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Cell Surface Sialylated N-Glycan Alterations during Development

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ABSTRACT

This brief survey focuses on the comparison of sialylated N-glycans of embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs) and of differentiated cells. In addition, the impact of sialic acid (Sia) deficiency on cell surfaces during development is summarized. The most common Sia is N-acetylneuraminic acid (Neu5Ac). The branched structures of complex- and hybrid- type N-glycans are the carrier for Sia. Transmembrane adhesive proteins, voltage-gated ion channels and many ligand-activated receptors are some examples of heavily sialylated N-glycan bearing membrane proteins. Their oligosaccharide extensions provide an important contribution to glycocalyx glycans. ESCs and iPSCs are characterized with high mannose-type and biantennary complex-type core structures. Two branches terminate with α2,6- linked Sia. MSCs contain high mannose, hybrid- and complex- type N-glycans. Linear poly-N-acetyllactosamine (poly-Galβ1-4GlcNAc, poly-LacNAc) chains are the characteristic structures. Both α2,3- and α2,6- linked Sias are seen in a species-specific manner in MSCs. α2,6- linked Sia is probably a marker associated with the multipotency of human MSCs. Differentiated healthy cells contain the most abundant 2-branched complex structures. The bisecting branch on the core structure appears as a differentiation marker. poly-LacNAc chains are terminated with α2,3- and α2,6- linked Sia, with the former being higher. poly-LacNAc sequences have a high affinity for β-galactoside recognizing lectin and galectin. Galectin forms a lattice structure with the N-glycans of glycoproteins anchored to the plasma membrane. The impact of N-glycan-galectin complexes in cell biology is summarized. Finally, the effect of reduced Sia on clearance of aged cells is explained. Experimental evidence for the masking role of Sia in the regulation of histolysis in aged cells is revealed.

Keywords: Sialic acid, sialylated N-glycan carriers, embryonic stem cell, mesenchymal stem cell, differentiated cell, sia deficiency, galectin lattice

INTRODUCTION

Glycosylation of the proteins starts with transferring the common N-glycan precursor to the growing peptide in the lumen of endoplasmic reticulum. The precursor (2 GlcNAc, 9 Man, 3 Glc) is attached in the amide nitrogen of the asparagine residue in the β-glycosidic linkage (GlcNAc β1-Asn) by oligosaccharyltransferase. Maturation reactions continue in endoplasmic reticulum and Golgi lumens by the successive actions of glycosidases and glycosyltransferases. The structure of the common glycan precursor changes. These enzymes cause the formation of high mannose-, hybrid-, and complex- types of N-linked oligosaccharides (Figure 1) (1-3). The branched structures of complex- and hybrid-type N-glycans are the carrier for Sia.

Many carrier proteins for sialylated N-glycans are present within the structure of the plasma membrane and extracellular matrix. The effects of sialylation on the structure/function of a glycoprotein is dependent on the localization of the Sia on the glycoprotein (4). Sialylated glycans significantly changes during development depending on the manner of cell and tissue type (5). Light and heavily sialylated plasma membrane glycoproteins are responsible for different functions. Transmembrane adhesive proteins (cadherins and integrins), voltage-gated ion channels (for Na^+, K^+ and Ca^{+2}) and many ligand-activated receptors (for EGF and others) are examples of heavily sialylated membrane glycoproteins. Cadherins are responsible for attachment to neighboring cells and provide a strong intercellular adhesion (6). Integrins mediate attachment to the ex-

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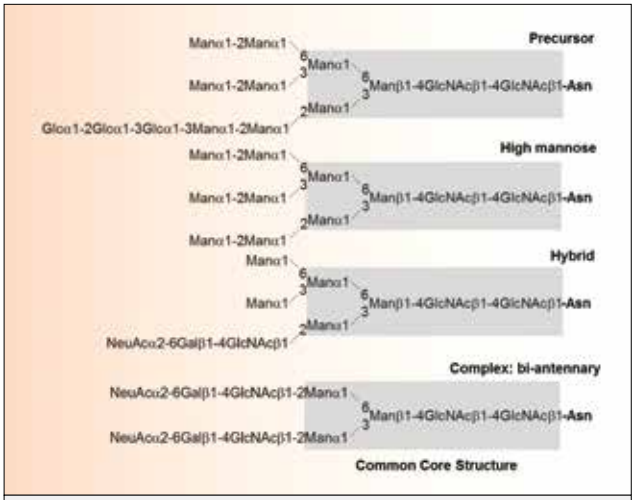

Figure 1. Precursor and basic types of N-glycans. The common core structure is shaded

tracellular matrix and transmit the signals across the plasma membrane in both directions (7). Voltage-gated ion channels are largely distributed in cell membranes and regulate membrane permeability for Na^+, K^+ and Ca^{+2} ions. Excitable cells in the nerve, skeletal and cardiac muscles play a significant role in regulating electrical excitability. In comparison with other membrane glycoproteins voltage-gated ion channels contain larger amount of Sia molecules (8).

Voltage-gated Na^+ channels (Na_v) are responsible for initiation, conduction and termination of the action potential in excitable cells. Sias alter Na_v gating activity in an isoform-specific manner. The number and location of sialylated chains and their attached pore forming or auxiliary subunits change the activity of Na_v (9,10). The comparison of N- and O- linked Sia contributions on channel gating is found to be similar (11) but, Sia and polySia have different effects on gating of the Na_v (12). Slight changes in channel function may cause several cardiac disorders (13).

Voltage-gated potassium channels (K_v) are responsible for repolarization of excitable cells (14). They contain significant amounts of Sias (8,15). Negative charges of Sias on N- or O-glycans cause conformational changes in the voltage-sensing domains but their effects on the channel gating mechanism don't appear the same (16). Cell surface expression of potassium channel $K_{v1.3}$ was investigated in tissue culture conditions on CHO pro 5 cells. Preventing N-glycosylation of $K_{v1.3}$ caused a significant decrease in its surface protein level. Supplementation of GlcNAc, L-fucose and Sia to the culture medium promoted $K_{v1.3}$ surface protein expression. Supplementation of GlcNAc lead to an increase in the degree of branching in the N-glycan core of $K_{v1.3}$. The increase in branching degree caused a decrease in internalization of $K_{v1.3}$ protein, but at the same time an increase in it's half-life on the cell surface (17). It is known that congenitally reduced sialylation causes altered gating of voltage gated Na^+ and K^+ channels, but the impact of reduced sialylation on cardiac electrical signals is not well known (18).

G-protein-coupled and ligand-activated receptors are other examples of sialylated membrane glycoproteins. In G-protein-coupled receptors, N-glycosylation and Sia have a role in the stabilization of the receptor dimers participating with disulfide bonding (19). Among the GFRs, EGFR is the most studied receptor, tyrosine kinase (20). For downstream signaling, the first necessity is the binding of the ligand, EGF, to its receptor. EGF-binding causes conformational changes of EGFR and it forms dimers. Dimerization leads to intracellular tyrosine kinase activation and subsequently auto-phosphorylation (21). EGFR bears twelve N-linked glycosylation sites on the extracellular region (22) and is heavily glycosylated (23). Sialylation diminishes EGFR activity by preventing its dimerization (24). Inhibition of sialylation on N-glycosylation is made by removing the Asn 420 and 579-linked glycans which causes ligand-independent dimerization (25). As well as, sialidase treatment promotes EGFR signaling (23). It is clear that sialylated N-linked glycans can affect the conformational stability of the channel and receptor proteins.

The presence of N-glycans on these heavily sialylated glycoproteins is important in cell physiology. They dynamically change by specific glycosyltransferases and glycosidases. In this way, biological events are regulated by covering or uncovering certain glycan sequences for recognition of specific lectins (galectins and siglecs) (26). Galectins with affinity for β-galactoside (Galβ1-4GlcNAc, LacNAc) form complexes with N-glycans on the cell surface of glycoproteins. They don't require a specific receptor and can bind with any of the suitable oligosaccharides from the cell surface or extracellular matrix (27). Galectin lattice regulates diffusion, selection, activation, arrest of T-cells, receptor kinase signaling and functionality of membrane receptors, glucose and amino acid transporters (28), and cell growth and differentiation (29).

Galectin-glycoprotein lattices control the organization of a plasma membrane domain like lipid rafts (30-33). Galectin lattices regulate lateral mobility of integrins (34), junctional stability of N-cadherins (32), receptor distribution at the cell surface (35), turnover of endocytic receptors (33), and intracellular signaling pathways (31,36-38). Galectins exhibit a remarkable functional diversity that participates in developmental processes, such as cell differentiation and pathophysiology, (39) cell adhesion and motility, regulation of immune homeostasis, and recognition of glycans on pathogens (26,28,40). It has been proposed that Galectin-glycoprotein lattices at the cell surface function as an "on and off switch" that regulates cell proliferation, differentiation, and survival (33). It appears that the cells may change cellular growth, differentiation, function, and probably pathologic transformation by altering the galectin glycoprotein lattice (35).

STRUCTURE, BIOSYNTHESIS AND FUNCTION OF SIALIC ACIDS

Sialic acids (Sias) are a large family of nine carbon monosaccharide sugars. The most common Sia is N-acetylneuraminic acid (Neu5Ac). Neu5Ac is the biosynthetic precursor for all other members of the family. It is usually found in a six-membered

ring configuration and the positions of carbon (C) 7-9 stay outside the ring as a glycerol side chain. C-1 has a carboxyl group, which is ionized at physiological pH to give a negative charge. C-5 determines the type of the four "core" Sia molecules. Presence of the N-acetyl group at the C-5 is known as Neu5Ac. Hydroxylation of the N-acetyl group at the C-5 produces N-glycolylneuraminic acid (Neu5Gc). De-acetylation of the N-acetyl group to an amino group forms neuraminic acid (Neu). The presence of a hydroxyl group at the C-5 produces 2-keto-3-deoxynonic acid (KDN) (41-44). Various substitutions (O-acetyl, O-methyl, O-sulfate and O-lactyl groups) of one or more of the hydroxyl groups at the 4-, 7-, 8- and 9- carbon positions of these "core" molecules form different modifications. Nearly fifty modifications exist in nature (42,45).

The sialylation pattern of a cell is regulated by three group enzymes; Sia Synthase, Sialyltransferase, and Sialydase or Neuraminidase. Sia synthase localized in the nucleus is responsible for the synthesis and availability of the activated Sia substrate, CMP-Sia. Sialyltransferase adds Sia during sialo-oligosaccharide biosynthesis using CMP-Sia as a donor, in trans-Golgi. The sialidase family separates from Sia during degradation of sialoglycoconjugates in lysosomes or endosomes (44). The specific sialylated pattern of a cell is produced by the activities of these three enzyme groups.

The C-2 on a Sia structure can form α glycosidic linkage with another sugar. The most common Neu5Ac can be found in four distinct linkage types to penultimate sugars. There are twenty known genes in mice and humans with encoding sialyltransferases for synthesizing these four major linkages. Some sialyltransferases (ST3GAL subfamily with six members) add Sia in an α2,3-linkage to Galactose (Gal), whereas others (ST6GAL subfamily with two members and ST6GALNAC subfamily with six members) add Sia α2,6-linkage to either Gal or GalNAc (N-acetyl-galactosamine). The fourth type of Sia linkage is directed by the polysialyltransferase family (ST8SIA subfamily with six members) which adds an α2,8 linked Sia to another Sia (4,46,47). Sialidase or neuraminidase enzymes are classified in four different groups, according to their subcellular localization, in mammals. The NEU1, NEU2, NEU3 and NEU4 enzymes are located in lysosomes, cytosol, plasma membranes, and lysosome/mitochondria (48-50). Combinations of different glycosidic linkages with the various substitutions produce structural diversity in hundreds of Sia molecules (51). This wide structural diversity of Sia molecules contributes to the enormous diversity of carbohydrate parts of proteins and lipids in cell membranes and secreted molecules (45).

Bio synthesis of Sia begins with the epimerization and subsequent phosphorylation of UDP-GlcNAc (Uridine Di Phosphate-N-Acetylglucosamine) to ManNAc-6-P (N-acetylmannosamine-six-phosphate), in cytosol. The bi-functional enzyme, UDP-N-acetylglucosamine 2-epimerase /N-acetylmannosamine kinase (GNE) catalyze these reactions. The enzyme Neu5Ac-9-P synthase combines with ManNAc-6-P and phosphoenol pyruvate to form Neu5Ac-9-P. Following dephosphorylation of Neu5Ac-9-P by Neu5Ac-9-P phosphatase, Neu5Ac is formed and transported into the nucleus. In the nucleus, Neu5Ac is changed to CMP-Neu5Ac by CMP-Neu5Ac synthetase and transported to the Golgi apparatus by the CMP-Sia transporter (16,52). Only the activated sugar is transported into the Golgi apparatus. In trans Golgi lumen, activated Sia serves as a substrate for sialyltransferases. Linkage-specific sialyltransferases add the Sia as a terminal unit to a selected glycan chain, depending on the availability of the preferred acceptor chain sequence (53,54).

The terminal location of Sia on the glycan chain facilitates the cell surface interactions between the cells and the immediate environment. These acidic monosaccharides act directly as a biological target for many pathogenic microorganisms. For example, human parainfluenza virus initiates an infection through Sia recognition and binding in a glycosidic linkage specific manner. α2,3- linked and α2,6- linked Sia residues are receptors for the type-1 and type-2 parainfluenza virus, respectively (55). In addition, Sias have a masking role, covering penultimate sugars. Sia covers penultimate galactose residue that is recognized by an asialoglycoprotein receptor. In a similar way, Sia covers penultimate LacNAc (Galβ1-4GlcNAc) that is recognized by a β galactoside recognition lectin, it is known as galectin. These two main functions of Sia participates in the regulation of many important events during development, such as cell communication, differentiation, aging, adhesion, migration, self/non-self-discrimination and many cell signaling events.

EMBRYONIC STEM CELL SIALYLATED N-GLYCANS

Stem cells are undifferentiated cells with a high capacity for self-renewal (proliferate indefinitely) and pluripotency (differentiated into three germ layers) (56). Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are valuable resources for cell replacement therapies. Determination of specific markers are required for effective utilization of stem cells and elimination of tumor initiating cells (57). For this purpose, cell surface glycan variations have been investigated to detect the most appropriate markers.

A study to determine the characteristic surface of glycosylation patterns was conducted with labeled lectin applications on glycosidase digested or nondigested cells by microscopic methods (58-60). Useful impressions of mainly terminal and some internal monosaccharide residues of oligosaccharide chains were obtained from these studies. Advanced analytical techniques including nuclear magnetic resonance (NMR) and mass spectrometry (61) and newly developed microarray technology (62,63) have provided additional structural information about stem cell glycosylation.

In human stem cells, the major pluripotency-specific N-glycosylation is made up of a high mannose-type and a biantennary complex-type core structure as determined with MALDI-TOF mass spectrometry NMR spectroscopy (61), flow cytometry and immunohistochemistry, (58, 64,65) and fluorescein labeled lectin staining (59). Upon induction of pluripotency, the occurrence of a significant increase in the high mannose-type N-gly-

cans (66,67) indicate that it is in an immature stage of N-glycoproteins (68,69). Biantennary complex-type core structures bear type 2 N-acetyl lactosamine (LacNAc, Galβ1-4GlcNAc) chains in hESCs (61). However, Type 1 LacNAc (Galβ1-3GlcNAc) is a characteristic feature of hiPSCs (62,68). The LacNAc chains are terminated with α2,6- and α2,3 linked Sias, but the α2,6- Sia linked higher in hESCs. In iPSCs, a linkage type of Sia changed from α2,3 to α2,6. However, large branched poly-LacNAc chains present in mouse ESCs suggest that it might participate in cell survival by increasing the interaction among membrane molecules (70). Complex terminal fucosylation is also characteristic of the N-glycosylation structure of hESCs (61,62,68). One terminal bears an α1,2-linked fucose residue (Fucα1-2Galβ1-4GlcNAc) while the other terminal bears an α1,3- or 1,4-linked fucose residue producing a Le^x structure (Gal β1-4 (Fucα1-3) GlcNAc). In addition, the most abundant fucose linkage in hESCs N-glycans is a core α1,6- linked fucose residue which links to the asparagine-linked GlcNAc residue.

MESENCHYMAL STEM CELL SIALYLATED N-GLYCANS

Mesenchymal stem cells (MSCs) are adult multipotent progenitor cells. They differentiate into mesenchymal cell lineages. Adipose tissue, bone marrow, and umbilical cord blood are harvested sources of MSCs (71,72). MSCs are considered very valuable cell sources for stem cell-based therapy because of the probability of teratoma formation in ESCs and iPSCS (73,74). The ability to adhere to plastic surfaces is one of the main characteristics of MSCs and it is also useful for glycoengineering studies (71).

High mannose-type N-glycans are characteristic for undifferentiated bone-marrow derived MSCs from humans (73,75) and equines (76). Biantennary N-glycans are detected in adipose tissue-derived hMSCs (72). Hybrid type N-glycans are found to be the less abundant type of N-glycosylation in undifferentiated and adipogenically differentiated hMSCs (73,75). In mice, an extraordinary branch is demonstrated by expression of GnT-V (the corresponding gene is Mgat5) in neural progenitor cells that have a self-renewal ability and multipotency (77). This is amazing because GnT-V is associated with oncogenic differentiation (78,79). Although a suggested function of GnT-V is related with cell growth and migration by regulating integrins (80) in cancer cells, expressed GnT-V in neural progenitor cells may perform a similar function during neural development and brain injury conditions (77). GnT-V and its reaction products appeared in proliferating cells. In differentiated cells, they were markedly reduced (77).

Linear poly-LacNAc chains terminated with Sia are the characteristic structures in undifferentiated hMSCs (75,73). Using mass spectrometry-based quantitative techniques, different amounts of α2,3-linked Sia obtained from the hMSCs expanded on different surfaces were detected. These results reveal that α2,3- sialylation participates in controlling hMSC multipotency by regulating cell adhesion (81). It is known that MSCs change differentiation potentials depending on the stiffness or chemically modified substrates (82,83). In addition, bone marrow-derived hMSCs, osteogenic precursors, and poly-LacNAc chains are terminated with α2,3- linked Sia (75).

Comparisons of lectin binding intensity of early (with differentiation ability) and late (without the ability to differentiate) passages of adipose derived hMSCs, using high density lectin microarray, demonstrated that early passage cells show stronger binding to specific lectins for α2,6-linked Sia (74). Bone marrow-derived hMSCs and cartilage tissue-derived chondrocytes also showed this similar binding activity, but no binding activity is indicated between α2,6-linked Sia and its specific lectins in human dermal fibroblasts. Using HPLC analysis combined with MS spectrometry, α2,6-linked Sia on sialylated N-glycans is detected at a higher percentage (24-28 %) in early passage cells than late passage cells (13-15 %) in adipose tissue-derived hMSCs (72). These results suggest that α2,6-sialylation is a marker associated with the differentiation potential in stem cells (74,72). The presence of α2,3- and α2,6 linked Sia on bone marrow derived macrophages and osteoclast precursors (MSCs) is demonstrated with lectin labeling in mouse (84). α2,3- linked Sia was detected throughout osteoclastogenesis, but α2,6-linked Sia disappeared at the terminal stage of differentiation. Desialylated cells, particularly α2,6-linked Sia-deficient cells do not develop into multinuclear osteoclasts. This suggests that α2,6-linked Sia may be involved in osteoclast differentiation and regulating the cell fusion process (84).

Fucosylated N-glycans of human bone marrow MSCs bear at least two or more fucose residues, one of which is a core fucose that is α1,6-linked to the asparagine-linked GlcNAc residue of the N-glycans (73). Using a cell microarray procedure, a core fucose addition to α1,3 terminal fucose and complex terminal fucose were detected on N-glycans MSCs of canine and ovine species (76). Finally, according to a study performed on veterinary species in bone marrow derived MSCs, terminal Sia linkage shows variability in a species-specific manner. For example, while the MSCs of canines contain more α2,3- linked Sia, equine MSCs have a higher density of α2,6- linked Sia (76).

DIFFERENTIATED CELL SIALYLATED N-GLYCANS

The dynamics of expression on cell surface glycans vary widely depending on cell types and stages of development. Characteristic changes during healthy or pathologic differentiation processes have been reported (85-87). Some glycan structures on ESCs, iPSCs and MSCs disappear and a new glycan profile occurs on differentiated cells. Alterations appear mainly in core branching, in poly-LacNAc extension and in terminal units, such as in Sia and fucose content.

High mannose-type N-glycans represent immature forms of N-glycans (65). In differentiated cells, the quantity of high mannose-type N-glycans decreases and complex/hybrid types of N-glycans start to appear in abundance (61,66,88). N-acetylglucosaminyltransferases (GlcNAcTs, GnTs) (as productions of Mgat genes) conduct the production of Tri and tetraantennary and more branched, complex N-glycans in Golgi apparatus. Sequential activity rules for the GnTs were established by Brockhausen

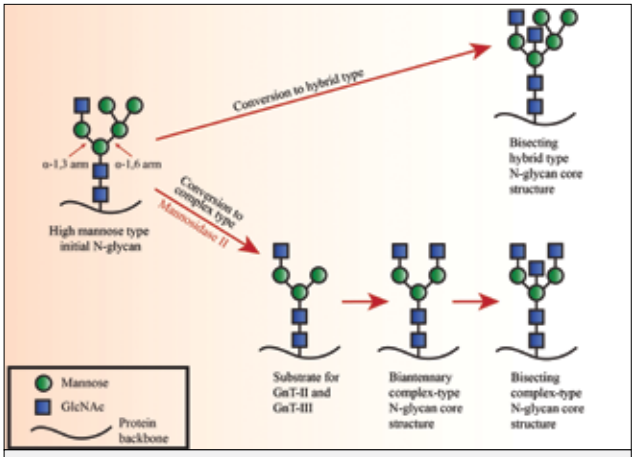

Figure 2. Two- three- and four branched N-glycan core structures, modified from Taniguci and Korekane (2011) with permission

et al. (89). These enzymes are responsible for the branching of the core structure *in vitro* (90-94).

"GnT-1 acts before all other GnTs and is responsible for the conversion from the high mannose-type to the hybrid and complex-types of N-glycans (Figure 2) (91). It catalyzes the formation of β1-2 linkage by transferring GlcNAc sugar to the Man residue on the α1-3 arm of the core structure with 5 mannose (Man). α-mannosidase II removes two Man residues on the α1-6 arm and a substrate for the GnT-II and GnT-III enzymes form. GnT-II controls the conversion of a hybrid type to complex type structures and catalyzes the β1-2 linkage by adding GlcNAc to the α1-6 arm on the core. Activity of GnT-II is a prerequisite for the GnT-IV, GnT-V and GnT-IX enzymes (95) that are responsible for cancer progression. GnT-III catalyzes the formation of β1-4 glycosidic linkage by transferring GlcNAc to the first Man residue on the core (Figure 2). The formation of a bisecting GlcNAc prevents subsequent processing and elongation of N-glycans (90) which inhibits the catalytic activity of the GnT-II, GnT-IV, GnT-V and FUT8 enzymes that are responsible for branching of the core structure *in vitro* (89,91).

Differentiated healthy cells contain the most abundant 2-branched complex structures on their N-glycoproteins. A few 3- and 4- branched glycans are also present (3). The distribution of PHA.E lectin ligands (58,59) and N-glycan signals (61) indicate the presence of bisecting GlcNAc on the N-glycan core of differentiated cells. During neural differentiation of murine iPSCs and ESCs, the bisecting N-glycan is upregulated and suggested as a differentiation marker (96). In caco-2 cell differentiation, the levels of bisecting N-glycan increase (88). Similarly, human induced pluripotent stem cell-derived cardiomyocytes also contain bisecting GlcNAc in relative abundance (97).

During primary human hepatocyte differentiation from hESCs, the first bi and triantennary complex N-glycans were found in hepatocyte-like cells (98). They were asialylated, monosialylated or fully galactosylated N-glycans. When they differentiated to primary human hepatocyte, bisialylated biantennary and trisialylated triantennary N-glycans were dominant. Similar changes observed during monocyte-derived macrophage differentiation show an increase particularly in triantennary glycans (99). One (Y101) of the mesenchymal stromal cell clones showed an abundant amount of complex N-glycan, during differentiation into osteoblasts (100). In addition, another mesenchymal stromal cell clone (Y202), which cannot differentiate into osteoblasts, showed a similar reduction in oligomannose glycan content when incubated in an osteogenic medium. Based on these observations, it suggests that expressed N-glycans, upon induction of differentiation, may be important for self-renewal rather than for cell fate determination (100).

Several N-glycans that have bisecting GlcNAc carry LacNAc repeats and a core fucose sugar in their glycomic profiles (101). The presence of the bisecting GlcNAc on the cell surface glycoproteins affects their interaction with galectins and siglecs, probably altering N-glycan conformation (92).

Characteristic changes occur on terminal sialylation and fucosylation of branched N-glycans during differentiation. Hybrid type N-glycan branches in a transition embryoid body bear both α2,6- and α2,3- linked Sia in humans (61). Differentiated chondrocytes have α2,6- linked Sias. The expression ratio of α2,6- to α2,3- linked Sias determines the differentiation status of chondrocytes (102). α2,6- sialylation decreases in adipogenesis (103) and osteoclastogenesis (84) in mouse but desialylated cells do not differentiate into osteoclasts despite the normal expression of an osteoclast marker.

During brain development, completely differentiated cells bear mostly α2,3- linked Sia as compared with α2,6- linked Sia, in rats (104). This development is explained with a linkage shift from α2,6- linked Sia to α2,3- linked during mouse brain development (105). This linkage shift may affect the biological functions of endogenous lectins, such as galectins and siglecs. The binding activity of galectins is greatly diminished when LacNAc chains are capped with α2,6- linked Sia (106,107).

N-GLYCAN-GALECTIN LATTICES

Galectins are soluble proteins and found within the cell, cell surface, and ECM as well as, in biological fluids. (108). They recognize and bind to β galactoside (Galβ1-4GlcNAc, LacNAc) sequences. LacNAc sequences are seen on poly-LacNAc extensions of tri- and tetraantennary N-glycan chains on cell membrane glycoproteins. In general, affinity of galectins to poly-LacNAc sequences is higher when compared with the affinity of LacNAc alone. However, galectin types (Gal-1, Gal-2, and Gal-3) display some differences in glycan binding properties. For example, only Gal-3 is bound to proximal LacNAc of poly-LacNAc extensions (109).

Galectin binding is influenced by N-glycan branching, LacNAc content and the balance of α2,3- and α2,6- linked terminal Sia (38). While Gal-1 is connected to only α2,3- sialylated poly-LacNAc, Gal-3 is connected to both α2,3- and α2,6- sialylated glycans (109). α2,6- sialylation alters binding of specific galectins, being consistent with biological function differences (35).

A detailed study on oligosaccharide binding specificity of galectins was performed using frontal affinity chromatography (106). Three (OH) groups on LacNAc, i.e. 4-OH and 6-OH of Gal, and 3-OH of GlcNAc are required for binding of galectins. In complex type N-glycans, no galectin could bind if 6-OH of Gal linked to a Sia. However, it did show that modified glycans such as α1-2 Fuc, α1-3- Gal, α1-3- GalNAc and α2,3- Sia have a preference for galectins (106).

Galectins released from cells are concentrated on the cell surface and generate galectin-glycan complexes. Gal-3, existing as a monomer in solution, produces a pentameric structure through self-associated intermolecular interactions and mediates crosslinking of proteins, forming a lattice organization (108, 110,111).

IMPACTS OF SIALIC ACID DEFICIENCY AT CELL SURFACES

Disorders at any stage of Sia biosynthesis, inactivation of the functional enzymes, such as GNE and activity of endogene sialydases cause Sia deficiency on cell surfaces. GNE (UDP-GlcNAc2-epimerase/ManNAc kinase) catalyzes the first two steps of Sia biosynthesis. Inactivation of GNE causes early embryonic lethality in mice (112-114). The GNE-deficient ESCs are not successful in the formation of embryoid bodies in the first day of culture. However, following the addition of a Sia, GNE-deficient ESCs form normal embryoid bodies (115). Proliferation of ESCs is correlated with GNE-expression and the cellular Sia concentration (113).

Comparison of the developmental profiles of wild type and GNE knockout mice indicates that GNE plays an important role in the development of excitable tissues (114). Genetic defects of the GNE cause GNE myopathy, a disease related with progressive muscle atrophy and weakness. Hyposialylation and production of reactive oxygen species, ROS, are correlated with muscle atrophy but an increase in sialylation causes a reduction in ROS. Depending on this relation, it suggests that Sia has a role as a ROS scavenger in skeletal muscles (116). In GNE myopathy, lectin binding and MS analysis show that GNE-deficient cells contain low levels of sialylation and distinct N-glycans, differentiated in branching of core structure and in poly-LacNAc extensions. These N-glycan chains display a binding affinity with galectin 1 (117).

The effect of desialylation was first described during a clearance of serum glycoproteins (118,119). At the same time, a hepatic galactose specific receptor (asialoglycoprotein receptor) from a rabbit liver was characterized (120) and a similar clearance mechanism for the desialylated cells, for erythrocytes in the rabbit was reported for the first time (121,122). According to a postulated hypothesis; a time-dependent loss of Sia residues uncovers the penultimate galactose residues. An asialoglycoprotein receptor, a lectin (123-125), in rat liver and peritoneal macrophages, recognizes and captures these galactose residues.

This receptor, a C-type lectin, is involved in the recognition and binding of terminal galactose on the glycan chains and is responsible for the clearance of asialoglycoproteins, and redetermined as an Ashwell-Morell Receptor (126). In a similar way, Sia residues of membrane glycoconjugates control the lifespan of erythrocyte. Desialylation of glycophorin (127) is responsible for the clearance of aged erythrocytes (128). Enzymatically desialylated erythrocytes in vitro show that the desialylation rate of aged cells is low but sufficient to lead to their capture by macrophages (124). Comparisons of quantified Sia on young and old cells claim that the decrease in Sia with cell aging may act as a senescent cell marker, capable of triggering their selective removal (129). Insufficient sialylation can causes rapid clearance of the cells. On the contrary, a long half-life is related to high level terminal Sia on the cell surfaces. This point is extremely important to the development of therapeutic glycoproteins (130).

In vitro desialylated platelets are also cleared rapidly from circulation similar to erythrocyte clearance (131,132). Platelets that lost the Sias during circulation are cleared by the hepatic endocytic Ashwell-Morell Receptor (133-136).

Sia depletion in aged cells is displayed in the human diploid fibroblastic cell line, TIG3. The young cells grow at a higher rate than aged cells in vitro conditions (137). The results obtained from lectin blot analysis of membrane glycoproteins show that the α2,6- sialylation, but not α2,3- sialylation, of N-glycans decreases markedly in the aged cells when compared to the young cells. The gene expression of the α2,6- sialyltransferase I (ST6Gall), which transfers Sia to the galactose residue of N-glycans, decreases in the aged cells which supports the results mentioned above. Some valuable information was obtained using lectin microarray. Human skin samples that taken from different age groups were tested for the glycan changes on diploid fibroblasts. α2,6- sialylated glycans, in particular, were found to differ between elderly and fetus derived cells at the early passage. In addition, both cell types exhibited sequentially decreasing α2,3- sialylated O-glycan structures (138). Comparisons of early and late passage cells by fluorescence activated cell sorting analysis using lectins, show that a decrease in sialylation and an increase in sialidase NEU1 occurs, in aged cells. Myofibroblast differentiation was inhibited by the reduction of sialylation. Using a sialidase inhibitor, a demonstration of restored myofibroblast differentiation in late passage fibroblasts provides evidence that Sias decrease in aging cells (139).

Additional evidence for the masking role and decreased capacity in aged cells of Sia were obtained from insect tissue, prothoracic glands, in *Galleria mellonella* (Lepidoptera). Prothoracic glands secreting ecdysone hormone in larval instars, are larval structures that disintegrate gradually by hemocytic autolysis in the pupal period and then disappear completely in the adult life of the insect. The presence of Sia on prothoracic glands was determined by electronic ionization mass spectroscopy, electron microscopy, and spectrophotometry (140). For investigation of the role of Sia during the degeneration process of prothoracic glands, neuraminidase digested larval glands (young cells) were incubated within the hemolymph collected from the same age larvae. Light and electron microscopic observations

showed an incomplete capsule formation by the accumulation of hemocytes around desialylated gland cells but not in the control group (141). Desialylated larval cells were recognized as a foreign structure by hemocytes. According to the tested idea, a progressive loss of Sia at distinct developmental stages uncovers the penultimate sugar in glycan chains, allowing for the adhesion of hemocytes during the degeneration period of the glands. The encapsulation and degeneration of experimentally created desialylated larval cells, like the behavior observed in pupal cells, constitute the evidence for the masking role of Sia in the larval period (141). Observation of similar results around experimentally created desialylated larval surfaces in nervous tissue (142), in corpus cardiacum-corpus allatum complex (143) and in testis (144) indicates that Sia acts as a mask for hemocytic receptors during the larval period under normal conditions. These studies provide an explanation for the functionality of Sia in insects, confirming that Sia is a universal molecule.

CONCLUSION

N-glycan patterns of the plasma membrane glycoproteins contribute a large amount to the glycosylation of the glycocalyx. Like a tag, the total N-glycan pattern on the cell surface produces specific markers for the types of embryonic differentiation and aging stages and physiological and pathological state of the cells. The determination of cell surface markers is essential for basic studies and clinical applications. For the effective use of stem cells in cell therapy, it is necessary to know the pluripotency and multipotency associated glycans, for discrimination from other differentiated cells (145-147).

An important function of N-glycan-galectin lattices is to regulate the differentiation mechanism. Lattice structures forming between LacNAc repeats on N-glycan branches, and galectins determine plasma membrane glycoprotein residency time by inhibiting endocytosis of them (31,34). It has been suggested that galectin mediated glycoprotein assemblies are responsible for the signaling, adhesion, migration, and proliferation in many cell types. N-glycan-galectin lattices that affect the activities of membrane glycoprotein can control the decision between cell growth and arrested growth by regulating receptor turnover (26,28,29,39). Since dysregulation of the N-glycan-galectin lattices is responsible for many chronic diseases, it should be targeted for development of new strategies in medical treatment.

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