Genetic mechanisms for the synthesis of fucosyl GM1 in small cell lung cancer cell lines

Noriyo Tokuda\(^2\), Qing Zhang\(^2\), Shoko Yoshida\(^2,3\), Susumu Kusunoki\(^2\), Takeshi Urano\(^2\), Keiko Furukawa\(^2\), and Koichi Furukawa\(^1,2\)

\(^2\)Department of Biochemistry II, Nagoya University School of Medicine, 65 Tsukumai, Showa-ku, Nagoya 466-0065, Japan; \(^3\)Department of Internal Medicine II, Nagoya City University School of Medicine, 1 Kawasaki, Mizuho-ku, Nagoya 467-8602, Japan; and \(^2\)Department of Neurology, Kinki University School of Medicine, 377-2 Ohno-higashi, Sayama, Osaka 589-8511, Japan

Received on November 26, 2005; revised on July 9, 2006; accepted on July 10, 2006

Fucosyl GM1 has been reported to be specifically expressed in small cell lung cancer (SCLC) cells. However, the genetic basis for the synthesis of fucosyl GM1 has not been investigated. We analyzed the glycosyltransferases responsible for the synthesis of fucosyl GM1 in SCLC cell lines. In four SCLC cell lines expressing fucosyl GM1, both FUT1 and FUT2 mRNAs were detected, indicating that either one or both of α1,2-fucosyltransferases may be involved in the expression of fucosyl GM1. However, three of these four lines contained function-loss mutations in the FUT2 coding region, suggesting that FUT1 is mainly involved in the α1,2-fucosylation of GM1. The expression levels of the GM1 synthase gene showed no correlation with those of fucosyl GM1, whereas the co-transfection of GM1 synthase cDNA with FUT1 or FUT2 into SK-LC-17 clearly enhanced the neo-expression of fucosyl GM1, indicating its essential role. In contrast, the co-transfection of GD3 synthase cDNA reduced the expression levels of fucosyl GM1 with FUT1 or FUT2. Consequently, FUT1 seems to mainly contribute to the expression of fucosyl GM1, although both FUT1 and FUT2 are capable of generating the antigen. These results should promote the functional analysis of fucosyl GM1 leading to the development of novel therapies for SCLC.

Key words: fucosyl GM1/fucosyltransferase/H enzyme/small cell lung cancer

Introduction

The nervous tissues of vertebrates are enriched with gangliosides, sialic acid-containing glycosphingolipids, that play crucial roles in the maintenance of the tissue integrity and normal function (Wiegandt, 1985). Some of them are also expressed in neuroectoderm-derived cancer cells, such as malignant melanomas (Portoukalian et al., 1976; Carubia, et al., 1984), neuroblastomas (Cheung et al., 1985), and gliomas (Fredman et al., 1986). Therefore, gangliosides such as GD3 and GD2 have been considered to be tumor markers and have been utilized as targets for immunotherapy (Houghton et al., 1985). Recent studies using cDNAs for the synthetic enzymes of gangliosides have revealed that those antigens are involved in the malignant properties of cancer cells, such as the prominent proliferation and invasiveness (Yoshida et al., 2001; Hamamura et al., 2005).

Fucosyl GM1 is a member of the gangliosides with a unique structure consisting of a part of GM1 and α1,2-fucosylated galactose at the nonreducing end (Klenk, 1942). This structure was detected in particular animal cells under developmental regulation (Suchy et al., 1988; Kusunoki et al., 1992) and in restricted sites of mammalian nervous tissues (Kusunoki and Inoue, 1991; Kusunoki et al., 1992) and has sometimes become one of the target antigens in autoimmune neuronal diseases (Yoshino et al., 1993; Yuki and Ariga, 1997). Fucosyl GM1 is one of major gangliosides in the rat pheochromocytoma cell line, PC12 (Ariga et al., 1988; Nishio et al., 2004), and its expression was further enhanced by the transfection of GM1 synthase cDNA in our study (Ferrari et al., 1995). However, no rigorous study on the function of fucosyl GM1 in nervous systems has ever been performed, in contrast to GM1 (Ferrari et al., 1995; Mutoh et al., 1995; Nishio et al., 2004) or b-series gangliosides (Fukumoto et al., 2000).

More than 10 years ago, Lindholm and others reported that fucosyl GM1 was expressed in human small cell lung cancer (SCLC) cells (Nilsson et al., 1986). Livingston and others extensively analyzed the expression pattern of fucosyl GM1 in human normal tissues and various cancer tissues (Brezicka et al., 1989; Zhang et al., 1997), showing that fucosyl GM1 was expressed in very few normal tissues and also in SCLC tissues. Consequently, fucosyl GM1 has been considered to be a candidate for a tumor marker for SCLC (Vangsted et al., 1994). Furthermore, Livingston and others have tried to develop a vaccine with fucosyl GM1 for the treatment of patients with SCLC and have reported significant responses of anti-fucosyl GM1 antibodies in the patients who underwent vaccination with fucosyl GM1 conjugated with KLH (Dickler et al., 1999). These results indicate that fucosyl GM1 can be a target antigen in antibody immunotherapy for SCLC. However, no studies have ever been performed on the mechanisms for the synthesis and expression of fucosyl GM1.

In the present study, glycosyltransferases responsible for the synthesis of fucosyl GM1 in SCLC cell lines were analyzed. Simultaneously, other factors that may affect the efficiency of the synthesis and expression of fucosyl GM1 were also investigated. These results should promote the functional analysis of fucosyl GM1 in human cancer cells and contribute to the development of novel therapies for the refractory disease.

© The Author 2006. Published by Oxford University Press. All rights reserved. For permissions, please e-mail: journals.permissions@oxfordjournals.org

\(^1\)To whom correspondence should be addressed; e-mail: koichi@med.nagoya-u.ac.jp
Results

Specific expression of fucosyl GM1 in SCLC cell lines

In a previous study, we reported that GM1 was present in almost all SCLC and non-SCLC cell lines (Figure 1A) (Yoshida et al., 2001). Furthermore, we analyzed the expression of fucosyl GM1 in 22 SCLC cell lines and 22 non-SCLC cell lines with flow cytometry (Figure 1A). It was demonstrated that four SCLC cell lines significantly presented this ganglioside on the cell surface (Figure 1B). Conversely, no non-SCLC cell lines had fucosyl GM1 on the cell surface. Furthermore, to determine whether this glycolipid exists in the cytosol, we attempted to detect fucosyl GM1 using immunocytochemistry. This ganglioside was only detected in the same four SCLC cell lines showing fucosyl GM1 expression on the cell surface (data not shown). These results were in accordance with the report that the expression of this antigen was specifically associated with SCLC cell lines (Nilsson et al., 1986; Brezicka et al., 1989; Zhang et al., 1997).

Expression levels of genes relevant to biosynthesis of fucosyl GM1

Fucosyl GM1 is generated by adding α1,2-linked fucose to the galactose of GM1 at the last step of biosynthetic pathway. In humans, FUT1 and FUT2 enzymes are known as functional α1,2-fucosyltransferases. To analyze the correlation between the expression of fucosyl GM1 and each of the α1,2-fucosyltransferases, we investigated the expression levels of FUT1 and FUT2 genes in the SCLC cell lines with northern blotting (Figure 2). FUT1 mRNA was detected in many cell lines, and FUT2 mRNA was weakly detected in only two SCLC cell lines. It is showed that all cell lines expressing fucosyl GM1 strongly expressed the FUT1 gene, and two of them, that is, SBC1 and NCI-H69, also expressed FUT2 gene. FUT1 was also expressed in some of the fucosyl GM1-negative cell lines.

Furthermore, the expression levels of the FUT1 and FUT2 genes were determined using a quantitative real-time reverse transcription–polymerase chain reaction (RT–PCR) analyzer. In this study, we used 11 SCLC cell lines, including the FUT1 gene-positive and FUT1 gene-negative lines indicated by northern blotting analysis. As shown in Figure 3, the expression levels of the FUT1 and FUT2 genes in fucosyl GM1-positive cell lines were relatively high. The statistical analysis of FUT gene expression levels between fucosyl GM1-positive and fucosyl GM1-negative groups revealed that p-value for FUT2 was 0.03 and that for FUT1 was 0.06. However, p-value for FUT1 was <0.001, when the value of ACC-LC-5 expressing extraordinarily high GD3 synthase was eliminated.

![Fig. 1.](https://academic.oup.com/glycob/article-abstract/16/10/916/782275)
Ganglioside GM1 is a precursor of fucosyl GM1, and the biosynthesis of GM1 is an essential step for the expression of fucosyl GM1. In addition, GD3 metabolism is also a crucial step for the biosynthetic pathway of fucosyl GM1, because GD3 synthase competes with GM1 synthase for the common precursor GM3 via GM2 (Figure 4). To clarify whether the activities of GM1 synthase and GD3 synthase affect the expression of fucosyl GM1 on the cell surface, we measured the expression levels of the GM1 synthase and GD3 synthase genes (Figure 3). There was no correlation between the expression levels of the GM1 synthase gene and those of fucosyl GM1 in the SCLC cell lines. As for GD3 synthase gene, it was hard to find statistical differences between the expression levels of the GD3 synthase gene and those of fucosyl GM1. This is because there were no cell lines that expressed significant levels of GD3 synthase in the fucosyl GM1-positive samples, and GD3 synthase levels were widely dispersed from zero to very high in the fucosyl GM1-negative samples. Thus, it was concluded that the expression levels of GD3 synthase gene largely affect the expression of fucosyl GM1, because fairly high levels of GD3 synthase expression resulted in no expression...
of fucosyl GM1, even if they showed high levels of α1,2-fucosyltransferases.

Expression of blood group antigens containing α1,2-linked fucose

Similar to fucosyl GM1, some of the histo-blood group antigens contain an α1,2-linked fucose, and they are also synthesized from the precursors by the FUT1 or FUT2 enzyme. To determine whether the high expression of the FUT1 and FUT2 genes affects the expression of those antigens, we analyzed the expression levels of H (type 1 and type 2), Lewis b, and Lewis y antigens on the cell surface with flow cytometry. As shown in Figure 5, the cell lines that showed relatively high levels of α1,2-fucosyltransferases expressed these antigens prominently. H Antigen was not detected on the NCI-H69 cell line, presumably as a result of complete metabolic conversion to Lewis b and/or Lewis y antigens. These results suggested that high levels of the α1,2-fucosyltransferase gene expression lead to the synthesis of histo-blood group antigens as well as fucosyl GM1 in SCLC cell lines.

Both FUT1 and FUT2 were capable to synthesize fucosyl GM1 in the transfectant cells

Although it was suggested that both FUT1 and FUT2 genes are involved in the synthesis of fucosyl GM1, it has not been clear whether FUT1 and FUT2 really have the ability to transfer fucose to GM1 in the SCLC cells. We examined this issue with the SK-LC-17 cell line, which does not express fucosyl GM1 and weakly expresses GM1. FUT1/FUT2 and GM1 synthase cDNAs were transiently introduced into SK-LC-17. Although fucosyl GM1 was not clearly expressed by the transfection with either FUT1 or FUT2 cDNA alone, and cells transfected with only GM1 synthase cDNA did not express fucosyl GM1 (Figure 6A), the co-transfection of FUT1 or FUT2 cDNA with GM1 synthase cDNA resulted in the definite expression of fucosyl GM1 on the cell surface (Figure 6B). Fucosyl GM1 expression was strongly inhibited by the co-transfection of GD3 synthase cDNA (Figure 6C). These results indicated that both FUT1 and FUT2 act onto GM1 to generate fucosyl GM1 in the presence of a sufficient amount of GM1, and GD3 synthase acts as a competitor in the biosynthesis of fucosyl GM1, even when GM1 synthase and α1,2-fucosyltransferase were sufficiently expressed.

Frequent mutations in the FUT2 gene in fucosyl GM1-positive cell lines

The inactivating mutations of the FUT1 and FUT2 genes have been reported. FUT1 inactivating mutations are very rare and responsible for the Bombay phenotype characterized by the lack of ABH antigen on erythrocytes and vascular endothelium (Kaneko et al., 1997). In contrast, FUT2 inactivating mutations are more frequent and responsible for the nonsecretor phenotype (Table I). Individuals with these FUT2 inactivated forms have been typed “se.” It is found in ~20% of the European and North American populations. A low-activity mutation, A385T, named sej, retains ~3% of the FUT2 activity (Kudo et al., 1996), and this
mutation is found in ∼16% of the Japanese population (Narimatsu et al., 1998). Then, we analyzed FUT2 gene mutations with direct sequencing of genome DNA from SCLC cell lines. In 21 cell lines, nine cell lines did not have any mutations (Type Se/Se), as summarized in Table II, and 11 lines had homozygous mutations (Type se/se or sej/sej). Just one cell line had a heterozygous mutation (Type Se/sej).

Among the fucosyl GM1-positive cell lines, two cell lines had low-functional FUT2 with A385T, and one cell line had inactivated FUT2 with G428A. Therefore, it was indicated that the expression of fucosyl GM1 does not depend on the activity of FUT2 enzyme in these cell lines. Thus, it is suggested that FUT1 enzyme is primarily involved in the expression of fucosyl GM1 on the cell surface in SCLC cell lines.

The FUT2 mutation, se/se and sej/sej, lead to the loss of fucosyl GM1 synthetic activity

To clarify whether the mutations, se/se and sej/sej, lead to the complete loss of fucosyl GM1 synthetic activity, we analyzed the expression patterns of fucosyl GM1 in the cells transfected with these mutant genes. Although the co-transfection...
of FUT2 wild-type Se/Se derived from SBC1 cell line and GM1 synthase cDNA resulted in the definite expression of fucosyl GM1 on the cell surface, the co-transfection of se/se derived from NCI-H69 cell line and GM1 synthase cDNA did not induce the expression of fucosyl GM1 on the cell surface at all (Figure 7). In the case of co-transfection of sej/sej derived from ACC-LC-48 cell line and GM1 synthase cDNA, the expression of fucosyl GM1 was observed, although the expression level was much lower than that by Se/Se. The transfection of sej/sej mutant cDNA derived from ACC-LC-170 cell line also resulted in a low expression level of fucosyl GM1 just as sej/sej from ACC-LC-48 cell line (data not shown).

**Discussion**

Fucosyl GM1 has been thought to be specifically expressed in SCLC cells in both cell lines and tumor tissues (Nilsson et al., 1986; Brezicka et al., 1989; Zhang et al., 1997). Our study results with cultured cell lines also indicated that fucosyl GM1 is specifically expressed in SCLC cells. Among glycosyltransferases involved in the synthesis of fucosyl GM1, the expression levels of the GM1 synthase gene did not show any correlation with the expression pattern of fucosyl GM1 in lung cancer cell lines. This might be because the GM1 synthase gene was broadly expressed in the majority of cell lines although at low levels, and it might be sufficient to provide some level of the precursor for the synthesis of fucosyl GM1. In contrast, the expression of FUT1 and/or FUT2 was considered to be essential for the synthesis of
fucosyl GM1, and the expression levels of the GD3 synthase gene oppositely affected fucosyl GM1 expression. In some cell lines in which the GM1 synthase level is relatively low, the co-transfection of GM1 synthase cDNA was sufficient for FUT1/FUT2 action to make cells express fucosyl GM1. In fact, GM1 synthase and either or both of FUT1/FUT2 effectively bring about fucosyl GM1 in SK-LC-17 cells, indicating its essential involvement in the synthesis of fucosyl GM1.

Both FUT1 and FUT2 are defined as α1,2-fucosyltransferases involved in the synthesis of blood group H antigen, that is, Fucα1,2Galβ1,3/4GlcNAc—structures on glycoproteins and glycolipids (Koda et al., 2001). They are differentially expressed among various tissues and utilize different acceptor structures. FUT1 is expressed in human erythrocytes and vascular endothelial cells and synthesizes type 2 H antigens using type 2 precursor structures. Very rare cases with function-loss mutations in the FUT1 gene have been called the Bombay or para-Bombay type, with the co-existence or noncoexistence of the FUT2 mutation, respectively (Koda et al., 2001). FUT2 is mainly expressed in secretions, the gastrointestinal tracts, bronchial tracts, and genital organs and catalyzes the synthesis of type 1 H antigens on mucins. This reaction is, therefore, essential for the synthesis of blood group-carrying mucins such as A, B, and H. Thus, FUT2 has been called Sec enzyme (secretion), because its activity determines the presence of blood group antigens in the saliva.

Preferential usages of type 2 and type 1 chains with FUT1 and FUT2, respectively, were also observed in the transfectant cells with cDNA expression plasmids of individual glycosyltransferases (Lopez-Ferrer and de Bolos, 2002; Mathieu et al., 2004). For example, the expression of FUT1 induced type 2 H and Lewis y or Lewis b antigens and the down-regulation of sialyl Lewis x but did not affect the expression of sialyl Lewis a. As for the synthesis of other type H antigens, such as type 3/4 chains, no definite findings about human fucosyltransferases have been reported, although some animal (rat and bovine) α1,2-fucosyltransferases have been known to prefer Galβ1,3GalNAc acceptor substrates (Barreaud et al., 2000; Sherwood et al., 2001). Bovine fut1 and fut2 synthesize fucosyl GM1 almost equally (Barreaud et al., 2000), whereas the human Se enzyme (FUT2) was reported to act on type 1 and type 3 chain acceptors (Clausen and Hakomori, 1989; Sarnesto et al., 1990). In fact, the preferential production of type 3 H chain with FUT2 has not been reported (Lofling et al., 2002). However, little is known about the α1,2-fucosyltransferase responsible for the synthesis of fucosyl GM1 in human tissues.

Thus, whether both FUT1 and FUT2 or either one of them is involved in the synthesis of fucosyl GM1 in SCLC is difficult to be clearly determined based on the obtained results and on the past findings. In fact, these two genes were up-regulated in fucosyl GM1-positive SCLC cell lines, and they behaved in a similar manner in the sublines generated from ACC-LC-170 (data not shown). Furthermore, both of them could induce the expression of fucosyl GM1, when co-transfected into a fucosyl GM1-negative cell line. This was more obvious when co-transfected with GM1 synthase cDNA. The fact that three of four fucosyl GM1-positive SCLC cell lines contained mutated FUT2 gene indicated that FUT1 might be responsible for the synthesis of fucosyl GM1 in vivo. Although many reports indicating that similar glycosyltransferase-like molecules function as a molecular chaperone of a real enzyme have recently appeared (Ju and Cummings, 2002), FUT1 seems capable of acting independently in SCLC cells. However, the possibility that FUT1 and FUT2 perform their function more efficiently when present together than when being isolated cannot be excluded, although no additive effects were observed when co-transfected.

As for affinities of the two enzymes toward GM1, there were already reports on the kinetics of FUT1 and FUT2 toward GM1. In bovine, it was shown that $K_m$ values of fut1 and fut2 for GM1 were 1.29 and 1.42 (mM), respectively (Barreaud et al., 2000). In mice, $K_m$ values of Fut1 and Fut2 toward GM1 were 909.1 and 500.0 (μM), respectively (Iwamori and Domino, 2004). All these data suggested that these two enzymes might not have so different affinities toward GM1.

The implications of the expression of α1,2-FUTs in malignant tumor cells have been studied using cloned animal and human cDNAs. In a rat colon carcinoma cell line, the reduction of α1,2-fucosyltransferases FTA or FTB (human FUT1 and FUT2 homologs) with antisense cDNA resulted in an increased tumorigenicity or reduced tumorigenicity, respectively (Hallouin et al., 1999), suggesting differential protein fucosylation among two fucosyltransferases leading to opposite effects. They also affected the sensitivity to apoptosis in the transfected cells (Goupille et al., 2000). In human pancreatic cancers, α1,2-fucosyltransferase activities were generally down-regulated (Mas et al., 1998), and the restoration of the activity with FUT1 cDNA decreased the adhesive and metastatic properties (Aubert et al., 2000). Thus, the expression of FUTs appears to affect the expression of ligands of selectins (Mathieu et al., 2004), thermosensitivity (Okamura et al., 2002), and metastatic properties (Aubert et al., 2000). However, precise α1,2-fucosylated structures should be various and have not been clarified in individual experiments.

Whether fucosyl GM1 plays key roles in malignant tumors has not been known at this moment. Because the generation of high expressants of fucosyl GM1 became possible based on the results obtained in this study, the implications of this antigen in the cell proliferation and invasion in SCLC cells will be easily investigated. Although targeted deletions of the FUT1 and FUT2 did not show any serious defects in mice (Domino et al., 2001), fucosyl GM1 has been considered to be involved in the signal control (Kosugi et al., 1987). Fucosyl GM1 has also been identified as a target molecule in neurological disorders caused by autoantibodies (Yoshino et al., 1993; Yuki and Ariga, 1997). These facts might suggest that fucosyl GM1 is an important functional molecule expressed on the cell surface of some neuronal cell groups, and it may also play critical roles in the malignant properties of human cancer cells.

The expression of fucosyl GM1 in human tissues should be very restricted compared with that in mice (Iwamori and Domino, 2004) according to the results of immunohistochemical analyses (Zhang et al., 1997). In mice, the $K_m$ values of
Fut1/Fut2 for GM1 are 5–10 times more than those for nLc4 and Lc4 (Iwamori and Domino, 2004). These findings suggest that antibody therapy might be effective against SCLC particularly because of its specific expression, as fucosyl GM1 can be expressed only when nLc4/Lc4 structures are less abundant and the GD3 synthase activity is not strong.

The discrepancy in the incidence of fucosyl GM1 expression between cell lines and tissue samples, as analyzed in this study and reported by Brezicka et al. (1989) and Zhang et al. (1997), respectively, is probably because of the changes in the cell phenotypes during adaptation to in vitro culture system after resection. Alternatively, fucosyl GM1 may be a cryptic antigen or may be present in the cytoplasm. Our restricted study of immunocytochemistry after fixation suggested that the former interpretation is more likely. Therefore, the possibility that fucosyl GM1 can be used as a target in the immunotherapy of SCLC, such as monoclonal antibodies (Brezicka et al., 1991) and vaccination (Krug et al., 2004), should be convincing, although this issue remains to be carefully investigated. Combined antibody therapy toward multiple tumor antigens might be more effective, as suggested in recent reports (Brezicka et al., 2000; Livingston et al., 2005).

Materials and methods

Cell lines and culture

All human lung cancer cell lines were kindly provided by Dr. T. Takahashi (Nagoya University, Nagoya). Among SCLC cell lines, all ACC-series cell lines (almost Japanese), NCI-N417, and SBC1 and SBC3 were derived from Asian patients. NCI-H69 was derived from a Caucasian patient. They were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), 100 U/mL of benzylpenicillin, and 100 μg/mL of streptomycin sulfate, at 37°C in a humidified 5% CO2 atmosphere.

Flow cytometry

The cell surface expression of glycolipids was analyzed with FACScan (Becton Dickinson, Mountain View, CA). The cells were incubated with anti-fucosyl GM1 monoclonal antibody CDR73–6 (Kusunoki et al., 1994), anti-Lewis b monoclonal antibody (Seikagaku, Tokyo, Japan), or anti-Lewis y monoclonal antibody (Seikagaku) for 45 min on ice and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgM antibody (Zymed, San Francisco, CA) or IgG antibody (Cappel, Durham, NC). To analyze the expression of GM1, we incubated cells with the cholera toxin (CT) B subunit–biotin conjugates (List Biological Laboratories, Campbell, CA) for 45 min on ice and then incubated with FITC-conjugated avidin (EY Laboratories, San Mateo, CA). Control samples were prepared using the second antibody alone. The intensity of staining was measured and presented in arbitrary units as the log of the fluorescence intensity.

Northern blotting

Total RNA was isolated from lung cancer cell lines with Trizol reagent (Invitrogen, Rockville, MD) according to the manufacturer’s instructions. Fifteen micrograms each of formamide-denatured total RNA was separated by an agarose gel and transferred onto a nylon membrane, GeneScreen Plus (Du Pont New England Nuclear, MA). After baking, the membrane was prehybridized for 3 h and hybridized with [32P]dCTP-labeled cDNA fragment of FUT1 or FUT2 as previously described (Yamashiro et al., 1993). The membrane was washed twice with 2× SSC and 1% SDS at 60°C twice, and finally once with 0.1× SSC at room temperature and then exposed to an imaging plate (Fujifilm, Kanagawa, Japan) for 15 h at room temperature. The hybridized bands were detected by BAS-2000 (Fujifilm). MCAS and WiDr were positive controls of the FUT2 gene, as reported (Koda et al., 1997).

Quantitative real-time RT–PCR

The RNA from each cell line was reverse-transcribed into first-strand cDNA with an oligo dT primer. The quantitative RT–PCR analysis was performed using a DNA Engine Opticon 2 System (Bio-Rad Laboratories, Hercules, CA) with a DynaMo SYBR Green qPCR Kit (Finnzymes Oy, Espoo, Finland) according to the manufacturer’s protocol. The amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was measured as an internal standard. The PCR was performed using primers specific to each gene through 40 cycles of 95°C for 20 s, 60°C for 10 s, and 72°C for 10 s after preincubation at 95°C for 10 min. The specific primers used for amplification had the following sequences: FUT1-F (5′-CTGCGCAGACTCTGAGTTCCC-3′), FUT1-R (5′-AGGCTTAGCCGAATGGCTGAA-3′), FUT2-F1 (5′-CTGGTGTTGCTAGTGCTT-3′), FUT2-R1 (5′-ACTCCACCATGGCTTTAAT-3′), GM1 synthase-F (5′-GTCGCCAGGGCGCGGGG-3′), GM1 synthase-R (5′-TCCTTGTTCAAGTTCTCGGA-3′), GM2 synthase-F (5′-AGAGGGTCAGGCAGATCTCA-3′), GM2 synthase-R (5′-CGGACTGTGTCTGCTGTT-3′), GM3 synthase-F (5′-AACCCAGAACACCTTTGCAC-3′), GM3 synthase-R (5′-TCACACCTCCTTGGAC-3′), GD3 synthase-F (5′-CAGCATAATTCCGGCAAGGT-3′), GD3 synthase-R (5′-ATTGCCACCAATCTCAGCA-3′), GAPDH-F (5′-GTCAGTTGGTGACCTGAC-3′), and GAPDH-R (5′-TGCTGTAGCAGGCGGCGTT-3′).

Polymorphism of the FUT2 gene

A part of coding region of the FUT2 gene (185–941) was amplified with PCR using genomic DNA isolated from lung cancer cell lines. PCR was performed with the following primers: FUT2-F2 (5′-GGAACCAGATGGGCGAGTAC-3′) and FUT2-R2 (5′-GCTGCTCTCTGCTTAAAGATT-3′). Then, the product was separated using an agarose gel, followed by extraction from the gel using a QIAEX II Gel Extraction Kit (Qiagen GmbH, Hilden). The purified products were sequenced with the dideoxy termination method using an ABI PRISM 3100 Gene Analyzer (Applied Biosystems, Foster City, CA).

Construction of the expression vectors

pCDM7-FUT1 and pcDNA1-FUT2 were kindly provided by Dr. J.B. Lowe (Larsen et al., 1990; Kelly et al., 1995). The expression vector pcDNA3.1-FUT1 was prepared by
inserting an XhoI–XbaI fragment from pCDM7-FUT1 into the XhoI–XbaI site of a pcDNA3.1 vector (Invitrogen). pMIMkneo-MIT-9 vector was prepared by inserting an XhoI–XbaI fragment from rat GM1 synthase cDNA clone pMIT-9 (Miyazaki et al., 1997) into the XhoI–XbaI sites of a pMIMkneo vector (presented by Dr. Maruyama at Tokyo Medical and Dental University).

Gene transfection

SK-LC-17 cells in a 60-mm plastic tissue-culture plate (Becton Dickinson) at a density of 3 × 10^5 cells/mL plate were transfected transiently with pMIMkneo-MIT-9, pcDNA3.1-FUT1, and/or pcDNAI-FUT2 using LipofectAMINE reagent (Invitrogen) according to the manufacturer’s instructions. After the cells were cultured for 60 h, the glycolipid antigens expressed on the cell surface were detected with flow cytometry analysis.

ACC-LC-170 subcloning

Subclones of the ACC-LC-170 parent cell line were established by the limited dilution method. The ACC-LC-170.10 subline with a low expression of fucosyl GM1 on the cell surface and the ACC-LC-170.31 subline with a high expression of this glycolipid based on the flow cytometry with monoclonal antibody CDR73–6 were established.

Acknowledgments

We thank Ms. Mizuno and Ms. Nakayasu for excellent technical assistance. This study was supported by a Grant-in-Aid for Scientific Research on Priority Area (14082102) from the Ministry of Education, Culture, Science, Sports and Technology of Japan.

Conflict of interest statement

None declared.

Abbreviations

FUT (or fut), fucosyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT–PCR, reverse transcription–polymerase chain reaction; SCLC, small cell lung cancer.

References


Klenk, E. (1942) Über die ganglioside, eine neue gruppe von zuckerhalti-
Kudo, T., Iwasaki, H., Nishihara, S., Shinya, N., Ando, T., Narimatsu, I.,
cloning sequence, and expression of a human GDP-L-fucose:
fucosyltransferase from human serum.
Purification of H gene-encoded beta-galactoside alpha, 1–2 fucosyl-
An amino acid region at the N-terminus of rat hepatoma alpha1–2 fucosyltransferase modulates enzyme activity and interaction with lipids; strong preference for glycosphingolipids containing terminal Galbeta1,3GlcNAc-structures. Biochemistry. 40, 5708–5719.
Kusunoki, S., Inoue, K., Iwashita, M., Nishihara, S., Ando, T., Nariamtsu, I.,
Nishio, M., Fukushima, S., Furukawa, K., Ichimura, A., Miyazaki, H.,
Yamashiro, S., Ruan, S., Furukawa, K., Tai, T., Lloyd, K.O., Shiku, H., and Furukawa, K. (1993) Genetic and enzymatic basis for the differen-
Yoshino, H., Ariga, T., Latov, N., Miyatake, T., Kushiy, K., Kasama, T.,
vous tissue is a target antigen in patients with autoimmune neuropa-
Yuki, N. and Ariga, T. (1997) Antibodies to fucangangliosides in neurolog-