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)

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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 K5 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 HST 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 of the HST2 gene. A 0.78-kbp EcoRI–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 BamHI–BamHI 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 B22 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 "H-labeled by nick translation to a specific activity of 1–5 × 106 dpm/μg and used for hybridization at a concentration of

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Abbreviation: FGF, fibroblast growth factor.
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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 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 EcoRI-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 EcoRI-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 EcoRI-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 EcoRI–EcoRI fragment of the HST1 gene; it did not hybridize to the 2.8-kbp and 0.8-kbp EcoRI–EcoRI fragments of the HST1 gene or the 8.0-kbp EcoRI–EcoRI 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.

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 EcoRI 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 EcoRI–EcoRI 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 EcoRI, respectively. Upon Bcl I digestion, human genomic DNA exhibited one band of 9.0 kbp with BB4 as a probe, whereas EcoRI-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 RET/PS1 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 HST1 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 EcoRI, 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
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, FGF-B, 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 q13.1 (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. 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.
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.

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