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cDNA cloning and chromosomal mapping of human *N*-acetylglucosaminyltransferase V+

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Human *N*-acetylglucosaminyltransferase V (GnT-V, EC 2.4.1.155) cDNA was isolated from a human fetal liver cDNA library. Oligonucleotide primers for polymerase chain reaction were designed according to the amino acid sequence of human GnT-V. Screening for the cDNA was carried out by plaque hybridization using PCR products of about 500 bp. Human GnT-V has 741 amino

+The nucleotide sequence data reported in this paper will appear in the DDJB, EMBL and GenBank Nucleotide Sequence Databases with the accession number D17716.

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Abbreviations: GnT-V, α1-6 mannoside β1-6*N*-acetylglucosaminyltransferase (EC 2.4.1.155); FITC, fluorescein isothiocyanate; PCR, polymerase chain reaction; BrdU, bromodeoxyuridine; RT-PCR, polymerase chain reaction following reverse transcription of RNA.

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acids and six putative N-glycosylation sites. The homology to rat GnT-V is 88% at the nucleotide level and is 97 % at the amino acid level, and there is one amino acid insertion. Using the cDNA clones as probe, five overlapping genomic clones have been isolated from a human phagemid DNA library. The GnT-V gene has been mapped to chromosome 2q21 using fluorescence in situ hybridization.

β1-6 *N*-acetylglucosaminyltransferase V (GnT-V), which catalyzes the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to α-D-6 mannoside, is one of the most important enzymes in the branching of asparagine-linked oligosaccharide. The enzyme also appears to play a significant role in malignant cells. Activation of GnT-V in the malignant transformation of rodent cells has been reported (1,2,3). And the metastatic potential of some tumor cells correlates strongly with their content of the enzymatic product of GnT-V (4). Furthermore we reported that GnT-V activity increased during rat hepatocarcinogenesis in correlation with the amount of GnT-V mRNA (5).

We previously cloned rat and human cDNAs of GnT-III (6,7), which catalyzes the biosynthesis of bisecting GlcNAc. In terms of substrate specificity, GnT-III competes with GnT-V in the trimming of complex type oligosaccharide. Like GnT-V, GnT-III exhibits changes in activity during carcinogenesis (5,8,9). The mechanisms by which these enzymes are regulated are therefore of great interest.

Recently we purified human GnT-V from a small cell lung cancer cell line, QG cells (10), and partially cloned the rat GnT-V cDNA (5). Here we report the cDNA sequence of human GnT-V and the chromosomal localization of the GnT-V gene.

MATERIALS AND METHODS

PCR Oligonucleotides for use as primers in PCR were synthesized based on the amino acid sequences of the tryptic peptides of human

GnT-V which had been reported in our previous paper (5). The oligonucleotide sequences of the 5' and 3' primers were ggaattcGA-RCCNGARTTYAAYCAYGC and caagcttATRAARAARTCNGTRTT (single letter code; R=A or G; Y=C or T; N=A or C or G or T), respectively. PCR was carried out using the cDNA from a human small cell lung carcinoma cell line as template. Forty cycles (94°C for 30 sec., 50°C for 30 sec. and 72°C for 90 sec.) were run using *Taq* polymerase (Perkin-Elmer Cetus). Products of approximately 500 bp in length were digested with EcoRI and HindIII, and subcloned into the Bluescript II KS+. The clones were then sequenced by the dideoxy chain termination method.

RT-PCR Oligonucleotide primers for reverse transcription and PCR amplification were designed based on the rat and human cDNAs. The primer sequences are summarized in Table I. RT-PCR was carried out using a GeneAmp RNA PCR Kit (Perkin-Elmer Cetus) according to the manufacturer's instructions.

Screening of human genomic library A human phagemid genomic DNA library prepared from peripheral blood cells was kindly donated by the Japanese Cancer Research Resources Bank. Eight independent inserts were obtained using the coding region of the human cDNA as the probe.

Southern blot analysis Two micrograms of phagemid DNA were digested with SalI or SalI-EcoRI and electrophoresed on a 0.6% agarose gel. The separated DNA fragments were transferred to Hybond N+ (Amersham), and hybridization-positive bands were detected with a Digoxigenin luminescence detection kit (Boehringer-Mannheim) using the coding region of the human cDNA as the probe.

Fluorescence in situ hybridization Two positive EcoRI fragments detected by the Southern blot analysis were subcloned into Bluscript II KS+. These two subcloned plasmids were labeled with biotin-16dUTP (Boehringer-Mannheim) using a nick translation labeling kit (Boehringer-Mannheim). Chromosome *in situ* suppression hybridization was performed on BrdU-incorporated metaphase chromosomes of a karyotypically normal male as described by Takahashi *et al.* (11) using 500 ng of each labeled DNA per chromosome slide as the probe, together with 1 mg of human Cot-1 DNA per slide as a competitor. Chromosomes were then counterstained with propidium iodide (PI). Hybridization signals detected with FITC-conjugated avidin (Vector Laboratories) were photographed under a fluorescence microscope equipped with a B-2A (for PI/FITC) or a B-3E (for FITC) filter.

RESULTS AND DISCUSSION

Nucleotide and amino acid sequence of human GnT-V

Many positive plaques were obtained from about 2 million plaques of a human fetal liver cDNA library (Clontech) using radio-labeled PCR product as a probe. Among these positive plaques, however, there were only two independent inserts, and neither of these inserts contained a terminal codon (Fig. 1). RT-PCR was then carried out using human RNA obtained from a neuroblastoma cell line, GOTO cells (12), as a template. Three pairs of oligonucleotide primers for the subsequent PCR were synthesized as shown in Table I. The RT-PCR procedure was carried out twice for each set of primers. The independently amplified products, RT-1 to RT-6, were subcloned into pT7Blue vector and sequenced. In each case, two

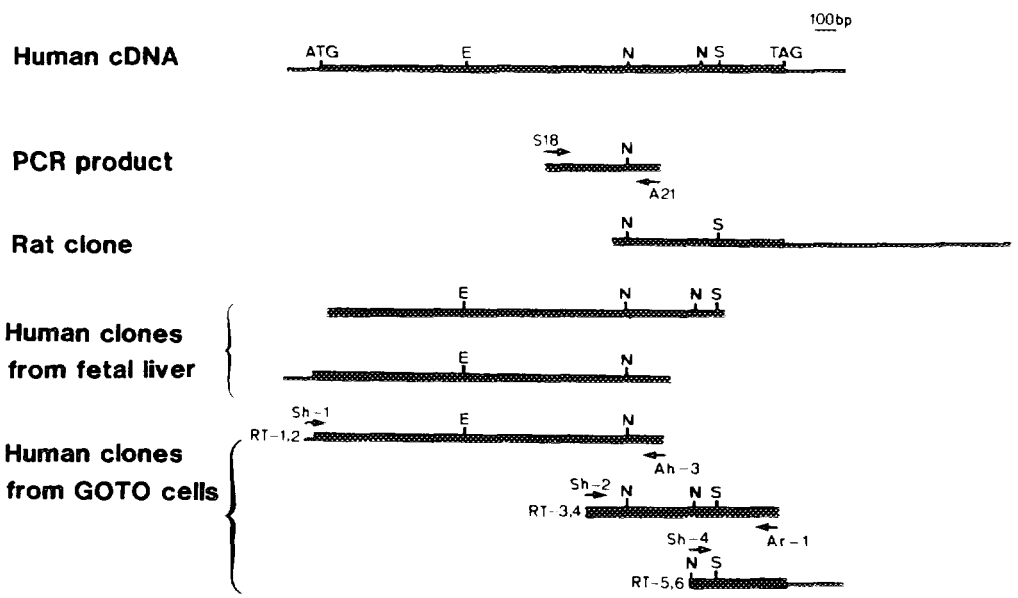

Fig. 1. Schematic structure of GnT-V cDNA. Arrows indicate the oligonucleotide primers used in PCR. The bold lines indicate the coding region of GnT-V. The letters E, N and S represent EcoRI, NcoI and SmaI restriction sites, respectively. The rat clone was obtained from a rat cDNA library (data not shown).

Table I. The RT-PCR primer sequences

product	reverse transcription primer	PCR primer
RT-1, 2	Ar-1, CAGTCTTTGCAGAGGGCC	Sh-1, GGTGAAGTTGCCAGAGAGCA Ah-3, CCAATGAAAAAGTCTGTGTT
RT-3, 4	Ar-4, GAATGAATCCAGGGTGGC	Sh-2, GTGGATAGCTTCTGGAAGAA Ar-1
RT-5, 6	Ar-5, CTCTGGTCAGAGTCCCTGA-CTGTCTTTCAG	Sh-4; CAGGACTTCTGCCATGGG Ar-4

The oligonucleotids were synthesized according the human cDNA sequence, Sh- and Ah-, or the rat cDNA sequence, Ar-.

amplified fragments obtained from one set of primers were identical. The cDNAs obtained from the human GOTO cells overlapped in sequence with the cDNAs obtained from the fetal liver library. No primer sequence was found in the 3'-regions of RT-5 and RT-6, and the 3'-region following the stop codon shared no homology with the same portion of the rat sequence. The reason for this result is still unknown.

Figure 2 shows the nucleotide and deduced amino acid sequences of human GnT-V. There is 88% homology between the human and rat nucleotide sequences in the coding region and there is 66% homology in the upstream regulatory regions (13). Figure 3 shows the amino acid sequence comparison between human and rat GnT-Vs. There is 97% homology between these amino acid sequences. The differences include a single amino acid insertion (Valine) in the neck region of human GnT-V.

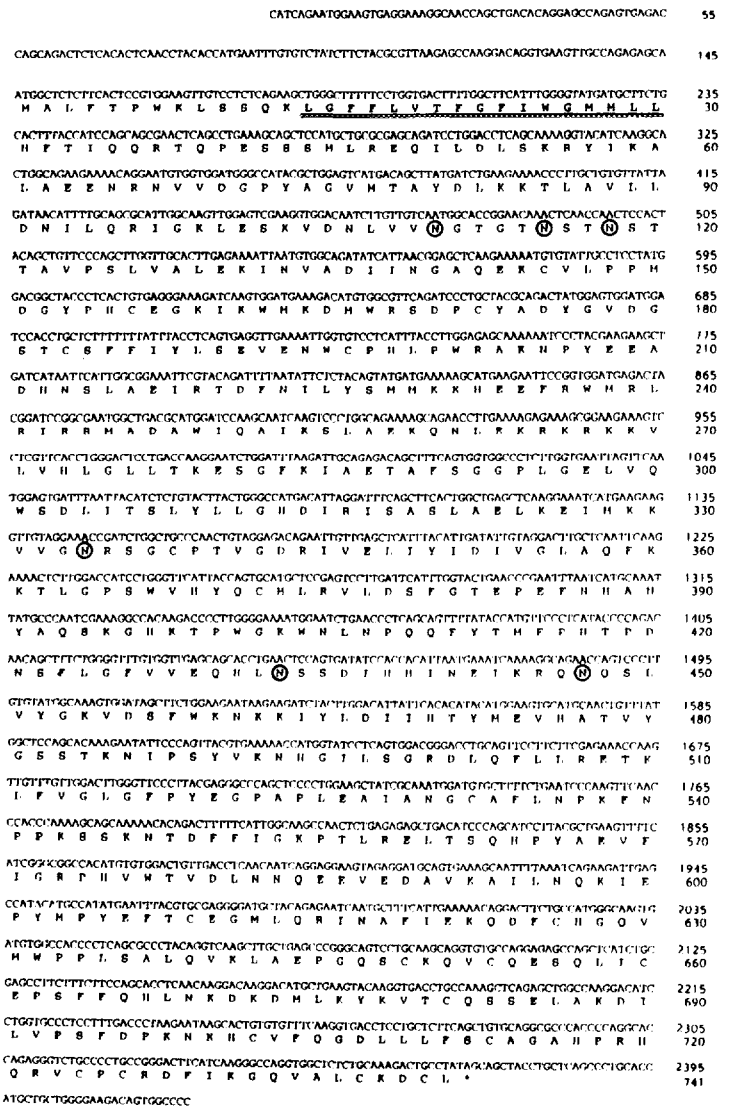

Fig. 2. Nucleotide and predicted amino acid sequence of human GnT-V. Single letter notation is used for the amino acids. The proposed transmembrane region is underlined. Aspargine residues that are circled are putative N-glycosylation sites.

Chromosomal mapping of human GnT-V

Two Southern blot analysis-positive phagemids were digested with SalI and SalI-EcoRI enzymes. After ethidium bromide

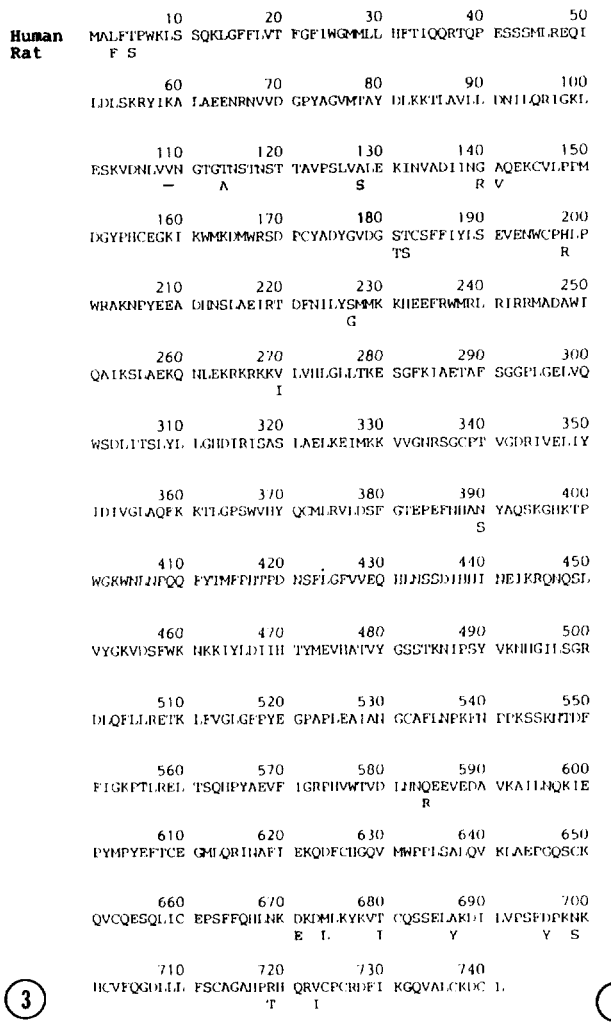

Fig. 3. Comparison of human and rat GnT-V amino acid sequences. Only the amino acid residues of the rat GnT-V sequence which differ from those of the human GnT-V sequence are shown. The symbol - in the rat sequence indicates a deletion in comparison with the human sequence.

Fig. 4. Southern blot analysis of human GnT-V genomic clones. Human phagemid clones C10 and C13 were treated with restriction enzymes and electrophoresed on a 0.6% agarose gel. Lanes 1 and 3, C10 and C13 DNA, respectively, treated with SalI. Lanes 2 and 4, C10 and C13 DNA, respectively, treated with SalI and EcoRI. A, ethidium bromide staining of the agarose gel; B, Southern blot analysis of the human phagemid clones for use in chromosomal mapping. The coding region of a human GnT-V cDNA was used as the probe.

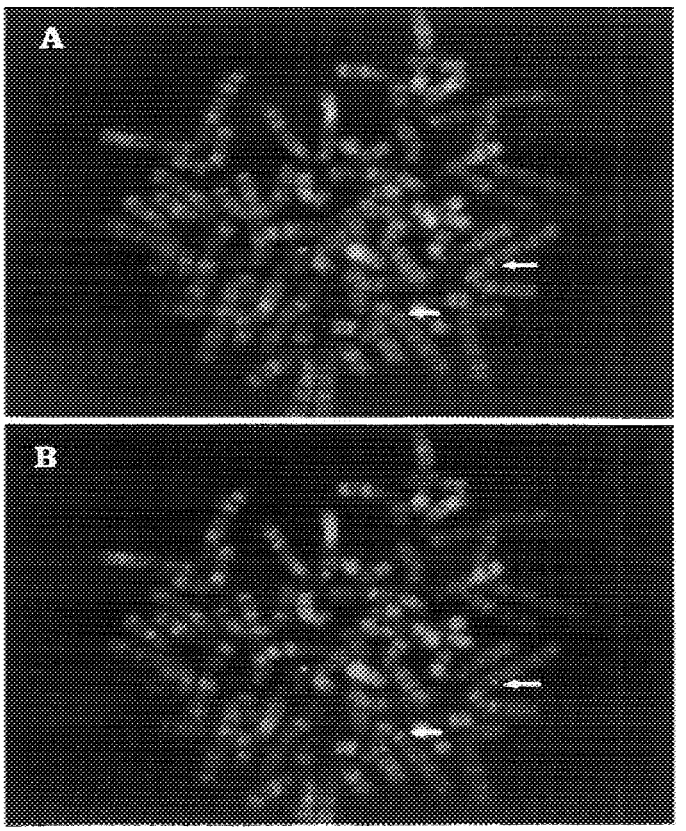

Fig. 5. Localization of C10 on chromosome 2q21 by fluorescence in situ hybridization. Hybridization-positive signals indicated by arrows were photographed under a fluorescence microscope with B-2E (A) and B-2A (B) filters. The location of the cloned sequence on chromosome 2q21 is schematically represented by the symmetric dots (C).

staining, the gel was blotted to a membrane, and another Southern blot analysis was done to identify the fragments containing exon (Fig. 4). Our data suggest that there are more than 11 exons (data not shown) in the GnT-V gene. Large numbers of exons and introns have also been found in the genes for α2-6 sialyltransferase (14), β1-4 galactosyltransferase (15) and α1-3galactosyltransferase (16).

An oligonucleotide fragment C10-1 from a genomic clone C10 was subcloned into Bluescript II KS+ vector. As shown in Fig. 5,

fluorescence *in situ* hybridization analysis was carried out using the subcloned plasmid as the probe, and a typical doublet signal was observed in a single location at 2q21. The same data was obtained using a subclone of C13-1 (data not shown).

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