Glycoconjugate Journal 21, 9–15, 2004

Regulation of integrin functions by N-glycans

Jianguo Gu and Naoyuki Taniguchi

Department of Biochemistry, Osaka University Graduate School of Medicine, B1, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Integrins are cell surface transmembrane glycoproteins that function as adhesion receptors in cell-ECM interactions and link matrix proteins to the cytoskeleton. Integrins play an important role in cytoskeleton organization and in the transduction of intracellular signals, regulating various processes such as proliferation, differentiation, apoptosis, and cell migration. Although integrin-mediated adhesion is based on the binding of α and β subunits to a defined peptide sequence, the strength of this binding is modulated by various factors including the status of glycosylation of integrin. Glycosylation reactions are catalyzed by the catalytic action of glycosyltransferases, such as *N*-acetylglucosaminyltransferase III, V and α1, 6 fucosyltransferase, etc., which catalyze the formation of glycosidic bonds. This review summarizes effects of the posttranslational modification of N-glycans of α3β1 and α5β1 integrins on their association, activation and biological functions, by using biochemical and genetic approaches.
Published in 2004.

Keywords: **N-glycosylation, Integrinα3β1, Integrinα5β1, glycosyltransferase, GnT-III, GnT-V**

Introduction

Most of secreted and cell surface proteins are glycosylated. Cell surface carbohydrates contribute to a variety of interactions between the cell and its extracellular environment. Because they are located on the outermost layer of the cell, carbohydrates are the first molecules to be encountered and recognized by the other cells, antibodies, invading viruses, and bacteria. Many secreted molecules such as hormones and toxins have also been reported to bind to carbohydrate receptors on the cell surface. The attached sugar chains have many biological functions, for example, cell-cell communication, signal transduction, protein folding and stability [1–3]. Given the role of glycosylation in biological functions, therefore, it is not surprising that aberrant glycosylation patterns can serve as markers for certain disease states including cancer metastasis, development and differentiation [4].

Integrins consist of α and β subunits. Each subunit has a large extracellular region, a single transmembrane domain and a short cytoplasmic tail (except for β4 integrin). The N-terminal domains of the α and β subunits associate to form the integrin headpiece, which contains extracellular matrix (ECM) binding site, whereas the C-terminal segments transverse the plasma membrane and mediate interactions with the cytoskeleton and with signaling molecules (Figure 1A). Based on extensive searches of the human and mouse genomic sequences, 18 α- and 8 β-subunits are known to assemble into 24 integrins. Among these integrins, 12 members containing β1 subunit have been found (Figure 1B). Each of these integrins appears to have a specific, nonredundant function. Genes for the β subunits and the α subunits have been knocked out. Each phenotype is distinct, and reflects the different roles of the various integrins [5]. In the case of the phenotype of β1 integrin knock-out mice, for example, preimplantation development is completely blocked. Recent studies have shown that growth factor-induced proliferation, cell-cycle progression and differentiation require the adhesion of cells to the ECM, a process that is mediated by integrins [6,7].

The majority of studies of integrin-mediated signaling events have been performed on cells that adhere to fibronectin (FN) through the α5β1 integrin, one of the best characterized integrins, that recognize the tripeptide sequence, Arg-Gly-Asp. This association between FN and α5β1 integrin is involved in regulating not only cell adhesion and migration, but also differentiation and apoptosis [8,9]. On the other hand, another of the major integrins in epithelial cells, α3β1 integrin, which mediates adhesion to basement membrane laminins, preferentially promotes cell migration and prevents apoptosis [10–12]. These cell surface integrins are all major carriers of N-glycans. Changes in the N-glycan structures of these integrins can also affect cell-cell and cell-ECM interactions, thereby affecting cell adhesion, migration and tumor malignancy [13–15]. In epithelial cells, a shift of N-glycans of integrin to the highly β1, 6 GlcNAc branched type via catalysis

To whom correspondence should be addressed: Jianguo Gu, Department of Biochemistry, Osaka University Graduate School of Medicine, B1, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: 81-6-6879-3421; Fax: 81-6-6879-3429; E-mail: jgu@biochem.med.osaka-u.ac.jp

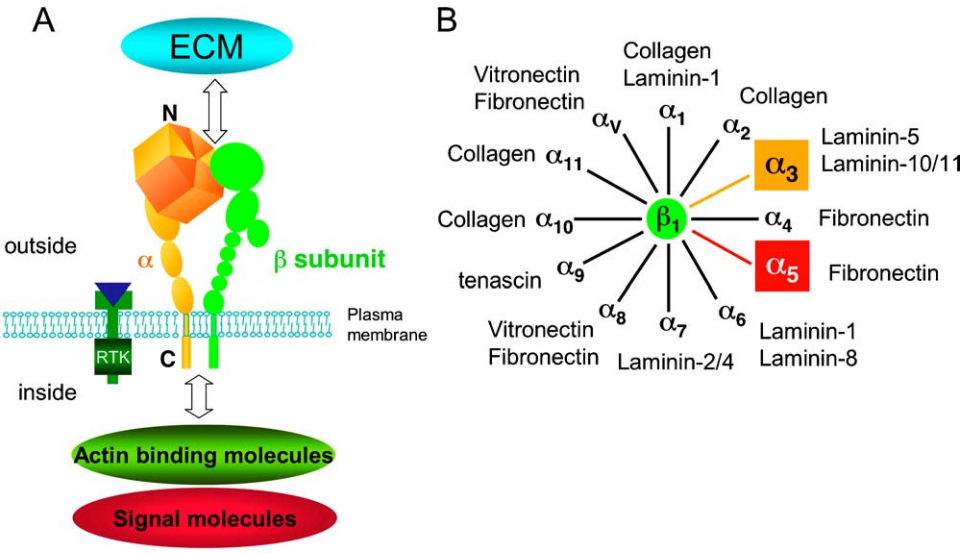

Figure 1. The integrin receptors and ECM. (A) Integrins are $\alpha\beta$ heterodimers. Each subunit has a large extracellular region and a short cytoplasmic tail (except for β4 integrin). The N-terminal domains of the α and β subunits associate to form the integrin headpiece, which is required for ECM binding, whereas the C-terminal domain mediates interactions with the cytoskeleton and signaling molecules. It is also clear that integrins synergize with other cell surface receptors including growth factor receptors to activate several signaling pathways, to affect cell shape, migration, proliferation and differentiation. RTK, receptor tyrosine kinase. (B) Among 24 integrins which have been identified so far, 12 members of β1 subunit-containing integrin have been found. They have relative ECM specificities. This review focuses mainly on α3β1 and α5β1 integrins.

by *N*-acetylglucosaminyltransferase V (GnT-V) leads to a decrease in cell adhesion, resulting in an increase in both cell motility and tumorigenicity [16–18]. Conversely, the modification of N-glycans with a bisecting GlcNAc catalyzed by *N*-acetylglucosaminyltransferase III (GnT-III) inhibits ligand binding ability, subsequently leading to the down-regulation of integrin-mediated signaling [19]. Although carbohydrate determinants such as sialyl Lewis A and sialyl Lewis X, which are frequently expressed on human cancer cells, are also involved in the adhesion of cancer cells to endothelial cells in the hematogenous metastasis of cancer [20], in this review, we will briefly overview the N-glycan structures of integirns and their related functions arising from recent studies, which provide insight into some long-standing questions concerning N-glycosylation functions.

Structures of N-linked oligosaccharides of the integrins

One of the most frequent biochemical alterations associated with tumorgenesis and metastasis is an altered expression and/or structure of cell-associated complex carbohydrates. It is noteworthy that poly-*N*-acetyllactosamine structures have been detected on a variety of cancer cells, and have been shown to reduce the extent of cell-cell and cell-ECM adhesion. Actually, α3β1 integrin, which is expressed by human colon carcinoma cells, is a major carrier of not only oncodevelopmental carbohydrate epitopes such as sialyl Lewis A and sialyl Lewis X, but β1, 6 branching N-glycans as well [21]. Based on the amino acid sequences, both integrin α3β1 and α5β1 contain fourteen and twelve potential asparagine-linked glycosylation sites on each of the α and β1 subunits (Figure 2), respectively. Determination of the structures of *N*-linked oligosaccharides on integrins had been believed to be a tedious work, since the purification of an integrin is difficult, and it is usually obtained in small quantity. However, Takahashi's group developed a sensitive analytical method using different properties of HPLC columns, and overcame this difficulty [22]. Finally, 35 different oligosaccharide structures have been identified in α5β1 integrin purified from human placenta, 10 of which were neutral, 6 mono-sialyl,

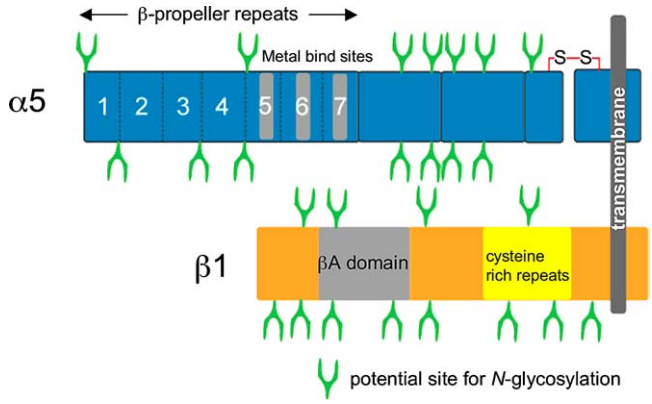

Figure 2. Schematic diagram of integrin α5 and β1 subunits. The potential sites for N-glycosylation are shown. The dark gray region in the β1 subunit indicates the βA domain; the white region indicates cysteine rich repeats.

10 di-sialyl, 7 tri-sialyl. The molar ratio of neutral and sialyl oligosaccharides was 20.8% and 77.7%, respectively, whereas high mannose-type oligosaccharides composed only 1.5% of the total. The most predominant structure was the biantennary di-α(2,3)-sialyl fucosyl sugar chains.

The structures of oligosaccharides of integrin $\alpha 3\beta 1$ from human ureter epithelium cells (HCV29) were characterized by means of matrix-assisted laser desorption/ionization mass spectrometry [23]. Most of the oligosaccharides were complex types with a wide heterogeneity including bi-, tri-, and tetra-antennary sugar chains, while high mannose-type structures were minor cmponents. These results, together with an analysis of oligosaccharides of integrin $\alpha 5\beta 1$, suggest that the N-glycosylation of native integrins is fully processed through the Golgi apparatus. Considering the fact that the oncogenic transformation of cells causes structural and functional changes in integrins [24], a complete characterization of the sites to which carbohydrate chains are attached, as well as structures of carbohydrate chains on each potential N-linked site, would be insightful.

N-glycosylation of integrins plays an important role in their biological functions

Glycosylation can act as a key regulatory switch for protein activity. This was clearly demonstrated by the discovery that the activity of the Notch receptor is directly modulated by O-glycosylation by the Fringe glycosyltransferase, ensuring correct development in Drosophila [25,26]. A treatment of purified integrin $\alpha 5\beta 1$ with N-glycosidase F, also known as PNGase F, which is an amidase which cleaves between the innermost GlcNAc and asparagines residures of N-glycans from N-linked glycoproteins, resulted in the blocking of $\alpha 5\beta 1$ binding to FN and the inherent association of both subunits [27], implicating that N-glycosylation is essential for functional integrin $\alpha 5\beta 1$.

A growing body of evidence indicates that the presence of the appropriate oligosaccharide can modulate $\alpha 5\beta 1$ integrin activation. When human fibroblasts were cultured in the presence of l-deoxymannojirimycin, an inhibitor of α-mannosidase II, which prevents N-linked oligosaccharide processing, immature $\alpha 5\beta 1$ integrin receptors appeared at the cell surface, and FN-dependent adhesion was greatly reduced [28]. In fact, an alteration of expression of 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 the oncogenic *Ras* gene, the cell spreading on FN was greatly enhanced due to an increase of $\beta 1$, 6 GlcNAc branched tri- and tetra-antennary oligosaccharides in $\alpha 5\beta 1$ integrins [15]. Similarly, characterization of carbohydrate moieties of integrin $\alpha 3\beta 1$ from non-metastatic and metastatic human melanoma cell lines showed that $\beta 1$, 6 GlcNAc branched structures were highly expressed in metastatic cells compared with non-metastatic cells [24], confirming the notion that the $\beta 1$, 6 GlcNAc branched structure acquires the properties of cancer invasion and metastasis. These cancer-associated glycan chains may modulate tumor cell adhesion by affecting the ligand binding properties of these integrins.

Furthermore, sialylation on non-reducing end of N-glycans of $\alpha 5\beta 1$ integrin plays an important role in cell adhesion. It has been reported that the hyposialylation of $\beta 1$ integrin contributed to an increase in the extent of FN binding in myeloid cells, in which the expression of the ST6Gal I sialytransferase was down-regulated by treatment with phorbol ester [29]. The similar phenomenon has also been observed in hematopotic or epithelia cells. The increased sialylation of the $\beta 1$ integrin subunit was correlated with decreased adhesiveness and metastatic potential [30–32]. However, on the other hand, the enzymatic removal of $\alpha 2$, 8-linked oligosialic acids in the $\alpha 5$ integrin subunit expressed in G361 melanoma cells inhibited cell adhesion to FN [33], supporting the observation that the N-glycans of α and β integrin subunits play distinct roles in cell-ECM interactions [34]. Collectively, these findings suggest that the interaction of integrin $\alpha 5\beta 1$ with FN is dependent on its N-glycosylation and the processing status of N-glycans.

The functions of integrins modulated by sugar remodeling

Oligosaccharide heterogeneity presents a challenge not only in deciphering a structure of interest, but also in identifying the multiple enzymes that are involved in its biosynthesis. The authors' group has been interested in tumor-associated changes in glycoproteins such as γ-glutamyltranspeptidase which plays a major role in the biosynthesis of glutathione [35] and found that bisecting GlcNAc residues are only found in enzymes purified from ascites hepatoma AH-66 but not in enzymes purified from normal rat livers [36]. In order to confirm the underlying mechanism, we purified and cloned the specific glycosyltransferase, GnT-III, responsible for producing the bisecting GlcNAc [37,38]. Until now, a large number of glycosyltransferases (about 170 genes) have been cloned as of this writing and some of their important functions are now understood. The authors' group has been interested in these glycosyltransferases, which are involved in the synthesis of branching N-linked sugar chains in glycoproteins, *i.e.* GnT-III, GnT-V [39–41] and $\alpha 1$, 6 fucosyltransferase (Fut8) [42–44] (Figure 3). These three branched N-glycans, bisecting GlcNAc, $\beta 1$, 6 GlcNAc branching and

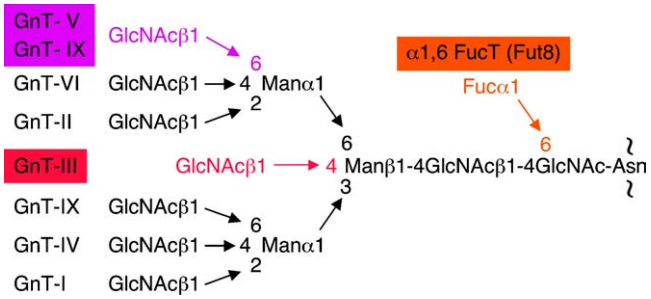

Figure 3. The authors' group has been interested in these four shaded glycosyltransferases, which are involved in the synthesis of branching N-linked sugar chains in glycoproteins.

core fucose (α1, 6 fucose), may play crucial roles in the inhibition of cancer metastasis, promotion of cancer invasion and metastasis, and growth and development, respectively.

The introduction of the bisecting GlcNAc results in the suppression of further processing and the elongation of N-glycans catalyzed by other glycosyltransferases, since they are not able to use the bisected oligosaccharide as a substrate [45]. Thus, GnT-III is generally regarded to be a key glycosyltransferase in N-glycan biosynthetic pathways. It is interesting to note that the metastatic capabilities of B16 mouse melanoma cells are down-regulated by the introduction of the GnT-III gene [46]. This anti-metastatic effect has been, in part, attributed to the effect of GnT-III on an increase in E-cadherin-mediated homotypic adhesion and the suppression of the phosphorylation of the E-cadherin-β-catenin complex on the cell-cell adhesion [47,48]. Recently, we found that the overexpression of GnT-III inhibited $\alpha5\beta1$ integrin-mediated cell spreading and migration, and the phosphorylation of the focal adhesion kinase [19]. The affinity of the binding of integrin $\alpha5\beta1$ to fibronectin was significantly reduced as the results of the introduction of the bisecting GlcNAc to the α5 subunit. Taken together, the overexpression of GnT-III inhibits tumor metastasis by at least two mechanisms: an enhancement of cell-cell adhesion and a down-regulation of cell-ECM adhesion (Figure 4).

To explore the possible mechanisms involved in increased β1,6 branched N-glycans on cell surface of metastatic cancer cells, Guo *et al.* transfected GnT-V cDNA into human HT1080 cells using retroviral vectors, and found that cell migration toward FN and invasion through the matrigel were both substantially stimulated in cells in which the expression of GnT-V was induced [17]. Increased branched sugar chains inhibited the clustering of the integrin $\alpha5\beta1$ and the organization of F-actin into extended microfilaments in cells plated on FN-coated plates, confirming the hypothesis that the degree of adhesion of cells to their ECM substrate is a critical factor in regulating the rate of cell migration, *i.e.*, migration is maximal under conditions of intermediate levels of cell adhesion [49].

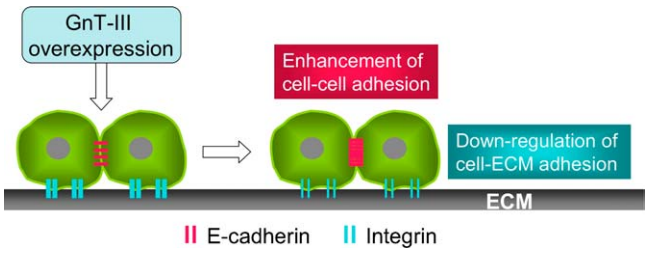

Figure 4. Possible mechanisms for the suppression of cancer metastasis by the overexpression of GnT-III. One is an enhancement in cell-cell adhesion by an increase in the stability of E-cadherin on the cell surface and the suppression of the phosphorylation of β-catenin. The other is the down-regulation of integrin-mediated cell-ECM adhesion.

Interestingly, these glycosyltransferases appear to selectively modify some N-glycans, but not entire sugar chains. There was no detectable expression of β1,6 branched structures on the α5 subunit purified from HT1080 cells before and after induction of GnT-V, but the branched structures on the β1 subunit were greatly increased after the induction of GnT-V. In contrast to the modification by GnT-V, the reactivity of E_4-PHA lectin was greatly enhanced in integring α5 subunit, but was barely detectable in the β1 subunit purified from GnT-III transfectants. To study mechanism of these selective modifications would be interesting.

Effects of N-glycosylation on cross-talk between growth factor receptors- and integrins-mediated signals

The remodeling of cell surface growth factor receptors and ECM receptors by modification of their oligosaccharide structures is associated with the function and biological behavior of tumor cells. Nerve growth factor has been shown to bind to its receptor, TrkA, on the surface of PC12 cells, resulting in TrkA dimerization and phosphorylation [50]. TrkA-mediated neurite outgrowth and its tyrosine phosphorylation are blocked as the result of the transfection of GnT-III to PC12 cells, suggesting that bisecting structures may participate in the regulation of TrkA functions [51]. It is well known that integrin-mediated cell adhesion functions cooperatively with growth factor receptors in the control of cell proliferation, cell differentiation, cell survival, and cell migration in epithelia cells and fibroblasts [7]. Because integrins and growth factor receptors share many common elements in their signaling pathways, it is clear that there are many opportunities for integrin signals to modulate growth factor signals. In fact, integrins enable growth factor signaling in many cases, that is, normal growth factor signaling does not occur unless cells are adhered to ECM or other cells through integrins.

The association of integrins with growth factor receptors is indicated by co-clustering and co-precipitation studies [52,53]. Not only physical association but also functional cooperation between integrins and growth factor receptors such as EGF receptor has been demonstrated. The mitogen-activated protein (MAP) kinase pathway provided the best characterized example of this principle, because a number of integrins and growth factor signals converge at multiple points [54]. Adding soluble mitogens to cells in suspension triggers the weak or transient activation of the Erk, compared with strong and sustained Erk activation observed in adherent cells. To examine whether these synergistic effects are also needed for differentiation, PC12 cells in a serum-free medium were plated on plastic dishes without an ECM-coating. Treatment with EGF or NGF alone failed to induce neurite formation in these cells [55], suggesting that the integration of signaling pathways trigged by receptor tyrosine kinases and integrins are required for the regulation of PC12 cell differentiation. Interestingly, EGF-induced neurite outgrowth through the Ras/MAP kinase activation

pathway, was completely blocked in GnT-III-transfected PC12 cells. The blocking was restored by the overexpression of a constitutively activated mitogen- or extracellular signal-regulated kinase kinase-1 (MEK1). These observations indicate that the modulation of N-glycan structures of integrins could control not only cell adhesion, as described above, but growth factor-mediated signals as well.

Many recent studies indicate that integrins are associated with various members of the tetraspanin family such as CD9 and CD82 [56]. Integrin $\alpha 3$ or $\alpha 5$ subunit associated with tetraspanin CD9 or CD82 strongly affect cell motility and may control cell malignancy. The formation of complexes of integrins and tetraspanin is affected by the N-glycosylation of both integrin and tetraspanins, as well as by gangliosides in the microdomain [57]. CD82 with complete N-glycosylation reduces its association with $\alpha 3$ or $\alpha 5$ integrin, whereas CD82 with incomplete N-glycosylation enhances their associations [58]. Conversely, the association of CD9 with the $\alpha 3$ or $\alpha 5$ integrin subunits is not influenced by N-glycosylation, since CD9 contains no N-glycosylation sites.

Future perspectives

It is well known that a large number of proteins undergo post-translational modification with corresponding changes in their structures and functions. Among the various posttranslational modification reactions of proteins, glycosylation is the most abundant, and nearly 50% of all proteins are thought to be glycosylated. As described above, modulation of the N-glycans of these integrins could significantly alter their biological functions including cell spreading, migration as well as signaling transduction. Integrins contain multiple potential N-linked glycosylation sites on each of α or β subunit, so it is essentially important to identify which sites of these integrins are occupied with N-glycans, which N-glycans are required for their functions, as well as which N-glycans are participated in association with other molecules such as growth factor receptors, for unraveling molecular mechanism of the inhibition of tumor metastasis, such as regulated by GnT-III.

References

1 Varki A, Biological roles of oligosaccharides: All of the theories are correct, *Glycobiology* **3**, 97–130 (1993).
2 Dwek RA, Glycobiology: "towards understanding the function of sugars", *Biochem Soc Trans* **23**, 1–25 (1995).
3 Saxon E, Bertozzi CR, Chemical and biological strategies for engineering cell surface glycosylation, *Annu Rev Cell Dev Biol* **17**, 1–23 (2001).
4 Dennis JW, Granovsky M, Warren CE, Protein glycosylation in development and disease, *Bioessays* **21**, 412–21 (1999).
5 Hynes RO, Integrins: Bidirectional, allosteric signaling machines, *Cell* **110**, 673–87 (2002).
6 Yamada KM, Miyamoto S, Integrin transmembrane signaling and cytoskeletal control, *Curr Opin Cell Biol* **7**, 681–9 (1995).
7 Schwartz MA, Ginsberg MH, Networks and crosstalk: Integrin signalling spreads, *Nat Cell Biol* **4**, E65–8 (2002).
8 Frisch SM, Ruoslahti E, Integrins and anoikis, *Curr Opin Cell Biol* **9**, 701–6 (1997).
9 Ruoslahti E, Integrin signaling and matrix assembly, *Tumour Biol* **17**, 117–24 (1996).
10 Kreidberg JA, Functions of alpha3beta1 integrin, *Curr Opin Cell Biol* **12**, 548–53 (2000).
11 Gu J, Sumida Y, Sanzen N, Sekiguchi K, Laminin-10/11 and fibronectin differentially regulate integrin-dependent Rho and Rac activation via p130(Cas)-CrkII-DOCK180 pathway, *J Biol Chem* **276**, 27090–7 (2001).
12 Gu J, Fujibayashi A, Yamada KM, Sekiguchi K, Laminin-10/11 and fibronectin differentially prevent apoptosis induced by serum removal via phosphatidylinositol 3-kinase/Akt- and MEK1/ERK-dependent pathways, *J Biol Chem* **277**, 19922–8 (2002).
13 Dennis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS, Beta 1-6 branching of Asn-linked oligosaccharides is directly associated with metastasis, *Science* **236**, 582–5 (1987).
14 Hakomori S, Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism, *Cancer Res* **56**, 5309–18 (1996).
15 Asada M, Furukawa K, Segawa K, Endo T, Kobata A, Increased expression of highly branched N-glycans at cell surface is correlated with the malignant phenotypes of mouse tumor cells, *Cancer Res* **57**, 1073–80 (1997).
16 Demetriou M, Nabi IR, Coppolino M, Dedhar S, Dennis JW, Reduced contact-inhibition and substratum adhesion in epithelial cells expressing GlcNAc-transferase V, *J Cell Biol* **130**, 383–92 (1995).
17 Guo HB, Lee I, Kamar M, Akiyama SK, Pierce M, Aberrant N-glycosylation of beta1 integrin causes reduced alpha5beta1 integrin clustering and stimulates cell migration, *Cancer Res* **62**, 6837–45 (2002).
18 Yamamoto H, Swoger J, Greene S, Saito T, Hurh J, Sweeley C, Leestma J, Mkrdichian E, Cerullo L, Nishikawa A, Ihara Y, Taniguchi N, Moskal JR, Beta1,6-*N*-acetylglucosamine-bearing N-glycans in human gliomas: Implications for a role in regulating invasivity, *Cancer Res* **60**, 134–42 (2000).
19 Isaji T, Gu J, Nishiuchi R, Zhao Y, Takahashi M, Miyoshi E, Honke K, Sekiguchi K, Taniguchi N, Introduction of bisecting GlcNAc into integrin alpha 5 beta 1 reduces ligand binding and down-regulates cell adhesion and cell migration, *J Biol Chem* **279**, 19747–54 (2004).
20 Hakomori S, Kannagi R, Glycosphingolipids as tumor-associated and differentiation markers, *J Natl Cancer Inst* **71**, 231–51 (1983).
21 Prokopishyn NL, Puzon-McLaughlin W, Takada Y, Laferte S, Integrin alpha3beta1 expressed by human colon cancer cells is a major carrier of oncodevelopmental carbohydrate epitopes, *J Cell Biochem* **72**, 189–209 (1999).
22 Nakagawa H, Zheng M, Hakomori S, Tsukamoto Y, Kawamura Y, Takahashi N, Detailed oligosaccharide structures of human integrin alpha 5 beta 1 analyzed by a three-dimensional mapping technique, *Eur J Biochem* **237**, 76–85 (1996).
23 Litynska A, Pochec E, Hoja-Lukowicz D, Kremser E, Laidler P, Amoresano A, Monti C, The structure of the oligosaccharides of alpha3beta1 integrin from human ureter epithelium (HCV29) cell line, *Acta Biochim Pol* **49**, 491–500 (2002).

24 Pochec E, Litynska A, Amoresano A, Casbarra A, Glycosylation profile of integrin alpha 3 beta 1 changes with melanoma progression, *Biochim Biophys Acta* **7**, 1–3 (2003).

25 Moloney DJ, Panin VM, Johnston SH, Chen J, Shao L, Wilson R, Wang Y, Stanley P, Irvine KD, Haltiwanger RS, Vogt TF, Fringe is a glycosyltransferase that modifies Notch, *Nature* **406**, 369–75 (2000).

26 Bruckner K, Perez L, Clausen H, Cohen S, Glycosyltransferase activity of Fringe modulates Notch-Delta interactions, *Nature* **406**, 411–5 (2000).

27 Zheng M, Fang H, Hakomori S, Functional role of N-glycosylation in alpha 5 beta 1 integrin receptor. De-N-glycosylation induces dissociation or altered association of alpha 5 and beta 1 subunits and concomitant loss of fibronectin binding activity, *J Biol Chem* **269**, 12325–31 (1994).

28 Akiyama SK, Yamada SS, Yamada KM, Analysis of the role of glycosylation of the human fibronectin receptor, *J Biol Chem* **264**, 18011–8 (1989).

29 Semel AC, Seales EC, Singhal A, Eklund EA, Colley KJ, Bellis SL, Hyposialylation of integrins stimulates the activity of myeloid fibronectin receptors, *J Biol Chem* **277**, 32830–6 (2002).

30 Pretzlaff RK, Xue VW, Rowin ME, Sialidase treatment exposes the beta1-integrin active ligand binding site on HL60 cells and increases binding to fibronectin, *Cell Adhes Commun* **7**, 491–500 (2000).

31 Kawano T, Takasaki S, Tao TW, Kobata A, Altered glycosylation of beta 1 integrins associated with reduced adhesiveness to fibronectin and laminin, *Int J Cancer* **53**, 91–6 (1993).

32 Dennis J, Waller C, Timpl R, Schirrmacher V, Surface sialic acid reduces attachment of metastatic tumour cells to collagen type IV and fibronectin, *Nature* **300**, 274–6 (1982).

33 Nadanaka S, Sato C, Kitajima K, Katagiri K, Irie S, Yamagata T, Occurrence of oligosialic acids on integrin alpha 5 subunit and their involvement in cell adhesion to fibronectin, *J Biol Chem* **276**, 33657–64 (2001).

34 Chammas R, Veiga SS, Travassos LR, Brentani RR, Functionally distinct roles for glycosylation of alpha and beta integrin chains in cell-matrix interactions, *Proc Natl Acad Sci USA* **90**, 1795–9 (1993).

35 Taniguchi N, Ikeda Y, gamma-Glutamyl transpeptidase: Catalytic mechanism and gene expression, *Adv Enzymol Relat Areas Mol Biol* **72**, 239–78 (1998).

36 Yamashita K, Hitoi A, Taniguchi N, Yokosawa N, Tsukada Y, Kobata A, Comparative study of the sugar chains of gamma-glutamyltranspeptidases purified from rat liver and rat AH-66 hepatoma cells, *Cancer Res* **43**, 5059–63 (1983).

37 Narasimhan S, Control of glycoprotein synthesis. UDP-GlcNAc:glycopeptide beta 4-N-acetylglucosaminyltransferase III, an enzyme in hen oviduct which adds GlcNAc in beta 1-4 linkage to the beta-linked mannose of the trimannosyl core of N-glycosyl oligosaccharides, *J Biol Chem* **257**, 10235–42 (1982).

38 Nishikawa A, Gu J, Fujii S, Taniguchi N, Determination of N-acetylglucosaminyltransferases III, IV and V in normal and hepatoma tissues of rats, *Biochim Biophys Acta* **1035**, 313–8 (1990).

39 Shoreibah MG, Hindsgaul O, Pierce M, Purification and characterization of rat kidney UDP-N-acetylglucosamine: alpha-6-D-mannoside beta-1,6-N-acetylglucosaminyltransferase, *J Biol Chem* **267**, 2920–7 (1992).

40 Gu J, Nishikawa A, Tsuruoka N, Ohno M, Yamaguchi N, Kangawa K, Taniguchi N, Purification and characterization of UDP-N-acetylglucosamine: alpha-6-D-mannoside beta 1-6N-acetylglucosaminyltransferase (N-acetylglucosaminyltransferase V) from a human lung cancer cell line, *J Biochem* **113**, 614–9 (1993).

41 Saito H, Nishikawa A, Gu J, Ihara Y, Soejima H, Wada Y, Sekiya C, Niikawa N, Taniguchi N, cDNA cloning and chromosomal mapping of human N-acetylglucosaminyltransferase V+, *Biochem Biophys Res Commun* **198**, 318–27 (1994).

42 Uozumi N, Yanagidani S, Miyoshi E, Ihara Y, Sakuma T, Gao CX, Teshima T, Fujii S, Shiba T, Taniguchi N, Purification and cDNA cloning of porcine brain GDP-L-Fuc:N-acetyl-beta-D-glucosaminide alpha1–>6fucosyltransferase, *J Biol Chem* **271**, 27810–7 (1996).

43 Yanagidani S, Uozumi N, Ihara Y, Miyoshi E, Yamaguchi N, Taniguchi N, Purification and cDNA cloning of GDP-L-Fuc:N-acetyl-beta-D-glucosaminide: alpha1-6 fucosyltransferase (alpha1-6 FucT) from human gastric cancer MKN45 cells, *J Biochem* **121**, 626–32 (1997).

44 Miyoshi E, Noda K, Yamaguchi Y, Inoue S, Ikeda Y, Wang W, Ko JH, Uozumi N, Li W, Taniguchi N, The alpha 1-6-fucosyltransferase gene and its biological significance, *Biochim Biophys Acta* **6**, 9–20 (1999).

45 Schachter H, Biosynthetic controls that determine the branching and microheterogeneity of protein-bound oligosaccharides, *Adv Exp Med Biol* **205**, 53–85 (1986).

46 Yoshimura M, Nishikawa A, Ihara Y, Taniguchi S, Taniguchi N, Suppression of lung metastasis of B16 mouse melanoma by N-acetylglucosaminyltransferase III gene transfection, *Proc Natl Acad Sci USA* **92**, 8754–8 (1995).

47 Yoshimura M, Ihara Y, Matsuzawa Y, Taniguchi N, Aberrant glycosylation of E-cadherin enhances cell-cell binding to suppress metastasis, *J Biol Chem* **271**, 13811–5 (1996).

48 Kitada T, Miyoshi E, Noda K, Higashiyama S, Ihara H, Matsuura N, Hayashi N, Kawata S, Matsuzawa Y, Taniguchi N, The addition of bisecting N-acetylglucosamine residues to E-cadherin down-regulates the tyrosine phosphorylation of beta-catenin, *J Biol Chem* **276**, 475–80 (2001).

49 Palecek SP, Loftus JC, Ginsberg MH, Lauffenburger DA, Horwitz AF, Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness, *Nature* **385**, 537–40 (1997).

50 Jing S, Tapley P, Barbacid M, Nerve growth factor mediates signal transduction through trk homodimer receptors, *Neuron* **9**, 1067–79 (1992).

51 Ihara Y, Sakamoto Y, Mihara M, Shimizu K, Taniguchi N, Overexpression of N-acetylglucosaminyltransferase III disrupts the tyrosine phosphorylation of Trk with resultant signaling dysfunction in PC12 cells treated with nerve growth factor, *J Biol Chem* **272**, 9629–34 (1997).

52 Miyamoto S, Teramoto H, Gutkind JS, Yamada KM, Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors, *J Cell Biol* **135**, 1633–42 (1996).

53 Sundberg C, Rubin K, Stimulation of beta1 integrins on fibroblasts induces PDGF independent tyrosine phosphorylation of PDGF beta-receptors, *J Cell Biol* **132**, 741–52 (1996).

54 Renshaw MW, Ren XD, Schwartz MA, Growth factor activation of MAP kinase requires cell adhesion, *EMBO J* **16**, 5592–9 (1997).

55 Gu J, Zhao Y, Isaji T, Shibukawa Y, Ihara H, Takahashi M, Ikeda Y, Miyoshi E, Honke K, Taniguchi N, Beta1,4-N-Acetylglucosaminyltransferase III down-regulates neurite outgrowth induced by costimulation of epidermal growth factor and integrins through the Ras/ERK signaling pathway in PC12 cells, *Glycobiology* **14**, 177–86 (2004).

56 Hakomori Si SI, Inaugural Article: The glycosynapse, *Proc Natl Acad Sci USA* **99**, 225–32 (2002).

57 Kawakami Y, Kawakami K, Steelant WF, Ono M, Baek RC, Handa K, Withers DA, Hakomori S, Tetraspanin CD9 is a "proteolipid," and its interaction with alpha 3 integrin in microdomain is promoted by GM3 ganglioside, leading to inhibition of laminin-5-dependent cell motility, *J Biol Chem* **277**, 34349–58 (2002).

58 Ono M, Handa K, Withers DA, Hakomori S, Glycosylation effect on membrane domain (GEM) involved in cell adhesion and motility: A preliminary note on functional alpha3, alpha5-CD82 glycosylation complex in ldlD 14 cells, *Biochem Biophys Res Commun* **279**, 744–50 (2000).

Received 23 March 2004: accepted 13 May 2004