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Short Communication

ASSIGNMENT OF THE HUMAN UDP-GalNAc:POLYPEPTIDE, *N*-ACETYLGALACTOSAMINYLTRANSFERASE-TYPE-2 GENE TO CHROMOSOMAL REGION 1q42 BY FLUORESCENCE *IN SITU* HYBRIDIZATION

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Mucin-type oligosaccharides have been assigned for rolls providing protection from proteolytic degradation and supplying the appropriate charge and water binding properties to mucus secretions. The initial reaction of O-linked oligosaccharide biosynthesis is the post-translational modification of the peptide by transferring an *N*-acetylgalactosamine (GalNAc) to a serine or threonine residue. The reaction is catalyzed by UDP-*N*-acetyl-alpha-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase (GalNAc-T) (EC 2.4.2.41). The addition of GalNAc to polypeptide can take place early in the rough endoplasmic reticulum or in the *cis* region of Golgi complex and carcinogenesis of the mucous membrane may alter the expression of this enzyme. Hence the mucin oligosaccharide structures are altered. This reaction is controlled at least by two different enzymes, GalNAc-T1 (type-1) and GalNAc-T2 (type-2), those are differently expressed in normal human cells, organs and cancer tissues (White *et al.*, 1995).

The cDNA encoding full-length bovine GalNAc-T1 was cloned from the library of bovine intestine, by synthetic oligonucleotide polymer of the amino-terminal amino acid sequence of bovine colostrum GalNAc-T1 (Homa *et al.*, 1993). We have cloned the human GalNAc-T1 from human colon tissues, by RT-PCR with the oligonucleotide primers synthesized based on the nucleotide sequence of bovine GalNAc-T1 cDNA. Very recently the similar construction of

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oligonucleotide probe was used for PCR to isolate the sequence from human salivary gland cDNA, and the human gene for GalNAc-T1 was reported to be located to chromosome 18 by Southern analysis of panels of human/rodent somatic cell hybrid DNAs (Meurer *et al.*, 1995). We have gained finer assignment of the gene to human chromosomal region 18q12.1 using fluorescence *in situ* hybridization (FISH) with R-banded chromosomes (Takai *et al.*, 1997). In the present study, using our cloned cDNA encoding the human GalNAc-T2 gene, like in the case of mapping of the human GalNAc-T1 gene, we determined the assignment of the human GalNAc-T2 gene to a human chromosome by FISH. The GalNAc-T1 nucleotide sequences of bovine and human, and human GalNAc-T2 nucleotide sequence can be referred to the GenBank™/EMBL Data Bank with accession numbers L07780, X85018 and X85019.

To clone a human GalNAc-T2 cDNA by RT-PCR, the oligonucleotides were designed based on the sequence of the human GalNAc-T2 cDNA as recently described (White *et al.*, 1995). The following two PCR primer pairs were used: (I) sense primer, 5′-CAGAATTCATGCGGCGGCGCCTCGC-3′ and antisense primer, 5′-CAGGATCCAAAGTTGTCCATATTAATGA-3′ corresponding to the nucleotide numbers of bovine GalNAc-T1 cDNA 1–20 and 783–764, respectively, (II) sense primer, 5′-CAGAATTCACTCGGGTTGTGTCACCCAT-3′ and antisense primer, 5′-CAGGATCCCTACTGCTGCAGGTTGAGCG-3′ corresponding to the nucleotide numbers 736–755 and 1697–1716, respectively. These contained *Bam*Hl and *Eco*RV sites in 5′ primer and 3′ primer, respectively. Total cellular RNA was isolated from the surgically resected human colon tissues by the guanidine isothiocyanated method. Amplification of a segment of mRNA was carried out using the following conditions: 94°C, 1 min; 55°C, 1 min; 74°C, 1.5 min, 40 cycles. The amplified two PCR fragments were subcloned into Bluescript (Stratagene, CA) using *Bam*Hl, *Eco*RI and internal *Cla*Il sites. A resultant cDNA clone of human GalNAc-T2 coding for the entire coding region was sequenced by the dideoxy chain termination method.

This 1.7 kb cDNA fragment of the human GalNAc-T2 gene was used as a probe for FISH. The probe DNA was labelled by nick-translation with biotin-16-dUTP (Boehringer) as described previously (Takai *et al.*, 1994).

R-banded human chromosomes were prepared from lymphocytes of a healthy male (Viegas-Pequignot and Dutrillaux, 1978; Takahashi *et al.*, 1990). The standard FISH methods (Lawrence *et al.*, 1988) were performed with some modification (Takahashi *et al.*, 1991; Takai *et al.*, 1994). The signal amplification procedure was carried by the previously reported methods (Pinkel *et al.*, 1986; Takai *et al.*, 1994). The slides were finally stained with propidium iodide. The stained chromosomes were observed using Nikon OPTIPHOT-2-EFD2 microscope (B-2A filter). Fuji chrome film (Sensia, ASA 100) was used for photography.

Among 50 human (pro)metaphase cells hybridized with the probe and microscopically observed, 32 cells revealed symmetrical double spots on the

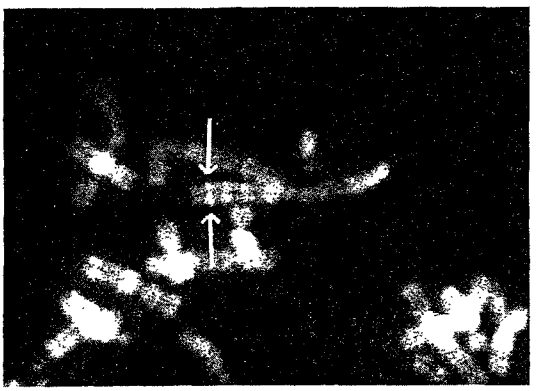

Fig. 1. Human R-banded chromosomes of partial metaphase cell stained with propidium iodide after fluorescence *in situ* hybridization with about 1.7 kb cDNA as a probe. Arrows indicate the symmetrical double spots of the human gene for GalNAc-T2 are located at the chromosomal region 1q42. This photograph was originally taken colored with Fuji chrome film without any image analyzer.

chromosome 1 at band q42 (Fig. 1). No symmetrical double spots were detected in other chromosomal regions with any prominent coincidence. Thus, we concluded that the human gene encoding GalNAc-T2 is located on human chromosomal region 1q42.

Some cases of stomach cancer have been reported to show chromosomal deletions or rearrangements at 1q42 region (Mitelman, 1994). Since the GalNAc-T is the key enzyme for the synthesis of mucin-type oligosaccharide, understanding on the alteration of the expression of GalNAc-T at the stage of gastrointestinal oncogenesis is of interest. Further studies on relationship between gastrointestinal carcinogenesis and GalNAc-T are currently underway.

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