Structural and Quantitative Evidence for Dynamic Glycome Shift on Production of Induced Pluripotent Stem Cells*§

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We recently reported that induced pluripotent stem cells (iPSCs) prepared from different human origins acquired similar glycan profiles to one another as well as to human embryonic stem cells. Although the results strongly suggested attainment of specific glycan expressions associated with the acquisition of pluripotency, the detailed glycan structures remained to be elucidated. Here, we perform a quantitative glycome analysis targeting both N- and O-linked glycans derived from 20187 human iPSCs and human dermal fibroblasts as undifferentiated and differentiated cells, respectively. Overall, the fractions of high mannose-type N-linked glycans were significantly increased upon induction of pluripotency. Moreover, it became evident that the type of linkage of Sia on N-linked glycans was dramatically changed from α-2-3 to α-2-6, and the expression of α-1–2 fucose and type 1 LacNAc structures became clearly apparent, while no such glycan epitopes were detected in fibroblasts. The expression profiles of relevant glycosyltransferase genes were fully consistent with these results. These observations indicate unambiguously the manifestation of a “glycome shift” upon conversion to iPSCs, which may not merely be the result of the initialization of gene expression, but could be involved in a more aggressive manner either in the acquisition or maintenance of the undifferentiated state of iPSCs. *Molecular & Cellular Proteomics 11: 10.1074/mcp.M112.020586, 1913–1923, 2012.

Induced pluripotent stem cells (iPSCs)† are genetically manufactured pluripotent cells obtained by the transfection of reprogramming factors. Such iPSCs were first reported in 2006 for the mouse (1) and in 2007 for humans (2, 3). Although iPSCs have already been used in the fields of drug development and disease models (4–7), basic aspects of iPSCs largely remain to be elucidated to provide us with a fuller understanding of their properties and for therapeutic applications to be developed in the field of regenerative medicine. These aspects include the need for a definitive system to be established to evaluate their properties; e.g., pluripotency, differentiation propensity, risk of possible contamination of xenografts, and even the potential for tumorigenesis. Cell surface glycans are often referred to as the “cell signature,” which changes dramatically depending on the cell properties and conditions (8) as a result of changes in gene expression, including epigenetic modifications of glycan-related molecules. Glycans, because of their outermost cell-surface locations and structural complexity, are considered to be most advantageous communication molecules, playing roles in various biological phenomena. Indeed, SSEA3/4 and Tra-1–60/81, which have been used to discriminate pluripotency, are cell surface glycan epitopes that respond to some specific antibodies (9–12).

Glycan-mediated cell-to-cell interactions have been shown to play important roles in various biological phenomena including embryogenesis and carcinogenesis (13–16). This might also be the case for the acquisition and maintenance of iPSC and ESC pluripotency, although there remains much to clarify concerning the roles of cell surface glycans in these events. Thus, the development of novel cell surface markers to evaluate the properties of iPSCs and ESCs is keenly required. Toward this goal, a glycomic approach has been made by several groups (17–20). In our previous study using an advanced lectin microarray technique (21), thirty-eight lectins capable of discriminating between iPSCs and SCs were statistically selected, and the characteristic features of the pluripotent state were obtained. The glycan profiles of the parent SCs, derived from four different tissues, were totally different from one another and from those of the iPSCs. Despite this observation, the technique used lacks the ability...
to determine detailed glycan structures or allow their quantification. For this purpose, a conventional approach based on high performance liquid chromatography (HPLC) combined with matrix-assisted laser desorption-ionization (MALDI) - time of flight (TOF) mass spectrometry (MS) was undertaken for both the definitive identification of glycan structures and their quantitative comparison, which remained unclear in the previous analysis (21).

We report here structural data on N-linked and O-linked glycans derived from the human iPSC 201B7 cell line (2) and human dermal fibroblasts (SC) representing undifferentiated and differentiated cells, respectively. For quantitative comparison, the glycans were liberated by gas-phase hydrazinolysis from similar numbers of cells (22–25) fluorescently tagged with 2-aminopyridine (2-AP) at their reducing terminus (26, 27), following which the derived pyridylaminated (PA-) glycans were purified by multiple-mode (i.e. anion-exchange, size-fractionation and reverse-phase) HPLC. Their structures were determined and quantified by HPLC mapping assisted with MALDI-TOF-MS and exoglycosidase digestion analyses. This report thus provides the first structural evidence showing the occurrence of a dynamic “glycome shift” upon induction of pluripotency.

EXPERIMENTAL PROCEDURES

Cells—As representative iPSCs, 201B7 cells, the first iPSCs established from human dermal fibroblasts by the Yamanaka group, were used (2). The cells were maintained in DMEM-F12 medium (Invitrogen) supplemented with 20% knockout serum replacement (KSR: Invitrogen), 0.1 mM 2-mercaptoethanol (Sigma-Aldrich), MEM non-essential amino acids (Invitrogen) and 10 ng/ml recombinant human basic FGF (Wako, Osaka, Japan) on mitomycin C-treated mouse embryo fibroblast feeder cells. As a counterpart SC, we chose normal human dermal fibroblasts (American Type Culture Collection: ATCC), which were maintained in fibroblast basal medium (ATCC) supplemented with fetal bovine serum (2%). For both differentiated fibroblasts (SC) and undifferentiated 201B7 (iPS) cells, two separate cultures of the same quantities were prepared for quantitative comparison, which remained unclear in the previous analysis (21).

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**RESULTS**

**Basic Strategy—Quantitative structural analysis of glycans from 20187 iPSC and human dermal fibroblasts (SC)-** A basic strategy for the construction of a comprehensive glycosylation “grand map” is shown in supplemental Fig. S1. Both N- and O-glycans were liberated from lyophilized iPSCs and SCs by gas-phase hydrazinolysis (22–25), and whole glycans comprising both intracellular (ER and Golgi) and surface membranous fractions were re-N-acetylated and labeled with 2-AP (26, 27). The derived PA-glycans were first separated by anion-exchange HPLC (supplemental Fig. S2A). Neutral glycans, which passed through the column (designated N fraction), and the adsorbed anionic glycans were fractionated and designated A1–A5 (according to the order of elution, supplemental Fig. S2A). The above fractions were then subjected to size-fractionation HPLC (for fractions N, A1 and A4, see supplemental Fig. S2B). When necessary, each fraction was further purified by reversed-phase HPLC (chromatogram not shown), and quantified on the basis of fluorescence intensity relative to an appropriate authentic standard (PA-Glc).

As regards neutral glycans, unambiguous assignment was made for glycans No.1–12, 14–26, and 29–30 by reversed-phase HPLC with MALDI-TOF MS confirmation. For others (i.e. 13, 27–28), serial exoglycosidase digestion was performed with β-galactosidase, β-1–3 galactosidase, β-N-acetylhexosaminidase, α-mannosidase, α-L-fucosidase and α-1–2, 1-L-fucosidase. For the product observed, the retention time was compared with the standard identified in the respective reaction mixtures. For example, a glycan fractionated as iPSC N-6–3 (No. 13) was treated with an α-fucosidase with broad specificity, and the retention time observed for the product agreed with that of M2B (glycan No.1), for which an authentic standard is available. As a result, for these efforts, 27 N-linked glycans were identified as neutral glycans in iPSCs (Table I).

Apparent contamination by high-molecular weight polyhexose was also observed in the iPSC N fraction, and attributed to components included in the medium used for iPSC preparation (data excluded from Table I). Likewise, 16 N-linked glycans were identified in SCs (Table I). In addition, O-linked glycans were analyzed under the conditions employed for hydrazinolysis (100 °C for 4 h) (details to be published elsewhere). From N fractions of iPSCs and SCs, 5- and 2- O-linked glycans were obtained, respectively (Table II).

Acidic glycans were further analyzed by a combination of sialidase digestion, MALDI-TOF MS and reversed-phase HPLC (supplemental Fig. S2). After digestion with universal α-2–3, -6 sialidase (Clostridium perfringens), the resultant products were passed through a Mono-Q column, analyzed by MALDI-TOF MS and reversed-phase HPLC, and their structures were determined as described above. The type of linkage of each sialic acid was determined by susceptibility to two sialidases, i.e. α-2–3–specific sialidase (cloned from Salmonella typhimurium) and the universal α-2–3, -6 sialidase from C. perfringens. As a result, 8 acidic N-linked glycans (No. 31–36, 39, 40) were identified in the iPSC A1 fraction, while 2 N-linked glycans (No. 37, 38) and 1 O-linked glycan (No. 53) were identified in the iPSC A4 fraction (Table I, II). Likewise, 2 N-linked glycans (No. 41, 42), and 2 N-linked glycans (No. 43, 44) and 1 O-linked glycan (No. 53) were identified in the SC A1 and A4 fractions, respectively (Table II). Notably, all of the acidic N-linked glycans derived from iPSCs had α-2–6–linked sialic acid, whereas those from SC had the α-2–3 linkage type (described later). On the other hand, no N-linked glycans were detected in substantial amounts (<1 pmol) in the A2, A3, or A5 fractions. Instead, 4 O-linked glycans (No. 50–52, 54) were identified in these fractions from both cell types (Table II). Although the five O-linked glycans included in the A2–A5 fractions (No.50–54) were common to iPSCs and SCs, the molar fraction (%) of the α-2–3 disialyl structure (No.53) was greatly reduced (from 11.5% to 1.4%) in iPSCs, whereas those of the other 4 sialo-glycans remained largely the same (described below).

To confirm the reproducibility of the data obtained by structural analyses, two separate experiments were performed for both iPSCs and SCs using the same amounts of cell preparations. As a result, the obtained glycan values were largely the same. Moreover, individual values for various glycans agreed well between assays (supplemental Table S1). Indeed, sufficiently high correlation coefficients, i.e. 0.9846 and 0.9811, were obtained for iPSC and SC preparations, respectively (supplemental Fig. S3). Therefore, the data provided in this work assure a sufficiently high accuracy to permit discussion of the issue of a glycome shift from a quantitative viewpoint. Tables I and II provide details of the average yield of each glycan in iPSC and SC preparations.
Evidence for the Glycome Shift—Based on the above results, we noted the significant changes in N- and O-glycosylation patterns from SCs to iPSCs. Bar graph representations were made to compare molar fractions (%) of each glycan between the iPSC and SC fractions (Fig. 1). The data obtained are also summarized according to sub-categories of glycan structures presented in Fig. 2 (details described below).

High-mannose-type N-linked Glycans—Total amounts of high-mannose-type N-linked glycans were found to increase considerably in iPSCs (86.1% molar fraction) compared with SCs (69.9%) (Fig. 2). The significantly higher content of this type of glycan in iPSCs is represented by the presence of Man9 (No.10 in Table I) and Man5 (No.5). In contrast, complex-type N-linked glycans became less evident in iPSCs. Based on the presumed glycan synthetic pathways (30), these differences can likely be attributed to down-regulation of the expression of β-N-acetyl-glucosaminytransferase I (MGAT1), which adds GlcNAc to Man5 as the first step in the biosynthesis of complex or hybrid-type glycans. It is also possible to speculate that Golgi α-mannosidase II is responsible for this structural change. In this regard, the expression of the MGAT1 gene was lowered 20-fold in iPSCs relative to SCs as shown in a previous DNA microarray analysis (21). If this is the case, it explains well why its substrate, Man5 (No.5), accumulates in iPSCs, while the levels of its downstream products (i.e. complex type glycans) decrease. On the other hand, we could not detect a significant level (>1 pmol) of the MGAT1 product, i.e. GlcNAcMan5, which is a substrate of the Golgi α-mannosidase II. Because this enzyme is specific to GlcNAcMan5 and its downstream products, but not to upstream products lacking GlcNAc, it is not clear whether Golgi α-mannosidase II is a

| No. | Structure | SC | iPSC | No. | Structure | SC | iPSC |
|-----|-----------|----|------|-----|-----------|----|------|
| 1   | Pa         | N-7.3 (5.0) | N-5-4 (3.3) | 15  | Pa         | N-8-3 (0.5) | 31  | Pa         | A1-1 (0.5) |
| 2   | Pa         | N-10-1 (0.4) | N-7-5 (0.3) | 16  | Pa         | N-8-5 (0.4) | 32  | Pa         | A1-4-1 (1.1) |
| 3   | Pa         | N-13-1 (0.4) | N-9-2 (0.4) | 17  | Pa         | N-10-6 (0.5) | 33  | Pa         | A1-4-3 (0.5) |
| 4   | Pa         | N-9-3 (0.5) | N-10-8 (0.8) | 18  | Pa         | N-10-3 (0.7) | 34  | Pa         | A1-7-3 (0.7) |
| 5   | Pa         | N-14-1 (0.6) | N-12-1 (5.6) | 19  | Pa         | N-11-2 (0.4) | 35  | Pa         | A1-7-1 (1.0) |
| 6   | Pa         | N-17-1 (13.0) | N-14-2 (8.9) | 20  | Pa         | N-13-2 (0.5) | 36  | Pa         | A1-7-3 (0.3) |
| 7   | Pa         | N-19-1 (5.4) | N-17-1 (6.9) | 21  | Pa         | N-13-3 (0.7) | 37  | Pa         | A4-2-2 (0.6) |
| 8   | Pa         | N-19-2 (3.6) | N-17-2 (2.8) | 22  | Pa         | N-13-4 (0.9) | 38  | Pa         | A4-2-3 (1.4) |
| 9   | Pa         | N-21-1 (14.3) | N-20-1 (17.6) | 23  | Pa         | N-13-6 (0.3) | 39  | Pa         | A1-5-1 (0.3) |
| 10  | Pa         | N-22-1 (14.2) | N-23-1 (20.8) | 24  | Pa         | N-16-3 (0.4) | 40  | Pa         | A1-6-2 (0.5) |
| 11  | Pa         | N-23-2 (0.4) | N-24-4 (4.2) | 25  | Pa         | N-19-3 (4.5) | 41  | Pa         | A1-1-2 (1.9) |
| 12  | Pa         | N-26-1 (2.9) | N-26-1 (2.9) | 26  | Pa         | N-19-4 (0.7) | 42  | Pa         | A1-2-1 (2.7) |
| 13  | Pa         | N-9-1 (10.8) | N-6-3 (9.3) | 27  | Pa         | N-19-5 (0.6) | 43  | Pa         | A4-2-2 (1.5) |
| 14  | Pa         | N-11-1 (1.8) | N-8-2 (2.8) | 28  | Pa         | N-21-2 (0.7) | 44  | Pa         | A4-2-3 (5.2) |

| No. | Structure | SC | iPSC | No. | Structure | SC | iPSC |
|-----|-----------|----|------|-----|-----------|----|------|
| 1   | Pa         | N-9-1 (10.8) | N-6-3 (9.3) | 27  | Pa         | N-19-4 (0.7) | 42  | Pa         | A1-2-1 (2.7) |
| 2   | Pa         | N-11-1 (1.8) | N-8-2 (2.8) | 28  | Pa         | N-19-5 (0.6) | 43  | Pa         | A4-2-2 (1.5) |

* The hyphenated alphanumeric codes comprise fractionation procedures in the order of anion-exchange HPLC, size-fractionation HPLC and reversed-phase HPLC.

* The numbers in parentheses are the percentage as the total amount of N-linked glycans. Data are the average of two separate experiments.
regulatory enzyme partaking in the observed change in high-mannose structures.

GlcNAc-exposed N-linked Glycans—The levels of GlcNAc-exposed complex type N-linked glycans (No. 15–23) were increased in iPSCs (5.3%), and essentially absent in SCs (Fig. 2, Table I). For this reason, a lowered expression of B4GALT, which transfers β1–4-linked Gal to GlcNAc residues, is considered likely. In support of this, previous gene expression profiling showed a 15-fold decrease in B4GALT1, which is a major B4GALT in iPSCs (21). This explains the fact that a series of agalactosylated glycans with exposed GlcNAc (No. 15–23) are accumulated in iPSCs. On the other hand, sialylation appears to proceed immediately after full galactosylation occurs, because the proportion of sialylated complex-type glycans (6.9%) is much higher than that of fully galactosylated but non-sialylated glycans (1.7%). Taken together, these results lead to the conclusion that structures of complex type glycans of iPSCs are restricted to particular forms of sialoglycans (see below) in contrast to the strong depression of complex type glycan biosynthesis.

Sialylation—Sialylated N-linked glycans constituted 6.9% of the total N-linked glycans prepared from each sample to be 100%. Glycan numbers correspond to those used in Tables I and II.

α2–6 Sialylation—Sialylated N-linked glycans constituted 6.9% of the total N-linked glycans in iPSCs. Notably, the identified Sia-linkage was exclusively of the α2–6 type (Table I and Fig. 2). This is in clear contrast to SCs, where sialylated N-linked glycans constituted 11.3% of the total

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**Table II**

| No. | Structure | SC  | iPSC  |
|-----|-----------|-----|-------|
| 45  | PA        | N-6-1 | 6.5%  | 13.3% |
| 46  | PA        | N-7-1 | 5.2%  |       |
| 47  | PA        | N-6-1 | 3.9%  |       |
| 48  | PA        | N-7-2 | 4.1%  |       |
| 49  | PA        | N-8-1 | 6.6%  |       |

a The hyphenated alphanumeric codes consist of fractionation procedures in the order of anion-exchange HPLC, size-fractionation HPLC, and reversed-phase HPLC.
b The numbers in parentheses are the percentage as the total amount of O-linked glycans. Data are the average of two separate experiments.

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**FIG. 1.** Comparison of (A) N-linked and (B) O-linked glycans prepared from iPSC and SC. The yields of each glycan were expressed as percentages, taking the total amounts of N-linked or O-linked glycans prepared from each sample to be 100%. Glycan numbers correspond to those used in Tables I and II.

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N-linked glycans, but were mostly of the α-2–3 type. Consequently, sialic acid in N-linked glycans of iPSCs was dramatically changed from the α-2–3 to the α-2–6 type, whereas total sialylation was reduced by 61%. Terminal features of iPSCs, including α-2–6 sialylation, are summarized in Table III along with their relation to previously reported lectin probes and glycosyltransferases (21).

Further to the above, essentially no α-2–3 Sia should be present in iPSCs with only α-2–6 linkage. To confirm this shift in Sia linkage, we analyzed total fractions containing Sia residues. In this way, unfractionated acidic fractions (iPSC A1-A5 and SC A1-A5) were treated with different types of sialidases, i.e. C. perfringens α-2–3, -6 and S. typhimurium α-2–3 sialidases, and the resultant products were analyzed by anion-exchange HPLC (Fig. 3). When A1 and A4 fractions (corresponding to N-linked monosialylated and disialylated fractions, respectively) were treated with α-2–3 sialidase, almost all of the acidic glycans derived from the SC fractions were sensitive, indicating that they consist mostly of α-2–3 linked monosialylated (A1) and disialylated (A4) glycans, respectively. In contrast, the corresponding monosialylated (A1) fraction of iPSCs was substantially resistant to α-2–3 sialidase digestion. On the other hand, iPSC A4 gave two peaks corresponding to neutral and disialylated glycans after α-2–3 sialidase treatment.

Sialidase analysis of other acidic fractions suggested that A2, A3 (monosialo-), and A5 (disialo-) fractions correspond to O-linked glycans No. 51, 52, and 54, respectively (Table II). However, the difference in the linkage type of sialic acid in O-linked glycans between iPSCs and SCs was relatively small except for that of No.53. Nevertheless, the total content of acidic O-linked glycans was lower in iPSCs (58.2%) compared with that of SCs (84.9%).

α-1–2 Fucosylation—Significant amounts of α-1–2 linked fucose were found in both N- (1.7%) and O-linked glycans (8.0%) from iPSCs, while none were detected in SCs. This finding agrees well with the results obtained by previous lectin microarray analysis, and with the up-regulated expression of the two fucosyltransferases responsible for α-1–2 fucosylation, FUT1 and FUT2 (21) (see also Table III).

Two α-1–2 fucosylated N-linked glycans (No.27 and No.35) were identified in iPSCs: briefly, a glycan fractionated as iPSC N-19–4 corresponded to difucosylated biantennary glycan (m/z, 2033.6 [M+Na]+ in positive ion mode) in MALDI-TOF MS. This glycan was treated with α-1–2 fucosidase and an α-fucosidase with broad specificity. As a result, the retention times observed for the products agreed with those of core-fucosylated and non-fucosylated biantennary glycans, respectively (supplemental Fig. S4). Thus, iPSC N-19–4 was identified as α-1–2 and core-fucosylated biantennary glycan.
TABLE III
Typical features of pluripotency-related glycan structures, and relation of glycan ligands to corresponding lectin probes and glycosyltransferase genes

| Terminal structurea | Typical structurea | Glycan No. | Lectin probeb | Glycosyltransferasec |
|---------------------|-------------------|------------|---------------|----------------------|
| Sia                 | α2-6              | 31-40      | SNA           | ST6GAL1              |
|                     |                   |            | SSA           | ST6GAL2              |
|                     |                   |            | TJA1          |                      |
|                     |                   |            | rPSL1a        |                      |
| Fuc                 | α1-2              | 27, 35     | rGC2          | FUT1                 |
|                     |                   |            | rBC2LCN       | FUT2                 |
| LacNAC linkage      | β1-3              | 28         | BPL           | B3GALT5              |
|                     |                   |            |               |                      |

a For definition of structural descriptions, see legends to Table I.
b Lectins effective for probing the presence of depicted glycosylation features or glycan ligands. Data from ref. (21).
c Glycosyltransferase genes, of which over-expressions are responsible for the depicted glycan structures. Data from ref. (21).

Fig. 3. Analysis of the linking mode of sialic acid contained in iPSC and SC acidic fractions. Each iPSC A1, A4, SC A1, and A4 fraction was treated with α2-3, -6 sialidase and α2-3 sialidase, and the reaction mixtures were analyzed by anion-exchange HPLC as described in "Experimental procedures." S0, S1, S2, and S3 are standard glycans binding sialic acid.
(No.27). Likewise, a glycan fractionated as iPSC A1–7–1 (No.35) was identified as α-1–2 and core-fucosylated, and α-2–6 monosialylated biantennary glycan (Table I). In contrast, no α-1–2 fucosylated N-linked glycan was detected in SCs.

Two fucosylated O-linked glycans were detected in iPSC N-6–1 (No.47) and N-7–2 (No.48) fractions (Table II). They gave the same m/z value (973.4 [M+H]+ in positive ion mode) corresponding to a fucosyl Gal-core 2 structure in MALDI-TOF MS, and their retention times shifted to that of Gal-core 2 (No.46) after treatment with α-1–2 fucosidase. However, only No. 47 was sensitive to the digestion with α-1–2 fucosidase, no N-19–5, of which the retention time agreed with an authentic 1920

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ciently quantitative nor reliably comparative for the purpose of drawing a “grand map” of glycoprotein glycans from iPSCs and SCs. A recent study focusing on enriched plasma membrane of hESCs succeeded in part in revealing a glycome shift. Unfortunately, the observed change was restricted to the major group of N-glycans, i.e. high-mannose-type glycans, which represent immature biosynthesis products of N-glycans (31). On the other hand, the present study targeted whole cell extracts, and provides various lines of evidence in support of a “glycome shift” across a wider dynamic range. Interpretation of the results clearly reveals the strong tendency to suppress the biosynthesis of complex-type N-glycans (Fig. 4): a series of high-mannose type glycans became evident in iPSCs (Fig. 2), which is attributed to the down-regulation of relevant ER α-mannosidases and subsequent β-N-acetyl-glucosaminyltransferase (GnT) I. Together with accumulation of the resultant Man5, a key structure to complex type maturation, the biosynthesis of downstream products was further depressed by the down-regulation of β-1–4 GaIT (21). All these events seem to set up a “prerequisite” stage before cells become undifferentiated and acquire pluripotency.

More critically, however, significant structural changes have been observed in the outermost domains of glycans in terms of α-2–6 sialylation, H-type fucosylation, and type 1 LacNAc structure (Fig. 2). Some N- and O-linked glycans in iPSCs have α-1–2 linked fucose, but no such glycans were detected in SCs. Another impressive feature of iPSCs is the emergence of type 1 LacNAc structures both in N- and O-linked glycans (No. 28 and 49, respectively). Notably, glycan 49 corresponds to an epitope structure recognized by Tra-1–60/81 in a recent report (11). Because the type 1 LacNAc is produced by β-1–3-GaIT, this is likely to be a key enzyme relevant to pluripotency, in addition to ST6Gal and FUT1/2. Interestingly, all of the structural features determined in the present study are in good agreement with our previous data (21) obtained by lectin microarray and gene expression analyses (Table III).

Although the biological significance of the present findings remain largely elusive, some speculation is possible. A linkage from α-2–3 to α-2–6 may affect the biological functions of endogenous lectins. Galectins, a group of soluble β-galactoside-binding lectins exhibit no or greatly diminished binding to β-galactosides (e.g. LacNAc) when capped with α-2–6 linked sialic acid (32, 33). In this context, it is worth mentioning that ST6Gal 1 is over-expressed in many types of human cancers, which led to the hypothesis that tumor cells are protected against galectin-mediated apoptosis via α-2–6 sialylation of surface receptors (34).

Fucosylation is also involved in many cell adhesion and signaling events (35, 36). The published gene expression profiles have demonstrated that the α-1–2 fucosyltransferase gene FUT1 is overexpressed in hESCs compared with embryoid body. In addition, FUT2, FUT4, and FUT8, encoding α-1–2, α-1–3, and α-1–6 fucosyltransferase enzymes, respectively, are expressed in hESC (17). In our previous study, we showed that
FUT1 and FUT2 are overexpressed in iPSCs, but that FUT8 expression is decreased compared with SCs (21). Notably, stem cell specific overexpression of FUT1 correlates well with our finding that more complex fucosylation takes place in N-linked glycans of iPSCs. FUT2 expressed in stem cells is considered to be responsible for the Fuc/H251–1–2 fucosylation of O-linked glycans based on its preference for type 1, 3, and 4 chains, which were scarcely detected in N-linked glycans in the present study. The decreased expression of FUT8 in iPSCs is consistent with the decreased core-fucosylation.

FIG. 4. A glycome “grand map” covering presumed biosynthetic pathways of N-linked glycans produced in iPSCs and SCs. Glycan numbers (1–44) correspond to those defined in Table I. The map is constructed based on the generally accepted biosynthetic pathways of N-linked glycans (30). Red and blue arrows indicate biosynthetic reactions depicted in iPSCs and SCs, respectively. Estimated percentages of respective biosynthetic reactions are shown in italicized figures near the arrows, where the starting precursor Man9Glc3GlcNAc2 (No. 12, 100%) is taken as 100%. Quantitative data are from Table I. Thick arrows indicate specific reactions exclusively detected in iPSCs. The following monosaccharide symbols are used to express glycan structures: green circle, Man; blue square, GlcNAc; yellow circle, Gal; blue circle, Glc; red triangle, Fuc; violet diamond, NeuAc.

FUT1 and FUT2 are overexpressed in iPSCs, but that FUT8 expression is decreased compared with SCs (21). Notably, stem cell specific overexpression of FUT1 correlates well with our finding that more complex fucosylation takes place in N-linked glycans of iPSCs. FUT2 expressed in stem cells is considered to be responsible for the α1–2 fucosylation of O-linked glycans based on its preference for type 1, 3, and 4 chains, which were scarcely detected in N-linked glycans in the present study. The decreased expression of FUT8 in iPSCs is consistent with the decreased core-fucosylation.
Recently, Tang and co-workers reported a monoclonal antibody raised against human pluripotent stem cells (hPSCs), including hESCs and human iPSCs (37). Interestingly, this antibody, designated SSEA-5 was shown to specifically bind to the H type 1 glycan, Fucα1–2–Galβ1–3–GlcNAc. This epitope coincides with the glycan structure recognized by a lectin, rBC2LCN, which was found to be the best probe lectin to evaluate the glyciomics of iPSCs and ESSCs (21). In this respect, using an advanced lectin microarray technique called the “antibody-overlay method,” we have recently identified podocalyxin as a likely glycoprotein carrying rBC2LCN glycan ligands (Tateno et al., unpublished results). Moreover, rBC2LCN was found to show substantial affinity to α1–2 fucosylated type 1 (Galβ1–3GlcNAc) and type 3 (Galβ1–3GalNAc) glycan structures. In effect, an O-glycan (No. 48) corresponding to the latter has been identified in the present study. Although biological functions of these type 1/3 H glycans remain to be elucidated, their possible involvement in pluriotency via homophilic carbohydrate-carbohydrate interaction is feasible, as has been identified in compaction at the morula stage of F9 embryonal carcinoma cells (38). On the other hand, expression of the ABO gene was low in normal individuals. Further study is necessary to examine these hypotheses concerning glycan functions in pluriotency.

In conclusion, of particular note are the α2–6 sialylation, α1–2 fucosylation and type 1 LacNAc characteristics identified here, which are specific to iPSCs. Although the functions of these modifications remain unclear, one key experiment to solve this question is to characterize glycoproteins carrying rBC2LCN glycan ligands, which include podocalyxin. The present observations provide strong focus and direction for future stem cell biology research.

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[5] This article contains supplemental Figs. S1 to S4 and Table S1.

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