Structure and Expression of H-type GDP-L-Fucose:β-D-Galactoside 2-α-L-Fucosyltransferase Gene (FUT1)

TWO TRANSCRIPTION START SITES AND ALTERNATIVE SPlicing GENERATE SEVERAL FORMS OF FUT1 mRNA

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The expression of the ABO antigens on erythrocyte membranes is regulated by H gene (FUT1)-encoded α(1,2)fucosyltransferase activity. We have examined the expression of the FUT1 in several tumor cell lines, including erythroid lineage and normal bone marrow cells, by Northern blot and/or reverse transcription-polymerase chain reaction (RT-PCR) analyses. RT-PCR indicated that bone marrow cells, erythroleukemic cells (HEL), and highly undifferentiated leukemic cells (K562) that have erythroid characteristics expressed the FUT1 mRNA while four leukemic cell lines did not. The FUT1 mRNA was also demonstrated in gastric, colonic, and ovarian (MCAS) cancer cell lines by RT-PCR. Northern blot analysis indicated that a 4.0-kilobase transcription start site was expressed in some of these tumor cell lines. Rapid amplification of 5′ cDNA end (RACE) analysis suggested that the FUT1 transcript had several forms generated by two distinct transcription start sites and alternative splicing. The results of RT-PCR using specific primers for each starting exon suggested that two transcription initiation sites (exon 1A and exon 2A) of the FUT1 were identified in gastric cancer cells and in ovarian cancer cells. Only exon 1A was identified as a transcription start site in another gastric cancer cell line, two colonic cancer cell lines, and in K562 cells, whereas only exon 2A was identified in HEL cells and in bone marrow cells. These two transcription start sites were located 1.8 kilobases apart. Therefore, two distinct promoters appeared to be present in the FUT1. The distinct promoters of the FUT1 and alternative splicing of the FUT1 mRNA may be associated with time- and tissue-specific expression of the FUT1.

The ABO(H) histoblood group antigens are oligosaccharides (1), and their biosynthesis is regulated by several glycosyltransferases that add monosaccharides to a precursor molecule in a sequential fashion (2, 3). The α(1,2)fucosyltransferase†

† This work was supported by a Grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: α(1,2)fucosyltransferase, GDP-β-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase; H enzyme, H gene-encoded α(1,2)fucosyltransferase; Se enzyme, Secretor gene-encoded α(1,2)fucosyltransferase; RT-PCR, reverse transcription-polymerase chain reaction; RACE, rapid amplification of cDNA ends; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; bp, base pair(s); kb, kilobase pair(s).
transcripts in several tumor cell lines including the erythroid lineage. We have identified two distinct transcription start sites of the FUT1 that were different from one previously described (18).

 MATERIALS AND METHODS

Cell Lines, RNA Isolation and Northern Blot Analysis—Human cancer cell lines MCAS (ovarian cancer), COLO201 and WiDr (colon cancer), KATOIII and MKN74 (gastric cancer), and HEL (erythroleukemia) cells were obtained from the Health Science Research Resources Bank, Osaka, Japan. Human hematopoietic cell lines K562 (chronic myelogenous leukemia, blast crisis), HL60 (promyelocytic leukemia), U937 (histiocytic lymphoma), BALL-1 (B-cell lymphoblastic leukemia), and MOLT-4 (T-cell lymphoblastic leukemia) cells were a kind gift from Dr. K. Sagawa (Kurume University). Total cytoplasmic RNA was isolated from these cells using the acid guanidinium thiocyanate/phenol/chloroform method (19). For Northern blot analysis, total RNA (20 µg) was denatured, digested with 10 µg of RNase A, and separated by electrophoresis on a 1.0% agarose gel and stained with ethidium bromide. No PCR specific to FUT1 was performed. The products were analyzed by 1.0% agarose gel electrophoresis and stained with ethidium bromide.

PCR Amplification—All PCR amplifications (22) were performed in a 50-µl reaction mixture containing 10 pmol of each primer, 1.2 units EX Taq DNA polymerase (Takara, Kyoto, Japan), 200 µM dNTP, and 1× EX Taq reaction buffer.

Reverse Transcription PCR (RT-PCR)—Synthesis of single strand cDNA of several kinds of cultured cells was performed on total RNA (20 µg) using SUPERSCRIPT preamplification system (Life Technologies, Inc.) according to the manufacturer instructions. 1 µl (total 20 µl) of resultant single strand cDNA was used as the template for PCR. The RT-PCR primers for the amplification of FUT1 (15) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene (24) are shown in Table I. The temperature profile of all PCR reactions was as follows: denaturation at 94 °C for 20 s, annealing at 65 °C for 1 min, extension at 72 °C for 1 min, and 25 cycles for G3PDH and 30 cycles for FUT1. The starting exon-specific RT-PCR was performed using different primer pairs for PCR. For the generation of the 5'-end of cDNA, RACE analysis was as follows: denaturation at 94 °C for 20 s, annealing at 65 °C for 1 min, and extension at 72 °C for 3 min, and 30 cycles. The 5'-RACE product was cloned into pGEM using the pGEM-T vector system I (Promega, Madison, WI) for sequencing.

PCR Amplification of the Promoter Regions and the Genomic DNAs of the FUT1—The genomic DNA of the FUT1 (from exon 1 to exon 3) was amplified by PCR using 100 ng of genomic DNA as the template. The 5'-Ex1 (upper primer, Table II) and FUT1 nest (lower primer, Table I, and see Fig. 3C) primers were used for amplification of the FUT1 (denaturation at 94 °C for 10 s, annealing and extension at 68 °C for 4 min, and 30 cycles). The PCR product of the FUT1 genomic DNA between exons 1 and 3 was cloned into pGEM DNA for sequencing.

Construction of Luciferase Reporter Gene Plasmid—To create constructs of the luciferase fusion genes, a 0.7-kb FUT1 exon 3 fragment (see Fig. 5) of pGEM containing the 2.8-kb PCR fragment between exons 1 and 3 of the FUT1 was subcloned into pGLO2-enhancer vector (Promega). The plasmid DNA was purified with Qiagen tips (Qiagen Inc., Chatsworth, CA) and transacted into MCAS cells using Trans-IT (Takara) as described previously (20, 21). After 48 h, the cells were lysed, and the luciferase activity was measured using the luciferase assay system (Promega) and a luminometer (Lumat LB9501, Berthold, Wildbad, Germany). The relative promoter activity was indicated by relative light units obtained from transfection with pGLO2-control vector as 100%. Transfection efficiency was normalized as described previously (21).

DNA Sequencing—Double-stranded plasmid DNA containing the FUT1 fragment was sequenced in both orientations using an AutoRead DNA sequencing kit and an ALF DNA sequencer (Pharmacia, Uppsala, Sweden) or using an ABI PRISM dye terminator cycle sequencing ready reaction kit and an ABI 373 sequencer (Applied Biosystems).

RESULTS

Expression of the FUT1 in Several Tumor Cell Lines—Northern blot analysis indicated that about 4.0 kb of the transcripts of the FUT1 were detected in total RNA from HEL and MCAS cells but not from WiDr cells (Fig. 1A). The transcript with the same size was also detected in total RNA from KATOIII cells but not from COLO201 and MKN74 cells (data not shown). However, the FUT1 transcript was detected in all these cell lines (Fig. 1B) and in undifferentiated leukemic K562 cells by RT-PCR analysis, whereas leukemic cells (HL60, U937, BALL-1, and MOLT-4) tested did not express the FUT1 (data not shown). The results indicated that epithelial cancer cells expressed FUT1 mRNA, while many leukemic cells did not express the mRNA. However, erythroid lineage HEL cells (25) and undifferentiated leukemia K562 cells, which has erythroid characteristics (26), expressed the FUT1 mRNA.

Identification of the 5’ cDNA End of the FUT1 Transcript by RACE Analysis—To isolate the 5’-end of FUT1 cDNA, RACE was performed using 1 µg of either poly(A)⁺ RNA from HEL or MCAS cells or of human bone marrow Marathon-Ready cDNA (Clontech). Several different sizes of 5’-RACE products of the FUT1 were amplified from cDNA prepared from bone marrow, HEL, and MCAS cells (Fig. 2). DNA sequence analysis of 15 clones of the 5’-RACE products of the FUT1 from each of the cells indicated that the bone marrow and the HEL cells had two and four different forms by alternative splicing with a single transcription initiation site, respectively, while the MCAS cells had seven forms with two distinct transcription initiation sites and alternative splicing (Fig. 3, B and C). Although we could not identify accurately the 5’-ends of the FUT1 by RACE analysis, the longest 5’-ends of each starting exon are shown in Fig.

## Table I

| Primer sequence |
|-----------------|
| Primer sequence |
| Position of primer |
| 5'-FUT1 | TGCCATCGTATGCCGGCCTGGCCTGGGAG | 324–366 bp of FUT1 |
| 3'-FUT1 | GGGGCCAACATTGTGCTCAGGAGAACAC | 1189–1213 bp of FUT1 |
| FUT1 nest | GGCCTCCCCTCGCCCGAGCCTGTCC | (∉126) (∉150) bp of FUT1 |
| 5’-G3PDH | TGAAGGTCGAGGTCAACCGATTGTTG | 11–36 bp of G3PDH |
| 3’-G3PDH | CATGTTGGCCATGAGGTCCACCAC | 970–993 bp of G3PDH |
Structure and Expression of FUT1

Identification of the FUT1 Gene Structure—To identify the FUT1 gene structure, the genomic DNA sequence between exons 1 and 3 of the FUT1 was amplified by PCR. DNA sequence analysis indicated that the first and second introns of the FUT1 were 1654 and 202 bp, respectively, and the all exon/intron junctions of these genes were compatible with GT/AG rule (Fig. 3C). The gene structure of the FUT1 is shown in Fig. 3A.

Analyses of DNA Sequence and Promoter Activity of the 5’-flanking Region of the Exon 2 of the FUT1—Since the 5’-flanking region of exon 2 (or first intron) of FUT1 appeared to act as a promoter, we isolated this region and DNA sequence analysis was performed. The 5’-flanking region of the exon 2 of the FUT1 contained a TATA-like sequence (27), three possible AP1 binding sites, two possible AP2 binding sites, two GATA consensus sequences, and a Myc consensus sequence (28) (Fig. 5).

The pGL2-enhancer vector containing 5’-flanking regions of the exon 2 of the FUT1 (between nucleotides –662 and 45 bp of the exon 2 of the FUT1, Fig. 5) showed promoter activity about 20-fold that of pGL2-control vector with SV40 early promoter and enhancer after transfection of each plasmid into MCAS cells (data not shown). The results suggested that this region acted as a promoter and that the exon 2A was one of transcription initiation sites of the FUT1 in MCAS cells.

Discussion

In the present study, we examined the structures of cDNA and genomic DNA of the FUT1. The FUT1 cDNA had several forms created by two distinct transcription initiation sites and alternative splicing of 5’-untranslated exons. Kelly et al. (18) have reported the transcription start site of FUT1 gene of A431 cells using the primer extension method. However, the transcription start site reported previously was present within exon 3C in this study, and the site was unlikely to be a transcription start site in MCAS and in HEL cells. The reason for this discrepancy is unknown, but one possibility may be that it was due to a difference in cell types.

A recent study has suggested that the re-expression of the H and H-related antigens such as Lewis b and Lewis y in colorectal tumors was regulated by H and Se enzymes (13). In addition, the expression of the FUT1 transcript was increasing during malignant transformation in colorectal mucosa (14). Our results indicated that the FUT1 transcript was expressed in not only colonic cancer cells but also gastric cancer cells. The expression of the H and H-related antigens in normal digestive mucosa, such as gastric and proximal colonic mucosa, are thought to be regulated by the Se enzyme but not the H enzyme (1). Our results suggest that the expression of the FUT1 mRNA and thereafter the expression of the H enzyme are increasing during malignant transformation in digestive mucosa.

The ABH antigens are expressed in embryonic but not in adult muscular, bone, and neuronal tissues (12). Recently, as well as the FUT1 gene, some other glycosyltransferase genes with distinct promoters and alternative 5’-ends have been re-

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**Table II**

Primer sequences for specific amplification of each starting exons of the FUT1

| Primer   | Primer sequence                  | Position of primer |
|----------|----------------------------------|--------------------|
| 5’-Ex1   | GAAAGTCCCTGACTGGAGTTGGCAG        | 1–25 bp of FUT1 exon 1 |
| 5’-Ex2   | ACTACCGTCTCTGGCGCTTGTAG          | 1–24 bp of FUT1 exon 2 |

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**Fig. 1. Expression of the FUT1 mRNA.** A, Northern blot analysis. 20 μg of total RNA prepared from each tumor cell line was divided into 10 μg/lane, electrophoresed, and transferred onto a nylon membrane. The membrane was then divided to prepare duplicate membranes. One membrane was used for Northern blot analysis, and the other membrane was stained by ethidium blue. The positions of 28 and 18 S ribosomal RNAs are indicated by arrows. B, RT-PCR analysis. Total RNA (2 μg) prepared from each tumor cell line was reverse-transcribed with oligo(dT) primer, and the resulting single strand cDNA from each membrane was used as a template for PCR analysis. The FUT1 amplification was performed for 30 cycles and the G3PDH for 25 cycles. PCR products were electrophoresed in a 1.2% agarose gel and stained by ethidium bromide. Endonuclease StyI-digested Lambda DNA (α/S) was used as a molecular size marker.

**Fig. 2. Rapid amplification of 5’ cDNA end of the FUT1.** Double strand cDNA for RACE was prepared using 1 μg of poly(A)+ RNA from each cell line or human bone marrow cells. The 5’-RACE products of the FUT1 from bone marrow, HEL cells, and MCAS cells were electrophoresed in a 1.0% agarose gel and stained by ethidium bromide. StyI-digested Lambda DNA (α/S) and MspI-digested pBluescript were used as molecular size markers. Some DNA fragment sizes are indicated.

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3C. To analyze the transcription initiation sites in each cell line, RT-PCR was carried out using a primer specific for each of the two distinct starting exons (5’-Ex1 primer for exon 1A or 5’-Ex2 primer for exon 2A, see Table II). The results also indicated that both exon 1A and exon 2A were transcription start sites in MCAS and KATOIII cells, while only exon 1A was a start site in MKN74, WiDr, and COLO201 cells (Fig. 4). In hematopoietic cells, only exon 1A was used as a transcription start site in K562 cells, while only exon 2A was used in HEL cells (Fig. 4). As in HEL cells, exon 2A but not exon 1A was a transcription start site of the FUT1 mRNA in normal bone marrow cells (Fig. 4). Since the mature peripheral leukocytes

(data not shown) and four leukemic cell lines did not express FUT1 mRNA by RT-PCR analysis, the results suggested that normal erythroid progenitor cells in bone marrow used only one promoter present in the 5’-flanking region of FUT1 exon 2.

**Fig. 3. Expression and Northern blot analysis of FUT1 in various cells.** A, Expression analysis was performed. Total RNA (2 μg) prepared from each tumor cell line was reverse-transcribed with oligo(dT) primer, and the resulting single strand cDNA from each membrane was used as a template for PCR analysis. The FUT1 amplification was performed for 30 cycles and the G3PDH for 25 cycles. PCR products were electrophoresed in a 1.2% agarose gel and stained by ethidium bromide. The positions of 28 and 18 S ribosomal RNAs are indicated by arrows. B, Northern blot analysis. Total RNA (2 μg) prepared from each tumor cell line was reverse-transcribed with oligo(dT) primer, and the resulting single strand cDNA from each membrane was used as a template for PCR analysis. The FUT1 amplification was performed for 30 cycles and the G3PDH for 25 cycles. PCR products were electrophoresed in a 1.2% agarose gel and stained by ethidium bromide. Endonuclease StyI-digested Lambda DNA (α/S) was used as a molecular size marker.

**Fig. 4. Rapid amplification of 5’ cDNA end of the FUT1.** Double strand cDNA for RACE was prepared using 1 μg of poly(A)+ RNA from each cell line or human bone marrow cells. The 5’-RACE products of the FUT1 from bone marrow, HEL cells, and MCAS cells were electrophoresed in a 1.0% agarose gel and stained by ethidium bromide. StyI-digested Lambda DNA (α/S) and MspI-digested pBluescript were used as molecular size markers. Some DNA fragment sizes are indicated.

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The ABH antigens are expressed in embryonic but not in adult muscular, bone, and neuronal tissues (12). Recently, as well as the FUT1 gene, some other glycosyltransferase genes with distinct promoters and alternative 5’-ends have been re-
ported, such as the α(2,6)sialyltransferase gene (29–31), mu-
rine β(1,4)galactosyltransferase gene (32), human N-acetyl-
glucosaminyltransferase V gene (33), α(1,3)fucosyltransferase (34)
gene, and the human β(1,4)-N-acetylgalactosaminyltrans-
ferase gene (35). Kozak (36) discussed that transcription of a
single gene by multiple promoters may provide additional flex-
ibility in the regulation of gene expression. Such promoters
could have tissue- and developmental stage-specific activity. In
fact, as shown in this study, the promoter usage and splicing
patterns of the FUT1 mRNA in erythroleukemic cells (HEL)
and in normal erythroid progenitor cells were different from
those in undifferentiated leukemic cells (K562), which has
erythroid characteristics, suggesting that changes in promoter
usage and splicing patterns appear during differentiation of
the erythroid lineage. Thus, the alternative use of multiple
promoters and alternative splicing of the glycosyltransferase
genes may be associated with tissue- and stage-specific glyco-
sylation patterns.

The tissue expression pattern of the ABO antigens is differ-
ent among vertebrate species (2). The expression of these an-
tigens in the digestive mucosa has been observed from amphib-
ians to higher mammals, while only human and some higher
anthropoid primates but not old world monkeys express these
antigens on erythrocyte membranes. Recently, rabbit homo-
louges of human FUT1, FUT2, and a pseudogene of FUT2
(Sec1) have been isolated (38, 39). In addition, we have had
indications that the old-world African green monkey had three
functional α(1,2)fucosyltransferases that were homolo-
goustohuman FUT1, FUT2, and Sec1.2 Thus, the expression of
ABO antigens in digestive mucosa was likely regulated by
α(1,2)fucosyltransferases encoded by the FUT2 homologues
in many vertebrate species. On the other hand, although enzy-
matic properties of the rabbit and monkey FUT1 homologue-
derived enzymes are similar to the H enzyme (38), they cannot
express ABO antigens on their erythrocyte membranes. A pos-

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2 Y. Koda, M. Soejima, and H. Kimura, unpublished results.

**Fig. 3. Gene structure of the FUT1 and 5′-untranslated region of the FUT1 transcripts.** A, the structure of genomic DNA of the FUT1.
Exons of the FUT1 are indicated as rectangles. The protein coding region is indicated by the shaded line in exon 4. The length of 300 bp is given
in the lower right corner. B, schematic illustration of seven forms of the 5′-untranslated region of the FUT1 transcripts of MCAS, HEL, and bone
marrow cells determined by 5′-RACE analysis. C, DNA sequences of the FUT1 exons 1, 2, and 3. The position of the nested primer for RACE is
indicated. All intron/exon junctions and exon/intron junctions are underlined. The intron sequences are indicated by small letters.
expression of the H and ABO antigens.

In the present study, we identified several forms of the FUT1 transcript generated by two transcription start sites and alternative splicing in 5’-untranslated exons. Our results suggested that dual promoters regulated the stage- and tissue-specific expression of the FUT1 transcript and thus regulated the expression of ABO and related histoblood antigens in many human tissues.

Acknowledgments—We are greatly indebted to Dr. Kimitaka Sagawa, Department of Blood Transfusion of our university for providing several leukemic cell lines.

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