Review

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A glycomic approach to the identification and characterization of glycoprotein function in cells transfected with glycosyltransferase genes

The transfection of glycoprotein glycosyltransferase genes into cells leads to modification of both the structure and function of the glycoproteins and as a result, changes in glycome patterns. *N*-glycan branching enzymes hold some promise as a model system for the identification of glycome patterns. Both *N*-acetylglucosaminyltransferase III and α1–6 fucosyltransferase are typical glycosyltransferases, which are involved in the branching of *N*-glycans. The resulting enzymatic products, bisecting *N*-GlcNAc and α1–6 fucose residues, are no longer modified by other glycosyltransferases and it is a relatively simple task to identify their modification by means of lectins. In this review, the glycome patterns of glycosyltransferase gene transfectants and the non-transfectants were compared by two-dimensional gel electrophoresis and lectin staining, and the biological significance of the two genes are described. Analyses of glycome patterns by transfecting glycosyltransferase genes will lead to new fields of study in the area of postgenome research.

Keywords: Glycome / Glycosyltransferase genes / α1–6 fucosyltransferase / Review PRO 0026

Contents

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Abbreviations: AFP, α-fetoprotein; **EGF**, epidermal growth factor; **GnT**, *N*-acetylglucosaminyltransferase; **FucT**, fucosyltransferase; **LCA**, Lens culinaris agglutinin; **E-PHA**, Erythroagglutinating Phytohemagglutinin; **GlcNAc**, *N*-acetylglucosamine

1 Introduction

The glycosylation of sugar chains represents a critically important post-translational modification reaction and is a target for future proteomic research. Carbohydrate moieties of glycoproteins, which are displayed on cell surface membranes, are changed during carcinogenesis and development [1]. These structural changes may occur as the result of alterations in the levels of glycosyltransferases, which are implicated in biosynthesis and trimming of the glycoforms. We call functional studies on the carbohydrate components of proteins, glycomics. This new field promises to provide fundamental answers in the area of functional genomics research (Drs. Vernon

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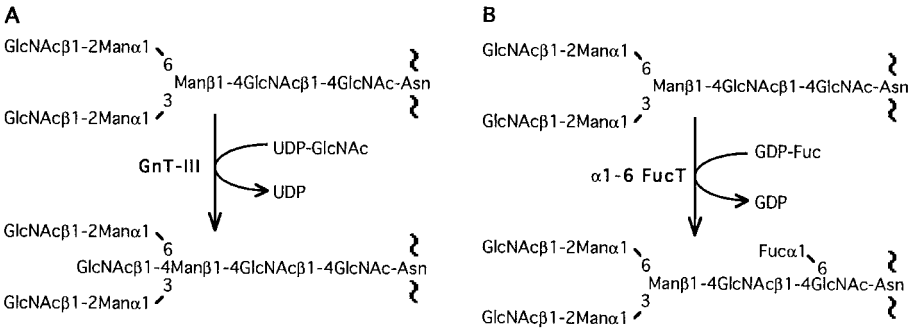

Figure 1. The catalytic reaction of GnT-III (**A**) and α1–6 FucT (**B**). GlcNAc, *N*-acetylglucosamine; Man, mannose; Fuc, fucose; GDP-Fuc, guanosinediphosphofucopyranoside; Asn, asparagine.

Reinhold, Harry Schachter, Andre Verbert, personal communication). Glycosylation can alter a proteins charge (due to sialylation or sulfation) and induce heterogeneous profiles as a consequence of differing glycoforms. To ascertain the functional relationship between a protein and its glycosylation, we overexpress a specific glycosyltransferase gene in various cells and tissues and characterize the altered products.

This review will focus on the use of UDP-*N*-acetylglucosamine:β-D-mannoside β1–4 *N*-acetylglucosaminyltransferase III (GnT-III) transfectants and α1–6 fucosyltransferase (α1–6 FucT) transfectants. The changes in glycosylation resulting from transfection of these glycosyltransferase genes will be discussed.

1.1 GnT-III and α1–6 FucT

GnT-III and α1–6 FucT catalyze addition of sugars, which give rise to bisecting GlcNAc and α1–6 fucosylated residues, respectively as shown in Fig. 1.

1.2 Typical 2-D gel electrophoresis of GnT-III and α1–6 FucT transfectants

The two glycosyltransferase genes, GnT-III and α1–6 FucT, were transfected into a human colon cancer cell line, WiDr and a human hepatoma cell line, Hep3B, respectively. As shown in Figs. 2 and 3, the electrophoretic patterns of glycoprotein extracts between transfectants and nontransfectants were not so different when measured by Coomassie staining. However, their glycosylation patterns of the glycoproteins were completely different as judged by lectin blot analysis. The lectins used in the staining are E-PHA (erythroaggultinating phytohemagglutinin) and LCA (lens culinaris agglutinin). E-PHA has a high affinity for bisected oligosaccharide structures and LCA preferentially binds to α1–6 fucosylated residues. There are a number of glycoproteins which undergo aberrant glycosylation upon glycosyltransferase gene transfection. In order to identify the target proteins in the transfectants, we have established various transfectants of GnT-III and α1–6 FucT *in vitro* and also transgenic animals which overexpress the above genes. In this review, the target proteins for glycosylation by the above genes and their pathophysiological significance are described.

2 *N*-Acetylglucosaminyltransferase III (GnT-III)

The bisecting GlcNAc is attached β1–4 to a mannose in the core region of *N*-glycans and is formed by the GnT-III enzyme. The enzyme was first described in hen oviduct [2] and a high level of activity has also been reported in various types of rat hepatoma [3, 4]. At least, six *N*-acetylglucosaminyltransferases [5] are known. The enzymatic properties and substrate specificity of GnT-I, GnT-II, GnT-III, GnT-IV and GnT-V have been characterized, as have their corresponding cDNAs [6]. Quite recently GnT-VI has been also characterized [7]. Each *N*-acetylglucosaminyltransferase is different in terms of protein structure as well as its enzymatic properties with respect to substrate specificity. No sequence homology is evident between these *N*-acetylglucosaminyltransferases, suggesting that the divergent points of the six types of *N*-acetylglucosaminyltransferases are quite old in the phylogenetic tree and the differing substrate specificity of these enzymes also supports the conclusion.

The GnT-III used here was purified from rat kidney by affinity chromatography using the substrate, biantennary sugar chain as a ligand [8]. The rat cDNA encodes 536 amino acids and there are epidermal growth factor (EGF)-like motifs which are structurally similar to those found in human β4 integrin in the amino acid sequences. The enzyme is a typical type II transmembrane protein with a cytoplasmic domain, a transmembrane anchor

domain, an extracellular stem region and a catalytic domain. It is well known that the bisecting GlcNAc structure affects the conformation of sugar chains, and once GnT-III acts on the biantennary sugar chains, other glycosyltransferases such as GnT-II, GnT-IV, GnT-V and α1–6 FucT will no longer act.

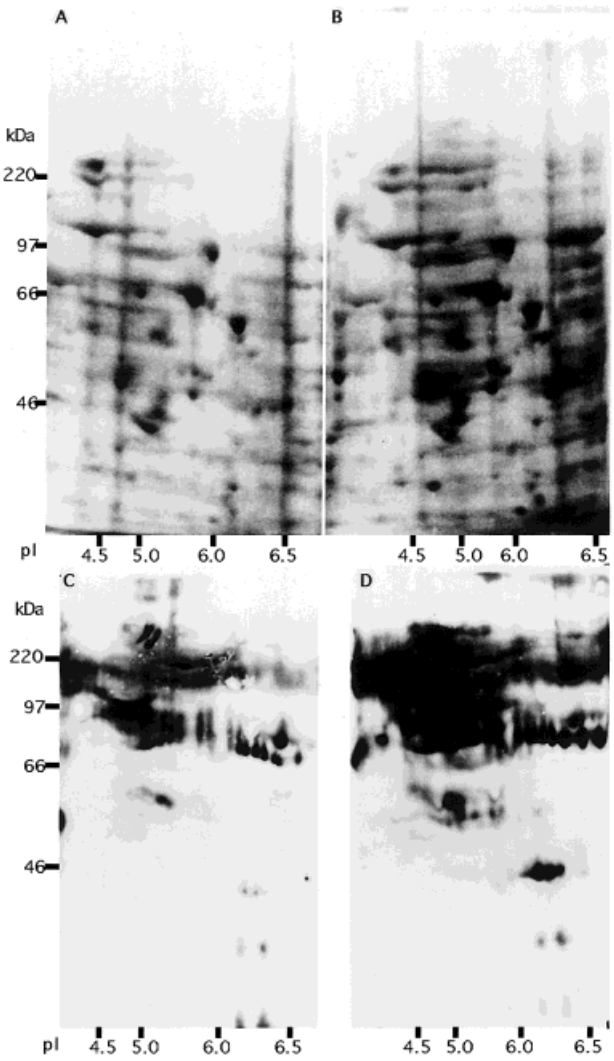

Figure 2. Two-dimensional IEF/SDS-PAGE profiles of total proteins from GnT-III nontransfected (A, C) and transfected (B, D) WiDr colon cancer cells. 1×10^7 cells were suspended in lysis buffer (9.5 M urea, 2% Triton X-100, 2% ampholine pH 3.5–10, 5% 2-mercaptoethanol), lysed at room temperature for 1 h and centrifugated at 10 000 $\times$ *g* for 20 min. After centrifugation, proteins of supernatant was loaded on a tube gel (8 M urea, 4% acrylamide, 2% Triton X-100, 2% Ampholine pH range 3.5–10) for first dimensional IEF. Electrophoresis was run at 400 V for 12 h at room temperature; 2-D 10% SDS-PAGE was run at 45 mA for 12 h at room temperature. The separated proteins were transferred onto PVDF membrane with 1.8 mA/cm^2 for 1 h. A and B are stained with Coomassie Brilliant Blue, C and D were stained with E-PHA lectin.

2.1 GnT-III and its glycosylation target proteins

2.1.1 γ-Glutamyltranspeptidase

The bisecting GlcNAc structure has been detected in various complex and hybrid types of *N*-glycans of various glycoproteins including IgG and γ-glutamyltranspeptidases [9–15]. The bisecting GlcNAc structure is abundant

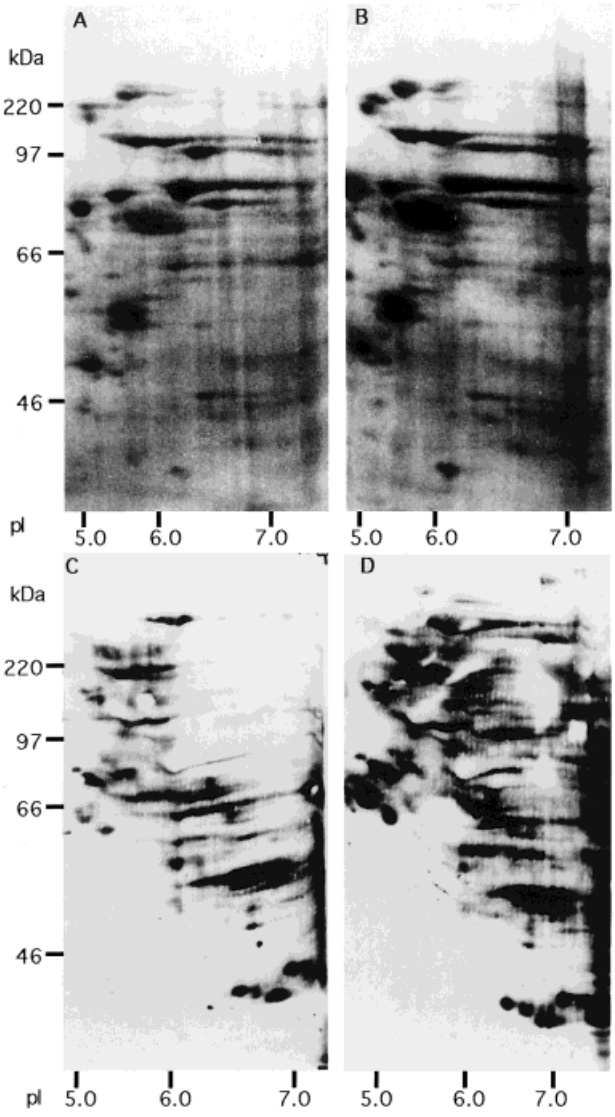

Figure 3. Two-dimensional IEF/SDS-PAGE profiles of total proteins from α1–6 FucT nontransfected (A, C) and transfected (B, D) Hep3B hepatoma cells. Electrophoresis was carried out at the same condition as in Fig. 2. A and B are stained with Coomassie Brilliant Blue, and C and D were stained with LCA lectin.

in the γ-glutamyltranspeptidase purified from rat primary hepatoma but is nearly absent in the same enzyme from normal rat liver [14]. γ-Glutamyltranspeptidase catalyzes the degradation of glutathione, is a typical type II transmembrane glycoprotein [16, 17], and is known to be highly expressed in the rat liver during chemical carcinogenesis [18, 19]. In a similar manner, the GnT-III activity is highly activated in hepatoma tissues [4] and their mRNA levels are also highly expressed, suggesting that γ-glutamyltranspeptidase is one of the endogenous natural acceptor molecules for GnT-III.

2.1.2 Bisecting GlcNAc and an endogenous lectin molecule

In order to determine whether or not bisecting GlcNAc residues are involved in the sorting of *N*-glycans, a hepatoma cell line, mRLN31 was chosen. A major reason for this choice is that these cells are sensitive to forskolin, a unique reagent which is widely used as an adenylyl cyclase activator to induce different kinds of proteins in many cell lines through the accumulation of cAMP. Forskolin was observed to induce GnT-III in normal hepatocytes as well as in hepatoma cells and resulted in an increase in bisecting GlcNAc in various glycoproteins in the tissues. However, even though increase of bisecting GlcNAc residues in various glycoproteins can be observed, secretory glycoproteins such as ceruloplasmin and α-fetoprotein (AFP) are secreted in a normal manner, whereas the extent of sorting of γ-glutamyltranspeptidase, lysosome associated glycoproteins 1 (LAMP-1) and β-glucuronidase that are localized in the cell surface are decreased [20].

Gao in our group recently identified the bisecting GlcNAc binding protein from porcine spleen microsome [21]. The protein was extracted from porcine spleen microsomes with Triton X-100 and then purified using DEAE cellulose and affinity chromatography conjugated with bisected biantennary sugar chains as a ligand. The purified protein binds to the surface of the GnT-III cDNA transfected K562 cells, which express a large amount of bisecting GlcNAc, in a dose dependent manner of the protein. The protein also inhibited the binding of E-PHA to the transfected cells. Microsequencing analysis of the peptide fragments, obtained from the digestion of the purified protein with Lysil endopeptidases (Seikagaku, Tokyo, Japan) and *Staphylococcus aureus* V8 protease revealed that the protein was homologous to annexin V (unpublished). This protein may function as a lectin, which is capable of recognizing bisecting GlcNAc-containing oligosaccharides.

2.1.3 E-Cadherin

We established a highly metastatic subclone, B16-hm, from low metastatic B16-F1 murine melanoma cells [22]. The staining with E-PHA yielded only a few faint signals for proteins from B16-hm cells and negative transfectants, whereas strong signals were observed for 80 kDa proteins from positive transfectants. These data show that the expression of GnT-III led to an increased level of synthesis of bisecting GlcNAc, which in turn, suppressed the formation of β1–6 tri- and tetra-antennary *N*-linked oligosaccharides. Structural analyses of the transfectants also provide support for this conclusion.

When positive transfectants were injected into syngeneic C57BL/mice, significantly fewer metastatic nodules were observed than with the parent cell and negative transfectants. These results demonstrate that GnT-III expression in the transfectants decreased the metastatic potential of B16-hm melanoma *in vivo*. E-cadherin, an important adhesion molecule is localized at cell-cell borders of the GnT-III transfectants [23]. The turnover and release of E-cadherin in the transfectants inhibited a release of E-cadherin from the cell surface, which resulted in an increased level and accumulation of E-cadherin molecules at the cell-cell contacts. The increased E-cadherin expression was accompanied by an increased cell aggregation in the transfectants. Moreover, tyrosine phosphorylation of β-catenin was also modulated by addition of bisecting GlcNAc structures on E-cadherin. Details of E-cadherin oligosaccharide story in terms of metastasis were described in [24]. Aberrant glycosylation of E-cadherin is one of the causes in suppression of lung metastasis of melanoma cells in GnT-III transfectants.

2.1.4 CD44 and hyaluronate

CD44, a cell-surface glycoprotein which is expressed on many different cell types, functions as an adhesion molecule for hyaluronate. The CD44 molecule, as deduced from cDNA sequences, contains several putative *N*-glycosylation sites [25]. The biological role of CD44 molecules has been analyzed in regard to tumor metastasis and lymphocyte function. A positive correlation for CD44 with metastasis in several tumor cell lines has been reported for both melanoma cells and lymphoma cells [26]. CD44 of mouse B16 melanoma cell does not contain bisecting GlcNAc structures because this cell does not have any GnT-III activity. In order to evaluate the effect of bisecting GlcNAc on the biological function of CD44 as a receptor for hyaruromic acid (HA), we examined the adhesion of positive transfectants to immobilized HA [27]. The time course for the adhesion to HA was significantly short, and was increased in both positive transfectants compared with parental cells and their mock transfectants. At 4 h after the incubation, approximately 57% of the positive transfectants had attached to the HA, while only 23% of the native B16-hm cells were attached at the same

time. An inhibitory antibody to CD44, KM 201 was found to block the adhesion of native B16-hm cells and positive transfectants to HA to an equal extent. In native B16-hm cells and in positive transfectants, adhesion to HA was completely blocked after treatment of the immobilized HA with hyaluronidase. The results of the adhesion assay and the blocking study, which involved the use of an inhibitory antibody, suggest that CD44 mediated the adhesion, and that the attachment is enhanced in positive transfectants.

2.1.5 Apolipoprotein B100

One strategy for analyzing the functional properties of a gene is to knock out the gene or to overexpress it *in vitro* or *in vivo*. In these strategies, the resulting phenotypes obtained by knocking out or by overexpression of the glycosyltransferase genes are sometimes due to a secondary effect which arises as a result of the modification of sugar chains. Therefore when the gene(s) are knocked out or overexpressed, it is always prudent to ascertain that the phenotypic changes are directly due to the genes of interest. In such cases, the identification of the target glycoproteins due to the knocked out gene or overexpressed gene should be a prerequisite. We have used this strategy for the overexpression of the GnT-III gene in mice or in several types of cells in order to better understand the role of the bisecting GlcNAc residue in glycoproteins. The bisecting GlcNAc is a unique sugar residue because surprisingly, many different types of phenotypic changes can be attributed to it *in vivo* and *in vitro*. Quite recently we established transgenic mice that specifically express GnT-III in hepatocytes. Normal mice hepatocytes do not produce GnT-III and it would be interesting to understand the biological significance of bisecting GlcNAc in hepatocytes if one can ectopically overexpress the gene. We found that the transgenic hepatocytes had a swollen oval-like morphology, and contained numerous lipid droplets [28]. A possible target protein could be apolipoprotein B100 (apo-B100). The reason for this is that bisecting GlcNAc structures accumulate and in the transgenic mice, serum triglycerides, β- and pre-β-lipoprotein formation and apolipoprotein B100 were significantly decreased compared with the same levels in nontransgenic mice. Apo-B100 is a glycoprotein that plays important structural roles in the formation of lipoprotein complexes. In the liver apo B100 is required for the assembly and secretion of very low density lipoproteins as well as low density lipoproteins. The aberrant glycosylation of apo-B100 may cause a decrease in the rate of release of lipoprotein and a concomitant accumulation of apo B100 in the liver.

2.1.6 α-Gal epitope

The major xenoantigen responsible for the discordant xenografts from pig to human is the α-Gal epitope (Gal-α1–3Gal-β1–4GlcNAc-R), which is expressed on the cells of most mammals including the pig. An overexpression of GnT-III in pig endothelial cells down regulates the expression of the Gal epitope by further processing of complex type sugar chains [29, 30]. In fact, nearly 80% of the Gal epitope can be eliminated, as evidenced by structural studies of the *N*-glycans (Koyota *et al*., unpublished). The major target proteins in the pig endothelial cells are not yet known, but the proteins with a molecular mass below 60 kDa were not expressed by lectin blotting analysis after GnT-III gene transfection.

2.1.7 Growth factor receptors, tyrosine receptor kinase and epidermal growth factor receptor

It is a well known fact that growth factor receptors are glycoproteins which have a number of N-linked oligosaccharides. In this section, the biological importance of oligosaccharides on the receptors are shown through a story of the modification of their sugar chain by GnT-III transfection. PC12 cells are known to differentiate into sympathetic neurons upon treatment with nerve growth factor (NGF). When GnT-III was transfected to this cell, GnT-III transfectants showed no responses to NGF treatment, resulting in neither differentiation nor decrease in cell growth rate. It is because GnT-III transfectants of PC12 cells showed no tyrosine phosphorylation of the TrkA/NGF receptor after NGF treatment [31]. When oligosaccharide structures of TrkA were analyzed by lectin blotting, the number of bisecting GlcNAc was markedly increased in GnT-III transfectants. Dimerization of TrkA after NGF treatment, judging by cross-linking, was also dramatically suppressed in GnT-III transfectants. These studies suggested that the transfection of GnT-III into the PC12 cells resulted in the aberrant glycosylation of Trk receptor and blocked the ligand-induced receptor dimerization. This may affect NGF-induced signaling in PC12 cells.

Several investigators have reported the role of *N*-linked sugar chains in the modulation of epidermal growth factor receptor (EGF-R) function. EGF-R has 12 potential sites of *N*-linked oligosaccharides. When these oligosaccharides were modulated by GnT-III transfection, the number of the active receptors was reduced in U373 MG glioma cells [32]. When one of these 12 potential sites was changed by site-directed mutagenesis methods, phosphorylation of EGF-R was constitutively enhanced without ligand stimulation [33]. Our recent data also indicates that GnT-III transfection increases the internalization rate of EGF/EGFR complexes and affects downstream signaling in HeLa cells [34]. While these phenomena were interesting, their biological meaning remains to be elucidated.

2.1.8 Tyrosinase

Tyrosinase is a rate-limiting enzyme, which plays a pivotal role in melanogenesis. It is a single chain glycoprotein that catalyzes the hydroxylation of tyrosine to β3,4 dihydorxypehnylalanine (DOPA) and the further oxidation of DOPA to DOPA quinione [35]. *N*-glycosylation has been reported to be essential for enzyme activity. We found that in B16-F10 melanoma cells which overexpress GnT-III, the melanin content and tyrosine hydroxylase activity were markedly increased [36]. Although the mechanisms are still unknown, the modification of tyrosinase activity by GnT-III gene transfection could be a novel approach in modulating the properties of malignant melanoma cells.

2.1.9 Hepatitis B associated antigen and prions

Hepatitis B virus (HBV) is a well-known virus which is causative for acute and chronic hepatitis. The HB611 cell lines in which the HBV gene was transfected into a human hepatoma cell line Huh6, produces a large amount of hepatitis B surface antigen, hepatitis B envelope antigen and HBV virion, the medium [37]. Gene transfection of GnT-III into HB611 cells resulted in the down-regulation of secretion of HBV-related antigens [38]. Surprisingly, this suppression of HBV expression was due to down-regulation of HBV-related mRNAs. While oligosaccharide structures of HB611 cells were totally changed by GnT-III transfection, suppression of gene expression of HBV mRNAs was not explained by the changes of oligosaccharides alone. Similar results were reported by Tanemura *et al.* [39]. Expression of α1–3 galactosyl transferase mRNA was suppressed by gene transfection of GnT-III in a porcine endothelial cell. As these reports suggested, the secondary effect of glyco-gene transfection should be kept in mind in any experiments. In normal prions (PrP(C)) the bisecting GlcNAc residue is high whereas in pathogenic prions (PrP(Sc)) the residue is small, indicating that GnT-III down-regulation is critical for the pathogenesis of prions and the replication pathway for prions [40]. These data suggest that some glycoproteins whose oligosaccharide structures are altered by the over-expression of GnT-III may control the expression of HBV associated antigen or the biosynthesis of pathogenic prions.

3 α1–6 Fucosyltransferase (α1–6 FucT)

α1–6 Fucosyltransferase (α1–6 FucT) catalyzes the transfer of fucose from GDP-Fuc to *N*-linked type complex glycoproteins (Fig. 1). The enzymatic products, α1–6 fucosylated (core fucosylated) *N*-glycans, are commonly observed in many glycoproteins [41]. In certain serum proteins, such as ceruloplasmin and transferrin, the content of α1–6 fucosylated *N*-glycans is quite low under normal conditions [42], but is increased in cases of malignancy [43]. An increased level of fucosylation of transformed or tumor cells has been also reported [44]. Voynow *et al.* [45] first succeeded in purifying and characterizing α1–6 FucT from cultured human skin fibroblasts of cystic fibrosis in 1991. Uozumi *et al.* [46] succeeded in the purification and cDNA cloning of α1–6 FucT from porcine brain using a GDP-hexanolamine-Sepharose 4B column. The apparent molecular mass, based on SDS-PAGE, was determined to be 58 kDa. Based on the cDNA sequence, α1–6 FucT contains 575 amino acids and no putative *N*-glycosylation sites. Although no sequence homology was found in the α1–6 FucT cDNA, compared with other members of the fucosyltransferase family, α1–6 FucT cDNA contains nine conserved amino acid sequences, which are completely consistent with the mammalian H/Se type fucosyltransferase [47]. α1–6 FucT appears to be a type II transmembrane protein, similar to other glycosyltransferases. Expression of α1–6 FucT mRNA is detected as a single 3.5 kb band in a variety of rat tissues with the exception of liver and pancreas [48]. Yanagidani *et al*. [49] succeeded in the purification and cDNA cloning of α1–6 FucT from a human gastric cancer cell line, MKN 45. The cDNA sequence showed that the homology to porcine brain α1–6 FucT is 92.2% at the nucleotide level and 95.7% at the amino acid level. Recently, α1–6 FucT has been purified from human blood platelets [50].

3.1 α1–6 FucT and glycosylation target proteins

Each glycosyltransferase has its target proteins whose biological function might be changed by oligosaccharide modification. In this section, target proteins of α1–6 FucT is discussed.

3.1.1 Platelet proteins

Previous study showed that platelets contain a large amount of α1–6 FucT. While the biological meaning of α1–6 FucT in platelets is unknown, most of the human serum enzyme is derived from platelets [51]. Even though the biological significance remains unknown, α1–6 fucosylated glycans are present in the GpIIb/IIIa complex, which is essential for fibrinogen binding [52]. In contrast, the activity of platelet α1–6 FucT is inversely related to blood platelet concentration [50]. Neither granulocytes, lymphocytes nor red cells significantly contribute to serum α1–6 FucT activity. It is interesting to note, however, that granulocytes enhance the thrombin-induced enzyme release from platelets. α1–6 FucT has been shown to be present in platelets of intermediate and high density but appears to be missing from the light ones, as judged by density gradient centrifugation. These results suggest

that platelet α1–6 FucT might serve as a marker for the ploidy level of megakaryocytes. α1–6 FucT, purified from platelets, showed slightly different characteristics from other α1–6 FucTs which have been purified from other sources.

3.1.2 α-Fetoprotein (AFP)

AFP is a fetal serum protein, the reappearance of which in mice with hepatoma has been demonstrated [53, 54], as well as in patients with hepatocellular carcinoma (HCC) and in rat serum treated with carcinogens [55]. AFP is a reliable tumor marker for primary hepatoma. However, AFP-positive cases of fulminant hepatitis and other hepatitis and liver cirrhosis have also been reported. The M_r of purified human AFP does not coincide with the number of amino acid residues, as deduced from cDNA sequence of mature AFP [56]. This difference can be accounted for by the N-glycosylation of asparagine 232 in the AFP amino acid sequence. The lectin-dependent fractionation of AFP was originally described by Breborowicz [57] and Miyazaki [58]. The use of Lens culinaris agglutinin (LCA), which is capable of preferentially recognizing α1–6 fucosylation on *N*-glycans and in crossed immune-affinoelectrophoresis, has demonstrated that AFP in the serum of patients with HCC has increased proportions of LCA-reactive AFP, whereas AFP in the serum of patients with chronic liver disease contained largely LCA-nonreactive AFP [59]. The LCA-reactive AFP was found to be a good marker for the differential diagnosis of HCC in the case of chronic liver disease and a good monitoring and prognosis marker for the early diagnosis of HCC [60–64].

3.1.3 Integrins

To understand the biological significance of the role of α1–6 FucT in hepatoma, especially in terms of metastasis, we established human hepatoma cell lines which express high levels of α1–6 FucT as a result of transfection with the α1–6 FucT gene. Using this system, we investigated intrahepatic metastasis after splenic injection into athymic mice [65]. Tumor formation in the liver was dramatically suppressed in the case of α1–6 FucT transfectants. Cell adhesion of mice hepatocytes to cultured nonparenchymal liver cells was significantly inhibited in the case of the α1–6 FucT transfectants. Two-dimensional electrophoresis followed by LCA lectin blot showed that certain glycoproteins (M_r 50,000–150,000, p*I* 4.8–5.5) were α1–6 fucosylated and might be linked to the suppression of intrahepatic metastasis. Adhesion to fibronectin was dramatically suppressed in the case of α1–6 FucT transfectants and certain proteins around 100 kDa were highly fucosylated. We therefore examined the functions of adhesion molecules and their oligosaccharide structures. Oligosaccharide structures on α2β1, α3β1, α5β1 and αvβ3 integrins and CD44 were investigated by immunoprecipitation followed by LCA blot. The α2β1 and α3β1 integrins were barely expressed or fucosylated in both control and α1–6 FucT transfectants. Increases in LCA binding to α5β1 integrin and CD44 were observed in the case of the α1–6 FucT transfectants. α1–6 fucosylation of CD44 minor bands was more prominent than that of the major band. In contrast, αvβ3 integrin was highly fucosylated in both control and α1–6 FucT transfectants. To determine the involvement of the α5β1 integrins in the adhesion to fibronectin, an anti-α5β1 integrin antibody was added, in order to inhibit cell attachment. Decreases in cell adhesion to fibronectin were observed in both control and α1–6 FucT transfectants. When high levels of the antibody were added, the cell adhesion approached the same level, suggesting that the difference in cell adhesion between control and α1–6 FucT transfectants was due to the α5β1 integrin.

3.1.4 Fibrinogen

Recently, several reports have appeared which focused on the role of α1–6 fucosylation in *N*-glycans. Stubbs [66] suggested that α1–6 fucosylation greatly influences the conformation and flexibility of the mannose α1,6 mannose antenna of the biantennary oligosaccharide from porcine fibrinogen. Stubbs's study is consistent with reports that α1–6 fucosylation is essential for the polysialylation of the neural cell adhesion molecule, which is catalyzed by a specific polysialic acid synthase [67] and is involved in the regulation of de-*N*-glycosylation by mammalian peptide *N*-glycosidase. These findings suggest that α1–6 fucosylation might play a role in the modification of protein and carbohydrate interactions.

3.1.5 Lysosomal acid lipase

Very recently, we have developed α1–6 FucT transgenic mice. α1–6 FucT transgenic mice, whose gene expression was regulated under the control of the β-actin promoter showed an abnormality in liver and kidney [68]. Levels of cholesterol esters and triglycerides were significantly increased in the liver of these tissues. We found that liver lysosomal acid lipase undergoes aberrant fucosylation and is inactivated, leading to a unique phenotype in which lipid is accumulated in the liver.

4 Conclusions

GnT-III and α1–6 FucT transfection provide a wide variety of information in glycobiology, although several problems remain unsolved. Since oligosaccharides of so many gly-

coproteins were changed by gene transfection of glycosyltransferases, secondary effects of cellular transformation could not be completely denied. To solve this problem, the most important thing is to analyze carefully a phenotype of glycogene-transfected cells. Target molecules seem to be different in each glycosyltransferase. 2-DE followed by lectin blot described in this review is one of the ways to search for these target molecules. We do not have any ideas on how to focus only one molecule having a specific sugar chain. Glycobiology is a very difficult area of biology. The introduction of a specific glycosyltransferase gene into cells and tissues may open up approaches to the study of glycomics and may lead to functional genomic studies in the post-genome era of research.

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