



Minireview

Core fucose and bisecting GlcNAc, the direct modifiers of the N-glycan core: their functions and target proteins

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ARTICLE INFO

Article history:

Received 15 March 2009

Received in revised form 13 April 2009

Accepted 29 April 2009

Available online 3 May 2009

Dedicated to Professor Dr. Hans Kamerling on the occasion of his 65th birthday

Keywords:

Glycosylation

N-Glycan

Core fucose

Bisecting GlcNAc

Fut8

GnT-III

ABSTRACT

Among the various posttranslational modification reactions, glycosylation is the most common, and nearly 50% of all known proteins are thought to be glycosylated. In particular, most of the molecules involved in cell–cell communication are glycosylated, and glycosylation is thus implicated in many physiological and pathological events, including cell growth, cell–cell adhesion, and tumor metastasis. As many of the glycosyltransferases are cloned, it is becoming possible to alter the oligosaccharide structures artificially and examine the effects. Among the glycosyltransferases involved in the biosynthesis of N-glycan branching, this review will focus on the function of Fut8 and N-acetylglucosaminyltransferase III, which directly modify the N-glycan core. It is suggested that these two glycosyltransferases are involved in the conformation and the function of the modified proteins including cell-surface receptors and adhesion molecules.

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Glycosyltransferases and N-glycan structure

It is well known that glycosylation affects many physicochemical properties of glycoproteins, such as conformation, flexibility, charge, and hydrophilicity. Thus, oligosaccharide modification affects biological processes including receptor activation, signal transduction, endocytosis, and cell adhesion, and leads to the regulation of many physiological and pathological events, including cell growth, migration, differentiation, tumor metastasis, and host–pathogen interactions.^{1–6} The structures of the oligosaccharides, which are determined by the activities of glycosyltransferases and glycosidases, often affect the function of the modified proteins. So far, over 180 glycosyltransferase genes have been identified,^{7,8} and by manipulating them, it is becoming possible to modify the oligosaccharide structures and examine the effects of the modification on certain events.^{9,10} Moreover, by identifying the target proteins that are directly associated with the phenotypic changes, we can actually determine the function of the specific oligosaccharide structure on the specific molecules. Recent trials for ‘functional glycomics’, which aim for a comprehensive understanding of the function of each oligosaccharide structure, are the key approaches for advanced biology in the post-genomic era.

We have isolated and cloned several important glycosyltransferases. Among them, this review will focus on the function of Fut8 and N-acetylglucosaminyltransferase III (GnT-III). Fut8 is an enzyme that catalyzes the introduction of fucose to position 6 of the initial N-acetylglucosamine (GlcNAc) residue of the N-glycan core, to produce ‘core fucose’^{11,12} (Fig. 1). The core fucosylation of α -fetoprotein is a well-known tumor marker for hepatocellular carcinomas. GnT-III is an enzyme that catalyzes the introduction of GlcNAc in a β -(1→4)-linkage to the mannose residue at the base of the trimannosyl core of the N-glycan, to produce a ‘bisecting GlcNAc’.^{13,14} Since an N-glycan with a bisecting GlcNAc is not the substrate of other glycosyltransferases such as GnT-II, IV, V, or Fut8,¹⁵ bisecting GlcNAc modification results in the suppression of further processing and elongation of N-glycans. Recently, Andre et al. have reported on the chemoenzymatic synthesis of an N-glycan with core fucose and/or bisecting GlcNAc, and on the making of artificially glycosylated BSA.¹⁶ On the other hand, we have been studying the function of core fucose and bisecting GlcNAc by examining Fut8 or GnT-III transfected cells, knockdown cells, and transgenic and knockout animals, and determining their target glycoproteins.^{10,17}

Here, we introduce some examples of target glycoproteins, whose core fucose or bisecting GlcNAc regulates their functions. It is suggested that the modulation of the N-glycan could control many biological reactions such as cell signaling and cell adhesion.

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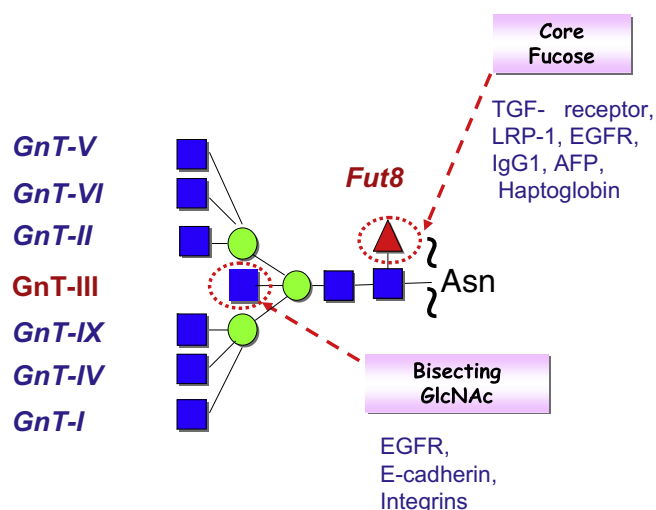


Figure 1. The structure of N-glycan branching formed by glycosyltransferases. GnT-III and Fut8 produce a bisecting GlcNAc and core fucose, respectively. GnT-III suppresses the elongation of N-glycans, since other glycosyltransferases are not able to act on N-glycans with a bisecting GlcNAc. The target proteins are also indicated. Blue Square, GlcNAc; Green Circle, Mannose; Red Diamond, Fucose.

1. EGFR

The epidermal growth factor receptor (EGFR) mediates the epidermal growth factor (EGF) and TGF α signalings, which stimulate cell growth and cell differentiation.¹⁸ The extracellular domain of EGFR contains 11 potential glycosylation sites, and it has been suggested that initial N-glycosylation is required for the proper sorting of EGFR to the membrane as well as for ligand binding.^{19–22} Particularly, the N-glycan on Asn-420 functions to suppress ligand-independent spontaneous oligomerization.²³

Core fucosylation is required for the binding of EGF to EGFR; the EGF-induced phosphorylation of EGFR was substantially blocked in Fut8 knockout cells, and downstream signaling such as Erk and Jnk activation was significantly suppressed.²⁴

For the effect of bisecting GlcNAc modification of the N-glycan of EGFR, we found that endocytosis of EGFR is upregulated and downstream Erk phosphorylation is increased in the GnT-III overexpressing HeLaS3 cells.²⁵ Later, Partridge et al. reported that EGFR with poly(*N*-acetylglucosamine)-containing N-glycans avoids constitutive endocytosis.²⁶ It is suggested that the cell surface residing lectin, galectin-3, preferentially binds to poly(*N*-acetylglucosamine) glycan-ligand on the EGFR, which is produced by GnT-V activity, and sustains the cell surface residency of EGFR. It is possible that our results with GnT-III derived from the same mechanisms; since the bisecting GlcNAc prevents further N-glycan processing as described in the previous section, increased GnT-III expression should diminish the formation of poly(*N*-acetylglucosamine) on EGFR, and consequently attenuate the binding to galectin-3 on the cell surface, which leads to the upregulation of endocytosis. In our results, upregulation of the endocytosis caused an increase in Erk phosphorylation, which was suppressed by the endocytosis inhibitor. It is contrary to the results of Partridge et al., in which an increase in endocytosis of EGFR was shown to reduce the nuclear translocation of phosphorylated Erk. Indeed, the relation between endocytosis of the receptor and downstream signaling is still under discussion; in general, ligand-induced receptor endocytosis attenuates growth factor signaling; however, accumulating evidence indicates that internalized receptors may activate specific signal-transduction pathways.^{27–30} It is also suggested that the extent of receptor phosphorylation after internalization is determined by the balance between the receptor tyrosine kinase and

phosphatase that are present in endosomes.²⁸ Thus, the difference between the two reports may result from some unknown parameter. Nonetheless, the regulation pattern of endocytosis of EGFR by the N-glycan structure is consistent in both reports, indicating that the mechanisms widely work out despite the difference in cell type. Similar signal regulation by galectin–N-glycan interaction is also reported for GnT-IVa glycosylation of Glut-2 and galectin-9,³¹ and we assume that similar mechanisms may be applied to many other cell-surface molecules (Fig. 2).

2. E-cadherin

E-cadherin is a 120 kDa type I membrane protein, which belongs to the class of calcium-dependent cell-adhesion molecules.³² It mediates cell–cell adhesion through the assembly of multiprotein complexes linked to the actin cytoskeleton.³³ The extracellular domain of human E-cadherin consists of five repeats of about 110 amino acid residues, referred to as EC1 through EC5, and contains four potential N-glycosylation sites, two each in EC4 and EC5. It is synthesized in the form of a precursor polypeptide that is glycosylated, and the precursor is then processed to the mature polypeptide.³⁴

We examined E-cadherin expression levels in primary colorectal cancer samples and found that they were significantly increased along with Fut8 expression.³⁵ A low-molecular-weight population of E-cadherin appeared, as well as normal-sized E-cadherin, only in the cancer samples. We established Fut8 transfectants using WiDr human colon carcinoma cells, and it was revealed that the low-molecular-weight population of E-cadherin was significantly increased in Fut8 transfectants in a dense culture, which resulted in an enhancement in cell–cell adhesion. The appearance of the low-molecular-weight population and increase in total expression levels of E-cadherin are consistent with Fut8 activity, which is confirmed by using Fut8 knockdown cells, Fut8^{−/−} cells, and mutated Fut8 transfectants. From the results of pulse–chase studies, it was indicated that core fucosylation regulates the processing of oligosaccharides and turnover of E-cadherin. Thus, core fucosylation of the N-glycan of E-cadherin is possibly involved in the regulation of E-cadherin expression and cell–cell adhesion in vivo.

E-cadherin is also a target protein of GnT-III. Overexpression of GnT-III in melanoma cells contributes to the stability of E-cadherin on the surface of the cell, as it prevents the degradation of E-cadherin.³⁶ GnT-III transfection also reduces the phosphorylation of β -catenin following stimulation by EGF or Src, and therefore, β -catenin remains in a tight complex with E-cadherin and is not translocated to the nuclei.³⁷ Thus, E-cadherin becomes resistant to proteolysis, and homophilic interactions are enhanced. These results partly explain the mechanisms by which GnT-III suppresses cancer metastasis.³⁸

3. Integrins

Integrins are a family of heterodimeric transmembrane receptors of the extracellular matrix.³⁹ They consist of α and β subunits, each of which has a large extracellular region, a single transmembrane domain, and a short cytoplasmic domain (except for the $\beta 4$ integrin). The N-terminal domains of the α and β subunits associate to form the integrin headpiece, which contains the extracellular matrix binding site. The C-terminal domains traverse the plasma membrane and mediate interactions with the cytoskeleton and with signaling molecules. Integrin engagement during cell adhesion leads to intracellular phosphorylation, such as phosphorylation of focal adhesion kinase (FAK), thereby regulating gene expression, cell growth, differentiation, and survival from apoptosis.⁴⁰

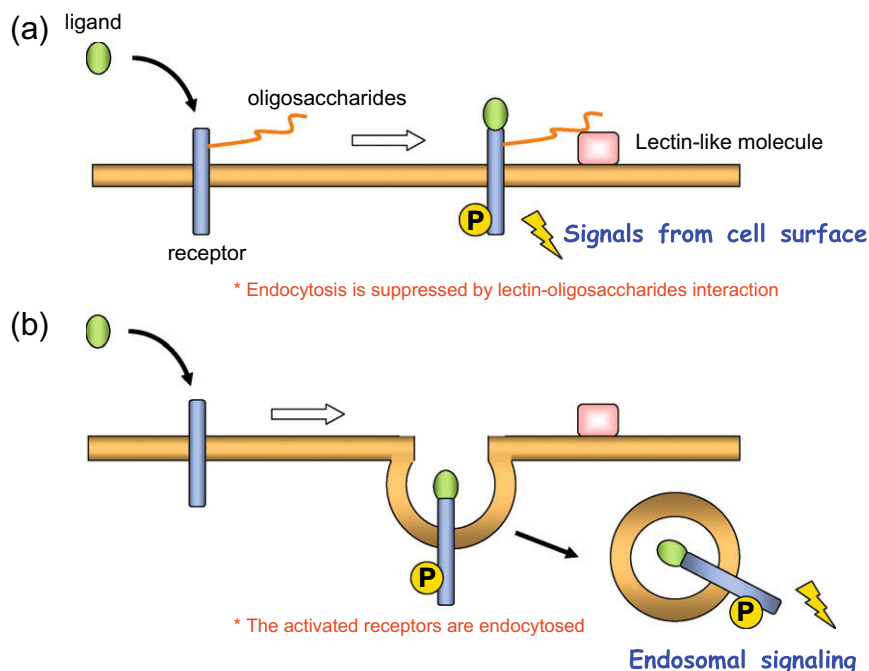


Figure 2. Hypothesis for the regulation of endocytosis of cell-surface receptors by glycosylation. (a) When N-glycans of cell-surface receptors are modified by a specific structure, they are retained on the cell surface through interaction with lectin-like molecules. (b) The receptors without such N-glycan are endocytosed and could be involved in the endosomal signaling.

Many studies report that the oligosaccharide can modulate integrin activation. An alteration in the expression of the N-glycans in $\alpha 5 \beta 1$ integrin could contribute to the adhesive properties of tumor cells and tumor formation. When NIH3T3 cells were transformed with Ras gene, cell spreading on fibronectin was significantly enhanced due to an increase in β -(1 \rightarrow 6)-GlcNAc branched tri- and tetra-antennary oligosaccharides of $\alpha 5 \beta 1$ integrins.⁴¹ Similarly, characterization of the carbohydrate moieties of integrin $\alpha 3 \beta 1$ from non-metastatic and metastatic human melanoma cell lines showed that β -(1 \rightarrow 6)-GlcNAc-branched structures were increased in metastatic cells,⁴² confirming that the β -(1 \rightarrow 6)-GlcNAc-branched structure leads to cancer invasion and metastasis properties. These cancer-associated glycan chains may modulate tumor cell adhesion by affecting the ligand-binding properties of these integrins.

Deletion of core fucosylation on $\alpha 3 \beta 1$ integrin, the major receptor of laminin 5, resulted in the downregulation of its function.⁴³ $\alpha 3 \beta 1$ -mediated cell migration on laminin 5 and focal adhesion kinase (FAK) phosphorylation induced by adhesion to laminin 5 are significantly reduced in Fut8^{-/-} cells. The reintroduction of Fut8 potentially restored both migration and signaling, indicating that core fucosylation is essential for the functions of $\alpha 3 \beta 1$ integrin.

The overexpression of GnT-III also suppresses $\alpha 3 \beta 1$ integrin-mediated cell migration on laminin 5,⁴⁴ as well as $\alpha 5 \beta 1$ integrin-mediated cell spreading, migration, and the phosphorylation of FAK.⁴⁵ The affinity of the binding of integrin $\alpha 5 \beta 1$ to fibronectin was significantly reduced as a result of the introduction of a bisecting GlcNAc to the $\alpha 5$ subunit. Thus, the overexpression of GnT-III inhibits tumor metastasis through at least two mechanisms: enhancement of cell-cell adhesion and downregulation of cell-ECM adhesion.

4. IgG

Most therapeutic antibodies that have been developed thus far are of the human IgG1 isotype. There are two N-linked glycosylation sites in the Fc region of human IgG. Biological activities referred to as 'effector functions' include antibody-dependent cel-

lular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) through the interaction of the Fc with either Fc γ receptors or complement components. Recent studies have shown that manipulation of the N-glycan may yield optimized ADCC. Since ADCC, a lytic attack on antibody-targeted cells, is considered to be a major mechanism responsible for the clinical efficacy of a number of therapeutic antibodies,^{46,47} this issue is important for clinical application.

It has been reported that deletion of the core fucose from Asn-297-linked N-glycan (Fc N-glycan) of the IgG1 molecule enhances ADCC activity by up to 50–100-fold.^{48,49} Fucose depletion from the Fc N-glycan is found to improve binding affinity to Fc γ RIIIa via an enthalpy-driven and association-rate-assisted mechanism.⁵⁰ The crystal structure analysis of human IgG1 has revealed that the Fc N-glycan is buried in the protein portion of the Fc and forms multiple noncovalent interactions with the C_{H2} domains.^{51–53} Multiple noncovalent interactions between the N-glycan and the protein are considered to exert a reciprocal influence on the each other's conformation, delicately affecting the binding affinity with Fc γ RIIIa.⁵⁴ Nonfucosylated IgG is considered to be safe, as it is naturally observed in human serum, and thus is the basis for a potential strategy for clinical application. It is also reported that the bisecting GlcNAc-modified N-glycan of IgG leads to increases in ADCC through a higher affinity for Fc γ receptor III of up to 10–20-fold.⁵⁵

5. Other target proteins for Fut8 and GnT-III

To define the physiological role of Fut8 more clearly, we investigated Fut8-null mice and found that core fucosylation of the TGF β receptor⁵⁶ and LRP-1⁵⁷ affects their activity. A total of 70% of Fut8^{-/-} mice died within three days after birth, and most of the survivors exhibited severe growth retardation and emphysema-like changes in the lungs. It was found that the activation of TGF β receptors was downregulated in Fut8^{-/-} cells.⁵⁶ It was also observed that loss of core fucosylation of low-density lipoprotein (LDL) and receptor-related protein-1 (LRP-1) significantly impairs the LRP-1 scavenging function, leading to an increase in the serum level of insulin-like

growth factor (IGF)-binding protein-3, which may be involved in growth retardation.⁵⁷

On the contrary, GnT-III-deficient mice were produced, and found to be viable and able to reproduce normally.⁵⁸ These mice also exhibited normal cellularity and morphology of organs. No alterations were apparent in circulating leukocytes or erythrocytes, or in serum metabolite levels that reflect kidney function. Thus, GnT-III and the bisecting GlcNAc in N-glycan appear to be dispensable for normal development and reproduction in mice.

Finally, we would like to mention the semi-comprehensive analysis of the cellular effect of bisecting GlcNAc.^{59,60} Using a promoter-reporter assay method, we examined transcription from the elements of AP-1, CRE, HSE, Myc, and NFκB in several cell lines induced by various types of stimulations.⁶⁰ Among those elements, we found that transcription from CRE was elevated twofold in GnT-III transfected Neuro-2a cells when stimulated with forskolin. We confirmed that the phosphorylation of CREB at Ser-133 was enhanced in the GnT-III transfectants. We focused on adenylyl cyclases that catalyze the synthesis of cAMP, and among the nine isoforms, we identified that adenylyl cyclase III is involved in the phenomena. By expressing adenylyl cyclase III along with GnT-III in the HEK-293 human embryonic kidney cell line, it was confirmed that bisecting GlcNAc modification of the N-glycan of adenylyl cyclase III upregulates the enzymatic activity.

Future perspective

As indicated above, core fucosylation and bisecting GlcNAc modulation of the N-glycans of the membrane protein could significantly alter their functions. It seems that signal regulation by an N-glycan structure often involves molecules that recognize the differences of oligosaccharides such as lectin-like molecules. It is also important to determine the conformational changes of glycoprotein caused by oligosaccharide modification. Integrated analysis of glycan from this new viewpoint will give further insight into molecular mechanisms that govern physiology and disease.

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