco*RI-digests of human DNA showed one band of 10 kbp with SS6 as a probe. DNA samples, containing the amplified *HST1* gene, from the primary tumor and lymph node metastasis of stomach cancer patient SC6 and A431 cells showed amplification of the *INT2* gene, with the degree of amplification being the same as that of the *HST1* gene. Neither the *HST1* gene nor *INT2* gene was amplified in the other 62 samples of DNAs tested. In the 3 DNA samples with coamplification of the *HST1* gene and the *INT2* gene, there was no amplification of the *HRAS* gene or the *ETS1* gene (data not shown), the chromosomal localizations of which are on the short arm and long arm of chromosome 11, respectively. The results indicate that the amplification was not due to an increase in number of chromosomes 11.

DISCUSSION

We have assigned the *HST1* gene to chromosome 11 based on the pattern obtained when a human HST1 cDNA probe was hybridized to a panel of DNAs isolated from mouse–human somatic cell hybrids. The gene was sublocalized to chromosome 11 at band q13.3 by *in situ* hybridization with a genomic fragment of the *HST* gene that hybridized to the *HST1* but not to the *HST2* gene.

Human DNA contained two types of DNA fragments hybridizing to the HST1 cDNA probe AA, which encompasses almost the entire sequence of open reading frame 1: one is the *HST1* gene and the other is the *HST2* gene. Upon digestion with *Eco*RI, the *HST1* gene generated 5.8-, 2.8-, and 0.8-kbp fragments hybridizing to the HST1 cDNA probe AA, and the *HST2* gene showed an 8.0-kbp fragment. The clones containing the *HST1* gene but not those with the *HST2* gene transform NIH 3T3 cells (unpublished data). The fact

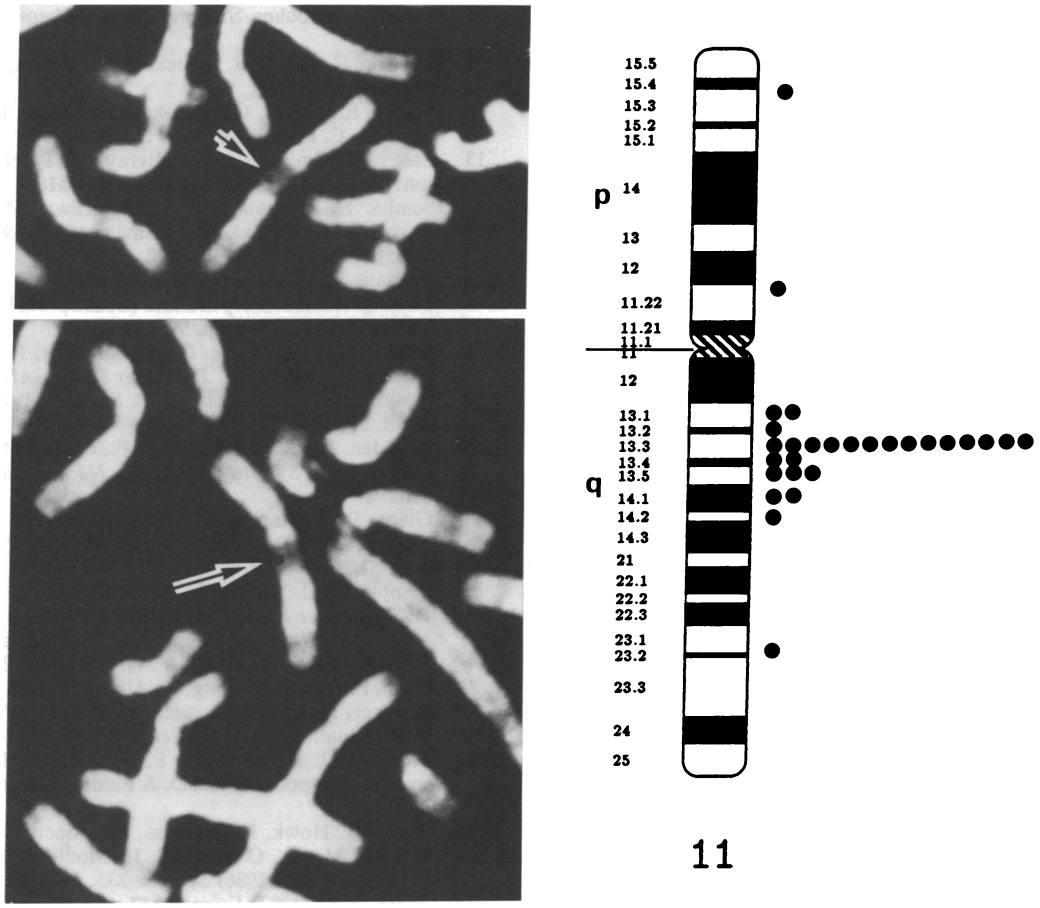

FIG. 2. Localization of the human *HST1* gene by *in situ* hybridization analysis. (*Left*) Prophasic chromosomes showing sublocalization of a silver grain on chromosome 11 at band q13.3 (arrow). (*Right*) Grain counts over prophasic chromosome 11, showing clustering of the grains on region q13.3.

that no amplification of the *HST2* gene was detected in DNA samples with amplified *HST1* gene further confirmed the presence of two distinct types of *HST* genes. The *HST2* gene might be a pseudogene or a novel gene related to the *HST1* gene. A genomic fragment of the *HST1* gene obtained directly from a human genomic library also had transforming activity (7, 9). This fragment had a coding sequence identical to that of the open reading frame 1 of the HST1 cDNA prepared from T361-2nd-1 cells. The deduced amino acid sequence of the *HST1*-encoded protein of 206 amino acid residues is 43%, 38%, and 40% homologous, respectively, to human basic FGF (22, 23), human acidic FGF (24), and mouse Int-2 protein (25) in selected regions. This group may constitute a family of genes encoding various types of growth factors (9, 26). There is, however, a distinct difference between the *HST1*-encoded protein and FGFs; the HST1 protein contains, but FGFs do not contain, a signal peptide, and this presence of the signal peptide may be essential for the acquisition of transforming activity (9). It was recently demonstrated that the gene for basic FGF, *FGFB*, when fused to a sequence for a signal peptide, transformed NIH 3T3 cells upon transfection (26).

Murine mammary tumor virus proviral DNA inserted in the mouse *Int-2* locus activates expression of the mouse *Int-2* gene located within this locus, whose protein product may be involved in the genesis of murine mammary carcinomas (10–13). The mouse *Int-2*-encoded protein has homology with the human *HST1*-encoded protein (9). The human *INT2* gene, the human homologue of the mouse *Int-2* gene, was previously mapped to chromosome 11 at band q13 (13), which was shown to contain the *HST1* gene in the present study. In three DNA samples, coamplification of the *HST1* gene and the *INT2* gene was detected, indicating that these two genes were

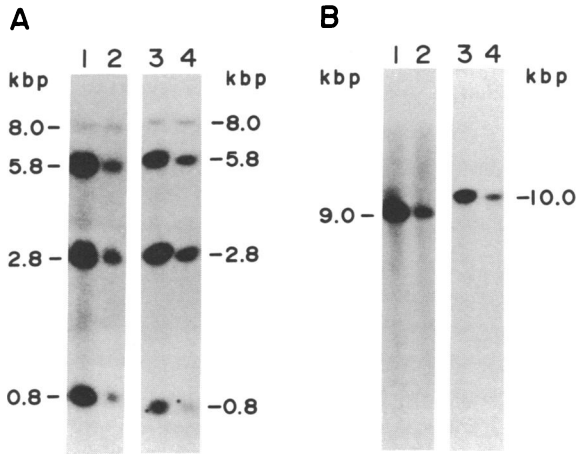

FIG. 3. Coamplification of the *HST1* gene and the *INT2* gene in tumors. Southern blot hybridization was performed on DNA samples. Lanes: 1, a lymph node metastasis of stomach cancer patient SC6; 2, noncancerous portion of the same patient; 3, A431 cells; 4, COLO 205 cells. (*A*) The HST1 cDNA probe AA was used as a probe to analyze *Eco*RI-digested DNAs. The 5.8-, 2.8-, and 0.8-kbp bands represent the DNA fragments of the *HST1* gene, whereas the 8.0-kbp band represents that of the *HST2* gene. (*B*) BB4 was used as an *INT2* probe for analysis of *Bcl* I-digested DNAs (lanes 1 and 2), and SS6 was used as an *INT2* probe for analysis of *Eco*RI-digested DNAs (lanes 3 and 4). *Hin*dIII-digested λ phage DNA was used as a size marker.

localized closely in chromosome 11 at band q13 and amplified together as one "amplicon" unit. It is possible that there may be a cluster of the *HST1*- or *INT2*-related genes in this region of chromosome 11q13, the products of which are factors involved in regulation of cell growth for various types of normal and malignant cells.

We thank Drs. G. Peters and C. Dickson for providing us human *INT2* probes, and the pathology staff of the National Cancer Center Research Institute for histological diagnoses of the specimens. This study was supported in part by Grants-in-Aid from the Ministry of Health and Welfare for a Comprehensive 10-Year Strategy for Cancer Control, Japan, and from the Ministry of Education, Science and Culture of Japan, and by the Princess Takamatsu Cancer Research Fund. M.W. received a Research Resident Fellowship from the Foundation for Promotion of Cancer Research, Japan.

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