The Relationship between the Branch-Forming Glycosyltransferases and Cell Surface Sugar Chain Structures†

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ABSTRACT: Many recombinant proteins developed or under development for clinical use are glycoproteins, and trials aimed at improving their bioactivity or pharmacokinetics in vivo by altering specific glycan structures are ongoing. For pharmaceuticals of glycoproteins, it is important to characterize and, if possible, control the glycosylation profile. However, the mechanism responsible for the regulation of sugar chain structures found on naturally occurring glycoproteins is still unclear. To clarify the relationship between glycosyltransferases and sugar chain branch structure, we estimated six glycosyltransferases' activities (*N*-acetylglucosaminyltransferase (GlcNAcTase)-I, -II, -III, -IV, -V, and β-1,4-galactosyltransferase (GalT)) which control the branch formation on asparagine (Asn)-linked sugar chains in 18 human cancer cell lines derived from several tissues. To visualize the balance of glycosyltransferase activity associated with each cell line, we expressed the relative glycosyltransferase activity in comparison to the average activity among the cell lines. These cell lines were classified into five groups according to their relative glycosyltransferase balance and were termed GlcNAcTase-I/-II, GlcNAcTase-III, GlcNAcTase-IV, GlcNAcTase-V, and GalT. We also characterized the structures of Asn-linked sugar chains on the cell surface of representative cell lines of each group. The branching structure of cell surface sugar chains roughly corresponded to the glycosyltransferase balance. This finding suggests that, for the sugar chain structure remodeling of glycoproteins, attention should be focused on the glycosyltransferase balance of host cells before introducing exogenous glycosyltransferases or down-regulating the activity of intrinsic glycosyltransferases.

Until now, many of the sugar chain structures present on various glycoproteins have been characterized. The *N*-glycan moieties of glycoproteins are related to several physical and biological processes such as secretion, lifetime, homing in on bone marrow, and active receptor binding (*1*−*5*). Sugar chains themselves also affect biological activity, immunogenicity, pharmacokinetics, solubility, immune cell trafficking, and protease resistance (*1*−*5*). During the past decade, various glycosyltransferase genes have been cloned (*6*−*10*). Recently, trials to control sugar chains using cloned glycosyltransferases have commenced to improve glycoprotein functions (*11*−*15*).

It has been suggested that cooperation between specific glycosyltransferases occurs to form specific sugar chain structures found on glycoproteins, although the precise mechanism responsible for this is unclear. Representative examples include recombinant erythropoietins produced by Chinese hamster ovary cells, baby hamster kidney cells, human B-lymphoblastoid Namalwa cells, and human lymphoblastoid RPMI1788 cells, each of which have different sugar chain structures (*16*−*19*). These phenomena have been detected in various combinations in another glycoproteins and in other cell lines (*20*−*22*). Erythropoietin has mainly tetraantennary sugar chains, but interferon-γ has mostly biantennary sugar chains, although both are produced by the same Chinese hamster ovary cell lines (*16*, *23*). Also, the alpha chains of follitropin, lutropin, and choriogonadotropin contain the same amino acid sequence but exhibit different glycosylation patterns despite being produced by Chinese hamster ovary cells (*24*). Thus, the sugar chains structures of glycoproteins are reflected in the balance of glycosyltransferases in host cells (*22*) and are independently regulated, differences resulting via unknown mechanisms, whose details remain unclarified.

To obtain more information regarding sugar chains present in various type cells, we attempted to measure the activities of six glycosyltransferases (i.e., GlcNAcTase-I, -II, -III, -IV, -V, and GalT)[1] in 18 human cell lines from several tissues, which are branch-forming enzymes of Asn-linked sugar

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chains. The results showed that the balance between these glycosyltransferases is different among the cell lines. To visualize the glycosyltransferase balance, we examined the relative glycosyltransferase activity by comparing the average activity among the cell lines. The cell lines were classified into five groups according to their relative glycosyltransferase balances: GlcNAcTase-I/-II, GlcNAcTase-III, GlcNAcTase-IV, GlcNAcTase-V, and GalT. We then characterized the structures of Asn-linked sugar chains on cell surface of a representative cell line from each group, since the sugar chain structures of cell surfaces reflect the glycosyltransferases' activities balance of each cell. The branching structure of cell surface sugar chains roughly corresponded to the glycosyltransferase activity balance.

Remodeling of sugar chain structures of glycoproteins is an attractive approach for modifying their functions and bioactivities. The results of our previous studies on human erythropoietin showed that the branching structure significantly affects its targeting in the body (*25*, *26*). However, the correlation between the in vivo activities of glycosyltransferases and the branch structures of sugar chains has not been clarified. Recently, Fukuta et al. showed that the structures of sugar chains of glycoproteins can be controlled by introducing glycosyltransferases into host cells (*11*−*14*). However, gene overexpression imposes a burden on host cells. Our results showed that the sugar chain structures of glycoproteins are systematically controlled via regulation of the balance of glycosyltransferase activities. Thus, the glycosylation of glycoproteins is reflected in the glycosyltransferase balance of host cells. We therefore propose that it is necessary to select appropriate host cell lines according to their glycosyltransferase balance to produce glycoproteins such as cytokines and enzymes for use in therapy.

EXPERIMENTAL PROCEDURES

Cell Lines. All cell lines used in this experiment were of human origin unless otherwise indicated. Urinary bladder carcinoma T-24, osteosarcoma HuO-3N1, breast cancer YMB-1, choriocarcinoma cell line BeWo, erythroid leukemia HEL, acute promyelocytic leukemia HL-60, acute lymphoblastoid leukemia BALL-1, Burkitt's lymphoma Namalwa and acute monocytic leukemia THP-1 cells were obtained from the Health Science Research Resources Bank (Osaka, Japan). Lung carcinoma A-549, colon adenocarcinoma CACO-2, cervical carcinoma HeLaS3, renal cell carcinoma OS-RC-2, hepatocellular carcinoma HepG2, eosinophilic leukemia EoL-1, chronic myelogenous leukemia KU-812, and T cell leukemia MOLT-4 cells were purchased from the RIKEN BioResource Center (Tsukuba, Japan), and Bowes human melanoma cell line was purchased from the American Type Culture Collection (Rockville, MD). BeWo cells were maintained in Ham's F-12 Kaighn's modified medium (Invitrogen, Carlsbad, CA) supplemented with 15% fetal bovine serum (FBS) (HyClone, Logan, UT). HEL, HL-60, EoL-1, KU-812, MOLT-4, BALL-1, THP-1, Namalwa, HuO-3N1, and OS-RC-2 cells were grown in RPMI 1640 medium (Invitrogen) supplemented with 10% FBS. T-24 cells were cultivated in Minimum Essential Medium (MEM) (Invitrogen) supplemented with 10% FBS, and HeLaS3 cells were cultivated in medium supplemented with 5% FBS. YMB-1 cells were cultured in MEM supplemented with 10% FBS, 1% nonessential amino acids (NEAA), and 4 mM HEPES, and CACO-2 cells were cultured in medium supplemented with 20% FBS and 1% NEAA. A-549, HepG2, and Bowes cells were maintained in Dulbecco's Modified Eagle MEM (DMEM) supplemented with 10% FBS. Above media was supplemented with 100 U/mL of penicillin and 100 μg/mL of streptomycin, and all cell lines were incubated in a 5% CO_2-humidified atmosphere at 37 °C.

Determination of the Activities of GlcNAcTases and GalT. The activities of GlcNAcTase-I and GlcNAcTase-II were measured following the method of Schachter et al. (*27*) except that Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-2-aminopyridine(PA) (core-PA for GlcNAcTase-I) and Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA (Gn(2)core-PA for GlcNAcTase-II) were used as substrates at a concentration of 0.8 mM, and the incubation time was 1 h. The activities of GlcNAcTase-III, -IV, and -V were examined according to the method of Oguri et al. (*28*), which is a modified method of Nishikawa et al. (*29*). The substrate Gn2(2′,2)core-PA was prepared as previously reported (*30*), and GalT activity was assayed indicated as below. The enzyme solution (3 μL) was incubated at 37 °C for 30 min with 10 mM HEPES buffer, pH 7.2, containing 0.8 mM substrate, such as GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-PA (Gn2(2′,2)-core-PA), 10 mM UDP-Gal, 33 mM NaCl, 3 mM KCl, 1.5% Triton X-100, 5.5 mM γ-lactone, and 10 mM $MnCl_2$ in a total volume of 20 μL.

Northern Blot Analysis. Poly(A^+)RNA was extracted from the 18 cell lines according to the Fast Track 2.0 kit manual (Invitrogen, Carlsbad, CA). Poly(A^+)RNA (2 μg) was denatured in formamide/formaldehyde followed by electrophoresis in a 1% agarose/formaldehyde gel. The separated RNA was then transferred to a Hybond N^+ nylon membrane (Amersham Biosciences, Piscataway, NJ). Several glycosyltransferase probes were prepared by PCR using [α-^{32}P]dCTP (Amersham Biosciences), for which the temperature cycling was 94 °C for 30 s, 60 °C for 75 s, and 72 °C for 2 min for 30 cycles after initial denaturation for 10 min at 95 °C using a Zymoreactor II (Atto, Tokyo, Japan). The PCR primers were constructed using published sequences available via Genbank or from previously published manuscripts. The primers used for PCR included the following: hGlcNAcTase-I, 5′-GGTGGAGAAAGTGAGGACCAATG-3′/5′-ACTGGAAGGTGACAATACCCCG-3′; hGlcNAcTase-II, 5′-ATACCTCAGACTGCTGCTGGACTC-3′/5′- CAGGTCTCTGGGACAATCTCTAGG-3′; hGlcNAcTase-III, 5′-ACGGCGTCCTTTTCCTCAAG-3′/5′-CGGTTCTCATACTGTCTGAAG-3′; hGlcNAcTase-IVa, 5′-GGCTATCACACCGATAGCTGGAG-3′/5′-TCCACCATTCCTTCTGCAACACC-3′; hGlcNAcTase-IVb, 5′-ACAACCCTCAGTCAGACAAGGAGG-3′/5′-GGTACCCTCAGAAGCCCGCAGCTT-3′; hGlcNAcTase-V, 5′-TGCGAGGGGATGCTACAGAGAATC-3′/5′-CCTTGTTGAGGTGCTGGAAGAAAG-3′; hGalT, 5′-GGTGTTTTTCACAGCCACGG-3′/5′-TGTCATCATCTTCTC-

[1] Abbreviations: GlcNAcTase, *N*-acetylglucosaminyltransferase; GlcNAcTase-I, UDP-GlcNAc-α1,3-Man-β1,2-GlcNAcTase; GlcNAcTase-II, UDP-GlcNAc-α1,6-Man-β1,2-GlcNAcTase; GlcNAcTase-III, UDP-GlcNAc-Man-β1,4-GlcNAcTase; GlcNAcTase-IV, UDP-GlcNAc-α1,3-Man-β1,4-GlcNAcTase; GlcNAcTase-V, UDP-GlcNAc-α1,6-Man-β1,6-GlcNAcTase; GalT, UDP-Gal-GlcNAc-β-1,4-galactosyltransferase; Asn, asparagine.

CTCCCCAG-3′; and hG3PDH, 5′-CCAAAATCAAGTGG-GGCGATG-3′/5′-CAGGAGGCATTGCTGATGATCTTG-3′. The blots were hybridized with each probe in Rapid-hyb buffer (Amersham Biosciences) at 65 °C for 3 h. Next, the filters were washed three times in 2× SSC/0.1% SDS at 65 °C for 15 min, once in 1× SSC/0.1% SDS at 65 °C for 30 min, and three times in 0.1× SSC/0.1% SDS at 65 °C for 20 min. Then, the relative emissions of several glycosyltransferase signals were detected using a BAS 2000 bioimaging analyzer (Fuji Film, Tokyo, Japan).

Materials and Enzymes. *Endo-β*-galactosidase from *Escherichia freundii*, *β*-galactosidase from the Jack bean, and α-fucosidase from the bovine kidney were purchased from Seikagaku Kogyo (Tokyo, Japan). Neuraminidase from *Arthrobactor ureafaciens* and 2-aminobenzamide (2-AB) were purchased from Nacalai Tesque (Kyoto, Japan), and 2,5-dihydroxybenzoic acid (DHB) was obtained from Aldrich Chemical (Milwaukee, WI).

Preparation of 2-AB−Labeled Sugar Chains. The cells were washed once with PBS (−) and treated with 0.1% EDTA/PBS (−) for 10 min at room temperature. After EDTA treatment, adherent cells were scraped off from dishes using a rubber policeman and washed five times with PBS (containing 1 mM $CaCl_2$). The cells were then treated with 1.5% glutalaldehyde in PBS for 60 min at room temperature, and their cell surface proteins were linked. Fixed cells were washed with chloroform/methanol (1:1) three times to remove lipid and then dried. The cell surface sugar chains on the fixed cells were digested with 2 U of *N*-glycanase in 10 mM Tris-HCl, pH 7.5, containing 0.1% SDS and 0.5% Nonidet P-40 for 24 h at 37 °C and then released into the buffer. Next, the cell surface sugar chains were reductively aminated with 2-AB in an acetic acid/dimethyl sulfoxide (3:7) solution containing sodium cyanoborohydride (*31*). Excess reagents were removed using a Superdex Peptide HR (Amersham Biosciences) column equilibrated with 50 mM pyridine acetate buffer, pH 5.0. The 2-AB−labeled oligosaccharides were collected from the flow-through fraction, and the fluorescence intensity was then monitored with an excitation wavelength of 330 nm and emission wavelength of 420 nm.

Preparation of Reducing Core Fragments. The 2-AB−labeled sugar chains were digested with neuraminidase (*Arthrobacter ureafaciens*), and desialylated neutral sugar chains were then separated by anion-exchange HPLC using a HiTrap Q column (1 mL, Amersham Biosciences). The elution was performed using an increasing linear gradient of sodium acetate from 2 to 160 mM (pH 5.0) for 60 min at 1 mL/min at room temperature. The contents of the flow-through fraction contained neutralized sugar chains, and these were sequentially digested by glycosidases to verify their structural identities. The 2-AB−labeled sugar chains were then desalted using a Sep-Pak C18 cartridge (Waters) and characterized as reducing core fragments.

Glycosidase Digestion. Enzymatic digestion of 2-AB−labeled sugar chains was performed using the following enzymes at 37 °C for 24 h. The 2-AB−labeled sugar chains were incubated with 0.2 U of neuraminidase (*Arthrobacter ureafaciens*) in 50 μL of 0.1 M ammonium acetate buffer, pH 5.0, with 20 mU of *endo-β*-galactosidase (*Escherichia freundii*) in 50 μL of 0.1 M ammonium acetate buffer, pH 5.6, with 20 mU of *β*-galactosidase (Jack bean) in 50 μL of 0.1 M citrate phosphate buffer, pH 4.1, or with 20 mU of α-fucosidase (bovine kidney) in 50 μL of 0.1 M citrate phosphate buffer, pH 5.0. The reaction mixture was then heated at 100 °C for 3 min to terminate digestion.

Analysis of Branch Structure. The core fragments were chromatographed on a normal-phase TSK gel Amide-80 (4.6 mm × 250 mm, Tosoh) column using a decreasing linear gradient of acetonitrile from 60% to 50% for 60 min in 50 mM ammonium acetate buffer, pH 4.0, at 1.0 mL/min at room temperature. The fragments were separated into bi-, tri-, and tetraantennary fractions, and the triantennary fraction was further separated into 6′,2′,2-type and 2′,4,2-type components using a reversed-phase TSK gel ODS 80Ts (4.6 mm × 150 mm, Tosoh) column. Elution was performed using an increasing linear gradient of acetonitrile from 0.5% to 7% for 40 or 50 min in 50 mM ammonium acetate buffer, pH 4.0, at 1.0 mL/min at room temperature. Each structure was confirmed by the retention time of standard sugar chains and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) spectra, where the spectra were analyzed using a Lasermat 2000 (Finnigan MAT, Hemel Hempstead, U.K.). Neutral oligosaccharides were positively ionized with a 2,5-dihydroxybenzoic acid (DHB) solution and observed as [M + Na] + ions. The DHB solution consisted of acetonitrile/distilled water (3:7) containing 1% (w/v) DHB and 0.01% (w/v) sodium dihydrogenphosphate dihydrate.

RESULTS

Enzyme Activities Related to the Branch Formation of Asparagine-Linked Sugar Chains in 18 Human Cell Lines. GlcNAcTases from -I to -V and GalT are involved in the branch formation of Asn-linked sugar chains. Table 1 shows the six glycosyltransferases activities for 18 human cancer cell lines. The GalT activity ranged from 4.52 to 73.19 (mean, 23.54) (nmol/h)/mg protein. The choriocarcinoma cell line BeWo exhibited the highest activity, and eosinophilic leukemia cell line EoL-1 exhibited the lowest activity. The GlcNAcTase-I activity ranged from 3.03 to 34.23 with a mean of 13.22 (nmol/h)/mg protein. BeWo cell line possessed the highest activity and Bowes malignant melanoma cell line the lowest. The GlcNAcTase-II activity ranged from 3.10 to 15.10 (mean, 9.29) (nmol/h)/mg protein, and differences in this activity among the cell lines were smallest compared to the other five glycosyltransferases. Acute monocytic leukemia cell line THP-1 exhibited the highest activity, and osteosarcoma cell line HuO-3N1 shows the lowest activity. The GlcNAcTase-III activity varied from under the limit of detectability to 10.17 (nmol/h)/mg protein with a mean of 1.31 (nmol/h)/mg protein. The BeWo cell line showed the highest activity, and the GlcNAcTase-III activity of eight cell lines (A549, YMB-1, HepG2, HuO-3N1, OS-RC-2, T-24, HeLaS3, and THP-1) was not detectable. An on−off mechanism may have been responsible for the activity of GlcNAcTase-III, because only specific cells express it. The GlcNAcTase-IV activity ranged from 0.08 to 5.25 (nmol/h)/mg protein with a mean of 0.72 (nmol/h)/mg protein, with the BeWo cell line having the highest activity followed by three cell lines having the lowest activity, lung carcinoma cell line A549, cervical carcinoma cell line HeLaS3, and chronic myelogenous leukemia cell line KU-812. The GlcNAcTase-V activity ranged from 0.11 to 0.94 (nmol/h)/

Table 1: Glycosyltransferases Activities of 18 Human Cell Lines[a]

cell line	specific activity ((pmol/h)/mg protein)						classification
	GalT	GlcNAcTase-I	GlcNAcTase-II	GlcNAcTase-III	GlcNAcTase-IV	GlcNAcTase-V	
Bowes	9520 ± 301	3030 ± 169	5190 ± 102	1230 ± 96	120 ± 10	550 ± 3	GlcNAcTase-III/-V
HuO-3N1	15210 ± 1240	5920 ± 758	3100 ± 255		80 ± 12	230 ± 32	GalT
A549	31520 ± 833	5190 ± 125	6040 ± 227		90 ± 16	170 ± 18	GalT
YMB-1	26240 ± 800	8850 ± 698	7510 ± 396		380 ± 52	420 ± 79	GalT
HepG2	23180 ± 583	22010 ± 2220	9830 ± 1000		2920 ± 56	940 ± 20	GlcNAcTase-IV/-V
OS-RC-2	53060 ± 1420	9520 ± 486	8220 ± 644		120 ± 21	460 ± 60	GalT
CACO-2	35970 ± 2200	19500 ± 389	11830 ± 281	5070 ± 371	1200 ± 130	250 ± 48	GlcNAcTase-III/-IV
T-24	12390 ± 564	12030 ± 916	10990 ± 1470		170 ± 30	230 ± 40	GlcNAcTase-I/-II
HeLaS3	35850 ± 2380	6470 ± 249	7960 ± 444		80 ± 8	300 ± 16	GalT/GlcNAcTase-V
BeWo	73190 ± 2290	34230 ± 1030	6230 ± 367	10170 ± 207	5250 ± 278	560 ± 14	GlcNAcTase-III/-V
THP-1	8630 ± 687	21880 ± 972	16010 ± 490		180 ± 10	150 ± 12	GlcNAcTase-I/-II
BALL-1	39110 ± 1440	18180 ± 1050	15100 ± 394	950 ± 43	100 ± 6	280 ± 20	GalT/GlcNAcTase-I/-II
MOLT-4	8980 ± 486	7540 ± 234	8980 ± 275	70 ± 30	1150 ± 43	220 ± 28	GlcNAcTase-IV
EoL-1	4520 ± 483	12010 ± 295	11830 ± 298	350 ± 48	120 ± 11	250 ± 30	GlcNAcTase-I/-II
KU-812	7510 ± 590	11390 ± 315	9230 ± 247	2940 ± 270	80 ± 4	110 ± 12	GlcNAcTase-III
HL-60	8580 ± 210	16220 ± 418	12740 ± 777	640 ± 81	550 ± 14	480 ± 15	GlcNAcTase-I/-II/-V
HEL	8170 ± 998	14060 ± 1180	10510 ± 478	2020 ± 154	240 ± 10	180 ± 5	GlcNAcTase-III
Namalwa	22040 ± 1170	9890 ± 313	5980 ± 425	60 ± 9	170 ± 20	330 ± 96	GalT/GlcNAcTase-V
average	23540	13220	9290	1310	720	340	

[a] Five GlcNAcTases and GalT activities were assayed as described in Experimental Procedures. Each result represented the mean ± SD of three different experiments. The specific activity of each enzyme was expressed as picomoles of products per hour of incubation per milligram of protein in the cell lysate.

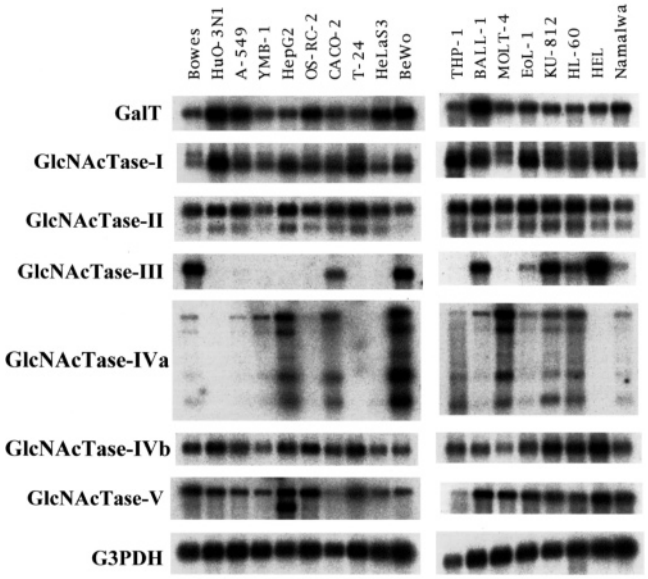

FIGURE 1: Northern blot analysis of six GlcNAcTases and GalT genes. Two micrograms of poly(A^+)RNA obtained from 18 human cell lines was subjected to electrophoresis using a 1% formaldehyde gel. The gel was then blotted onto positive charged nylon membranes and probed with cDNA for each glycosyltransferase and *G3PDH*. The bands produced by autoradiography were subsequently visualized with a Fuji BAS2000 Bio-imaging analyzer.

mg protein with a mean of 0.34 (nmol/h)/mg protein, and the hepatocellular carcinoma cell line HepG2 had the highest activity and the KU-812 cell line the lowest.

Northern Blot Analysis of mRNA of Enzymes Related to the Branch Formation of Asparagine-linked Sugar Chains. The mRNA expression levels of the enzymes corresponded to the enzyme activities among the 18 cancer cell lines (Figure 1). Specifically, the mRNA levels of *GlcNAcTase-III* and *GlcNAcTase-IVa* were well-correlated with their enzyme activities. However, several slight discrepancies occurred for a few cases for unknown reasons. Since *GlcNAcTase-IVb* mRNA expression was likely constitutive among the cell lines, it appeared that the enzyme activity of GlcNAcTase-IV depended on the *GlcNAcTase-IVa* mRNA expression level. Only HepG2 cells with the highest GlcNAcTase-V activity exhibited an extra band (Figure 1).

Selection of Representative Cell Lines According to the Relative Glycosyltransferase Balance. To easily visualize the expression pattern of the six glycosyltransferases, we attempted to produce a radial graph. First, we calculated the mean value for each glycosyltransferase activity (Table 1), and then we determined the deviation of each enzyme from the mean value such that X_i = (raw data)/(mean value), where *i* is each of GlcNAcTase-I, -II, -III, -IV, -V, and GalT. If a cell line had average activity for the six glycosyltransferases, the graph would become an equilateral hexagon with a scale of one. By comparing the graphs between the 18 cell lines, we noted that they could be roughly divided into five groups, a GalT−type, a GlcNAcTase-I/-II−type, a GlcNAcTase-III−type, a GlcNAcTase-IV−type, and a GlcNAcTase−V type, and also a complex group as shown in Table 1. Figure 2 shows seven representative cell lines according to their relative glycosyltransferase balances.

Structural Analysis of the Sugar Chains on Cell Surfaces of Seven Representative Human Cell Lines. The sugar chains released from the cell surfaces of each cell line were labeled with 2-aminobenzamide (2-AB) and then desialylated and purified. Then, the neutralized sugar chains were sequentially digested with *Escherichia freundii endo-β*-galactosidase, Jack bean *β*-galactosidase, and bovine kidney α-fucosidase. The glycosidases-digested sugar chains were next size-fractionated by normal-phase HPLC and separated into bi-, tri-, and tetraantennary fractions. The triantennary fraction was further separated into GlcNAc*β*1-6(GlcNAc*β*1-2)Man*α*1-6(GlcNAc*β*1-2Man*α*1-3)Man*β*1-4GlcNAc*β*1-4GlcNAc-2AB, named 6′,2′,2-type, and GlcNAc*β*1-2Man*α*1-6[GlcNAc*β*1-2(GlcNAc*β*1-4)Man*α*1-3]Man*β*1-4GlcNAc*β*1-4GlcNAc-2AB, 2′,4,2-type, by reversed-phase HPLC. Each structure was confirmed by the retention time for reversed-phase HPLC of standard sugar chains and by their MALDI-TOF MS

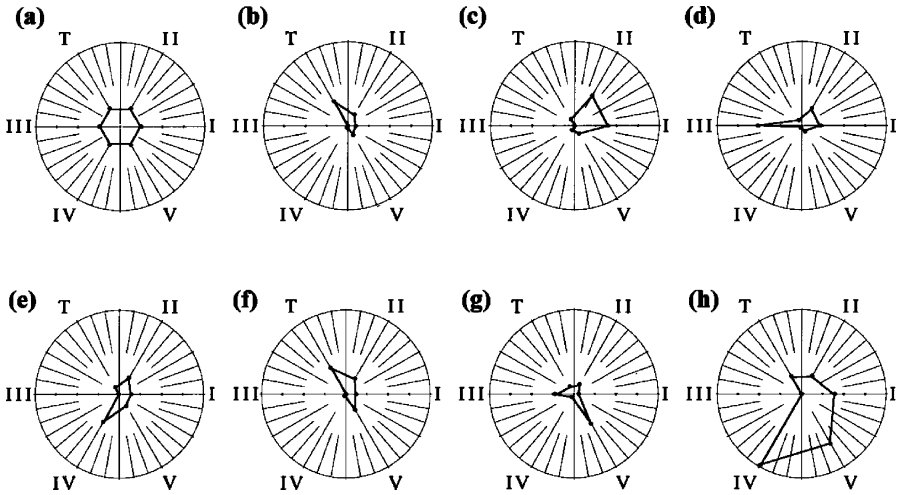

FIGURE 2: Seven representative cell lines according to their relative glycosyltransferase balances. The mean value for each glycosyltransferase activity was calculated and is shown in Table 1, and the X_i value (raw data/mean value) [i = GlcNAcTase-I, -II, -III, -IV, -V, and GalT] for each cell line was examined and plotted on a hexaradial scale and then aligned. (a) The standard type is shown and indicates an equilateral hexagon with a scale of one. (b) GalT-type, A549; (c) GlcNAcTase-I/-II−type, THP-1; (d) GlcNAcTase-III−type, KU-812; (e) GlcNAcTase-IV−type, MOLT-4; (f) GalT/GlcNAcTase-V−type, HeLaS3; (g) GlcNAcTase-III/-V−type, Bowes; (h) GlcNAcTase-IV/-V−type, HepG2.

Table 2: Profiles of Glycoforms for Cell Surface Sugar Chains of Seven Types of Cell Lines

	cell line						
	A549	THP-1	KU-812	MOLT-4	HeLaS3	Bowes	HepG2
biantenna (%)	58.9	52.1	85.0	50.2	31.0	41.5	20.0
triantenna 6′,2′,2 (%)	10.8	15.6	3.4	4.9	22.9	16.2	6.9
triantenna 2′,4,2 (%)	5.5	7.8	5.6	24.0	3.1	1.8	9.6
tetraantenna (%)	24.8	24.5	6.0	21.0	43.0	39.5	63.5
bisecting (+) (%)			37.0			29.0	
classification	GalT	GlcNAcTase-I/-II	GlcNAcTase-III	GlcNAcTase-IV	GalT/GlcNAcTase-V	GlcNAcTase-III/-V	GlcNAcTase-IV/-V

spectra. The assignment of branching structures and the percentages of sugar chains obtained from each cell surface is summarized in Table 2. A549 cells belong to the GalT type group and contain comparatively rich biantennary sugar chains. Its triantennary sugar chains were found to be composed of 6′,2′,2/2′,4,2, 2:1. THP-1 belongs to the GlcNAcTase-I/-II−type group and also contains a little more biantennary sugar chains. The KU-812 cell line was classified as a GlcNAcTase-III−type, exhibited relatively high GlcNAcTase-III activity, and possessed 85% biantennary sugar chain structure and 40% bisecting GlcNAc structure. Its triantennary sugar chain content was only 9% and was composed of 6′,2′,2/2′,4,2, 5:8. MOLT-4 cells belonged to the GlcNAcTase-IV−type group, and the triantennary sugar chains were composed of 6′,2′,2/2′,4,2, 1:5, while HeLaS3 cells belong to the GalT/GlcNAcTase-V−type, and their triantennary sugar chains were composed of 6′,2′,2/2′,4,2, 7:1. The quantity of tetraantennary sugar chains that they possessed was moderately abundant. The Bowes cells belonged to the GlcNAcTase-III/-V−type group, and its triantennary sugar chains were composed of 6′,2′,2/2′,4,2, 9:1. Since this cell line also contains somewhat abundant tetraantennary sugar chains, the amount of which was less than for HeLaS3 cells despite its higher GlcNAcTase-IV and GlcNAcTase-V activities, it had a rather high GlcNAcTase-III activity and 30% of sugar chains contained a bisecting GlcNAc structure. The HepG2 cells belonged to the GlcNAcTase-IV/-V−type group, and their triantennary sugar chains were composed of 6′,2′,2/2′,4,2, 7:10. Since this cell line has extremely high GlcNAcTase-IV and GlcNAcTase-V activities, about 64% of its sugar chains contained a tetraantennary structure and biantennary structure was only present in 20% of the chains.

DISCUSSION

Glycosylation, the addition of sugar residues to a peptide backbone, is a significant post-translational modification in eukaryotic cells and plays a role in defining the properties of glycoproteins (*1*−*5*). From the analysis of transgenic and gene targeting mice, it has been suggested that Asn-linked sugar chains play a role in development, physiology, and the immune system (*32*). Attempts to remodel the sugar chains of glycoproteins with an aim of improving their functions are ongoing. To meet this objective, it is essential to fully establish a technique for sugar chain remodeling. From the findings of approaches which aim to introduce exogenous glycosyltransferases or knock out unique glycosyltransferases, it is apparent that the sugar chains structures of glycoproteins can be modified by altering the balance of glycosyltransferases activities (*11*−*15*, *33*). Structural analysis of the sugar chains of human chorionic gonadotropin (hCG) has revealed that abnormal biantennary structures are present on hCG in the urine of choriocarcinoma patients (*34*, *35*), and these abnormal biantennary structures, including those of the GlcNAcβ1-4Manα1-3 group express abnormally GlcNAcTase-IV activity. We recently found that among two *GlcNAcTase-IV* genes in human tissues, only the *GlcNAcTase-IVa* gene is strongly expressed in choriocarcinoma cells (*36*). This indicates that the sugar chain structure is altered by extreme intrinsic changes in the balance of glycosyltransferases without artificial manipulation of glycosyltransferases

and suggests the potential for sugar chain structural modification and thus the alteration of the glycosyltransferase activity balance. Furthermore, Raju et al. and Hamako et al. determined that species-specific variations exist with respect to the glycosylation of immunoglobulin G (*37, 38*). This suggests that the glycosyltransferases activities of B cells are different among mammalian species. Also, when different host cells produce the same glycoprotein, the sugar chain structure has been altered (*16*−*22*). From these findings, we believe that the balance of glycosyltransferases activities among several cell lines are considerably different.

To confirm our hypothesis, we attempted to measure the activities of six glycosyltransferases, GlcNAcTase-I, -II, -III, -IV, -V, and GalT, in 18 human cell lines derived from several tissues, which are glycosyltransferases that are branch-forming enzymes of Asn-linked sugar chains. Our results showed that the balance of these glycosyltransferases activities is different among the cell lines (Table 1). The mRNA level of each enzyme shown in Figure 1 was generally correlated with the actual enzyme activity (Table 1). In a previous study, we showed that the *GlcNAcTase-IV* gene, which contributes to the elevated GlcNAcTase-IV activity, is *GlcNAcTase-IVa* (*36*). Among the 18 human cell lines, we also found that cells with high GlcNAcTase-IV activity always abundantly expressed *GlcNAcTase-IVa*, whereas those with weak enzyme activity showed almost undetectable levels of *GlcNAcTase-IVa* expression. From visualization results of the glycosyltransferase balance, we determined that many cell lines could be classified into five groups and combinations of groups according to their relative glycosyltransferase activity (Table 1). We then picked a representative cell line from each group (Figure 2) and examined the structures of Asn-linked sugar chains on their cell surfaces. The results showed that the branching structures of complex-type sugar chains on the cell surfaces are reflected on the degree of glycosyltransferase activity balance for each cell line (Table 2). Specifically, the ratio of 6′,2′,2/2′,4,2 and the content of bisecting GlcNAc structure resembled to the ratio of the GlcNAcTase-V activity−GlcNAcTase-IV activity and the intensity of the GlcNAcTase-III activity, respectively. Cell lines with a GlcNAcTase-III activity below the detection limit did not contain a bisecting GlcNAc structure (Table 2). This result is useful for selecting host cells which produce glycoproteins that have no bisecting GlcNAc structure. Since the acceptor substrate specificity of GlcNAcTase-III was broad and the addition of the bisecting GlcNAc eliminated the potential for α-mannosidase-II, GlcNAcTase-II, GlcNAcTase-IV, GlcNAcTase-V, and core α 1,6-fucosyltransferase to act subsequently (*39, 40*), the biantennary structure content of KU-812 cells with high GlcNAcTase-III activity was over 80%. We expected to observe a branch-suppressing effect in GalT-type cell lines, but the extent of the effect was lower than our expectations. Fukuta et al. found that the branching of sugar chains on human interferon-γ is suppressed in β1,4-GalT-enhanced clones (*13*), and thus, the suppression of branching may require higher GalT activity in these cells compared to the GalT activity required in A549 cells.

The remodeling of sugar chain structures of glycoproteins is an attractive approach for altering their functions and bioactivities. Recently, Fukuta et al. showed that the structure of sugar chains of glycoproteins can be controlled by introducing glycosyltransferases into host cells (*11*−*14*). For the case of exogenous glycosyltransferase transfection, the golgi localization of introduced glycosyltransferases is unknown and cannot be regulated. Nevertheless, for the sugar chains structures, a resemblance between the natural balance of intrinsic glycosyltransferases and acquired balance by introduced exogenous glycosyltransferases can be observed. However, although gene overexpression is very effective, it imposes a burden on host cells. Our observations suggest that the sugar chain structures of glycoproteins are systematically controlled by regulating the balance of glycosyltransferases activity. From our findings, we believe that examining the glycosyltransferases' activity balance of host cells before manipulating glycosyltransferase genes can aid in decreasing the excessive introduction of exogenous glycosyltransferases. In the present study, since delicate up- or down-regulation of glycosyltransferase activity was difficult, we believe that the utilization of a host cell's glycosyltransferase balance may provide a shortcut for the production of glycoproteins which remodel sugar chain structures. Although the productivity of donor cells is critical for selecting host cells which produce objective glycoproteins, to avoid the excessive introduction of exogenous glycosyltransferases, an understanding of the glycosyltransferase activity of host cells is important.

In this study, we tried to group 18 human cell lines and achieve a good correlation between cell surface sugar chain structures and the balance of glycosyltransferase activity. In the future, the relationship may be further clarified when additional data for other cell lines is available. At present, although few findings between branching structures and biological functions were made, if important roles of specific branching structures were found out, the neoglycoprotein with controlled branching structure would be need soon. We believe that it is necessary to select appropriate host cell lines according to their glycosyltransferases' activity balance to produce glycoproteins with an objective sugar chain structure. Our method of grouping according to their glycosyltransferases' activity balance is useful to select appropriate host cells.

REFERENCES

1. Kobata, A. (1992) Structures and functions of the sugar chains of glycoproteins, *Eur. J. Biochem. 209*, 483−501.
2. Varki, A. (1993) Biological roles of oligosaccharides: all of the theories are correct, *Glycobiology 3*, 97−130.
3. Helenius, A., and Aebi, M. (2001) Intracellular functions of N-linked glycans, *Science 291*, 2364−2369.
4. Rudd, P. M., Elliott, T., Cresswell, P., Wilson, I. A., and Dwek, R. A. (2001) Glycosylation and the immune system, *Science 291*, 2370−2376.
5. Takeuchi, M., and Kobata, A. (1991) Structures and functional roles of the sugar chains of human erythropoietins, *Glycobiology 1*, 337−346.
6. Amado, M., Almeida, R., Schwientek, T., and Clausen, H. (1999) Identification and characterization of large galactosyltransferase gene families: galactosyltransferases for all functions, *Biochim. Biophys. Acta 1473*, 35−53.
7. Fukuda, M., Bierhuizen, M. F., and Nakayama, J. (1996) Expression cloning of glycosyltransferases, *Glycobiology 6*, 683−689.
8. Tsuji, S. (1996) Molecular cloning and functional analysis of sialyltransferases, *J. Biochem. (Tokyo) 120*, 1−13.
9. Taniguchi, N., and Ihara, Y. (1995) Recent progress in the molecular biology of the cloned *N*-acetylglucosaminyltransferases, *Glycoconjugate J. 12*, 733−738.

10. Field, M. C., and Wainwright, L. J. (1995) Molecular cloning of eukaryotic glycoprotein and glycolipid glycosyltransferases: a survey, *Glycobiology 5*, 463−472.
11. Fukuta, K., Abe, R., Yokomatsu, T., Kono, N., Asanagi, M., Omae, F., Minowa, M. T., Takeuchi, M., and Makino, T. (2000) Remodeling of sugar chain structures of human interferon-γ, *Glycobiology 10*, 421−430.
12. Fukuta, K., Abe, R., Yokomatsu, T., Omae, F., Asanagi, M., and Makino, T. (2000) Control of bisecting GlcNAc addition to N-linked sugar chains, *J. Biol. Chem. 275*, 23456−23461.
13. Fukuta, K., Abe, R., Yokomatsu, T., Minowa, M. T., Takeuchi, M., Asanagi, M., and Makino, T. (2001) The widespread effect of β1,4-galactosyltransferase on *N*-glycan processing, *Arch. Biochem. Biophys. 392*, 79−86.
14. Fukuta, K., Yokomatsu, T., Abe, R., Asanagi, M., and Makino, T. (2000) Genetic engineering of CHO cells producing human interferon-γ by transfection of sialyltransferases, *Glycoconjugate J. 17*, 895−904.
15. Weikert, S., Papac, D., Briggs, J., Cowfer, D., Tom, S., Gawlitzek, M., Lofgren, J., Mehta, S., Chisholm, V., Modi, N., Eppler, S., Carroll, K., Chamow, S., Peers, D., Berman, P., and Krummen, L. (1999) Engineering Chinese hamster ovary cells to maximize sialic acid content of recombinant glycoproteins, *Nat. Biotechnol. 17*, 1116−1121.
16. Sasaki, H., Bothner, B., Dell, A., and Fukuda, M. (1987) Carbohydrate structure of erythropoietin expressed in Chinese hamster ovary cells by a human erythropoietin cDNA, *J. Biol. Chem. 262*, 12059−12076.
17. Nimtz, M., Martin, W., Wray, V., Klöppel, K.-D., Augustin, J., and Conradt, H. S., (1993) Structures of sialylated oligosaccharides of human erythropoietin expressed in recombinant BHK-21 cells, *Eur. J. Biochem. 213*, 39−56.
18. Yanagi, H., Yoshima, T., Ogawa, I., and Okamoto, M. (1989) Recombinant human erythropoietin produced by Namalwa cells, *DNA 8*, 419−427.
19. Cointe, D., Béliard, R., Jorieux, S., Leroy, Y., Glacet, A., Verbert, A., Bourel, D., and Chirat, F. (2000) Unusual N-glycosylation of a recombinant human erythropoietin expressed in a human lymphoblastoid cell line does not alter its biological properties, *Glycobiology 10*, 511−519.
20. Lifely, M. R., Hale, C., Boyce, S., Keen, M. J., and Phillips, J. (1995) Glycosylation and biological activity of CAMPATH-1H expressed on different cell lines and grown under different culture conditions, *Glycobiology 5*, 813−822.
21. Chan, A. L., Morris, H. R., Panico, M., Etienne, A. T., Rogers, M. E., Gaffney, P., Creighton-Kempsford, L., and Dell, A. (1991) A novel sialylated *N*-acetylgalactosamine-containing oligosaccharide is the major complex-type structure present in Bowes melanoma tissue plasminogen activator, *Glycobiology 1*, 173−185.
22. Ohno, M., Nishikawa, A., Koketsu, M., Taga, H., Endo, Y., Hada, T., Higashino, K., and Taniguchi, N. (1992) Enzymatic basis of sugar structures of α-fetoprotein in hepatoma and hepatoblastoma cell lines: correlation with activities of α1-6 fucosyltransferase and *N*-acetylglucosaminyltransferases III and V, *Int. J. Cancer 51*, 315−317.
23. Mutsaers, J. H. G. M., Kamerling, J. P., Devos, R., Guisez, Y., Fiers, W., and Vliegenthart, J. F. G. (1986) Structural studies of the carbohydrate chains of human γ-interferon, *Eur. J. Biochem. 156*, 651−654.
24. Amoresano, A., Siciliano, R., Orru, S., Napoleoni, R., Altarocca, V., De Luca, E., Sirna, A., and Pucci, P. (1996) Structural characterization of human recombinant glycohormones follitropin, lutropin and choriogonadotropin expressed in Chinese hamster ovary cells, *Eur. J. Biochem. 242*, 608−618.
25. Takeuchi, M., Takasaki, A., Inoue, N., Strickland, T. W., Kubota, M., Wada, M., Shimizu, R., Hoshi, S., Kozutsumi, H., Takasaki, S., and Kobata, A. (1989) Relationship between sugar chain structure and biological activity of recombinant human erythropoietin produced in Chinese hamster ovary cells, *Proc. Natl. Acad. Sci. U.S.A. 86*, 7819−7822.
26. Misaizu, T., Matsuki, S., Strickland, T. W., Takeuchi, M., Kobata, A., and Takasaki, S. (1995) Role of antennary structure of N-linked sugar chains in renal handling of recombinant human erythropoietin, *Blood 86*, 4097−4104.
27. Schachter, H., Brockhausen, I., and Hull, E. (1989) High-performance liquid chromatography assays for *N*-acetylglucosaminyltransferase involved in *N*- and *O*-glycan synthesis, *Methods Enzymol. 179*, 351−397.
28. Oguri, S., Minowa, M. T., Ihara, Y., Taniguchi, N., Ikenaga, H., and Takeuchi, M. (1997) Purification and characterization of UDP-*N*-acetylglucosamine: α1,3-D-mannoside β1,4-*N*-acetylglucosaminyltransferase (*N*-acetylglucosaminyltransferase-IV) from bovine small intestine, *J. Biol. Chem. 272*, 22721−22727.
29. Nishikawa, A., Gu, J., Fujii, S., and Taniguchi, N. (1990) Determination of *N*-acetylglucosaminyltransferase III, IV, and V in normal and hepatoma tissue of rats, *Biochim. Biophys. Acta 1035*, 313−318.
30. Tokugawa, K., Oguri, S., and Takeuchi, M. (1996) Large scale preparation of PA-oligosaccharides from glycoproteins using an improved extraction method, *Glycoconjugate J. 13*, 53−56.
31. Inoue, N., Watanabe, T., Kutsukake, T., Saitoh, H., Tsumura, H., Arai, H., and Takeuchi, M. (1999) Asn-linked sugar chain structures of recombinant human thrombopoietin produced in Chinese hamster ovary cells, *Glycoconjugate J. 16*, 707−718.
32. Hennet, T., and Ellies, L. G. (1999) The remodeling of glycoconjugates in mice, *Biochim. Biophys. Acta 1473*, 123−136.
33. Keusch, J., Lydyard, P. M., and Delves, P. J. (1998) The effect on IgG glycosylation of β-1,4-galactosyltransferase-1 activity in B cells, *Glycobiology 8*, 1215−1220.
34. Endo, T., Nishimura, R., Mochizuki, M., Kochibe, N., and Kobata, A. (1988) Altered glycosylation is induced in both α- and β-subunits of human chorionic gonadotropin produced by choriocarcinoma, *J. Biochem. (Tokyo) 103*, 1035−1038.
35. Mizuochi, T., Nishimura, R., Derappe, C., Taniguchi, T., Hamamoto, T., Mochizuki, M., and Kobata, A. (1983) Structures of asparagine-linked sugar chains of human chorionic gonadotropin produced in choriocarcinoma, *J. Biol. Chem. 258*, 14126−14129.
36. Takamatsu, S., Oguri, S., Minowa, M. T., Yoshida, A., Nakamura, K., Takeuchi, A., and Kobata, A. (1999) Unusually high expression of *N*-acetylglucosaminyltransferase-IVa in human choriocarcinoma cell line: a possible enzymatic basis of the formation of abnormal biantennary sugar chain, *Cancer Res. 59*, 3949−3953.
37. Raju, T. S., Briggs, J. B., Borge, S. M., and Jones, A. J. S. (2000) Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics, *Glycobiology 10*, 477−486.
38. Hamako, J., Matsui, T., Ozeki, Y., Mizuochi, T., and Titani, K. (1993) Comparative studies of asparagine-linked sugar chains of immunoglobulin G from eleven mammalian species, *Comp. Biochem. Physiol. B106*, 949−954.
39. Priatel, J. J., Sarker, M., Schachter, H., and Marth, J. D. (1997) Isolation, characterization and inactivation of the mouse Mgat3 gene: the bisecting *N*-acetylglucosamine in asparagines-linked oligosaccharides appears dispensable for viability and reproduction, *Glycobiology 7*, 45−56.
40. Schachter, H. (1986) Biosynthetic controls that determine the branching and microheterogeneity of protein-bound oligosaccharides, *Biochem. Cell Biol. 64*, 163−181.

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