Effects of dibutyryl cAMP and bromodeoxyuridine on expression of N-acetylglucosaminyltransferases III and V in GOTO neuroblastoma cells

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The sugar chain structures of the cell surface change dramatically during cellular differentiation. A human neuroblastoma cell line, GOTO, is known to differentiate into neuronal cells and Schwannian cell-like cells on treatments with dibutyryl cAMP and bromodeoxyuridine, respectively. We have examined the expression of UDP-N-acetylglucosamine: β-D-mannoside β-1,4N-acetylglucosaminyltransferase III (GnT-III: EC 2.4.1.144) and UDP-N-acetylglucosamine: α-6-D-mannoside β-1,6N-acetylglucosaminyltransferase V (GnT-V: EC 2.4.1.155), two major branch forming enzymes in N-glycan synthesis, in GOTO cells on two distinct directions of differentiation.

In neuronal cell differentiation, GnT-III activity showed a slight increase during initial treatment with Bt2cAMP for 4 days and decreased drastically after the fourth day, but the mRNA level of GnT-III did not show a decrease but in fact a slight increase. GnT-V activity increased to approximately two- to three-fold the initial level with increasing mRNA level after 8 days, and lectin blot analysis showed an increase in reactivity to Datsura stramonium (DSA) of the immunoprecipitated neural cell adhesion molecule (NCAM). In Schwannian cell differentiation, the activity and mRNA level of GnT-III showed no significant change on treatment with BrdU. GnT-V activity also showed no change in spite of the gradual increase in the mRNA level. These results suggest that the activation of GnT-V during neuronal cell differentiation of GOTO cells might be a specific change for branch formation in N-glycans, and this affects the sugar chain structures of some glycoproteins such as NCAM.

Keywords: glycosyltransferase, N-acetylglucosaminyltransferase, neuroblastoma, cell differentiation, cAMP, bromodeoxyuridine

Abbreviations and trivial names: GnT, N-acetylglucosaminyltransferase; Bt2cAMP, N6, O6-dibutyryl cAMP; BrdU, bromodeoxyuridine; DSA, Datsura stramonium; NCAM, neural cell adhesion molecule; PAGE, polyacrylamide gel electrophoresis.

Introduction

The oligosaccharide structures of the cell surface undergo significant changes during embryogenesis, differentiation and malignant transformation [1, 2]. These structural changes are closely related to the changes in and balance of glycosyltransferase and glycosidase activities. UDP-N-acetylglucosamine: β-D-mannoside β-1,4N-acetylglucosaminyltransferase III (GnT-III) and UDP-N-acetylglucosamine: α-6-D-mannoside β-1,6N-acetylglucosaminyltransferase V (GnT-V) play important roles in the biosynthesis of N-glycan branches. GnT-III makes a bisecting-GlcNAc structure in the tri-mannose cores of N-glycans [3], and is implicated in cell differentiation and malignancy-associated changes of N-glycans [4–7]. GnT-V makes a β1-6 branch on the α1-6 mannosides of N-glycans [8], and the β1-6 branch structure is thought to be highly associated with metastatic potential [6, 7, 9]. Both the GnT-III [10, 11] and GnT-V [12, 13] genes have already been cloned. These branching patterns of N-glycans directly influence subsequent processing of sugar chain structures.

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The relation between cell differentiation and glycosyltransferase activities has been investigated by several groups. GnT-II to V activities significantly increased upon the differentiation of human colonic adenocarcinoma CaCo-2 cells [14]. The elevation of GnT-III activity in differentiated CaCo2 cells suggested that this enzyme might be partly responsible for the decreased synthesis of polyactosamine-glycans upon differentiation, because GnT-III activity is known to inhibit further branching and galactosylation [15, 16]. On the other hand, the differentiation of murine embryonal carcinoma F9 cells was closely associated with the induction of multiple glycosyltransferase activities, the most pronounced increases being in GnT-V and Core2GnT, which control the levels of β1-6GlcNAc-branched N- and O-linked oligosaccharides, respectively, which are good substrates for polyactosamine elongation [17]. These observations suggest the importance of the branch-forming glycosyltransferases (i.e. GnT-III, IV and V), because the branch-formation of N- and O-linked oligosaccharides is markedly influenced upon cell differentiation. The glycosyltransferase changes during cell differentiation, however, seem to differ with each specific cell type.

To investigate the changes of GnT-III and GnT-V expression during neural cell differentiation, we examined GnT-III and V activities, and mRNA expression of cultured human neuroblastoma GOTO cells [18], that are known to differentiate into neuronal cells with Bt2cAMP [19], and into Schwannian cell-like cells with BrdU [20].

Materials and methods

Cell culture

Human neuroblastoma GOTO cells were obtained from the Japanese Cancer Research Resources Bank (Tokyo). GOTO cells were cultured in 1:1 (v/v) RPMI1640 and Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and kanamycin (0.1 mg ml⁻¹) under a humidified atmosphere of 95% air and 5% CO₂. The cells were plated at 0.5-1 × 10⁵ cells per ml cm⁻². After 24 h subculture, the cells were induced by adding 1 mM Bt2cAMP [19] or 5 μg ml⁻¹ BrdU [20]. Both cultures were refed with fresh medium containing the same reagents every 48 h. The cells were cultured in the presence of the differentiating agents for 8-10 days and then harvested for analyses.

Glycosyltransferase assays

Cultured cells were harvested by scraping, washed twice with phosphate-buffered saline (PBS), pH 7.2, and then resuspended in 0.1 ml PBS. After sonication, GnT-III and GnT-V activities in cell lysates were determined. The GnT-III and GnT-V assays were carried out as described previously using a fluorescence-labelled sugar chain, GlcNAcβ1 - 2Manα1 - 6(GlcNAcβ1 - 2Manα1 - 3)Manβ1 - 4GlcNAcβ1 - 4GlcNAc-PA (pyridylamino) as a substrate [21]. Protein concentrations were determined with a BCA protein assay kit (Pierce) using bovine serum albumin as a standard.

Northern blot analysis

Total RNA was prepared from the cells according to the method of Chomczynski and Sacchi [22]. Twenty μg of RNA was electrophoresed on a 1% agarose gel and then transferred onto a Zeta-probe membrane (BioRad) by capillary action. The membranes were hybridized for 18 h with ³²P-labelled human GnT-III cDNA, a fragment lying between Sac I sites [11] or human GnT-V cDNA [13] as a probe at 42 °C in a prehybridization solution comprising 50% formamide, 6XSSC, 15 mM sodium citrate and 150 mM NaCl, pH 7.0, 5 × Denhardt's solution, 0.5% SDS and 10% polyethylene glycol 8000. The membranes were rinsed for 30 min at 55 °C in 2XSSC and 0.1% SDS, then rinsed once more for 10 min at room temperature in 2XSSC without SDS, and then exposed to X-ray film (Kodak) with an intensifying screen at -80 °C for 2-7 days. The densitometric measurements representing GnT-III or GnT-V were normalized as to those of the respective β-actin or 28S rRNA band.

Immunoprecipitation of NCAM (neural cell adhesion molecule)

Cultured cells were harvested, washed twice with ice cold PBS, and then incubated for 30 min on ice with 0.5 ml of the lysis solution, i.e. 20 mM Tris/Cl (pH 8.0) containing 133 mM NaCl, 10% glycerol, 1% NP-40, and aprotinin (1 μg ml⁻¹). The lysates were centrifuged at 15 k rpm for 15 min at 4 °C. The supernatants were precleared using normal rabbit serum and protein-G-Sepharose 4FF (50% suspension, Pharmacia). After preclearing, the lysates were incubated for 3 h at 4 °C with an anti-human NCAM (CD56) monoclonal antibody (Becton Dickenson): 10 μg. Protein-G-Sepharose 4FF was added, and then the mixture was incubated for 1 h at 4 °C. After centrifugation at 2000 rpm for 5 min, the Sepharose was washed with the lysis buffer four times. The immunoprecipitated NCAM protein was eluted by boiling in Laemmli's sample solution (62.5 mM Tris/Cl (pH 6.8), 2% SDS, 10% glycerol and 0.002% bromophenol blue) including 5% 2-mercaptoethanol. The eluted samples were subjected to 6% SDS-PAGE and then analysed by lectin blotting as described below.

Lectin blot analysis

NCAM proteins were immunoprecipitated from control GOTO cells and GOTO cells treated with Bt2cAMP (8 days) or BrdU (10 days) as described above. The eluted NCAM samples were electrophoresed on a 6% SDS-PAGE gel. After electrophoresis, the gel was soaked in...