Vol. 190, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1993 Pages 42-46

HUMAN α-1,3 FUCOSYLTRANSFERASE (FucT-VI) GENE IS LOCATED AT ONLY 13 Kb 3' TO THE LEWIS TYPE FUCOSYLTRANSFERASE (FucT-III) GENE ON CHROMOSOME 19

Shoko Nishihara[1], Mariko Nakazato[1], Takashi Kudo[1,2], Hirohisa Kimura[1], Takao Ando[3], and Hisashi Narimatsu*[,1]

[1]Division of Cell Biology, Institute of Life Science, Soka University, 1-236 Tangi-cho, Hachioji, Tokyo 192, Japan

[2]Department of Microbiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

[3]Basic Research Division, BML Inc., 361-6 Matoba, Kawagoe, Saitama-ken 350, Japan

Received November 16, 1992

SUMMARY: Human α-(1,3/1,4) fucosyltransferase (FucT-III) gene and α-1,3 fucosyltransferase (FucT-VI) gene were found in the 38-kb fragment isolated from a human cosmid library. These genes were present 13 kb apart in tandem orientation. The FucT-III gene has already been mapped on the Lewis locus of chromosome 19. This demonstrated that FucT-VI gene was localized close to the Lewis locus on chromosome 19.

Fucosylated oligosaccharides show precise developmental and tissue-specific expression patterns (1,2) and are expected to play an important role in various biological phenomena, for example, the compaction in mammalian embryogenesis (3,4) and the adhesion of neutrophils to inflamed endothelial cells (5). The expression of the fucosylated oligosaccharides is considered to be regulated by expression of fucosyltransferases. Four or five types of α-1,3 fucosyltransferase have been expected in human tissues by biochemical and genetic observations (2,6). One α-(1,3 /1,4) fucosyltransferase (FucT-III) and two α-1,3 fucosyltransferase (FucT-IV and FucT-V) have already been cloned by Lowe et al. (7 - 10). However the fucosyltransferase which determines SSEA-1 during embryogenesis or the ligand for the ELAM-1 on the inflamed endothelial cells has not been assigned yet. Recently a new candidate of the fucosyltransferase seemingly responsible for the ELAM-1 ligand, FucT-VI, has been isolated by pcr methods using homologous sequence to FucT-III (11). The chromosome mapping for FucT-VI gene has not been done yet.

*To whom correspondence should be addressed.

Abbreviations: FucT, fucosyltransferase; SSEA-1, stage-specific embryonic antigen-1; ELAM-1, endothelial leukocyte adhesion molecule-1; pcr, polymerase chain reaction; ORF, open reading frame; Lex, Lewis x; sLex, sialyl-Lewis x.

0006-291X/93 $4.00

We at first tried to clone FucT-III gene from the human leukemia cell line cosmid library by synthetic oligonucletide probes. During the screening a single cosmid clone, pWE-HFT1, spanning the 38 kb insert was isolated and it encoded both FucT-III and FucT-VI genes. In this communication we demonstrate that FucT-VI gene is located at a distance of approximately 13 kb downstream from FucT-III gene on chromosome 19 in tandem orientation.

MATERIAL AND METHODS

Human genomic library and screening. The human genomic cosmid library was provided by Dr. H. Inoko (Tokai University, School of Medicine). The library was screened with the following radiolabeled oligonucleotide probes which had the partial sequences of the FucT-III coding region:

A 5'-ACCCATGGATCCCCTGGGTGCAGC-3'
B 5'-AGACACGGTCATCGTGCACCACTG-3'
C 5'-CTCTCAGGTGAACCAAGCCGCTATG-3'
D 5'-TGAAGTATCTGTCCAGGGCTTCCAG-3.

Approximately 6 X 10^6 bacterial colonies that carried recombinant cosmids were screened with a mixture of four oligonucleotides for colony hybridization. Filters were hybridized for 16 hr. at 42 °C in 50 % formamide, 3.2 X SSPE, 1 X Denhart's solution , 1% SDS, and 200 μg/ml denatured salmon sperm DNA. The filters were rinsed two times for 5 min. each at room temperature in 2 X SSPE, 0.1% SDS and then washed two times for 30 min. at 65 °C in 1 X SSPE, 0.1% SDS. A single independent hybridization-positive colony was isolated after tertiary screening. The cosmid DNA was characterized by restriction endonuclease digestions and Southern blot analyses.

Southern blot analysis. Cosmid DNA was digested with restriction endonucleases, fractionated by a 0.5% or 0.8% agarose gel electrophoresis, and subjected to Southern blot analysis. Southern blots were hybridized and washed exactly same as in case of the screening and probed with the synthetic oligonucleotides, A, B, C, and D.

DNA sequencing analysis. Cosmid DNA was digested with SmaI. The 3.0 kb fragment including FucT-III gene and the 4.2 kb fragment including FucT-VI gene were subcloned into pBluescript SK(-). Then each fragment was subcloned into M13mp18 or mp19 vector with the respective enzyme sites and sequenced by dideoxy chain termination method.

RESULTS AND DISCUSSION

All of the fucosyltransferase genes reported to date have no intron. Three α-1,3 fucosyltransferases (FucT-III, FucT-IV, and FucT-V) form a gene family sharing the highly homologous sequence to each other (7 - 10). We at first intended to clone FucT-III gene by screening the human genomic library with synthetic oligonucleotide probes which were the partial sequences of FucT-III coding region. Southern blots analyses on the isolated clone, pWE-HFT1, revealed two hybridization-positive SacI-fragments (20kb and 3.2kb). As FucT-III, FucT-IV, and FucT-V have been reported to posess no SacI site in their coding region (7 - 10), pWE-HFT1 was expected to encode two kinds of fucosyltransferase gene. Two hybridization-positive SmaI-fragments (3.0kb and 4.2kb) were subcloned into pBluescript SK(-) and the restriction mapping analysis was performed on each fragment. As shown in Fig.1, the mapping of 3.0kb SmaI-fragment was completely identical to that of FucT-III gene and the mapping of 4.2 kb SmaI-fragment suggested that it encoded a novel α–1,3

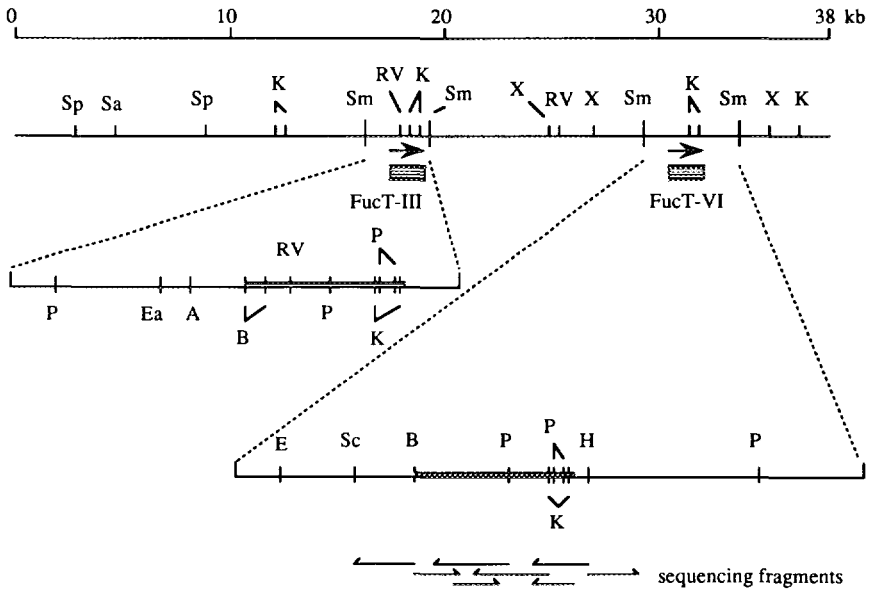

Figure 1. Restriction map of the 38 kb insert of pWE-HFT1. The striped boxes indicate the ORF positions of FucT-III and FucT-VI. Restriction sites are indicated for AccI (A), BamHI (B), EagI (Ea), EcoRI (E), EcoRV (RV), HindIII (H), KpnI (K), PstI (P), SacI (Sc), SalI (Sa), SmaI (Sm), SpeI (Sp), and XhoI (X).

fucosyltransferase gene having a high degree of sequence homology to FucT-III. Then sequence analysis was carried out for each fragment. In the 3' portion of 3.0kb SmaI-fragment, the ORF of FucT-III was identified. The 4.2 kb SmaI-fragment encoded a single long ORF whose sequence deduced 91% amino acid homology to FucT-III. During preparation of this manuscript, an ORF sequence of a new member of α–1,3 fucosyltransferase family, named as FucT-VI, was reported (11). It was isolated by the pcr method, so that its flanking sequences were not reported. The sequence of the ORF in the 4.2 kb fragment was identical with the ORF sequence of FucT-VI, except for one nucleotide unrelated to amino acid substitution. As shown in Fig.2, the 5'- and the 3'-noncoding region of FucT-VI gene did not show high homology to FucT-III gene in contrast with the ORF. The flanking sequences of FucT-VI in Fig.2 would be useful to specify its transcript.

To determine the position and the orientation of each gene, the restriction endonuclease mapping of pWE-HFT1 were performed by Southern blot analyses. The distance between FucT-III gene and FucT-VI gene was about 13 kb and they were arranged in tandem (Fig.2). The FucT-III has been mapped on the Lewis locus of chromosome 19 (7). These facts demonstrated that FucT-VI gene was localized close to the Lewis locus on chromosome 19. The FucT-V gene is also on Chromosome 19 (10). Three of four cloned genes having α-1,3 fucosyltransferase activity are located on chromosome 19. This fucosyltransferase family might be generated by gene duplication.

In general glycosyltransferases have highly restricted substrate specificity. Each linkage is considered to require each enzyme. No transferase can utilize more than one type of sugar donor. They can clearly recognize even subtle differences among acceptor molecules and

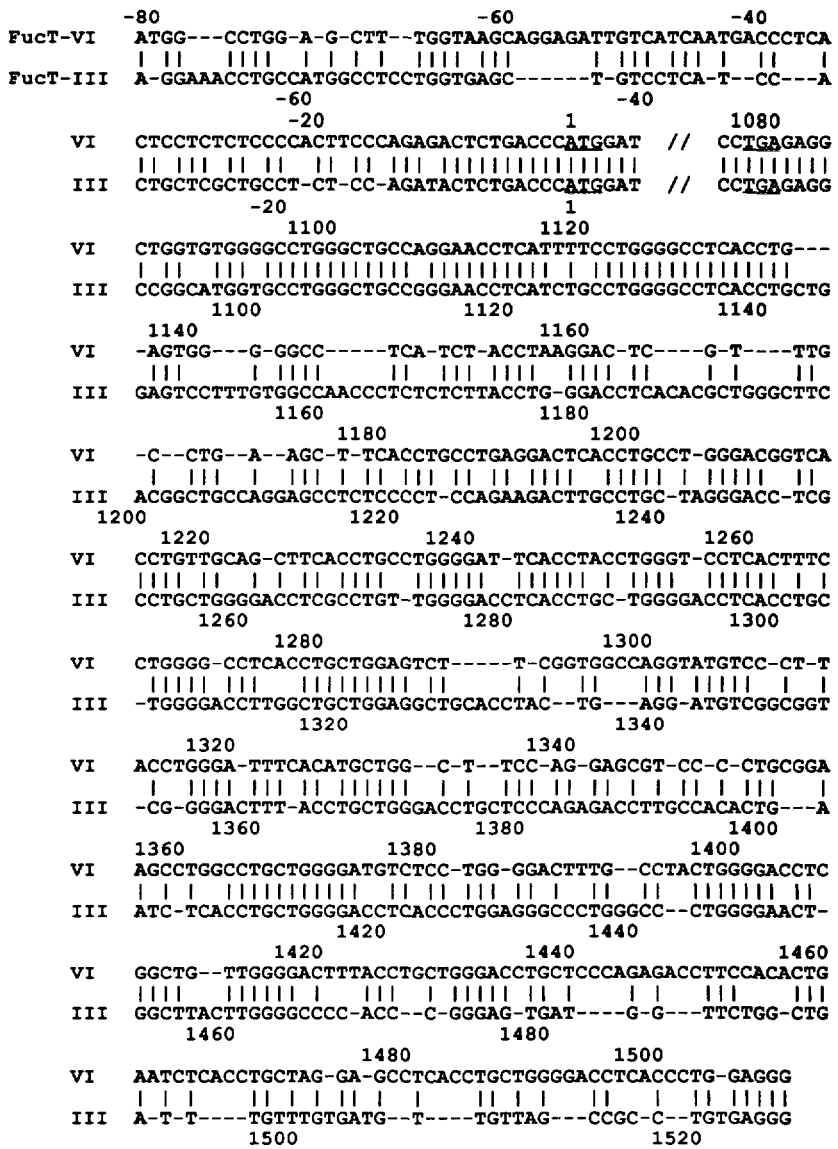

Figure 2. Comparison of the 5'- and 3'-noncoding region DNA sequences of FucT-VI and FucT-III. The underlined tri-nucleotides indicate the initiation and the stop codons of ORFs.

different intersugar linkages. Fucosyltransferases also have restricted specificities (2, 6). The ELAM-1 ligand and the SSEA-1 epitope have been reported to share sLex structure (5) and Lex structure (4), respectively. While FucT-IV on chromosome 11 can not synthesize sLex (8), three fucosyltransferases on chromosome 19, FucT-III, FucT-V, and FucT-VI, can determine sLex. Considering that FucT-VI expression in myeloid cells has been reported (11), it is a probable candidate of the fucosyltransferase responsible for the ELAM-1 ligand. However it is unknown which of four fucosyltransferases contributes to the appearance of SSEA-1 during embryogenesis.

To solve above questions, the availability of the upstream regions of these genes will permit us to analyze the tissue-specific or stage-specific transcriptional regulation of fucosyltransferase genes.

Acknowledgments: We thank Dr. H. Inoko (Tokai University, School of Medicine) for providing us with the human genomic cosmid library. We also thank Dr. K. Hattori and Ms. Y. Toyama for technical assistance of sequencing.

REFERENCES

1. Muramatsu, T., Condamine, H., Gachelin, G., and Jacob, F. (1980) J. Embryol. Exp. Morph. **57**, 25-36.

2. Macher, B. A., Holmes, E. H., Swiedler, S. J., Stults, C. L. M., and Srnka, C. A. (1991) Gycobiology **1**, 577-584.

3. Solter, D., and Knowles, B. B. (1978) Proc. Natl. Acad. Sci. **75**, 5565-5569.

4. Gooi, H. C., Feizi, T., Kapadia, A., Knowles, B. B., and Solter, D., and Evans, M. J. (1981) Nature **292**, 156-158.

5. Springer, T. A., and Lasky, L. A. (1991) Nature **349**, 196- 197.

6. Mollicone, R., Candelier, J.-J., Mennesson, B., Couillin, P., Venot, A. P., and Oriol, R. (1992) Carbohydr. Res., **228**, 265-276.

7. Kukowska-Latallo, J. F., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1990) Genes Dev. **4** 1288-1303.

8. Lowe, J. B., Kukowska-Latallo, J. F. , Nair, R. P., Larsen, R. D., Marks, R. M., Macher, B. A., Kelly, R. J., and Ernst, L. K. (1991) J. Biol. Chem. **266**, 17467-17477.

9. Kumar, R., Potvin, B., Muller, W. A., and Stanley, P. (1991) J. Biol. Chem. **266**, 21777-21783.

10. Werston B. W., Nair, R. P., Larsen, R. D., and Lowe, J. B. (1992) J. Biol. Chem. **267**, 4152-4160.

11. Koszdin, K. L., and Bowen, B. R. (1992) Biochem. Biophys. Res. Commun. **187**, 152-157.