A novel strategy for mammalian cell surface glycome profiling using lectin microarray

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The glycome represents the total set of glycans expressed in a cell. The glycome has been assumed to vary between cell types, stages of development and differentiation, and during malignant transformation. Analysis of the glycome provides a basis for understanding the functions of glycans in these cellular processes. Recently, a technique called lectin microarray was developed for rapid profiling of glycosylation, although its use was mainly restricted to glycoproteins of cell lysates, and thus unable to profile the intact cell surface glycans. Here we report a simple and sensitive procedure based on this technology for direct analysis of the live mammalian cell-surface glycome. Fluorescent-labeled live cells were applied in situ to the established lectin microarray consisting of 43 immobilized lectins with distinctive binding specificities. After washing, bound cells were directly detected by an evanescent-field fluorescence scanner in a liquid phase without fixing and permeabilization. The results obtained by differential profiling of CHO and its glycosylation-defective mutant cells, and splenocytes of wild-type and β1-3-N-acetylglucosaminyltransferase II knockout mice performed as model experiments agreed well with their glycosylation phenotypes. We also compared cell surface glycans of K562 cells before and after differentiation and found a significant increase in the expression of O-glycans on differentiated cells. These results demonstrate that the technique provides a novel strategy for profiling global changes of the mammalian cell surface glycome.

Keywords: glycome/lectin microarray/live cells/profiling

Introduction

Mammalian cells in nature are covered with a dense and complex array of glycans called the glycocalyx, which comprises of various forms of glycoconjugates (glycoproteins, glycolipids, and proteoglycans). It has been assumed that different cell types express distinct sets of glycans, and that glycosylation of a single cell type significantly changes during cell development and differentiation (Priatel et al. 2000; Moody et al. 2001; Amado et al. 2004; Comelli et al. 2006). Altered glycosylation is also a typical feature during malignant transformation (Dennis et al. 1999a, b). In fact, certain types of glycan structures represent epitope markers for tumor progression such as sialyl lewisα and sialyl lewisβ. The functional consequences of these glycosylation changes are likely to be recognized by endogenous lectins that regulate a myriad of cellular processes (Kim and Varki 1997; Aarnoudse et al. 2006; Toscano et al. 2007).

Lectins have long been used as useful tools to characterize cell surface glycans because of their substantial selectivity (“lectus” in Latin meaning gather or select) in terms of branching, linkage, and terminal modifications of complex glycans (Sharon and Goldstein 1998; Sharon and Lis 2004). They are also effective in a wide variety of biochemical and cell biological assays, including blood-group typing, tissue staining, lectin-probed Western blotting, and flow cytometry. Alternatively, as immobilized forms, they are frequently used in affinity purification of glycoproteins and glycopeptides. A technique called glyco-catch is a lectin-based systematic approach for identification of glycoproteins, glycosylation sites, and glycan profiles (Hirabayashi et al. 2002; Kaji et al. 2003).

In 2005, a novel lectin-based glycan profiling method called lectin microarray was developed that allows rapid and sensitive analysis of complex glycan structures (Hirabayashi 2004). Pilobello et al. (2005) and Rosenfeld et al. (2007) developed lectin microarrays with 9 and 24 immobilized lectins, respectively, to which specific binding of glycoproteins was easily observed without glycan liberation. From a technical viewpoint, these methods require washing steps to remove unbound proteins, since their detection methods, often those used for DNA and antibody microarrays, require dry conditions. In contrast to antibody-antigen interactions, generally lectins have low affinity to their ligands (Kd > 10−6 M) and would not be expected to bind with sufficient avidity to withstand washing steps to remove unbound protein (Blixt et al. 2004). For this reason, Kuno et al. (2005) developed an alternative, sensitive lectin array system based on an evanescent-field detection principle that assures detection of even weak interactions between lectins and monovalent oligosaccharides, in a liquid phase at equilibrium. Furthermore, Ebe et al. (2006) demonstrated that this method is also applicable to the analysis of crude glycoproteins extracted from mammalian cells. However, it is not applicable to the analysis of glycolipids, one of the other important types of glycoconjugate at the cell surface. In this context, Pilobello et al. (2007) recently reported a ratiometric two-color lectin microarray to detect both glycoproteins and glycolipids simultaneously (Pilobello et al. 2007). However, the highly multivalent and diverse nature of glycans fabricated on each cell surface is destroyed during application of all of the above methods using cell lysates. Results thus obtained would fail to profile intact glycans, which is critical to the elucidation of cell signaling events occurring at the cell surface.
Glycomics is the systematic study of all glycans expressed in a cell. Till date, there has been no high-throughput technology available to analyze such cellular glycomes directly. In this report, we describe a novel strategy for mammalian cell surface glycome profiling using a lectin microarray that provides a snapshot of the cell surface glycans.

Results
A proposed strategy for direct profiling of the mammalian cell surface glycome

Figure 1A shows a basic strategy for a live cell-targeted lectin microarray system. 43 lectins with binding specificities for biologically relevant glycan structures of glycoconjugates were chosen and were covalently attached to epoxy-activated glass slides. The lectin microarray format and glycan-binding specificities of lectins used in this article are shown in Figure 1B and Supplementary Table 1, respectively. Monosaccharide symbols are listed in Figure 1C. The activity and quality of the lectin array were confirmed using various serum glycoproteins, neoglycoproteins, and monovalent oligosaccharides (data not shown) (Kuno et al. 2005). For live cell labeling, Cell-Tracker Orange CMRA reagents were used, which were converted to fluorescent derivatives once inside the cells. Chinese hamster ovary (CHO) cells used as a model cell line were incubated at 37°C with varying concentrations of CMRA (0–10 µM) in RPMI1640. Cells were immediately fluorescence-labeled, reaching a plateau after 30 min (see Supplementary Figure 1). Cells used throughout this study were labeled with 10 µM of CMRA by incubating at 37°C for 30 min.

The CMRA-labeled cells were allowed to bind to the lectin array at 4°C for 1 h. This was followed by washing with PBS by gravity under static conditions, bound cells were directly detected by an evanescent-field fluorescence scanner in PBS/BSA without fixing and permeabilization. Mammalian cell surface glycans were then analyzed from the patterns of fluorescence signals obtained.

To validate the system, we examined the binding of CHO cells to the lectin array. Varying numbers of CHO cells (0–3×10^5/well) were applied on the array, washed with PBS, and bound cells were detected by the evanescent-field fluorescence scanner. The signal intensities of the 43 lectins are visually different (Figure 2A). This indicates that CHO cells were specifically and selectively bound to the lectin-immobilized spots. That is, intense signals were observed on the spots corresponding to MAL, reaching saturation at 3×10^5/well, whereas less intense signals were detected on those of ECA, ABA, and HHL (Figure 2B). The fluorescence intensities were gradually amplified by increasing the number of cells applied to the array (Figure 2A and B). Little or no nonspecific binding on glass slides and control BSA spots were observed.

In the next step, to confirm the specificity of these interactions, an inhibition assay was performed using a disaccharide competitor, Galβ1-4Glc (lactose). As shown in Figure 2C and D, cell binding to both lactose binders (ECA, RCA120) and terminal βGal-, βGalNAc-binders (BPL, TJA-II, PNA, WFA, HPA, VVA, SBA) was specifically inhibited by the presence of 0.1 M lactose (shown in Yellow), whereas no inhibitory effect was observed on other lectins, indicating that the signals obtained were due to specific interactions between immobilized lectins and cell surface glycans.
Fig. 2. Validation of the system. (A) Dose-dependent fluorescent signals of CHO cells observed with the lectin array. Varying numbers of CMRA-labeled CHO cells were allowed to bind with lectin array (0–3 × 10^5 cells/well) and bound cells were detected by the evanescent-field fluorescent scanner. Data were analyzed with the Array Pro analyzer ver. 4.5 and the net intensity for each spot was calculated by subtracting background from signal intensity. The net fluorescence intensities of four lectins (MAL, ECA, ABA, and HHL) are shown in (B). Data are the average ± S.D. of triplicate determinants. (C) Carbohydrate inhibition assay. CHO cells in PBS/BSA were allowed to bind with the lectin array (1 × 10^5 cells/well) either in the presence or absence of 0.1 M lactose and bound cells were scanned with the evanescent-field fluorescent scanner. (D) Data were analyzed as described in Materials and Methods. Yellow indicates the signals inhibited by 0.1 M lactose. Data are the average ± S.D. of triplicate determinations.

Differential profiling of CHO and its glycosylation-defective mutant cells

After the basic validation above, we examined whether or not the observed cell binding to each lectin spot properly reflects the mammalian cell surface glycome. In Figure 3A, representative N-glycans, mucin-type O-glycans, and glycolipids synthesized by CHO cells are illustrated (Patnaik et al. 2006). According to literature, CHO cells express complex-, hybrid-, and high mannose-type N-glycans, but without modification by bisecting GlcNAc and sulfation (Campbell and Stanley 1984; Sasaki et al. 1987; Lee et al. 2001). As regards O-glycans, major structures stem from the core 1 disaccharide (Galβ1-3GalNAc),
Fig 3. Differential profiling of CHO and its glycosylation-defective mutants. (A) Predicted representative structures of N-glycans, mucin O-glycans, and glycolipids synthesized in the cell lines are shown by monosaccharide symbols in Figure 1A. (B) CHO cells and their glycosylation-defective mutants, Lec2, Lec8, and Lec1 in PBS/BSA were allowed to bind to the lectin array (1 × 10⁵ cells/well) and bound cells were scanned with the evanescent-field fluorescent scanner. (C) Data were analyzed as described in Materials and Methods. Data are the average ± S.D. of triplicate determinants.
CHO cells are bound to both asialo complex-type N-glycan binders (PHA-L, ECA, RCA120, PHA-E, DSA) and to high mannose-type N-glycan binders (NPA, ConA, GNA, HHL), but to lesser extents. This observation indicates that CHO cells predominantly express complex-type N-glycans consistent with the previous report (Sasaki et al. 1987). One of the characteristic features of CHO cells is that they express high density of α2,3-linked sialic acids (Sia), but not α2,6Sia (Sasaki et al. 1987; Lee et al. 1989). Thus, strong signals were observed for an α2,3Sia-binder (MAL), but not for α2,6Sia-binders (SNA, SSA, TJA-I). Signals were also observed for core fucose (Fucα1-6GlcNAc) binders (PSA and LCA) as well as broader fucose (Fuc) binders (AOL and AAL). On the other hand, essentially no binding was observed for UEA-I that specifically binds to α1-2Fuc, consistent with the previous reports that CHO cells do not express α1-2-(H), α1-3-(Leα), or α1-4-(Leβ) linked Fuc (Howard et al. 1987). CHO cells were also bound to core 1-binders (BPL, ABA, Jacalin, ACA, MPA) and sialo-mucin binders (MAH and WGA), supporting previous reports that CHO cells express core 1 and its disialylated form (Sasaki et al. 1987). In this context, the signals for Tn (GlcNAcα1-Ser/Thr) binders (WFA, HPA, VVA, SBA) were also observed, indicating that CHO cells express Tn, which is commonly expressed in mucin-type glycoproteins (Sasaki et al. 1987).

The profile obtained for CHO cells was compared with that of their glycosylation-defective mutants, Lec2, Lec8, and Lec1 determined similarly in the previous study (Ebe et al. 2006). As shown in Figure 3B, these mutant cells showed specific binding to lectin spots with low background. As expected from the representative glycans expressed in these cells (Figure 3A), Lec1 and Lec8 showed visually different profiles compared with wild-type (WT), whereas Lec2 showed much similarity. From a quantitative viewpoint, correlation coefficients of Lec1 and Lec8 relative to WT were 0.26 and 0.29, respectively, whereas that of Lec2 relative to WT was 0.75 (meaning close similarity) (Ebe et al. 2006).

Detailed interpretation of the results: Lec2 having a CMP-sialic acid transporter-deficient mutation is known to express lower amounts of sialylated glycans (Figure 3C) (Stanley et al. 1980; Eckhardt et al. 1998). In fact, the signals for Sia binders (MAL and MAH) were decreased, but significant amounts of α2,3Sia are still expressed in this cell line as previously described (Kaneko et al. 2007). Due to the reduced incorporation of sialic acid, enhanced signals were observed instead for Gal/GalNAc-specific O-glycan binders. Unexpectedly, enhancement was not observed in the signals for asialo (terminal βGal-containing N-glycan) binders (PHA-L, ECA, RCA120, PHA-E, DSA). A similar observation was made by a recent flow cytometry analysis that only a slight increase was detected in the binding of βGal-specific lectins, galectins, to Lec2 cells in comparison with WT (Patnaik et al. 2006). Interestingly, the signals for an α2-6Sia binder, TJA-I, were significantly increased, whereas other α2-6Sia binders were not. This could be due to binding affinity of TJA-I to the core 1 structure (Galβ1-3GalNAc) in a multivalent form.

Secondly, Lec8 has a deletion mutation in the Golgi UDP-Gal transporter, and hence, expresses much reduced galactosylated glycoconjugates (Figure 3C) (Stanley et al. 1980; Oelmann et al. 2001). As expected, Lec8 showed a significant decrease in the signals for βGal binders (PHA-L, ECA, RCA120, PHA-E, DSA), whereas compensated signals were observed for an agalactosylated glycan (GlcNAc) binder, GSL-II, as well as Tn (GalNAc) binders (HPA, VVA, DBA, SBA, GSL-I) (Oelmann et al. 2001; Nakamura-Tsuruta et al. 2006). Unexpectedly, enhanced signals were obtained for an α2-6Sia binder, SNA. Although SNA binds α2-6Sia with high affinity, it also binds to Tn structure when it is presented in a highly multivalent form (data not shown). Thus, the signals are not due to nonspecific binding of Lec8 cells to the immobilized lectin.

Finally, Lec1, being a β1-2-N-acetylgalactosaminyltransferase I (GnT-I)-deficient mutant, lacks both complex and hybrid type N-glycans, while expression of high-mannose type N-glycans is increased in a compensative manner (Robertson et al. 1978; Chaney and Stanley 1986; Chen and Stanley 2003). Consistently, a significant increase was observed for the signals of mannoside-binders (GNA and HHL), whereas the signals for asialo binders (PHA-L, ECA, RCA120, PHA-E, DSA) decreased substantially. The signals for a series of O-glycan binders showed an apparent increase, possibly because of total depression of complex-type N-glycans. In this regard, O-glycans and/or glycolipids might be spatially more exposed at the surface of Lec1 mutant cells as a result of the absence of much larger complex-type N-glycans, i.e., liberation from steric hindrance. If this is the case, such an observation has been first made by the present analysis most probably because previous lectin microarrays could not detect intact glycans expressed on cells (Angeloni et al. 2005; Kuno et al. 2005; Ebe et al. 2006). Increased signals for the GlcNAc-binder, GSL-II, might also be explained by the increased accessibility of this lectin to GlcNAc-containing glycoconjugates other than N-glycans such as the core 2 structure (Datti and Dennis 1993).

Differential profiling of splenocytes from WT and β3GnT2-knockout mice

As an extension, we applied the system to differential profiling of primary cells. For this, mouse splenocytes were prepared from both WT and β1-3-N-acetylgalactosaminyltransferase II (β3GnT2) knockout (KO) mice that lacks poly-N-acetyllactosamine (LacNAc) chain elongation on N-glycans (Togayachi et al. forthcoming) (Zhou et al. 1999), which can readily be detected by the poly-LacNAc-binders, LEL, and STL (Figure 4A) (Patnaik et al. 2006). As expected, binding was observed for WT splenocytes to these lectins (Figure 4B). Compared with WT, however, splenocytes derived from β3GnT2 KO mice showed a significant decrease in the signals for LEL and STL in a specific manner, because no particular difference was observed for other lectins except for a slight decrease in the signals of ABA. Notably, signals for PHA-L, a representative lectin binding to tetraantennary N-glycans, are similar for the WT and β3GnT2-KO mice. These results were confirmed by flow cytometry using LEL and PHA-L as probes (Figure 4C). Therefore, N-glycan branching features are likely to be almost the same between the two, but elongation events are likely to be different.
Glycome profiling using lectin microarray

**Fig. 4.** Differential profiling of splenocytes of WT and β3GnT2 knockout mice. (A) Predicted representative structures of N-glycans synthesized in the cells are shown by monosaccharide symbols in Figure 1B. (B) Splenocytes from WT and β3GnT2 KO mice were allowed to bind with lectin array (1 x 10^6 cells/well) and bound cells were scanned with the evanescent-field fluorescent scanner. Data were analyzed as described in Materials and Methods. Data are the average ± S.D. of triplicate determinants. (C) Splenocytes of WT and β3GnT2 knockout mice stained with LEL or PHA-L were analyzed by flow cytometry. Data are shown by mean fluorescence intensity (MFI).

**Glycome profiling of K562 cells during differentiation**

Having demonstrated that the glycan (cell) profiling system is useful for direct analysis of both cultured and primary cells, we finally investigated the effect of a chemical stimulant on developing cells. For this model experiment, K562 cells, which represent a cell line of a progenitor of erythroblasts, and which can be differentiated into erythroid lineages by the addition of sodium butyrate (NaBu), were used. Thus, K562 cells (1 x 10^6) were cultured for 4 days in 10 mL of Ham’s F12 media, either in the presence or absence of 1 mM NaBu, and their glycan expression patterns were compared (Gahmberg et al. 1984).

**Fig. 5.** Glycome profiling of K562 cells during differentiation. K562 cells before and after induction of differentiation with sodium butyrate (K562 + NaBu) were allowed to bind with the lectin array (1 x 10^5 cells/well) and bound cells were scanned with the evanescent-field fluorescent scanner. Data were analyzed as described in Materials and Methods. Data are the average ± S.D. of triplicate determinants.

As shown in Figure 5, NaBu-treated cells showed a significant increase in the signals for core 1 (Galβ1-3GalNAc), Tn (GalNAc), and sialomucin relative to untreated cells, whereas no substantial difference was observed for the other lectins. K562 cells are known to express a family of sialoglycoproteins that contain a large number of O-linked glycans (Gahmberg et al. 1984). Among them, glycophorin A is a major sialoglycoprotein containing as many as 15 O-glycans, together with a single N-glycan. Previously, Gahmberg et al. reported that O-glycosylation of glycophorin A in K562 cells is increased after stimulation induced by NaBu (Gahmberg et al. 1984), demonstrating that the system is fully applicable for analyzing dynamic glycome changes during differentiation.

**Discussion**

Although there are various advanced technologies for structural glycomics such as mass spectrometry, liquid chromatography, capillary electrophoresis, affinity chromatography, NMR, and glycan microarray, none of these have enabled direct analysis of the mammalian cell surface glycome at a cellular level. The method described in this paper is an extension of a previous technique, lectin microarray, by which the cell surface glycome cannot be analyzed. The developed procedure, consisting of metabolic fluorescent labeling and the evanescent-field fluorescent detection principle, is of novel power for live cell profiling. This extension provides various practical and potential advantages, which have not been attained previously: (i) a complete
set of cell surface glycans (glycome) can be analyzed in an intact state, (ii) a relatively low number of cells ($1 \times 10^5$–$1 \times 10^6$ cells per 135 spots) is required, (iii) experiments are carried out in a rapid and high-throughput manner (2–3 h after cell preparation), and (iv) cells remain viable throughout analysis, and thus can potentially be used in further functional analyses, such as cell signaling and apoptosis. The use of an evanescent-field fluorescent scanner enables liquid-phase observation of cell binding to the array, whereas other published methods require dried sample for detection (Pilobello et al. 2005; Zheng et al. 2005; Hsu et al. 2006; Lee et al. 2006), which considerably deforms cell-surface architecture which is an important structural element for the recognition of endogenous lectins (Collins and Paulson 2004; Collins et al. 2004; Nimrichter et al. 2004). In high throughput, the current system is superior to conventional flow cytometry. A number of lectin-binding assays can be carried out at once in a rapid and sensitive manner. Although the emerging MS imaging is also a highly informative method, its application is, at present, largely restricted to relatively small ionizable molecules.

Possible limitations of the present live cell-targeted lectin microarray include: (i) similarly to flow cytometry, absolute amounts of glycans cannot be determined directly from the signal intensities, although this limitation is common to all other lectin microarray systems, (ii) accurate glycan structures cannot be determined, (iii) cells are not amenable to long-term storage, and (iv) since washing steps are required before detection, weakly bound cells may be washed off the lectin microarray and thus glycans expressed at low levels may not be detected. However, the purpose of the technique is not to determine accurate glycan structures, but to obtain information of functional cell surface glycans that are recognized by lectins in living systems. In this context, it is rather interesting that signals are changed due to the disposition of the target glycans because of the altered accessibility of immobilized lectins.

Although the live cell-targeted microarray analysis resulted in reasonable glycome profiles of cultured cells as well as primary cells, reflecting their reported glycosylation phenotypes, unexpected signals were also observed. One of the probable reasons for this is the unknown specificity of the immobilized lectins to cell surface glycans in a multivalent form. Although the detailed specificities for monovalent oligosaccharides of the lectins used in this article have been elucidated by means of frontal affinity chromatography (Nakamura et al. 2005), those for multivalent glycans at the cell surface have never been studied.

Glycobiologists have long believed that glycosylation features significantly differ depending on cell types and states. In other words, cellular glycomes should be differentiated from each other (Yim et al. 2001), but there have been no high-throughput techniques available to demonstrate this. Now that a rapid, sensitive, and high-throughput method has been developed, the idea has strong experimental support as is evident from Supplementary Figure 2. A diverse set of cells clearly shows distinct signal patterns, indicating that each has its own “signature” on lectin microarray. Thus, glycome profiling by the developed method will be extremely useful for analysis of cell surface glycome remodeling during cell differentiation, cell identification, and quality control in various contexts of biology, medicine, and industry. The strategy presented in this article should be applicable to functional studies of endogenous lectins and their target cells.

### Materials and methods

#### Cell culture

CHO cells and their glycosylation-defective mutants, Lec1, Lec2, and Lec8 were cultured in RPMI1640 containing 10% fetal calf serum (FCS), penicillin (100 U/mL), and streptomycin (100 µg/mL). For Lec8, proline was included in the culture media. K562 cells were cultured in Ham’s F12 media containing 10% FCS, penicillin (100 U/mL), and streptomycin (100 µg/mL). K562 cells were induced to differentiate for 4 days with 1 mM sodium butyrate (NaBu). The differentiation was estimated visually from the color of cells due to hemoglobin synthesis. The β1-3-N-acetylgalactosaminyltransferase II (β3GnT2) knockout (KO) mice were generated by a random mutagenesis method based on trapping with the retroviral vector and back-crossed onto a C57BL/6N (Charles River Japan Inc., Kanagawa, Japan). The β3GnT2 KO mice lack poly-LacNAc synthase that results in a large decrease of poly-LacNAc on N-glycans. All mouse manipulations were approved by the Animal Care and Use Committee of the AIST.

#### Fluorescent staining

Cells ($1 \times 10^7$/mL) were incubated for 30 min at 37°C in RPMI1640 containing 10 µM of Cell-Tracker CMRA (Invitrogen, Carlsbad, CA). After washing, cells were resuspended in chilled PBS/BSA (10 mM phosphate–buffered saline pH 7.0 containing 10 mg/mL BSA).

#### Lectin microarray

The lectin microarray was prepared as previously described with minor modification (Kuno et al. 2005). Lectins were dissolved in spotting solution (Matsunami, Osaka, Japan) at a concentration of 1 mg/mL (MAH, MAL, SNA, SSA, TJA-I, UDA) or 0.5 mg/mL (other lectins) and spotted on an epoxy-coated glass slide (Schott, Mainz, Germany) using a noncontact microarray printing robot, Microsys 4000 SYSTEM (Genomic Solutions Inc., Ann Arbor, MI) with a spot diameter size of 500 µm spaced at 650 µm intervals. After spotting, excess nonimmobilized lectins were removed by washing with 10 mM Tris-buffered saline, pH 7.4 containing 1% (v/v) Triton-X (TBST). The glass slide was incubated in a chamber (>80% humidity) at 25°C for 18 h for lectin immobilization. After incubation, a silicone rubber sheet with seven wells was carefully attached to the glass slide, and blocked with 100 µL of blocking solution (StabiGuard Choice; SurModics Inc., Eden Prairie, MN) at 20°C for 1 h. Each lectin was spotted in triplicate. Glycan-binding specificities of the lectins are listed in Supplementary Table 1.

#### Lectin array hybridization

CMRA-labeled cells suspended in PBS/BSA were added to each well of a glass slide (100 µL/well) and incubated at 4°C for 1 h. After unbound cells were separated from the wells by gravity in cold PBS, bound cells with lectins immobilized on a glass slide were detected with an evanescent-field fluorescence scanner, SC-Profiler (Moritex Co., Tokyo, Japan) under Cy3 mode. Data were analyzed with the Array Pro analyzer ver. 4.5 (Media Cybernetics, Inc., Bethesda, MD). The net intensity for each spot was calculated by subtracting background from signal intensity. Data are shown as the ratio of fluorescence intensities...
of the 43 lectins relative to the maximal fluorescence intensity on the lectin array.

Supplementary data
Supplementary data for this article is available at www.glycob.oxfordjournals.org.

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Conflict of interest statement
None declared.

Abbreviations
β3GnT2, β1–3-N-acetylglucosaminyltransferase II; CHO, Chinese ovary cells; Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; KO, knockout; LacNAc, N-acetyllactosamine; Man, mannose; NaBu, sodium butyrate; PBS/BSA, 10 mM phosphate-buffered saline pH 7.0 containing 10 mg/ml BSA; Sia, sialic acid; TBST, NaBu, sodium butyrate; PBS/BSA, 10 mM phosphate-buffered saline pH 7.0 containing 10 mg/ml BSA; Sia, sialic acid; TBST, Tris-buffered saline, pH 7.4 containing 1% (v/v) Triton-X; T, Thomsen-Friedenreich; WT, wild-type.

References


