

FUT2 gene involved in expression of H blood group antigen on surface of human tumor cell lines BEL-7404, SGC-7901, and SPC-A-1

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KEY WORDS ABO antigens; fucosyltransferases; FUT2 gene; cultured tumor cells

ABSTRACT

AIM: To investigate the expression of blood group H antigen on the surface of several human tumor cell lines such as BEL-7404, SPC-A-1, and SGC-7901. **METHODS:** *In vitro* and *in vivo* expression of the blood group H antigen was analyzed with flow cytometry and immunohistochemistry. Expression of FUT2 gene was determined by RT-PCR, Southern blot, and restriction digestion. **RESULTS:** Flow cytometric analysis showed that SGC-7901 cells, BEL-7404 cells, and SPC-A-1 cells had mean fluorescence intensity (MFI) values of 162 ± 43 , 81 ± 25 , and 28 ± 17 , respectively. DNA fragments of about 1.0 kb in length were obtained by RT-PCR from RNA isolated from these cells and were detected by FUT2-specific [α -³²P]dATP labeled DNA probes. **CONCLUSION:** Human tumor cell lines BEL-7404, SGC-7901, and SPC-A-1 all expressed blood group H antigen on their cell membranes *in vitro* and *in vivo*, but their expression levels varied significantly between different cell lines. FUT2 gene expression resulted in the production of these antigens on the cell membrane.

INTRODUCTION

The antigens of the human ABO blood group system are carbohydrate molecules constructed by the sequential action of a series of distinct glycosyltransferases. The terminal step in this pathway, catalyzed by the allelic glycosyltransferase products of the ABO locus, requires the expression of a precursor molecule called the H antigen.

The blood group H antigen is a fucosylated structure of the form $\text{Fuc}\alpha 1-2\text{Gal}\beta-$, whose expression is determined by GDP-*L*-fucose; β -*D*-galactoside 2- α -*L*-fucosyltransferase [α (1,2) FT; EC 2.4.1.69]. These enzymes catalyze a transglycosylation reaction between their sugar nucleotide substrate GDP-*L*-fucose and oligosaccharide acceptor substrates with terminal type I (Gal β 1-3GlcNAc-) or type II (Gal β 1-4GlcNAc-) moieties⁽¹⁾.

Recently, more attention was paid to the significance of the blood group H antigen in metastasis formation⁽²⁾ and tumorigenicity⁽³⁻⁵⁾ in cancer research. However, most of these results were obtained using tumor cell lines of rodents which had significant difference from humans, including cell surface carbohydrate structures⁽⁶⁾. To further investigate the function of H antigen in tumor development on human genetic backgrounds, a tumor cell line of human origin with a relatively high level expression of H antigen was needed. In addition, the gene responsible for H antigen expression represents tools to address this question by genetic approaches that perturb H antigen expression and should be determined. In this study, we examined several human tumor cell lines.

MATERIALS AND METHODS

Cell culture All cells were from Type Culture Collection of Chinese Academy of Sciences. Mouse NIH3T3 cells and human BEL-7404 cells (liver cancer) were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, BRL) with 10 % heat-inactivated newborn bovine serum (NBS). B16 cells (mouse melanoma), SPC-A-1 cells (human lung cancer), and SGC-7901 cells (human stomach cancer) were maintained in RPMI-1640 (Gibco, BRL) supplemented with 10 % NBS.

Flow cytometry Cells (1×10^6) were detached from the tissue culture flasks by brief treatment with edetic acid 5 mmol/L in phosphate-buffered saline and col-

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lected by centrifugation. After incubation with fluorescein isothiocyanate-conjugated lectin *Ulex europaeus* I (FITC-UEA-I; Sigma) which detects the H antigen⁽⁷⁾ at 4 °C in the dark for 1 h, flow cytometric analysis was performed with a Becton Dickinson FACScan cytometer. Mouse NIH3T3 and B16 cells were used as negative control.

RNA isolation, RT-PCR, and semiquantitative RT-PCR Total RNA was isolated from culture cells using TRIzol total RNA isolation reagent according to the manufacturer's instructions (Life Technologies). The first-strand cDNA were synthesized at 37 °C for 1 h by Moloney murine leukemia virus reverse transcriptase (superscript; Life Technologies) in the presence of dNTP 200 μmol/L, DTT 0.01 mol/L, RNase 20 u with random hexamers in a total volume of 20 μL. The RT-PCR primers, which were designed according to human FUT2 cDNA sequence⁽⁸⁾ for the amplification of FUT2 cDNA coding region, were as follows: P1 5'-CGG GAA TTC AGC CAT GCT GGT CGT TCA G-3' (28-mer, 59 - 78); P2 5'-CAA CTC GAG CTA TTA GTG CTT GAG TAA GG-3' (29-mer, 1079 - 1096). They yielded an RT-PCR product of 1.057 kb in length.

PCR amplification was performed using 1 × PCR reaction buffer, dNTP 400 μmol/L, 50 pmol of each primer, and 1.5 u Taq DNA polymerase in a total volume of 50 μL. The reaction mixture was overlaid with mineral oil and subjected to denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 70 s, 40 cycles for RT-PCR and 25 cycles for semiquantitative RT-PCR using a Perkin Elmer DNA Thermal Cycler 480. The products of PCR were analyzed by 0.7 % agarose gel electrophoresis and stained with ethidium bromide.

To quantitate PCR amplification of mRNA sequences, a relatively invariant β-actin mRNA was used as an internal standard in semiquantitative RT-PCR⁽⁹⁾.

Southern blot Southern blot analysis was performed according to the standard procedures⁽¹⁰⁾ using FUT2 cDNA released from plasmid pcDNA3-SeFT⁽¹¹⁾ as probes. The probes were labeled using Random Primed DNA Labeling kit (Boehringer Mannheim, GmbH, Germany) with [α -³²P]dATP. PCR products separated by agarose gel electrophoresis were transferred by capillary transfer onto Hybond-N membranes (Amersham) and then were subsequently hybridized with probes. After being rinsed in 2 × SSC (standard saline citrate) and 0.5 % SDS (sodium dodecylsulfate) at 25 °C, the blots were washed at 68 °C for 1 h in 0.1 × SSC and 0.5 %

SDS, exposed to X-ray film and analyzed by autoradiography.

Immunohistochemistry Pathogen-free female nude mice (BALB/c), 6 weeks old, were obtained from Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China, Certificate No 122) and were injected sc with 1 × 10⁷ tumor cells. After 4 weeks, the tumor was surgically removed from mice, embedded in Tissue-TekR OCT Compound (SAKURA, USA), and snap-frozen in liquid nitrogen. Sections (12 μm) were prepared and fixed in 4 % formaldehyde, and then quenched by 0.3 % H₂O₂ for 5 min to remove endogenous peroxidase activity, and washed in PBS. Non-specific binding was blocked by preincubation with blocking buffer containing 1 % bovine serum albumin. Sections were then stained with biotinylated UEA-I lectin (at concentration of 5 mg/L, Sino-American Biotech Com) at 37 °C for 60 min. After three washes, the sections were incubated with streptavidin-peroxidase 2 mg/L (Sigma) at 37 °C for 30 min before addition of diaminobenzidine (DAB).

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed by *t*-test.

RESULTS

In vitro and in vivo expression of H antigen on the surface of cells Flow cytometric analysis showed that SGC-7901, BEL-7404, and SPC-A-1 cells had MFI (mean fluorescence intensity) values of 162 ± 43, 81 ± 25, and 28 ± 17, respectively. In contrast, as negative control mouse cell lines NIH3T3 and B16 melanoma cells had MFI values of only 3 ± 1 (Tab 1). These data indicated that the human tumor cell lines were all intensely stained by UEA-I lectin which could recognize H antigen specifically.

Tab 1. Flow cytometric MFI values for the binding of FITC-UEA-I lectins to the cells. *n* = 3 experiments. $\bar{x} \pm s$. **P* < 0.01 vs control.

Cell lines	MFI
Control (B16)	3 ± 1
Control (NIH3T3)	3 ± 1
SGC-7901	162 ± 43 ^c
BEL-7404	81 ± 25 ^c
SPC-A-1	28 ± 17 ^c

To analyze *in vivo* expression of H antigen on the cell surface, experimental tumors were established with SGC-7901, BEL-7404, SPC-A-1, and B16 cells in nude mice (4 mice for each cell line group). Tumor tissues were sectioned and then incubated with biotinylated UEA-1 lectin and the UEA-1 lectin staining of cell membrane was observed in tissue sections isolated from human SGC-7901, BEL-7404, and SPC-A-1 cell tumors but not from mouse B16 cell tumor (Fig 1).

Expression of FUT2 gene in human tumor cell lines DNA fragments with the expected size of about 1.0 kb were obtained by RT-PCR from human SGC-7901, BEL-7404, and SPC-A-1 cells, but not from mouse NIH3T3 and B16 melanoma cells (Fig 2A), and were detected by FUT2 specific probes in Southern blot (Fig 2B). These fragments were digested by *Bam*HI into 0.87 kb and 0.66 kb fragments after 1.4 % agarose gel electrophoresis (Fig 3). This result is in accordance with *Bam*HI mapping of the FUT2 cDNA coding region^[8]. Semiquantitative RT-PCR was used to assess

the level of FUT2 mRNA in the cells. The results showed that FUT2 mRNA in SPC-A-1 cells were less in quantity than those in BEL-7404 and SGC-7901 cells, but no significant difference in levels of FUT2 mRNA was observed between BEL-7404 cells and SGC-7901 cells (Fig 4).

DISCUSSION

The present study shows that human tumor cell lines SPC-A-1, BEL-7404, and SGC-7901 all constitutively express the H blood group antigen on the cell membrane *in vitro* as well as *in vivo*, but the level of H antigen varies significantly from one tumor cell line to another. Human stomach cancer cell line SGC-7901 expressed the highest level of H antigen in all the cell lines tested.

Several lines of evidence have indicated that at least two distinct (H type and Secretor type) $\alpha(1,2)$ FTs are present in human tissues^[12,13]. The H gene (FUT1)-encoded $\alpha(1,2)$ FT (H type) is thought to regulate the

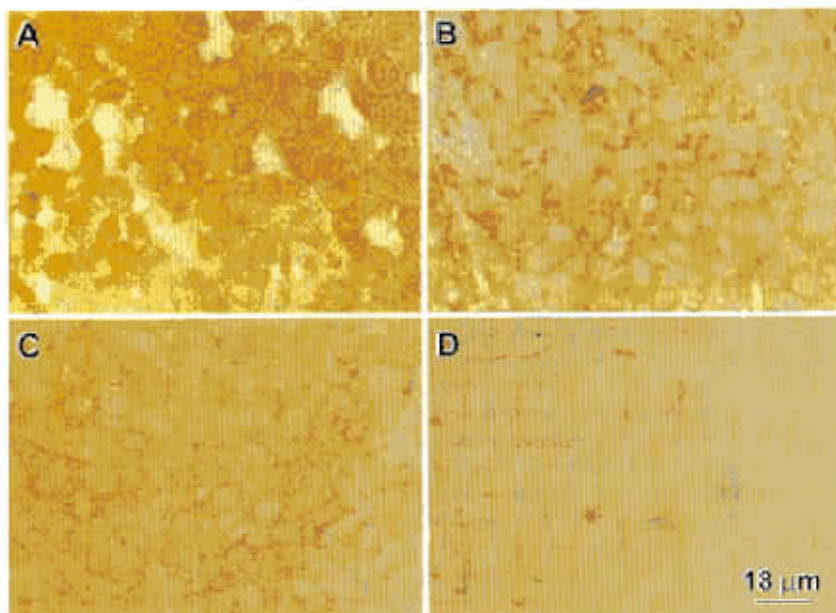


Fig 1. Immunohistochemical analysis with the biotinylated UEA-1 lectin of tumor tissues established with SGC-7901 (A), BEL-7404 (B), SPC-A-1 (C), and B16 (D) cell lines, respectively. $\times 500$.

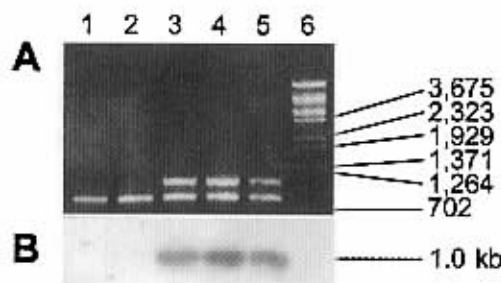


Fig 2. (A) RT-PCR amplification for detecting the FUT2 mRNA. (B) Southern blot analysis of specificity of RT-PCR products. 1) B16; 2) NIH3T3; 3) BEL-7404; 4) SGC-7901; 5) SPC-A-1; 6) λ DNA/Eco91I.

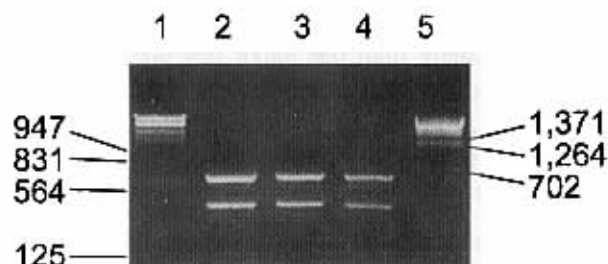


Fig 3. BamH I digestion analysis of RT-PCR products of about 1.0 kb in length. 1) λ DNA/EcoR I + Hind III; 2) BEL-7404; 3) SGC-7901; 4) SPC-A-1; 5) λ DNA/Eco91I.

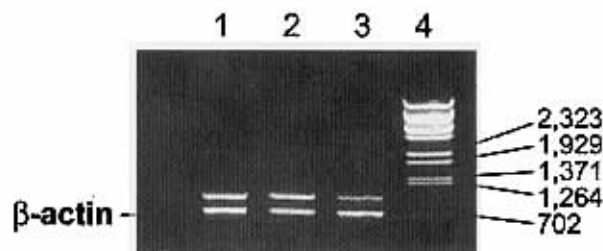


Fig 4. Results of semiquantitative RT-PCR. 1) BEL-7404; 2) SGC-7901; 3) SPC-A-1; 4) λ DNA/Eco91I.

expression of H antigen and thereafter A and/or B antigens in the erythroid lineage and in vascular endothelia, while the Secretor gene (FUT2)-encoded $\alpha(1,2)$ FT (Secretor type) is thought to regulate the expression of these antigens in the secretory glands and the digestive mucosa. Rouquier *et al*^[14] had indicated that a FUT2 transcript could be detected in poly (A)-rich RNA from normal small intestine, colon, and lung tissues. Koda *et al*^[15] detected the transcripts of FUT2 by Northern blot and RT-PCR analysis of the total RNA from ovarian cancer MCAS cells, colonic cancer cells WiDr and COLO201, and gastric cancer cells KATOIII and MKN74.

Here, the FUT2 mRNA was also detected by RT-PCR analysis of the total RNA from stomach cancer cells SGC-7901, liver cancer cells BEL-7404, and lung cancer cells SPC-A-1. These suggest that FUT2 gene contributes to the expression of H antigen on these cell membranes.

In addition, semiquantitative RT-PCR was performed to explore the mechanism by which the H antigen was expressed at a lower level on SPC-A-1 and BEL-7404 cells when compared with SGC-7901 cells. The results showed that reduced expression of H antigen on SPC-A-1 cells was accompanied by a decrease in the amount of FUT2 mRNA. This might represent an overall reduction of endogenous $\alpha(1,2)$ FT enzyme activity as suggested by Tanemura *et al*^[16] in the case of $\alpha(1,3)$ -galactosyltransferase. However, this could only partly explain why the level of H antigen was lower on the SPC-A-1 cell surface considering of that no significant difference in the amount of FUT2 mRNA levels was observed between BEL-7404 cells and SGC-7901 cells, although the H antigen level on the SGC-7901 cell surface was 2-fold of that on the BEL-7404 cells.

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FUT2 基因参与人肿瘤细胞系 BEL-7404、SGC-7901 和 SPC-A-1 上 H 血型抗原的表达

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关键词 ABO 抗原; 岩藻糖基转移酶类; FUT2 基因; 培养的肿瘤细胞

目的: 调查 H 血型抗原在人肿瘤细胞系 BEL-7404、SPC-A-1 和 SGC-7901 上的表达. **方法:** 免疫组织化学和流式细胞术分析细胞上 H 抗原在体内外的表达. RT-PCR, Southern 印迹和限制性酶切分析确定 FUT2 基因在细胞中的表达. **结果:** SGC-7901, BEL-7404 和 SPC-A-1 细胞表面 H 抗原表达的平均荧光强度分别为 162 ± 43 , 81 ± 25 和 28 ± 17 . 并且用 RT-PCR 从这些细胞中扩增出一约 1.0 kb DNA 条带, 此条带能被 FUT2 特异核酸探针检出. **结论:** 人肿瘤细胞系 BEL-7404, SPC-A-1 和 SGC-7901 于体内外在细胞表面均表达 H 抗原, 但表达水平在细胞之间变化显著. FUT2 基因的表达在细胞膜上产生这些抗原.

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