Metabolic glycoengineering of mesenchymal stromal cells with N-propanoylmannnosamine

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There is an increasing interest in the modification of cell surface glycosylation to improve the properties of therapeutic cells. For example, glycosylation affects the biodistribution of mesenchymal stromal cells (MSCs). Metabolic glycoengineering is an efficient way to modify the cell surface. The mammalian biosynthetic machinery tolerates the unnatural sialic acid precursor, N-propanoylmannnosamine (ManNProp), and incorporates it into cell surface glycoconjugates. We show here by mass spectrometric analysis of cell surface N-glycans that about half of N-acetyllactosamine was replaced by N-propanoylneuraminic acid in the N-glycans of human umbilical cord blood-derived MSCs supplemented with ManNProp. In addition, the N-glycan profile was altered. ManNProp-supplemented cells had more multiply fucosylated N-glycan species than control cells. The fucosylated epitopes were shown to be present in a predominance of the sLex epitope in these cells. The amounts of tri- and tetra-antennary and polylactosamine-containing N-glycans also increased in ManNProp supplementation. In accordance with previous studies of other cell types, increased expression of the sLex epitope in ManNProp-supplemented MSCs was demonstrated by flow cytometry. In light of the N-glycan analysis, the sLex epitope in these cells is likely to be carried by O-glycans or glycolipids. sLex has been shown to target MSCs to bone marrow, which may be desirable in therapeutic applications. The present results represent the first structural analysis of an N-glycome of ManNProp-supplemented cells and demonstrate the feasibility of modifying cell surface glycosylation of therapeutic cells by this type of metabolic glycoengineering.

Keywords: glycoengineering / mesenchymal stromal cell / N-propanoylmannnosamine / sialic acid / umbilical cord blood

Introduction

Mesenchymal stromal cells (MSCs; also called mesenchymal stem cells) are multipotent cells that can be harvested from umbilical cord blood (UCB), bone marrow or adipose tissue. MSCs are characterized by their ability to adhere to plastic and to differentiate along osteogenic, adipogenic and chondrogenic lineages, and by the expression pattern of a certain set of surface markers (positive for CD105, CD73 and CD90; negative for CD45, CD34, CD14 or CD11b, CD79a or CD19 and human leukocyte antigen (HLA) class II) (Dominici et al. 2006). MSCs are being explored to regenerate damaged tissue and treat inflammation, resulting from cardiovascular disease and myocardial infarction, brain and spinal cord injury, stroke, diabetes, cartilage and bone injury, Crohn’s disease and graft-versus-host disease (Malgieri et al. 2010).

MSCs show a characteristic glycosylation profile (Heiskanen et al. 2009), and certain glycan structures such as GD2 ganglioside (Martinez et al. 2007) and the i blood group epitope (Hirvon et al. 2012) can be used to differentiate MSCs from other cell types. The glycosylation of stem cells can be altered to optimize their therapeutic effects. For example ex vivo enzymatic fucosylation of MSCs to create selectin ligands directs the cells to bone marrow and primes them for transendothelial migration (Sackstein et al. 2008; Thankamony and Sackstein 2011).

The glycosylation of cells can also be modified by metabolic glycoengineering during culture (Du et al. 2009). The glycan biosynthetic machinery tolerates certain unnatural modifications of the metabolic substrates. Although nonnatural analogs of N-acetyllactosamine, N-acetylgalactosamine (GlcNAc) and l-fucose have been successfully incorporated into cell surface glycoconjugates, the efforts in metabolic glycoengineering concentrate on the sialic acid biosynthetic pathway, due to both the biological importance of sialylated glycan epitopes and the remarkable permissivity of sialyltransferases for modified substrates (Campbell et al. 2007). Analogues of N-acetyllactosamin (ManNAc), N-acetylgalactosaminic acid (Neu5Ac) and cytidine monophospho-Neu5Ac can be fed into the biosynthetic flux and become incorporated into cell surface glycoconjugates.
Nonnatural sialic acids created by metabolic glycoengineering affect many aspects of cell biology. They can enhance the immunogenicity of glycan epitopes (Hayrinen et al. 1995; Krug et al. 2012), modulate viral binding (Keppler et al. 1995), alter cell adhesion by activating integrins (Villavicencio-Lorini et al. 2002) and affecting levels of adhesion molecules such as carcinoembryonic antigen-related cell adhesion molecule 1 (Horstkorte et al. 2001) and sialyl Lewis x (sLex) (Horstkorte et al. 2004), inhibit contact-dependent growth control (Wieser et al. 1996), stimulate axonal growth (Büttner et al. 2002) and induce neuronal differentiation (Schmidt et al. 1998; Kontou et al. 2008). Metabolic influx of ManNAc analogs can also be used to introduce chemically reactive groups in sialic acid-containing glycans, which is useful in analyzing and imaging the glycome with fluorescent dyes (Prescher and Bertozzi 2005; Du et al. 2009). In this study, we present the effects of metabolic glycoengineering with N-propanoylmannosamine (ManNProp) on the glycosylation of UCB-derived MSCs. To our knowledge, these data represent the first mass spectrometric structural analysis of glycans derived from the surface of metabolically glycoengineered cells.

Results

Culture of UCB-MSCs in the presence of sialic acid precursors

MSC lines were prepared from UCB and cultured in standard conditions (Laitinen et al. 2011) until Passage 4, after which the cells were grown in the presence of 5 mM ManNAc, 5 mM ManNProp or without supplementation in standard medium for 2–3 days for mass spectrometric analysis and at least 4 days for flow cytometry studies. Supplementation with ManNAc or ManNProp did not cause changes in the viability, proliferation rate, morphology, surface marker expression or differentiation capacity of UCB-MSCs (Supplementary data, Figure S1). The cell line used has inherently low expression level of CD90, but it did not change with supplementation.

Expression of glycan epitopes on metabolically glycoengineered MSCs

Metabolically glycoengineered MSCs were stained with lectins and antiglycan antibodies and analyzed by flow cytometry to determine the expression of specific glycan epitopes (Figure 1). No difference between control cells, ManNAc-supplemented cells and ManNProp-supplemented cells was seen in the expression of α2,3- or α2,6-linked sialic acid (Maackia amurensis lectin 1 (MAL-1), Sambucus nigra agglutinin (SNA) and Limax flavus agglutinin (Sambucus nigra agglutinin)), Lewis x (LTA) or the stage-specific embryonic antigen 4 (SSEA-4) epitope. There was slight increase in α1,2-linked fucose (Ulex europaeus agglutinin (UEA-1) staining). The intensity of staining with anti-sLex antibody clones CSLEX-1 (anti-sLex) and CHO-131 (anti-core 2 sLex) was significantly higher in ManNProp-supplemented MSCs than in control cells and ManNAc-supplemented cells. The binding specificity of the anti-sLex antibodies was validated by sialidase treatment (Figure 2). Western blot analysis showed several proteins bearing sLex and core 2 sLex in UCB-MSCs (Figure 3). The intensity of staining by anti-sLex of a 200 kDa protein was particularly enhanced in ManNProp-supplemented cells.

Cell surface N-glycan profile of metabolically glycoengineered UCB-MSCs

Given the problems with availability and specificity, in addition to inherent limitations of the technique, profiling with lectins and antiglycan antibodies only gives a limited and inconclusive view of cell surface glycosylation. For a more thorough glycosylation analysis of metabolically glycoengineered UCB-MSCs, the cell surface N-glycan profile was analyzed by mass spectrometry (MS). Cell surface proteins were biotinylated on intact adherent UCB-MSCs, captured on streptavidin, and N-glycans were released from them for analysis. Permethylated and reduced cell surface N-glycans were first separated by nanoscale liquid chromatography (LC) and subsequently analyzed by high-resolution tandem mass spectrometry (MS/MS) using
an electrospray source in positive-ion mode. The N-glycan profiles were derived from LC-MS/MS data by using the in-house-developed software GlycanID (Peltoniemi et al. 2013). The ManNProp-supplemented UCB-MSCs showed an overall cell surface N-glycan profile typical of UCB-MSCs, where sialylated and core-fucosylated complex-type N-glycans dominate, whereas high mannose and hybrid N-glycans are present as minor components (Figure 4). The overall N-glycan profile of ManNProp-supplemented cells (Figure 4A) was similar to those of ManNAc-supplemented cells (Figure 4B) and control cells (Figure 4C), except for the replacement of Neu5Ac by N-propanoylneuraminic acid (Neu5Prop). When the intensities of the Neu5Ac and Neu5Prop forms of each composition were summed up and compared between the different culture conditions, the total amounts of most of the main components of the cell surface N-glycan profile, like the biantennary nonfucosylated and monofucosylated species, were approximately same in all the three cell culture conditions. The sialylation degree of the cell surface N-glycome was calculated by comparing the amount of sialic acid with the amount of potential sialylation sites. The sialylation degree was 46% in ManNProp-supplemented and control cells and 51% in ManNAc-supplemented cells.

Neu5Prop replaces Neu5Ac in ManNProp-supplemented cells
N-propanoylneuraminic acid differs from Neu5Ac by mass addition of 14 Da, and can thereby be distinguished in the mass spectrometric glycan profile (Figure 5). N-propanoylneuraminic acid partly replaced Neu5Ac in cells supplemented with ManNProp. All compositions containing sialic acid displayed the appearance of the same composition with the mass addition corresponding to the difference between acetyl- and propanoyl groups. Sixty-two percent of Neu5Ac was replaced by Neu5Prop in ManNProp-supplemented cells. The Neu5Prop incorporation occurred evenly across the N-glycome.

The amounts of multiply fucosylated N-glycans and large N-glycans increase by ManProp supplementation
The signal intensities of the compositions with more than one fucose and 0–1 sialic acids were higher in ManNProp-supplemented cells than in control cells or ManNAc-supplemented cells (Figure 6). Glycan compositions containing more than one fucose comprised 4% of all cell surface N-glycans in control cells and in cells supplemented with ManNAc, and 6% in cells supplemented with ManNProp. In addition, the signal intensities of the compositions where $H > 5$ and $N > 4$ (tri- and tetra-antennary N-glycans and/or polylactosamine-containing N-glycans) were higher in ManNProp-supplemented cells than in control cells or ManNAc-supplemented cells (Figure 7). For simplicity, a single-letter code is used for monosaccharides—$H$:Hex, $N$:HexNAc, $F$:Deoxyhexose, $G$:Neu5Gc, $P$:Neu5Prop, $S$:Neu5Ac and $X$: sialic acid. Large ($H > 5$, $N > 4$) N-glycans comprised 19% of all N-glycans in control cells and in ManNAc-supplemented cells and 23% in ManNProp-supplemented cells.

Structural analysis of multiply fucosylated and large N-glycans
The MS/MS fragmentation spectra of specific glycan precursor ions were annotated using the GlycoWorkbench software (Ceroni et al. 2008). The MS/MS spectra of P1H5N4F2 contained signals corresponding to sialylated nonfucosylated lactosamine ($m/z$ 861.43) and fucosylated lactosamine (Lex, Lea or blood group H; $m/z$ 660.32), but not sialylated and fucosylated lactosamine (sLex/a). Characteristic fragments arising from core fucose were also present ($m/z$ 717.38, $m/z$ 490.20) (Figure 8). The MS/MS spectra of S1H5N4F2 showed the
same fragments. The fragmentation pattern of H5N4F2 also indicated the presence of both core fucose and either the Lewis x/a or H epitope. Likewise, H5N4F3 gave characteristic fragments of core fucose and Lewis x/a or H epitope. The fragmentation pattern of P1H6N5F2 indicated the presence of core fucose, sialylated lactosamine, fucosylated lactosamine and nonsialylated, nonfucosylated lactosamine, but again no sLex was observed (data not shown).

N-glycan compositions larger than biantennary-type may be either tri- or tetra-antennary, or contain polylactosamines. Analysis of the MS/MS spectra of H6N5F1 indicated the presence of both core fucose, sialylated lactosamine, fucosylated lactosamine and nonsialylated, nonfucosylated lactosamine, but again no sLex was observed (data not shown).

Expression of genes related to fucosylation
To provide a mechanistic explanation for the altered glycosylation of ManNProp-supplemented UCB-MSC's, the expression levels of fucosyltransferases 1-11, fucosidases 1 and 2, fucose-2-phosphate guanyl transferase and guanosine diphosphate-fucose transporter in ManNProp-supplemented cells, ManNAc-supplemented cells and control cells were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). No significant differences were seen in the expression levels of these genes (Supplementary data, Figure S2).

Discussion
We report here the analysis of N-glycans and the expression of certain glycan epitopes on MSCs metabolically glycoengineered by supplementation with ManNProp. While the incorporation of Neu5Prop into the cell surface glycoconjugates in ManNProp supplementation has been documented for many cell types as analyzed by quantitation of sialic acid released from the cell surface, this study represents the first structural report of an N-glycome where Neu5Ac has been partly replaced by Neu5Prop.

The degree of incorporation (62% of Neu5Ac replaced by Neu5Prop) was similar to that reported for other cell types, typically 30–70% (Gaggiannis et al. 2007), except for cells lacking the bifunctional UDP-N-acetylglycosamine 2-epimerase/N-acetylmannosamine kinase, where incorporation is more efficient (85%) due to lack of endogenously formed ManNAc (Mantey et al. 2001). The replacement of Neu5Ac by Neu5Prop occurred evenly over the N-glycome, which suggests that the different sialyltransferases involved in sialylating N-glycans have equal affinities for ManNAc and ManNProp.

It is intriguing that the metabolic influx of ManNProp caused glycosylation changes beyond the incorporation of Neu5Prop, and even changes in nonsialylated epitopes. The increased expression of the sLex epitope upon ManNProp supplementation has been reported previously in human promyelocytic-60 cells (Horstkorte et al. 2004). The increased sLex expression could be attributed to enhanced affinity of the sialyltransferase for ManNProp or decreased activity of sialidases toward Neu5Prop-containing glycans, although the similar overall sialylation level in ManNAc- and ManNProp-supplemented cells

Fig. 5. Sialylated N-glycans in metabolically glycoengineered UCB-MSCs. The data are derived from LC-MS/MS analysis. Black bars represent control cells; gray bars ManNAc-supplemented cells and white bars ManNProp-supplemented cells. The same compositions with different sialic acid species are boxed. Monosaccharide abbreviations: S, N-acetylneuraminic acid, P, N-propanoylneuraminic acid; H, hexose; N, N-acetylhexosamine; F, deoxyhexose. Monosaccharide symbols: solid diamond, N-acetylneuraminic acid; striped diamond, N-propanoylneuraminic acid; white circle, galactose; gray circle, mannose; black square GlcNAc; triangle, fucose.
does not support this idea. Moreover, the altered expression of nonsialylated fucosylated epitopes, polylactosamines and tri- and tetra-antennary N-glycans cannot be explained by direct effect of the modified sialic acid on the glycosyltransferases or glycosidases. Thus, the signaling pathways triggered by ManNProp that lead to altered glycosylation remain to be elucidated. Regulation of glycosyltransferase gene expression by ManNProp is a possible mechanism. ManNProp supplementation has been shown to activate signal transduction pathways and alter gene expression in PC12 cells (Kontou et al. 2008; Horstkorte et al. 2010). However, we did not see any significant changes in the expression levels of genes related to fucosylation upon ManNProp supplementation.

There is an apparent discrepancy between the N-glycan structures analyzed by LC-MS/MS and the glycan epitopes detected by flow cytometry. While increased fucosylation in ManNProp-supplemented cells is detected by both methods and the slight increase in LTA-1 binding supports the mass spectrometric finding of increased expression of structures containing fucosylated nonsialylated epitopes, the marked increase in the intensity anti-sLex antibody staining is not supported by mass spectrometric findings in the cell surface N-glycome. This could be explained by the expression of the sLex epitope on O-glycans and/or glycosphingolipids which were not analyzed by MS in the present study. sLex is known to be expressed on O-glycans on other cell types such as leukocytes. One of the antibodies used here to detect sLex, CHO-131, is directed toward sLex in the context of a core 2 O-glycan, while the fine specificity of CSLEX-1 is less well known. Alternatively, sLex may be carried on such a rare population of N-glycans that it is under the detection limit of MS. Biologically relevant glycan epitopes that can be detected by antibodies may be invisible in the overall glycan profile of cells, but detectable in the glycosylation analysis of a single glycoprotein from the same cell, as is the case of sLex on P-selectin glycoprotein ligand 1 in WEHI-3 cells (Kawar et al. 2008). However, it is not known whether the presence of Neu5Prop instead of Neu5Ac alters the affinity of the antibodies for their epitopes, which should be kept in mind when interpreting the flow cytometry results.

Increased expression of the sLex epitope has been shown to confer MSCs with bone marrow tropism (Sackstein et al. 2008), which could be beneficial in bone repair applications. Increasing the amount of sLex on the cell surface also primes
the cells for transendothelial migration (Thankamony and Sackstein 2011), which is needed for entry into the site of inflammation or injury in any intravenous application. The amount of cell surface sLex has been increased by ex vivo enzymatic fucosylation of MSCs (Sackstein et al. 2008). The present observations demonstrate that metabolic glycoengineering may provide a cheaper and easier way to increase the amount of sLex epitopes on the MSC surface. However, the possible immunogenicity of Neu5Prop epitopes needs to be taken into account and controlled, if ManNProp-supplementedMSCs are intended for therapy.

The N-glycome of ManNProp-supplemented cells displayed an unexpected increase in the intensities of signals arising from large N-glycans (H > 5 and N > 4). Analysis of MS/MS spectra indicated that the N-glycan species responsible for these signals arise from both polylactosamines and tri- and tetra-antennary N-glycans. Polylactosamines are a typical feature of UCB-MSC N-glycosylation, and their expression wanes when the cells differentiate (Hirvonen et al., 2012). Polylactosamines and tri- and tetra-antennary N-glycans are the preferred ligands for certain members of the galectin family of N-acetyllactosamine-binding lectins. The affinity of galectin-3 for its ligands increases when multiple consecutive lactosamine units are present, as in polylactosamines (Stowell et al. 2008). Galectin-1 preferentially binds N-acetyllactosamine on tri- and tetra-antennary N-glycans (Hirabayashi et al. 2002). The biological functions of galectins are numerous and span a wide variety of cell types (Rabinovich and Toscano 2009). Notably, MSCs express galectin-1 and galectin-3 at high levels and have been suggested to be responsible for the immunosuppressive properties of MSCs (Sioud 2011). Therefore, enhancing the expression of galectin ligands and thus the tethering of galectins on the cell surface may influence the immunosuppressive properties of MSCs, which may render them more effective in anti-inflammatory applications such as cell therapy for graft-versus-host disease. Interestingly, ManNProp supplementation has been shown to upregulate galectin-3 expression in PC12 cells (Horskorte et al. 2010).

The present results show that metabolic glycoengineering with N-propanoylmannosamine (ManNProp) can be used to alter the glycosylation of UCB -derived MSCs. Metabolic glycoengineering could be used to change glycosylation into therapeutically beneficial direction, for example, to enhance desired biodistribution by enhanced expression of sLex or to increase the tethering of the immunomodulatory mediators galectin-1 and galectin-3 on the cell surface by increasing the density of their ligands. The principle of altering the properties of stem cells by incubating them with nonnatural ManNAc analogs has been demonstrated by inducing neuronal differentiation of human embryoid body-derived stem cells by N-thioglycolylneuraminic acid (Sampathkumar et al. 2006). Metabolic glycoengineering is a cost-effective and robust way to alter the properties of therapeutic cells.

Materials and methods
UCB -derived MSCs
UCB units were obtained via the Finnish Cord Blood Bank, Finnish Red Cross Blood Service, Helsinki, Finland. Voluntary donors gave informed consent and the study protocol was accepted by the ethical review boards of the Helsinki University Central Hospital and the Finnish Red Cross Blood Service. Cord blood was collected after normal vaginal delivery as described (Kekanainen et al., 2006). MSC lines were prepared as described (Laitinen et al. 2011). The cells were grown at 37°C in the presence of 5 mM ManNAc (Sigma-Aldrich, St. Louis, MO), 5 mM ManNProp (synthesized as described in (Keppeler et al. 1995)), or without supplementation in standard medium (Laitinen et al. 2011) for at least 4 days prior to flow cytometry analysis and for 2–3 days prior to MS analysis. UCB-MSCs were characterized to display MSC-like expression of surface markers by flow cytometry with fluorescent isothiocyanate (FITC-), PE- and APC-conjugated antibodies against CD13, CD14, CD19, CD29, CD34, CD45, CD73, CD90, CD105, HLA-DR and HLA-ABC (Dominici et al. 2006). Anti-CD90 (clone 5E10) was from Stem Cell Technologies (Vancouver, BC, Canada) or BD (Franklin Lakes, NJ). Anti-CD73, anti-HLA-ABC, anti-CD13, anti-CD14, anti-CD19, anti-CD45, and anti-HLA-DR, anti-CD29 and anti-CD105 were from BD. Anti-CD34 was from Miltenyi Biotech (Bergisch-Gladbach, Germany). The cells were shown to be able to differentiate into osteogenic, adipogenic and chondrogenic lineages as described (Suila et al. 2011). The cells used in this study were at Passage 5.

Flow cytometry
FITC-conjugated MAL-1 and SNA were from Vector laboratories (Burlingame, CA). FITC-conjugated LFA and LT Awere from Eyr laboratories (San Mateo, CA). FITC-conjugated UEA-1 was from Sigma-Aldrich. Anti-SSEA-4, clone MC-813-70, was from Millipore. Anti-sLex, clone CSLEX-1, was from R&D Systems (Minneapolis, MN) and anti-core 2 sLex, clone CHO-131, was from BD. The cells were stained with 30 μg/mL (anti-SSEA-4) or 15 μg/mL (CSLEX-1 and CHO-131) of the antiglycan antibodies followed by 7.5 μg/mL of FITC-conjugated rabbit anti-mouse IgM (Jackson ImmunoResearch, West Grove, PA) or 20 μg/mL of Alexa Fluor 488-conjugated goat antimouse IgG (Life Technologies, Grand Island, NY); or 10–20 μg/mL of the FITC-conjugated lectins. Unstained cells were used as controls for the lectin stainings, and cells stained with secondary antibody only for the antibody stainings. The cells were analyzed by the software packages FACS Aria and FACSDiv™ Version 5.0.2 (Becton Dickinson, San Jose, CA, USA).

Desialylation assay
UCB-MSCs were grown for 2 days in ManNAc or ManNProp or regular media and quickly detached from cell culture plates by trypsin. 500,000 cells were incubated with 200 μM Vibrio Cholerae neuraminidase (Sigma-Aldrich) or control media in 37°C for 2 h (the cells were suspended every 30 min). After the incubation, the cells were washed, fixed with PFA, stained with antibodies for FACS analysis using standard protocols and analyzed by FACS Aria and FACSDiv™ Version 5.0.2 (Becton Dickinson).

Western blot
Western blot was performed according to standard protocols using BioRad Mini-Protean Tris/Glycine/eXtended ready gels
4–20% and polyvinylidene difluoride (BioRad, Hercules, CA, USA). The filter was detected using the following antibodies: anti-core-2-sLex (clone CHO131) from R&D Systems, anti-sLex (CD15s) from BD Pharmingen and HRP-conjugated antimouse antibody from BioRad.

**Mass spectrometric analysis of cell surface glycans**

The cell surface proteins on intact cells were biotinylated essentially as described earlier (Scheurer et al. 2005). Hereafter, the labeled proteins were harvested with streptavidin-coupled magnetic beads (Dynabeads MyOne Streptavidin T1, Invitrogen, Carlsbad, CA, USA), reduced and alkylated as described earlier (Kinter and Sherman 2005). Digestion was carried out with immobilized TPCK trypsin (Pierce, Rockford, IL) at 37°C overnight with rapid shaking. Cell surface protein linked N-glycans were released from tryptic peptides by protein N-glycanase F (Sigma-Aldrich). After overnight incubation at +37°C, the peptide material was removed from the reaction mixture by C18 ZipTip (Millipore, Billerica, MA). The released N-glycans were reduced by using NaBH4 in 10 mM NaOH. The reduced N-glycan alditols were desalted by using graphitized carbon pipette tips (TopTip, Glygen, Columbia, ND) and permethylated by a solid-phase spin-column technique essentially as described (Kang et al. 2008).

Permethylated glycans were loaded to a reversed-phase precolumn (NanoEase Atlantis dC18, 180 µm × 23.5 mm, Waters, Milford, MA) and separated in a reversed-phase analytical column (PeptideMap 100, 75 µm × 150 mm, Thermo Fisher Scientific, Inc., Waltham, MA) with linear gradient of acetonitrile. Ultimate 3000 LC instrument (Thermo Fisher Scientific, Inc., Waltham, MA) was operated in nano scale with a flow rate of 0.3 µL/min. Eluted glycans were introduced to Linear Trap Quadrupole Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA) via an electrospray chip interface (Nanomate Triversa, Advion Biosciences, Inc., Ithaca, NY) in positive-ion mode. Based on full MS scan, six MS/MS data-dependent scans were acquired on Orbitrap-detector.

**RNA isolation, cDNA synthesis and qRT-PCR reactions**

RNA was isolated from pairs of flash-frozen UBC-MSCs (4–7 × 10^5 cells each) with or without treatment (ManNac or ManNProp) using RNeasy Plus Mini RNA isolation kit (Qiagen, Hilden, Germany) as described previously (Nairn et al. 2010). The isolated RNA samples were checked for contaminating genomic DNA using a cdNA synthesis reaction without the presence of reverse transcriptase. Superscript III First Strand Synthesis kit (Invitrogen) was used to prepare template cdNA as previously described (Nairn et al. 2010). Template cdNA reactions contained 0.5 µg of total RNA in a 20 µL reaction volume with an equal volume of oligo-dT and random hexamer primers and completed reactions were diluted 1:10 prior to addition to qRT-PCR reactions. The methods for primer design and validation in addition to the details of reaction setup were described previously (Naim et al. 2010). Briefly, triplicate reactions of 5 µL each consisted of 50% iQ™ SYBR® Green Supermix (BioRad), 25% diluted cdNA template and 25% primer pair mix (500 nM each primer, 125 nM final concentration, Eurofins MWG Operon) and were run in a RealPlex2 Mastercycler (Eppendorf) as described (Naim et al. 2010). The relative transcript abundance for each gene was calculated using the ΔΔCt method (Livak and Schmittgen 2001) using ACTB (β-actin) as the normalization gene as previously described (Nairn et al. 2010).

**Supplementary data**

Supplementary data for this article is available online at http://glycob.oxfordjournals.org/.

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**Abbreviations**

GlcNAc, N-acetylgalcosamine; LC, liquid chromatography; LFA, Limax flavus agglutinin; LTA, Lotus tetragonolobus agglutinin; MAL-1, Maackia amuriensis lectin 1; ManNac, 2-acetamido-3-O-methyl-3-deoxy-2-manno-2-ulosonic acid; ManNProp, 2-acetamido-3-O-(3-propionylmannosamido)-3-deoxy-2-manno-2-ulosonic acid; MAL-1, Maackia amuriensis lectin 1; ManNProp, 2-acetamido-3-O-(3-propionylmannosamido)-3-deoxy-2-manno-2-ulosonic acid; qRT-PCR, quantitative real-time polymerase chain reaction; sLex, sialyl Lewis x; SNA, Sambucus nigra agglutinin; SSEA-4, stage-specific embryonic antigen 4; UCB, umbilical cord blood; UEA-1, Ulex europaeus agglutinin.

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