TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Review Article

Turk J Biol
(2014) 38: 754-771
© TÜBİTAK
doi:10.3906/biy-1406-94

Glycosylation changes leading to the increase in size on the common core of *N*-glycans, required enzymes, and related cancer-associated proteins

Sabire KARAÇALI*, Savaş İZZETOĞLU, Remziye DEVECİ
Department of Biology, Molecular Biology Section, Faculty of Science, Ege University, Bornova, İzmir, Turkey

Received: 30.06.2014 • **Accepted:** 09.09.2014 • **Published Online:** 24.11.2014 • **Printed:** 22.12.2014

Abstract: Glycan parts of glycoconjugates on the surfaces of cells regulate many kinds of interactions between the cells and their immediate environments. Alterations in glycosylation on the cancer-associated glycoproteins are responsible for changes in their molecular interactions and biological functions. Glycosylation changes occur in the core and/or at the nonreducing end of the oligosaccharide chains of *N*-glycans. In this review, we focus on the branching of the common core structure of *N*-glycans, the responsible enzyme, and the extensions of some of the branches causing size increases on the surface of tumor cells. Abnormal branching, elongation of the branches, and increasing size of the common core of *N*-glycans are the typical features of these changes and are related with malignant transformations. Seven *N*-acetylglucosaminyltransferases (GnTs) (GnT-I, GnT-II, GnT-III, GnT-IV, GnT-V, GnT-VI, and GnT-IX) and α1,6-fucosyltransferase (FUT8) initiate the new branches on the core. GnT-IV, GnT-V, and GnT-IX initiate the branches available for poly-LacNAc extensions, which are responsible for tumor progression and metastasis. GnT-III prevents the catalytic activity of GnT-II, GnT-IV, GnT-V, and FUT8 to form branching and elongation of the branches. The contributions of GnT-III and the other enzymes to the cancer progression are in conflict with each other. While GnT-III prevents cancer, the others increase metastasis. The function of FUT8 is related to signal transduction and its activity is higher in tumor tissue than in healthy tissue. The impact of glycosylation changes on some of the cancer-associated proteins (growth factor receptors, adhesion and signal molecules, CD147, TIMP-1, and matriptase) is also summarized.

Key words: *N*-Glycan core branching, glucosyltransferases, FUT8, poly-LacNAc, galectin-3, adhesion and signal molecules, growth factor receptors, CD147, TIMP-1, matriptase

1. Introduction

In a 2009 special issue of *Biochimica et Biophysica Acta* dedicated to Dr Eric Berger, a colleague explained how his interest in glycosylation defects in human diseases began and how Berger, a medical doctor, had become a glycobiologist with the identification of the first disease caused by a glycosylation disorder in 1978 (Hennet, 2009). In the following years, the description of many human diseases related to glycosylation defects gradually increased. Altered *N*-glycosylation patterns of proteins have been described with increasing age and in several diseases including cancer (Ruhaak et al., 2011).

The cell surface cover, the glycocalyx, is composed of glycan chains (oligosaccharides or polysaccharides), parts of glycoconjugates found within the structure of the plasma membrane and the extracellular matrix (ECM). Glycoconjugates are hybrid molecules including glycoproteins, proteoglycans, glycosphingolipids, and glycophosphatidyl inositol anchors (Figure 1). Light and heavily glycosylated plasma membrane glycoproteins have different functions. They are the molecules indicating differentiation (Feizi, 1981, 1985, 1987, 1991), blood groups in ABO and Rh systems (Eyers et al., 1994; Fredriksson et al., 2010), normal and cancer stem cells (Yin et al., 1997; Irollo and Pirozzi, 2013), and tumor-associated antigens (Huang et al., 2013; Saldova et al., 2013b). Membrane receptors for growth factors (Matsumoto et al., 2008; Wu et al., 2013; Tan et al., 2014) and for Delta/Serrata (Takeuchi and Haltiwanger, 2014) bear glycans affecting the sensitivity of the cells to their molecular targets. Membrane transporters such as Na^+-K-ATPase (Tokhtaeva et al., 2010), the ATP-binding cassette (Hollenstein et al., 2007), glucose transporters (Haga et al., 2011), and transmembrane glycoproteins such as cadherin and integrin, playing an important role in cell adhesion and signaling (Zhao et al., 2008; Bassagañas et al., 2014), and a transmembrane serine protease involved in epithelial homeostasis in both health and disease

* Correspondence: sabire.karacali@ege.edu.tr

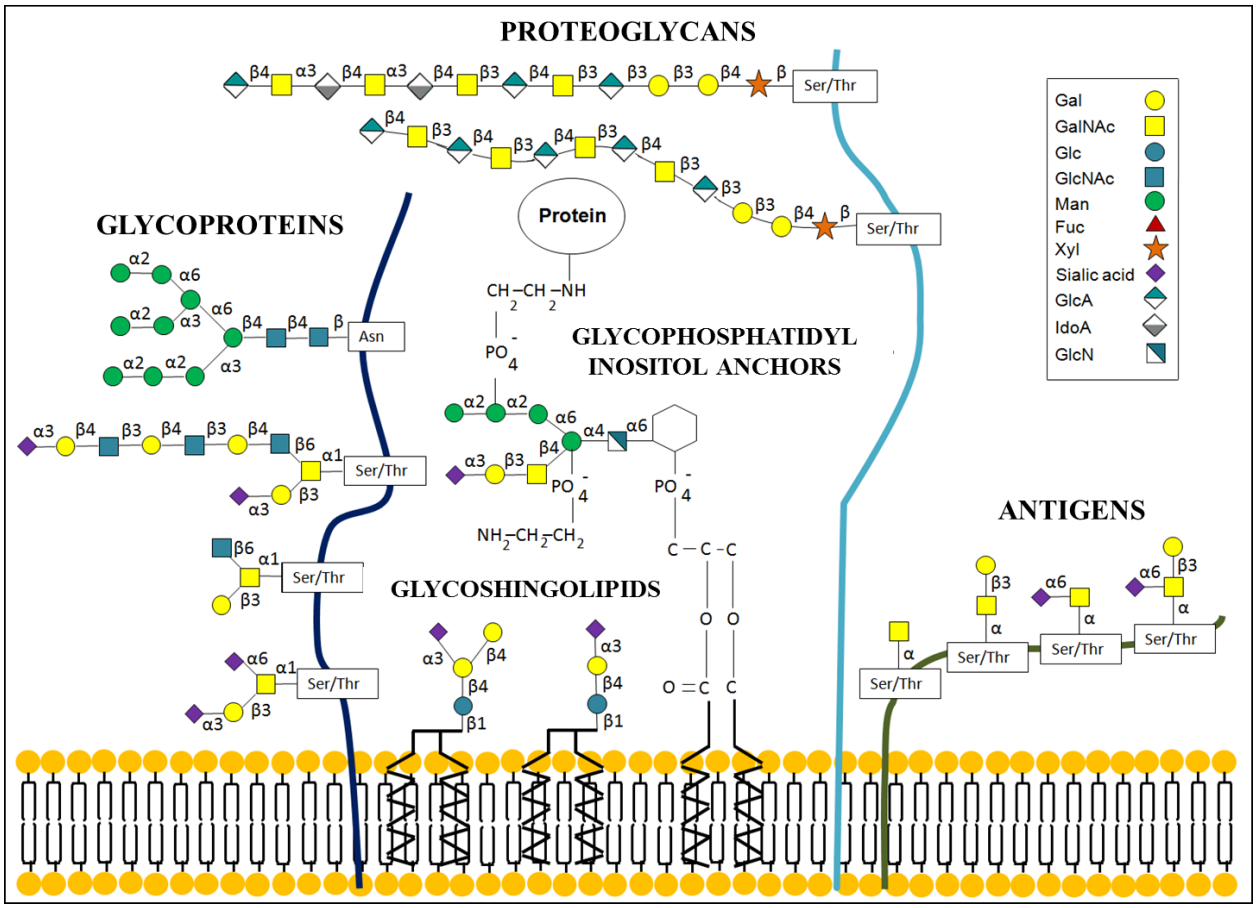

Figure 1. Cell surface glycoconjugates, modified from Varki et al. (2009) with permission.

situations (Gray et al., 2014) are all glycoproteins. Glycan parts of the cell-surface glycoconjugates have a function in self- and nonself-discrimination (Karaçalı et al., 2000; Bayro and Deveci, 2006; Eratak and Karaçalı, 2006; Özkan and Karaçalı, 2006). The glycan structures also decorate signal and carrier molecules in serum. Follicle-stimulating hormone (Lombardi et al., 2013), luteinizing hormone (Mi et al., 2014), sex hormone-binding globulin (Sumer-Bayraktar et al., 2012), and coagulant Factor VIII (Canis et al., 2012) are some such examples.

The cell-surface glycoproteins are responsible for many kinds of interactions between cells and their immediate environments. Free or membrane-associated glycan-binding proteins (lectins, galectins, siglecs, and mannose-binding free and membrane-attached receptors) play critical roles in these interactions. Events mediated by these interactions are recognition, antirecognition, adhesion, signal transduction, endocytosis, vesicle releasing, and migration between the organelles and the cells, on both molecular and cellular levels. Characteristic surface glycosylation patterns, glycotypes, providing a molecular signature, serve to discriminate the differentiated cells from each other.

Glycosylation changes affecting the disruption of normal cellular interactions play an important role in determining cell fate during embryonic and pathological development (Karaçalı, 2003; Varki et al., 2009; Dodla et al., 2012; Nairn et al., 2012). The alterations occurring on the glycans of cell-surface glycoconjugates, such as signaling and attaching and anchoring molecules, receptors, ligands, enzymes, differentiation and cancer-associated antigens, antibodies, and membrane transporters, dramatically change the normal cellular interactions between cells and their microenvironments. A significant relationship between the alteration of cell-surface glycan profiles and cancer progress has been described (Borzym-Kluczyk et al., 2012; Saldova et al., 2013a; Zhang et al., 2014). The modifications of cell-surface glycosylation are responsible for the behavioral changes of tumor cells, including invasion and metastasis (Li et al., 2010; Kang et al., 2011; Taniguchi and Korekane, 2011; Tian and Zhang, 2013; Zhang et al., 2013; Christiansen et al., 2014; Häuselmann and Borsig, 2014).

During the last few years, glycosylation abnormalities in genetic diseases have been highlighted as 'congenital disorders of glycosylation' (Cylwik et al., 2013a, 2013b;

Jaeken, 2013; Rosnoblet et al., 2013). Distribution of significant glycan structures on tumor cells makes the carbohydrates attractive targets as cancer biomarkers (Meany and Chan, 2011; Tuccillo et al., 2014) for the new and much publicized objective of personalized therapy (Contessa et al., 2008; Padler-Karavani, 2014) as well as for the development of anticancer vaccines (Hakomori, 2001; Li et al., 2010) and drugs (Kok and Sietsma, 2004; Gerber-Lemaire and Juillerat-Jeanneret, 2010).

Alterations in glycosylation occur in different parts (core and/or antennae) of glycan structures of glycoconjugates of tumor cells, and in various forms (Brooks et al., 2002; Varki et al., 2009; Karaçalı et al., 2011). In general, an increased number of branches on the core structures and their extensions by addition of new monosaccharides cause the formation of heavily glycosylated glycoconjugates. The addition of new epitopes on proximal and distal parts of oligosaccharide chains and the alterations in linkage types at terminal monosaccharides alter the adhesive interactions of the cells. These alterations occur on the glycans of *N*-linked glycoproteins, *O*-linked glycoproteins (mucins and proteoglycans), and glycosphingolipids. In this review we focus particularly on the branching and elongation of oligosaccharide chains occurring in the common core structure of *N*-glycans on cell-surface glycoconjugates in cancer cells. These changes are responsible for the increase in size and the occurrence of metastatic phenotypes. Responsible enzymes and their key targets associated with cancer are also addressed.

2. Alterations on the common core of *N*-glycans

2.1. Branching on the common core structure

Dolichol-linked precursors, common precursors of *N*-glycans consisting of 14 glycan units (2GlcNAc, 9Man, 3Glc), are formed at the initial synthesis stage of all *N*-linked glycoproteins (Brooks et al., 2002; Varki et al., 2009; Taylor and Drickamer, 2011). First the *N*-acetylglucosamine (GlcNAc) sugar of the precursor is attached to the amide nitrogen of the asparagine residue in the β-glucosidic linkage (GlcNAcβ1-Asn) by the oligosaccharyltransferase. After transferring the precursor to the growing peptide, maturation reactions of the glycans start in the lumen of the rough endoplasmic reticulum and continue in Golgi compartments. Glycosidases and glycosyltransferases successively modify the structure of the precursor. The differential actions of these enzymes cause the formation of high-mannose and hybrid and complex types of *N*-linked oligosaccharides (Figure 2). All of these types

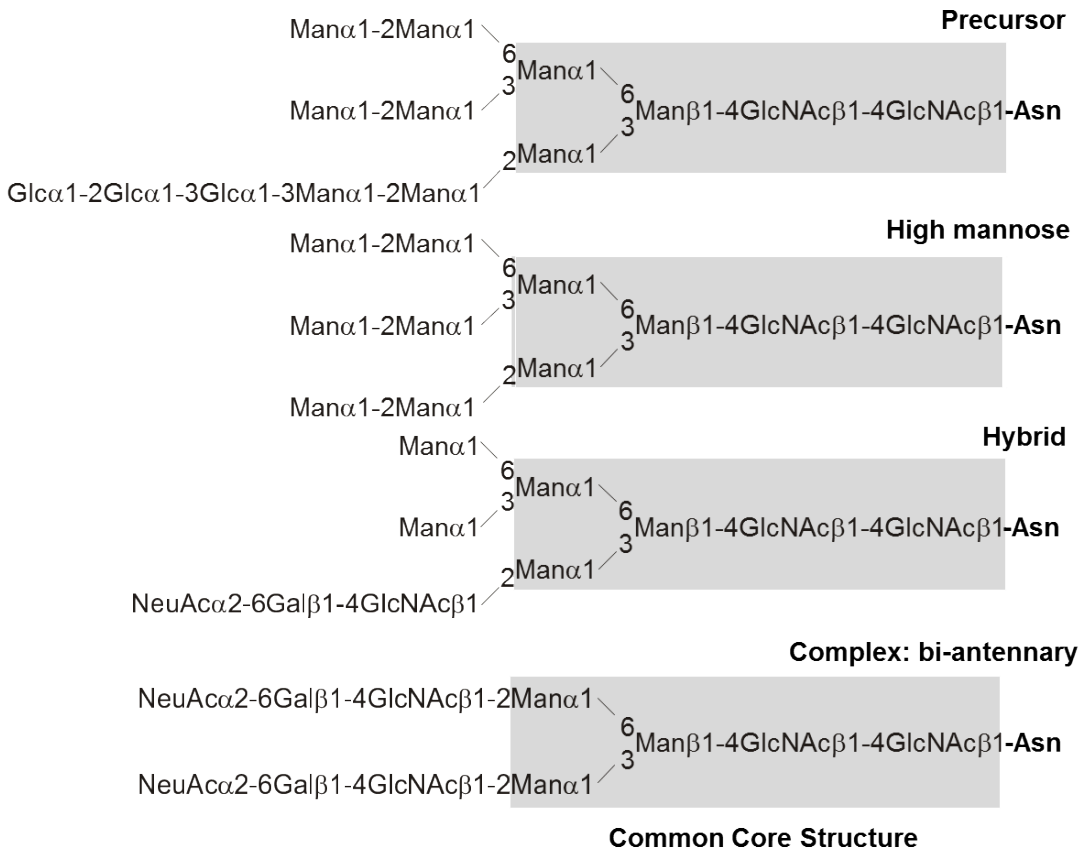

Figure 2. The precursor and the basic types of *N*-glycans. The common core structure is shaded.

have a common core structure consisting of 3 mannose (Man) residues and 2 GlcNAc residues.

Appropriate variations occurring on the glycans direct the protein folding, sorting, correct targeting, half-life, and a number of molecular interactions (adhesion, receptor-ligand binding, and activation) of glycoproteins. Characteristic combinations of these glycan types appear on the surfaces of each differentiated cell. Differentiated healthy cells, together with a few 3- and 4-branched glycans, contain most abundantly 2-branched complex structures on their *N*-glycoproteins.

In tumor cells, increased branching on the core of *N*-glycans is a typical feature, except for truncated expression of oligosaccharides (Brooks et al., 2002; Varki et al., 2009). Two Man residues are attached to the first Man residue by 2 different linkages (α1-6 and α1-3), forming the initiation of 2 basic arms on the core structure. Specific *N*-acetylglucosaminyltransferases (GlcNAc-Ts, GnTs) catalyze the transfer of GlcNAc sugar from the active donor, UDP-GlcNAc, to specific positions on the core mannoses of *N*-glycans via a specific glycosidic linkage. Three, 4, and more branches can be started by the activity of a particular GnT on the core structure (Chen et al., 2009). Each of the attached GlcNAc sugars provides a new substrate for the succeeding glycosylation and their number determines the number of branches or antennaries originating from the common core structure on hybrid and complex *N*-glycans. The enzymes catalyzing glycosidic linkages at the starting points of the new branches are seen in Figure 3.

The enzymes responsible for the addition of the new branches and for the increase in core size are well characterized by several GnTs and a fucosyltransferase (FUT8). In vertebrates, 7 different GnTs (productions of *Mgat* genes), indicated as GnT-I, -II, -III, -IV, -V, -VI, and -IX (Figure 3), have been determined to be involved in the initiation of the branching of the complex *N*-glycan core structure (Taniguchi and Korekane, 2011; Takamatsu et al., 2013).

Galactosyltransferases and sialyltransferases are the other important enzymes. One (Taylor and Drickamer, 2011) or more (Antonopoulos et al., 2012) branches are extended by the addition of galactose and GlcNAc residues, which produce the polylactosamine (poly-LacNAc) extensions. Correlations between the originating branches of poly-LacNAcs and regulation of tumor development, invasion, metastasis, aggressiveness, and survival have been investigated (Seto et al., 2013). Sialic acid attached like a cap at the nonreducing end of the oligosaccharide chains prevents further elongation of the chains.

2.1.1. *N*-Acetylglucosaminyltransferases (GlcNAcTs, GnTs)

Sequential activity rules for the GnTs that initiate the branching of the complex *N*-glycan core structure were established by Brockhausen et al. (1988). GnT-I and GnT-II are involved in initiating the synthesis of the various branches of complex *N*-glycans.

GnT-I, encoded by the *Mgat1* gene (Kumar and Stanley, 1989), acts before all the other GnTs. GnT-I is required for the conversion from the high-mannose type (with 5 Man residues) to the hybrid and complex types (with 3 Man residues) of *N*-glycans (Yip et al., 1997; Chen et al., 2002; Taniguchi and Korekane, 2011). It catalyzes the formation of β1-2 linkage by transferring a GlcNAc sugar to the Man residue on the α1-3 arm of the core structure with 5 mannoses (Figure 4). Two Man residues on the α1-6 arm are removed by catalytic activity of α-mannosidase II. This structure is the substrate for the GnT-II and GnT-III enzymes. Mutation on the corresponding gene, *Mgat1*, causes embryonic lethality (Loffe and Stanley, 1994; D'Agostaro et al., 1995).

GnT-II controls the conversion of hybrid type to complex type structures (D'Agostaro et al., 1995; Ye and Marth, 2004). GnT-II recognizes the structure formed by the catalytic activity of α-mannosidase II and catalyzes the β1-2 glycosidic linkage by adding a GlcNAc to the α1-6 arm on the core; a 2-branched core structure is formed (Figure 4). Activity of GnT-II is prerequisite (Zhang et al., 2000) for the GnT-IV, GnT-V, and GnT-IX activities that are responsible for cancer progression. Mutation on corresponding gene *Mgat2* causes a number of abnormalities in early stages of development (Wang et al., 2001).

GnT-III (the corresponding gene is *Mgat3*) catalyzes the formation of β1-4 glycosidic linkage by transferring a bisecting GlcNAc to the first Man residue on the core (Figures 3 and 4). The bisecting GlcNAc is found in various hybrid and complex *N*-glycans. The presence of a bisecting GlcNAc prevents subsequent processing and elongation of *N*-glycans, inhibiting 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 (Brockhausen et al., 1988; Isaji et al., 2010; Taniguchi and Korekane, 2011; Miwa et al., 2012; Xu et al., 2012). However, a contrary suggestion is also present. The glycomic profiles of several *N*-glycans having a bisecting GlcNAc revealed that they carry lactosamine (LacNAc) repeats and also a core fucose (Fuc) sugar (North et al., 2010). The presence of the bisecting GlcNAc on the cell surface glycoproteins, such as E-cadherin and integrins, probably alters *N*-glycan conformation, which affects their interaction with carbohydrate-binding proteins, such as galectins and siglecs. The bisecting GlcNAc of *N*-glycans on adhesion and signal molecules regulates cellular signaling and tumor progression by modulating *N*-glycan/galectin interactions (Miwa et al., 2012)

Overexpression of GnT-III increases the bisected *N*-glycans but reduces the β1-6 GlcNAc branching structures on target glycoproteins. Knockdown of

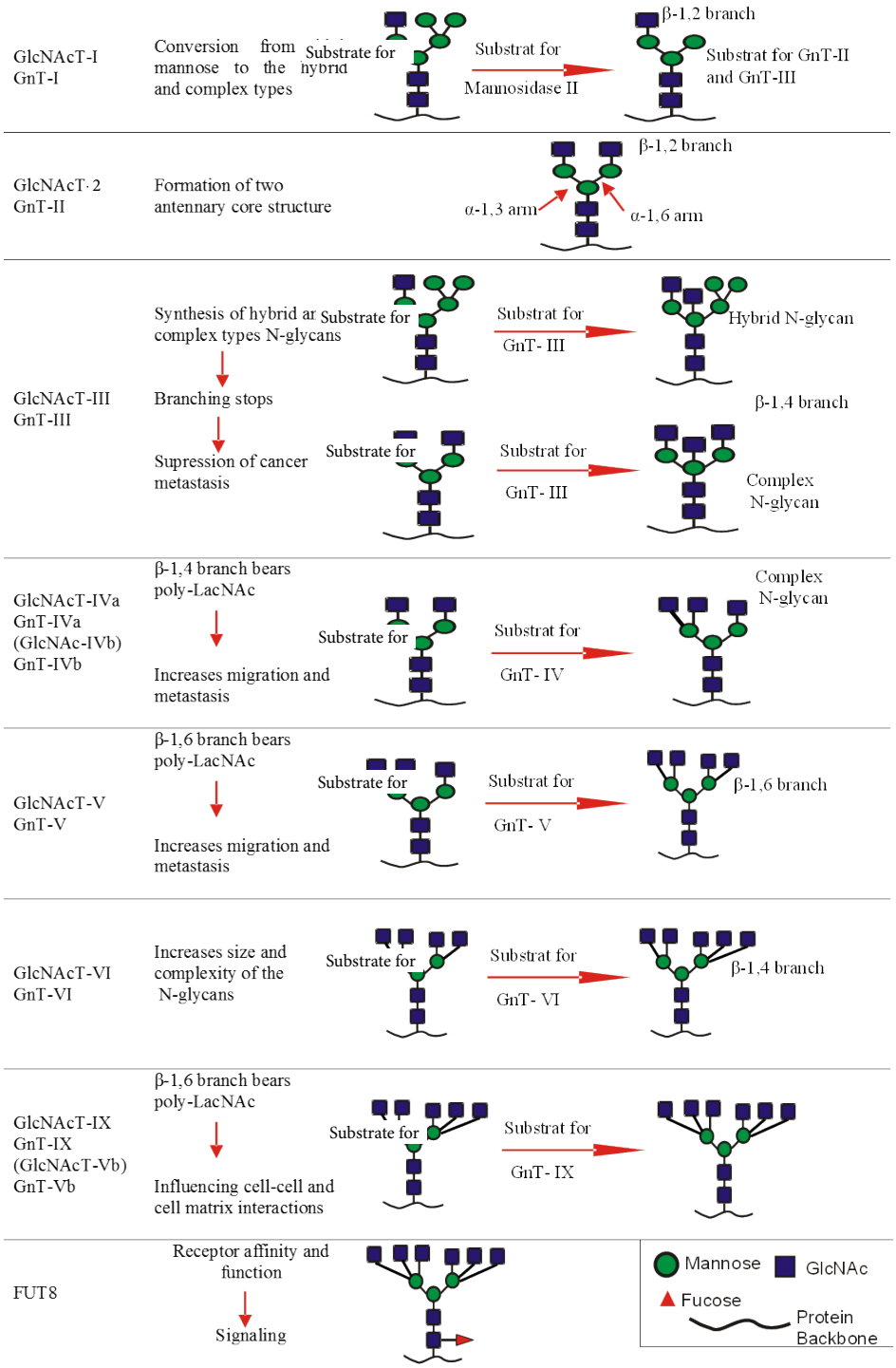

Figure 3. The branches on the common core structure of *N*-glycans. The glycosyltransferases that initiate formation of the branches and the known branches that bear poly-LacNAcs, and related enzymes, are indicated. Modified from Taniguchi and Korekane (2011) with permission.

endogenous GnT-III expression results in increased cell migration (Taniguchi and Korekane, 2011). As a result, the enzyme suppresses the integrin-mediated cell motility and has an inhibitory effect on cancer metastasis (Kariya et al., 2010; Taniguchi and Korekane, 2011; Xu et al., 2012). The *Mgat3* gene has a tissue-specific expression pattern. High expression levels appear particularly in mouse brain and kidney (Miwa et al., 2012)

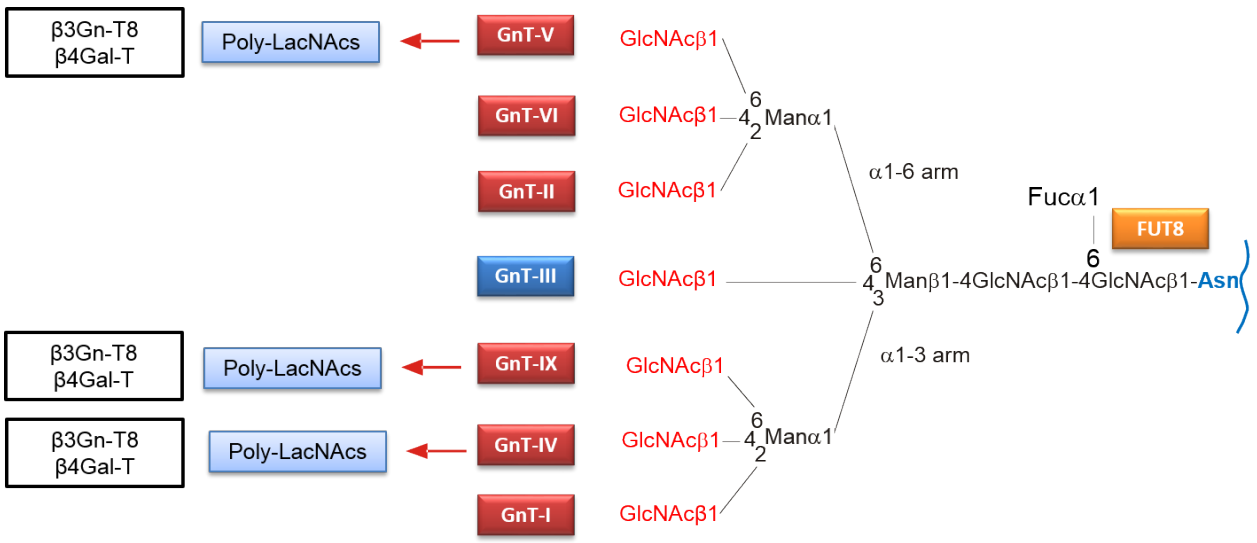

Figure 4. Branching on the common core structure of *N*-glycans. Sequential activities of responsible GnTs. Modified from Taniguchi and Korekane (2011) with permission.

GnT-IV (the corresponding gene is *Mgat4*) catalyzes the formation of β1-4 glycosidic linkage by transferring a GlcNAc sugar to the α1-3 Man arm of the *N*-glycan core. The enzyme acts on biantennary complex-type *N*-glycan and forms the 3-antennary core structure (Figures 3 and 4). The third branch extends by addition of LacNAc repeats, poly-LacNAcs that bear galectin-binding epitopes (Takamatsu et al., 2010, 2013; Taniguchi and Korekane, 2011). Secreted galectins are bound to β-galactoside sugar on poly-LacNAcs and cross-link glycoconjugates on the cell surface and in the ECM by producing a lattice formation. The galectin-glycoprotein lattice structure controls glycoprotein activity.

GnT-IV has 2 isoenzymes, GnT-IVa and GnT-IVb, and they both contribute to the galectin-mediated glycoprotein clustering on the cell surface (Takamatsu et al., 2013). In humans, GnT-IV isoenzymes have the same specificities but different affinities for sugar donors and acceptors. GnT-IVa has higher affinities and is more active than GnT-IVb (Oguri et al., 2006). The results obtained from GnT-IVb-deficient and both GnT-IVa- and GnT-IVb-deficient mice showed that GnT-IVb is expressed in various organs, whereas GnT-IVa expression is restricted to the gastrointestinal tissues (Takamatsu et al., 2010). GnT-IVa is expressed in malignant and premalignant trophoblastic cells but not in normal or benign cells (Niimi et al., 2012). On the contrary, in pancreatic cancer, the increased expression of GnT-IVb was found in tumor tissue, whereas GnT-IVa expression was found in surrounding normal tissues (Ide et al., 2006).

β1-Integrin and a cancer-associated antigen, extracellular matrix metalloproteinase (MMP) inducer CD147, are the target proteins of the enzyme GnT-IVa. Overexpression of GnT-IVa causes an increase in branching of complex *N*-glycans on CD147, leading to enhanced cell migration and metastatic capabilities. However, downregulation of GnT-IVa reduces the branching and decreases cell migration and metastasis (Fan et al., 2012). In GnT-IVb-deficient mice, total enzymatic activity is preserved at a level comparable to the wild type due to upregulated *Mgat4a* expression (Niimi et al., 2012). Transcription factor Ets-1 is responsible for this compensation. Ets-1 evolves from GnT-IVb deficiency and induces the expression of GnT-IVa and GnT-V enzymes (Niimi et al., 2012).

GnT-V (the corresponding gene is *Mgat5*) is an enzyme that has dual functions as a membrane-bound glycosyltransferase and a soluble angiogenic factor (Saito et al., 2002; Nakahara et al., 2006). The membrane-bound form of GnT-V catalyzes the formation of β1-6 linkage by transferring a GlcNAc sugar to the α1-6 arm of the *N*-glycan core (Figures 3 and 4). This added GlcNAc residue provides an initial substrate to form the poly-LacNAc extension. Expression of poly-LacNAcs on the *N*-glycan core, which is often modified with Fuc and sulfate residues (Mitsui et al., 2012) on the surfaces of many cancer cells, indicates their association with cellular differentiation and oncogenesis (Hua et al., 2012; Gao et al., 2014).

A close relationship between the metastatic potential of tumor cells and formation of poly-LacNAc extensions has been reported (Chakraborty and Pawelek, 2003; Pinho et al., 2013; Pocheć et al., 2013; Seto et al., 2013; Tanaka et al., 2013). The degree of the invasiveness and metastatic potential appears related to the amounts of poly-LacNAc chains. Comparison of the common glycoproteins from primary and metastatic melanoma cell lines shows that

the metastatic melanoma cells bear larger amounts of poly-LacNAc chains than the primary cells (Kinoshita et al., 2014). Observation of an inhibition in invasion and metastasis events following downregulation of GnT-V in BGC 829 cells (Huang et al., 2014) supports that suggestion. There are several supporting reports indicating that the gene of GnT-V, *Mgat5*, is correlated with metastasis. Tumor growth and metastasis were suppressed by knockout of *Mgat5* in animal studies (Demetriou et al., 1995; Yao et al., 1999; Granovsky et al., 2000; Yamamoto et al., 2000; Ihara et al., 2002; Saito et al., 2002; Tsui et al., 2008).

GnT-IX was recently designated (Inamori et al., 2003) as GnT-Vb (Kaneko et al., 2003) enzyme forming a β1-6 branched structure (Figures 3 and 4) on the α1-3 arm of the *N*-glycan core (Taniguchi and Korekane, 2011). In the brain, GnT-IX catalyzes the branched form of *O*-mannosyl glycan structures, as well (Kanekiyo et al., 2013). Brain-specific gene expression of this enzyme is regulated by epigenetic histone modification (Kizuka et al., 2011, 2014; Korekane et al., 2013).

2.1.2. α1,6-Fucosyltransferase (FUT8)

FUT8 (α1,6-fucosyltransferase) catalyzes the transfer of a Fuc sugar from the active sugar donor, GDP-fucose, to the first GlcNac residue linked to the asparagine residue of hybrid and complex *N*-glycan cores. FUT8 activity and increased core fucosylation play an important role in cancer development (Bernardi et al., 2013). In general, FUT8 activity is higher in tumor tissue than in healthy tissue and is related to sex, type of growth, and tumor stage (Muinelo-Romay et al., 2008). Overexpressed FUT8 and increased core fucose were observed in several malignant human cancers, such as in the serum of prostate (Saldova et al., 2011) and ovarian (Saldova et al., 2013a) cancer cells in cell lines of nonsmall cell lung cancer (Chen et al., 2013), and in tissues of colorectal (Muinelo-Romay et al., 2008) and hepatocellular (Li et al., 2013; Yin et al., 2014) carcinomas. However, increased levels of tetraantennary glycan without core fucosylation were also observed in hepatocellular carcinoma (Mehta et al., 2012).

Knockdown FUT8 in aggressive lung cancer cell lines significantly inhibits their malignant behaviors (Chen et al., 2013). In contrast, the level of FUT8 protein was decreased in gastric tumor tissues compared to the adjacent nontumor tissues (Zhao et al., 2014). Decreased core fucosylation in both tissue and serum from gastric cancer patients may result from the decreased expression of FUT8 (Liu et al., 2013). The results of glycoproteomic and microarray analyses show that core fucose regulates the function of proteins associated with malignancy. Cell-surface antigens, antibodies, receptors, sugar transporters on Golgi membranes, and adhesion molecules (E-cadherin and integrins) are modified by FUT8 (Chen et al., 2013). Core fucosylation of several growth factors has been demonstrated to be required for signal transduction and alters the sensitivity for ligands. Core fucosylation of *N*-glycans of epidermal growth factor receptor (EGFR) is necessary for the binding of epidermal growth factor (EGF) (Wang et al., 2006). FUT8 promotes EGFR dimerization and phosphorylation (Liu et al., 2011) as well as cellular growth (Matsumoto et al., 2008). Knockdown FUT8 leads to a decrease in the growth response. Decreased core fucosylation of EGFR causes a reduced activation of EGF-induced phosphorylation of the EGFR in gastric cancer (Zhao et al., 2014). Core fucose is required for the ligand binding affinity and function of TGF-β1 receptor (Wang et al., 2005; Venkatachalam and Weinberg, 2013).

Adhesion molecules, E-cadherin and integrins, play an important role in cancer development and progression. FUT8 regulates E-cadherin-mediated cell adhesion and is expressed in metastatic lung cancer cell (Geng et al., 2004). The increase of core fucosylation of E-cadherin leads to strengthened cell–cell adhesion (Osumi et al., 2009). Core fucosylation is essential for integrin-mediated cell migration and signal transduction (Zhao et al., 2008). FUT8 plays an important role in embryonic development; 70% of FUT8-deficient [FUT8 (-/-)] mice that lack the core fucose structure die within 3 days after birth. The others may survive for several weeks, but they show growth retardation. In embryonic fibroblasts from FUT8 (-/-) mice, the levels of bisecting GlcNAc on β1-integrin and N-cadherin were increased. The responsible enzyme, GnT-III, that inhibits cell migration in metastasis is regulated by FUT8 deficiency in vivo (Kurimoto et al., 2014).

All these alterations, with the increase in size and structural complexity of the *N*-glycan core, cause functional changes of the glycans on the surface cover of the cells. In general, the degree of branching is very closely related to tumorigenesis. The increase in *N*-glycan core branching and the formation of long linear or branched poly-LacNAcs cause tumor progression. Reduced *N*-glycan branching degree retards tumor progression (Mehta et al., 2012). Briefly, GnT-I and GnT-II are prerequisites in the biosynthesis of highly branched *N*-glycans. GnT-III prevents cancer of the cells. GnT-IV, GnT-V, GnT-IX, and FUT8 are responsible for tumor progression and metastasis.

2.2. Elongation of the oligosaccharide chains, poly-LacNAcs, and galectin-3

Carbohydrate chains on all types of glycoconjugates carry the repeats of *N*-acetyllactosamine (Gal-GlcNAc, LacNAc), poly-LacNAc extensions. Branching and composition of *N*-glycan cores affect the extent of poly-LacNAc chains. They are found more often in tetraantennary and triantennary *N*-glycans. The branch with β1-6 glycosidic linkage catalyzed by GnT-V on the α1-6 arm and 2 branches catalyzed by GnT-IX (GnT-Vb, isoenzyme of GnT-V) and

by GnT-IV on the α1-3 arm are available to carry poly-LacNAc chains (Figure 3). According to the prevalent hypothesis, the poly-LacNAc extension is attached to one of the specific branches on the common trimannosyl core structure. However, the poly-LacNAc chains were equally distributed on all branches and not selectively enriched on a specific *N*-glycan branch in activated cytotoxic T lymphocytes after antigenic stimulation (Antonopoulos et al., 2012). Two kinds of poly-LacNAc chains are known, indicated as type I and type II according to linkage types. Type II is the most common chain form and the linkages are Galβ1-4GlcNAc and GlcNAcβ1-3Gal in LacNac repeats. In type I, the linkages are Galβ1-3GlcNAc and GlcNAcβ1-4Gal in LacNAc repeats.

Linear and branched poly-LacNAcs form i-histo and I-blood group antigens, respectively (Twu et al., 2010) (Figure 5). These are present on the surfaces of red blood cells and other somatic cells. Poly-LacNAc structures are important ligands for galectin-mediated cell adhesion to ECM proteins, such as laminin and fibronectin (Sauerzapfe et al., 2009). Extended and branched poly-LacNAcs cause the formation of multiantennary complex type *N*-glycans of enormous size. The enzyme required for branching is β1-6 branching glycosyltransferase (IGnT-V, GCNT2). Correlations between the originating branches of poly-LacNAc and regulation of tumor development, invasion, metastasis, aggressiveness, and survival have been investigated (Seto et al., 2013). The increase in branching and in extension of poly-LacNAc is associated with tumor cell metastasis (Nabi and Dennis, 1998; Ishida et al., 2005; Hua et al., 2012; Peng et al., 2012; Liu et al., 2014). In contrast, reduced degrees of branching and extension of poly-LacNAc chains retard tumor progression and metastasis (Togayachi et al., 2010; Liu et al., 2011, 2014; Shen et al., 2011).

Required enzymes for the formation of poly-LacNAc are β1,4-galactosyltransferases (β4GalT) and β1,3-*N*-acetylglucosaminyltransferase (β3GnT). The length of poly-LacNAc extensions changes depending on the repeating action of these 2 transferases (Nabi and Dennis, 1998). β4GalT is present in a unique form in all cells. However, the number of known β3GnT genes is 8 in mice and they are expressed in a tissue-specific manner (Henion and Schwarting, 2014). Each of the 8 determined β3Gn-Ts enzymes (Seko and Yamashita, 2005; Peng et al., 2012; Henion and Schwarting, 2014) synthesizes a different glycan type. β3Gn-T8 (homolog to β3Gn-T2) (Togayachi et al., 2010) has a central role in carcinogenesis and catalyzes the formation of poly-LacNAc on β1-6 branches of *N*-glycans (Hoja-Łukowicz et al., 2013; Liu et al., 2014; Ni et al., 2014). β3Gn-T2 regulates the expression of extended poly-LacNAc chains that are essential for axon guidance and neuronal survival in the olfactory epithelium (Henion and Schwarting, 2014). β3Gn-T8 and β3Gn-T2 have the same specificity to act efficiently on tetraantennary and triantennary *N*-glycan cores (Seko and Yamashita, 2005). The mixing of β3Gn-T8 and β3Gn-T2 in vitro forms a heterocomplex and its enzymatic activity is greatly enhanced compared to the individual enzymes (Seko and Yamashita, 2005).

The branched and extended structure of poly-LacNAc chains is responsible for lattice formation with galectins. The galectin-glycoprotein lattice structure controls the activity of glycoproteins at the cell surface by regulation of their membrane localization, lateral mobility, and clustering. The lattice formation inhibits endocytosis (Grigorian et al., 2009), which is important for receptor turnover. Galectin-glycoprotein lattices can regulate the duration of signaling and receptor turnover. Thus, they can control the decision between cell growth and arrest (Rabinovich et al., 2007). Long poly-LacNAc chains with additional sialyl Lewis^x epitopes are highly metastatic, while short poly-LacNAc chains with many more sialyl Lewis^x epitopes are not metastatic (Srinivasan et al., 2009). This is probably related to the presence of the lattice formation for poly-LacNAc chains of different lengths.

A chemotherapeutic agent, 5-FU, inhibits the expression of β3Gn-T8, and this causes a reduction of poly-

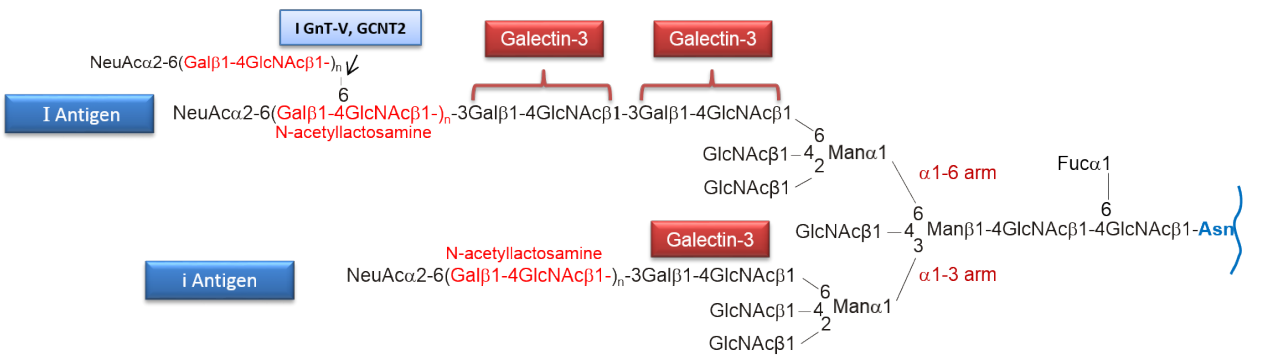

Figure 5. i and I antigens on poly-LacNAc chains. Galectin-3 recognizes proximal LacNAc units.

LacNAc on CD147 in colon cancer cells (Gao et al., 2014). The levels of β3Gn-T8 and poly-LacNAc chains gradually increase from low to high metastatic potential in colorectal cancer cell lines. A positive correlation between β3Gn-T8 expression and highly glycosylated CD147 indicates that β3Gn-T8 plays a critical role in the metastasis of colorectal cancer (Lu et al., 2014; Ni et al., 2014).

Galectins are soluble proteins recognizing and binding β-galactosides on poly-LacNAc extensions of the *N*-glycan core. They are found in the nucleus, cytoplasm, cell surface, and ECM, as well as in biological fluids (Argüeso and Panjwani, 2011). Different galectins have different specificities for oligosaccharides. Galectin-3 (Gal-3) binds proximal LacNac repeats on the poly-LacNAc chains of *N*-glycan cores (Stowell et al., 2008). 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 (Chiu et al., 2010; Argüeso and Panjwani, 2011). Galectin-glycan lattices create homotypic or glycoprotein complexes that are involved in cellular signaling related to a variety of cell functions, including cell adhesion, migration, invasion, angiogenesis, immune functions, apoptosis, and endocytosis (Garner and Baum, 2008; Nangia-Makker et al., 2008; Chiu et al., 2010; Çay, 2012). During the ECM remodeling, new Gal-3-glycan lattices occur and mediate new interactions (Lagana et al., 2006; Reticker-Flynn et al., 2012; Priglinger et al., 2013). Galectin-glycoprotein lattices control the organization of plasma membrane domains like lipid rafts and the direction of targeted delivery of glycoproteins to the cell surface. They determine the duration of signaling by inhibiting the endocytosis of glycoprotein receptors (such as growth factors) from the cell surface (Garner and Baum, 2008).

The GnT-V expression-dependent Gal-3-TGFβR lattice preserves growth factor receptor densities at the level necessary for invasive phenotypes in transformed cell surfaces (Rabinovich et al., 2007). GnT-IVa expression-dependent lattice formation increases the cell-surface half-life of glucose transporter 2 on pancreatic β-cells, probably by inhibiting receptor endocytosis (Ohtsubo et al., 2005; Rabinovich et al., 2007). These points indicate that lattice structures are involved in cellular signaling in various ways depending on the origin of the poly-LacNAc chains.

3. Changes in cancer-associated proteins

It appears that the enzymes (GnT-IV, GnT-V, and GnT-IX) initiating branches that possess poly-LacNAc chains on the core of *N*-glycans affect the same proteins involved in cancer development. GnT-V has a number of target proteins involved in tumor progression. Well-defined substrate proteins of GnT-V are growth factor receptors (such as EGF and TGF-β), adhesion and signaling molecules (cadherin and integrins), tumor-associated antigen (CD147), tissue inhibitor of metalloproteinase-1 (TIMP-1), membrane-bound serine protease (Matriptase), and lysosomal-associated membrane proteins 1 and 2 (Lamp-1 and Lamp-2). Each one contributes in a distinct manner to tumor progression and metastasis (Ochwat et al., 2004; Siddiqui et al., 2005; Kim et al., 2008; Taniguchi and Korekane, 2011; Drake et al., 2012; Christiansen et al., 2014). Expression of β1-6 GlcNAc branching on these substrate glycoproteins is related to a variety of tumors in malignant transformation (Ihara et al., 2002). Depending on the structural changes of *N*-glycans in these target proteins, different implications of GnT-V have been reported in cancer metastasis.

The occurrence of poly-LacNAc chains is very important for tumor progression and metastasis. The branched structure catalyzed by GnT-V on several glycoproteins has received particular attention in the literature. GnT enzymes have different affinities for the same common core substrate. This produces a restriction mechanism among the GnTs. For example, GnT-I and GnT-II have nearly 250- and 20-fold higher affinity for UDP-GlcNAc than GnT-V, respectively (Chen et al., 2009). Higher expression of GnT-I reduces GlcNAc branching on the core of *N*-glycans by reducing the availability of UDP-GlcNAc to GnT-IV and GnT-V.

3.1. Growth factor receptors

Growth factor receptors (GFRs) are synthesized in the cytoplasm and then transported toward the plasma membrane within the vesicles originating from Golgi membranes (Luo et al., 2013; Katsuda et al., 2014). It has been suggested that *N*-linked glycosylation is required for the successful cell-surface transportation and sensitivity of the TGF-β receptor in gastric carcinoma cell lines (Kim et al., 2012). *N*-glycans on GFRs such as EGF, TGF-β, IGF, and PDGF are modified by overexpression of GnT-V and high affinity ligands, poly-LacNAcs, for galectins are generated on tumor cells (Lajoie et al., 2007). The increase of β1-6 branches bearing poly-LacNAcs has an influence on ligand binding, dimerization, and promotion of function of EGFRs (Guo et al., 2004; Takahashi et al., 2004).

The formation of the lattice between increased poly-LacNAc chains and Gal-3 causes the inhibition of receptor endocytosis (Partridge et al., 2004; Häuselman and Borsig, 2014), the prolongation of receptor signaling (Kimura et al., 2012), and the promotion of cell proliferation. EGFR signaling in tumor cells is regulated by the competition between the galectin lattice and oligomerized caveolin-1 microdomains for EGFR (Lajoie et al., 2007). Morphological changes and cell detachment from the matrix occur after receptor stimulation (Guo et al., 2007). The cell detachment from the matrix is closely associated with tumor cell migration (Wang et al., 2009; Pocheć et

al., 2013). Results obtained from studies using knockout GnT-V and antisense cell lines support this suggestion (Seberger et al., 1999; Guo et al., 2001, 2007, 2010). Knockout of GnT-V by siRNA expression causes lowered expression of β1-6 branches on EGFR *N*-glycans without any effect on EGFR expression level (Guo et al., 2007). The EGFR signaling pathway maintains a balance among cell proliferation, differentiation, and apoptosis and thus plays an important role in the development and progression of several human carcinomas (Al Moustafa et al., 2012). Since occurrence of the epithelial-mesenchymal transition phenotype is initiated via EGFR signaling (Huang et al., 2013), downregulation of GnT-V has particular importance.

3.2. Adhesion and signal molecules

The modification of *N*-linked glycans on adhesion molecules such as E-cadherin and integrins can change their functions (Pinho et al., 2011). Overexpression of GnT-V provides the formation of β1-6 branches that bear a poly-LacNAc extension. This branch is the cause of E-cadherin-mediated tumor invasion (Pinho et al., 2013). Similarly, increased expression on β1-6 branching on *N*-glycans of β1-integrin inhibits the formation of fibronectin receptor α5β1. This deficiency causes a decrease in ECM adhesion and an increase in cell motility (Siddique et al., 2005). In the case of a decrease in GnT-V activity, an enhancement of integrin α5β1-dependent vascular endothelium adhesion and subsequent transmigration occur (Yang et al., 2012). These results indicate that *N*-glycan modification of the adhesion and signal molecules has an important function for migration and invasion activities of tumor cells.

3.3. Tumor-associated antigen (CD147)

CD147, a tumor-associated antigen, is a transmembrane protein and a member of the immunoglobulin receptor family and is highly expressed on the cell surface of various tumor cells (Bai et al., 2014). The role of CD147 in tumorigenesis is related to the inducement of MMP expression. It stimulates the secretion of MMPs from fibroblasts to degrade the basement membrane and the ECM, to facilitate cancer cell penetration, migration, metastasis, and angiogenesis (Weidle et al., 2010; Chen et al., 2012; Huang et al., 2013; Zhao et al., 2013). The degradation of the ECM and the cell adhesion contacts, and the formation of blood vessels, are the main events during metastasis that are initiated with CD147. The stimulating effect of CD147 on the production of MMPs reaches the target cells by the vesicles. CD147 is released by an extracellular vesicle shedding mechanism and transported within the vesicle membrane. Although vesicle shedding is common in normal cells, it occurs at much higher rates in tumor cells (Redzic et al., 2013). Thus, the activation of MMPs is triggered by GnT-V via CD147 (Lee et al., 2013). Released CD147 contributes to the cells undergoing an epithelial-to-mesenchymal transition by activating local MMPs (Siu et al., 2013).

Overexpression of GnT-V results from the increase of both CD147 and MMPs. CD147 contains high mannose and complex type *N*-glycans bearing poly-LacNAc extensions on β1-6 branches of the core structure. Heterogeneous glycosylation of CD147 causes remarkable variations in its size. According to the results from site-mutated glycosylation studies, *N*-glycans of CD147 contribute to its MMP-inducing activity and the most highly glycosylated form of CD147 is more effective. Because β1-6 branched glycans are high-affinity ligands for Gal-3, extracellular Gal-3 triggers the clustering of membrane glycoproteins that contain poly-LacNAc extensions. Gal-3 interacting with poly-LacNAc on the CD147 and integrin β1 of retinal pigment epithelial cells is responsible for modified cell behavior (Priglinger et al., 2013). Aberrant β1-6 branching glycans on CD147 probably play an important role in the biological activity of CD147 (Zheng et al., 2006), which has been considered as a potential tumor marker (Chen et al., 2012; Huang et al., 2013).

3.4. Tissue inhibitor metalloproteinase-1 (TIMP-1)

Tissue inhibitor of metalloproteinase-1 (TIMP-1) is also a target protein for GnT-V.

TIMP-1 regulates the activity of MMPs (Groblewska et al., 2012) and serves as a biomarker in gastric cancer (Grunnet et al., 2013). Polylactosamination on the β1-6 GlcNA branch and sialylation on TIMP-1 are both characteristic in human colon cancer cells, WiDr, in which GnT-V was overexpressed (Kim et al., 2008). Glycosylation of TIMP-1 participates in the regulation of interaction between MMPs (Kim et al., 2012). The aberrant glycosylation of TIMP-1 is closely correlated with invasive and metastatic potentials of colon cancer cells by producing a weaker inhibition of MMPs, both in vivo and in vitro. Thus, the function of TIMP-1 is associated with the inhibition of MMPs, thereby blocking tumor cell migration and invasion. However, independent of their inhibitory activity on MMPs, TIMPs also have direct cellular functions in normal tissue physiology and disease progression. A novel therapeutic approach to cancer treatment, involving the normalization of the tumor microenvironment including normal ECM components, was postulated (Stetler-Stevenson and Gavil, 2014). Involving the ability of TIMP-1 to act as a signaling molecule with cytokine-like activities (Ries, 2014) support this idea.

3.5. Membrane-bound serine protease (matriptase)

The other target protein for GnT-V is a type II transmembrane serine protease, matriptase, alternatively known as membrane-type serine protease-1 (MTSP-1). Expression of matriptase in a variety of normal tissues and especially in epithelial tissues (Takeuchi et al.,

2000) suggests that this protease could regulate different biological events (Ihara et al., 2004). The function of matriptase is associated with epithelial homeostasis in both health and disease situations (Gray et al., 2014). Matriptase participates in tumor growth and progression through the activation of 2 important cancer invasion effectors, hepatocyte growth factor (HGF) and urokinase plasminogen activator (uPA), on the surface of cancer cells (Qiu et al., 2007; Kotthaus et al., 2010; Owen et al., 2010). These proteins are involved in growth and motility of cancer cells, particularly carcinomas, and in the vascularization of tumors (Benaud et al., 2002). Proteases mediate the degradation of ECM and intercellular adhesive structures to allow penetration and migration of the cells into the extracellular angiogenic factors. Matriptase contributes to these processes (Uhland, 2006). Although proteolytic activity in the close environment of the cells is essential for tissue homeostasis, development, and repair, the incorrect regulation of proteolysis can cause malignant transformation (List et al., 2006; Bugge et al., 2007; List, 2009). Matriptase positively regulates carcinoma metastasis by activating the single-chain latent forms of uPA and HGF and converting them into biologically active forms (Suzuki et al., 2004; Kilpatrick et al., 2006; Qiu et al., 2007; Lee et al., 2010). A direct relationship between matriptase and GnT-V appears in human cancer tissues (Ihara et al., 2004; Ito et al., 2006). Matriptase with β1-6 GlcNAc catalyzed by GnT-V becomes resistant to autodegradation and trypsin digestion. *N*-glycosidase F-treated matriptase shows a greatly reduced resistance to degradation. The active matriptase is rapidly inactivated by hepatocyte growth factor activator inhibitor-1 (Chu et al., 2014).

All these alterations occurring in the *N*-glycan common core structure of cancer-associated proteins cause changes in their molecular interactions and functions.

4. Conclusion

Markedly extensive efforts have been made to understand the biological significance of protein glycosylation in cancer in recent years. In cancer progression and metastasis, the enzymes responsible for branching of the core structure and in formation and extension of poly-LacNAcs play very important roles. Elucidation of the interactions between their molecular structures and the functions of the associated enzymes, which are also glycoproteins, will make important contributions to a better understanding of tumor formation, progression, metastasis, and retardation.

References

Al Moustafa AE, Achkhar A, Yasmeen A (2012). EGF-receptor signaling and epithelial-mesenchymal transition in human carcinomas. Front Biosci 4: 671–684.

Antonopoulos A, Demotte N, Stroobant V, Haslam SM, van der Bruggen P, Dell A (2012). Loss of effector function of human cytolytic T lymphocytes is accompanied by major alterations in *N*- and *O*-glycosylation. J Biol Chem 287: 11240–11251.

Argüeso P, Panjwani N (2011). Focus on molecules: galectin-3. Exp Eye Res 92: 2–3.

Bai Y, Huanh W, Ma LT, Jiang JL, Chen ZN (2014). Importance of *N*-glycosylation on CD147 for its biological functions. Int J Mol Sci 15: 6356–6377.

Bassagañas S, Carvalho S, Dias AM, Pérez-Garay M, Ortiz MR, Figueras J, Reis CA, Pinho SS, Peracaula R (2014). Pancreatic cancer cell glycosylation regulates cell adhesion and invasion through the modulation of α2β1 integrin and E-cadherin function. PLoS One 9: e98595.

Bayro İ, Deveci R (2006). *Galleria mellonella* (Lepidoptera)'nın gelişen testislerinde sialik asidin rolü. İzmir, Turkey: Ege Üniversitesi Fen Bilimleri Araştırma Projesi (in Turkish).

Benaud CM, Oberst M, Dickson RB, Lin CY (2002). Deregulated activation of matriptase in breast cancer cells. Clin Exp Metastasis 19: 639–649.

Bernardi C, Soffientini U, Piacente F, Tonetti MG (2013). Effects of microRNAs on fucosyltransferase 8 (FUT8) expression in hepatocarcinoma cells. PLoS One 8: e76540.

Borzym-Kluczyk M, Radziejewska I, Darewicz B (2012). Glycosylation of proteins in healthy and pathological human renal tissues. Folia Histochem Cytobiol 50: 599–604.

Brockhausen I, Narasimhan S, Schachter H (1988). The biosynthesis of highly branched *N*-glycans: studies on the sequential pathway and functional role of *N*-acetylglucosaminyltransferases I, II, III, IV, V and VI. Biochimie 70: 1521–1533.

Brooks SA, Dwek MV, Schumacher U (2002). Functional and Molecular Glycobiology. 1st ed. Oxford, UK: BIOS Scientific Publishers Ltd.

Bugge TH, List K, Szabo R (2007). Matriptase-dependent cell surface proteolysis in epithelial development and pathogenesis. Front Biosci 12: 5060–5070.

Canis K, McKinnon TA, Nowak A, Haslam SM, Panico M, Morris HR, Laffan MA, Dell A (2012). Mapping the *N*-glycome of human von Willebrand factor. Biochem J 447: 217–228.

Çay T (2012). Immunhistochemical expression of galectin-3 in cancer: a review of the literature. Turk Patoloji Derg 28: 1–10.

Chakraborty AK, Pawelek JM (2003). GnT-V, macrophage and cancer metastasis: a common link. Clin Exp Metastasis 20: 365–373.

Chen CY, Jan YH, Juan YH, Yang CJ, Huang MS, Yu CJ, Yang PJ, Hsiao M, Hsu TL, Wong CH (2013). Fucosyltransferase 8 as a functional regulator of nonsmall cell lung cancer. P Natl Acad Sci USA 110: 630–635.

Chen H, Lam Fok K, Jiang X, Chan HC (2012). New insights into germ cell migration and survival/apoptosis in spermatogenesis: lessons from CD147. Spermatogenesis 2: 264–272.

Chen HL, Li CF, Grigorian A, Tian W, Demetriou M (2009). T cell receptor signaling co-regulates multiple Golgi genes to enhance *N*-glycan branching. J Biol Chem 284: 32454–32461.

Chen S, Tan J, Reinhold VN, Spence AM, Schachter H (2002). UDP-*N*-acetylglucosamine:alpha-3-D-mannoside beta-1,2-*N*-acetylglucosaminyltransferase I and UDP-*N*-acetylglucosamine:alpha-6-D-mannoside beta-1,2-*N*-acetylglucosaminyltransferase II in *Caenorhabditis elegans*. Biochim Biophys Acta 1573: 271–279.

Chiu CG, Strugnell SS, Griffith OL, Jones SJ, Gown AM, Walker B, Nabi IR, Wiseman SM (2010). Diagnostic utility of galectin-3 in thyroid cancer. Am J Pathol 176: 2067–2081.

Christiansen MN, Chik J, Lee L, Anugraham M, Abrahams JL, Packer NH (2014). Cell surface protein glycosylation in cancer. Proteomics 14: 525–546.

Chu LL, Xu Y, Yang JR, Hu YA, Chang HH, Lai HY, Tseng CC, Wang HY, Johnson MD, Wang JK et al. (2014). Human cancer cells retain modest levels of enzymatically active matriptase only in extracellular milieu following induction of zymogen activation. PLoS One 9: e92244.

Contessa JN, Bhojani MS, Freeze HH, Rehemtulla A, Lawrence TS (2008). Inhibition of *N*-linked glycosylation disrupts receptor tyrosine kinase signaling in tumor cells. Cancer Res 68: 3803–3809.

Cylwik B, Lipartowska K, Chrostek L, Gruszewska E (2013a). Congenital disorders of glycosylation. Part II. Defects of protein O-glycosylation. Acta Biochim Pol 60: 361–368.

Cylwik B, Naklicki M, Chrostek L, Gruszewska E (2013b). Congenital disorders of glycosylation. Part I. Defects of protein *N*-glycosylation. Acta Biochim Pol 60: 151–161.

D'Agostaro GA, Zingoni A, Moritz RL, Simpson RJ, Schachter H, Bendiak B (1995). Molecular cloning and expression of cDNA encoding the rat UDP-*N*-acetylglucosamine: alpha-6-D-mannoside beta-1,2-*N*-acetylglucosaminyltransferase II. J Biol Chem 270: 15211–15221.

Demetriou M, Nabi IR, Coppoliono M, Dedhar S, Dennis JW (1995). Reduced contact-inhibition and substratum adhesion in epithelial cells expressing GlcNAc-transferase V. J Cell Biol 130: 383–392.

Dodla MC, Young A, Venable A, Hasneen K, Rao RR, Machacek DW, Stice SL (2012). Differing lectin binding profiles among human embryonic stem cells and derivatives aid in the isolation of neural progenitor cells. PLoS One 6: e23266.

Drake PM, Schilling B, Niles RK, Prakobphol A, Li B, Jung K, Cho W, Braten M, Inerowicz HD, Williams K et al. (2012). Lectin chromatography/mass spectrometry discovery workflow identifies putative biomarkers of aggressive breast cancers. J Proteome Res 11: 2508–2520.

Eratak B, Karaçalı S (2006). *Galleria mellonella* (Lepidoptera)'da metamorfoz geçiren corpus cardiacum corpus allatum (CC-CA) kompleksinde sialik asidin rolü. İzmir, Turkey: Ege Üniversitesi Fen Bilimleri Araştırma Projesi (in Turkish).

Eyers SA, Ridgwell K, Mawby WJ, Tanner MJ (1994). Topology and organization of human Rh (rhesus) blood group-related polypeptides. J Biol Chem 269: 6417–6423.

Fan JH, Wang SJ, Yu SJ, He JN, Zheng WL, Zhang JN (2012). *N*-Acetylglucosaminyltransferase IVa regulates metastatic potential of mouse hepatocarcinoma cells through glycosylation of CD147. Glycoconj J 29: 323–334.

Feizi T (1981). Antibodies to defined carbohydrate sequences in immunological disorders of man. Med Biol 59: 131–133.

Feizi T (1985). Carbohydrate antigens in human cancer. Cancer Surv 4: 245–269.

Feizi T (1987). Significance of carbohydrate components of cell surfaces. Ciba Found Symp 129: 43–58.

Feizi T (1991). Carbohydrate differentiation antigens: probable ligands for cell adhesion molecules. Trends Biochem Sci 16: 84–86.

Fredriksson SA, Podbielska M, Nilsson B, Krotkiewska B, Lisowska E, Krotkiewski H (2010). ABH blood group antigens in *N*-glycan of human glycophorin A. Arch Biochem Biophys 498: 127–135.

Gao L, Shen L, Yu M, Ni J, Dong X, Zhou Y, Wu S (2014). Colon cancer cells treated with 5-fluorouracil exhibit changes in polylactosamine-type *N*-glycans. Mol Med Rep 9: 1697–1702.

Garner OB, Baum LG (2008). Galectin-glycan lattices regulate cell-surface glycoprotein organization and signalling. Biochem Soc Trans 36: 1472–1477.

Geng F, Shi BZ, Yuan YF, Wu XZ (2004). The expression of core fucosylated E-cadherin in cancer cells and lung cancer patients: prognostic implications. Cell Res 14: 423–433.

Gerber-Lemaire S, Juillerat-Jeanneret L (2010). Studies toward new anti-cancer strategies based on alpha-mannosidase inhibition. Chimia (Aarau) 64: 634–639.

Granovsky M, Fata J, Pawling J, Muller WJ, Khokha R, Dennis JW (2000). Suppression of tumor growth and metastasis in Mgat5-deficient mice. Nat Med 6: 306–312.

Gray K, Elghadban S, Thongyoo P, Owen KA, Szabo R, Bugge TH, Tate EW, Leatherbarrow RJ, Ellis V (2014). Potent and specific inhibition of the biological activity of the type-II transmembrane serine protease matriptase by the cyclic microprotein MCoTI-II. Thromb Haemost 112: 402–411.

Grigorian A, Torossian S, Demetriou M (2009). T-cell growth, cell surface organization, and the galectin-glycoprotein lattice. Immunol Rev 230: 232–246.

Groblewska M, Siewko M, Mroczko B, Szmitkowski M (2012). The role of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in the development of esophageal cancer. Folia Histochem Cytobiol 50: 12–19.

Grunnet M, Mau-Sørensen M, Brünner N (2013). Tissue inhibitor of metalloproteinase 1 (TIMP-1) as a biomarker in gastric cancer: a review. Scand J Gastroenterol 48: 899–905.

Guo HB, Johnson H, Randolph M, Nagy T, Blalock R, Pierce M (2010). Specific posttranslational modification regulates early events in mammary carcinoma formation. P Natl Acad Sci USA 107: 21116–21121.

Guo HB, Randolph M, Pierce M (2007). Inhibition of a specific *N*-glycosylation activity results in attenuation of breast carcinoma cell invasiveness-related phenotypes: inhibition of epidermal growth factor-induced dephosphorylatıon of focal adhesıon kinase. J Biol Chem 282: 22150–22162.

Guo HB, Zhang Y, Chen HL (2001). Relationship between metastasis-associated phenotypes and *N*-glycan structure of surface glycoproteins in human hepatocarcinoma cells. J Cancer Res Clin Oncol 127: 231–236.

Guo P, Wang QY, Guo HB, Shen ZH, Chen HL (2004). *N*-acetylglucosaminyltransferase V modifies the signaling pathway of epidermal growth factor receptor. Cell Mol Life 61: 1795–1804.

Haga Y, Ishii K, Suzuki T (2011). *N*-glycosylation is critical for the stability and intracellular trafficking of glucose transporter GLUT4. J Biol Chem 286: 31320–31327.

Hakomori S (2001). Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. Adv Exp Med Biol 491: 369–402.

Häuselmann I, Borsig L (2014). Altered tumor-cell glycosylation promotes metastasis. Front Oncol 4: 1–15.

Henion TR, Schwarting GA (2014). *N*-linked polylactosamine glycan synthesis is regulated by co-expression of β3GnT2 and GCNT2. J Cell Physiol 229: 471–478.

Hennet T (2009). How does a medical doctor become a glycobiologist. Biochim Biophys Acta 1792: 824.

Hoja-Łukowicz D, Link-Lenczowski P, Carpentieri A, Amoresano A, Pocheć E, Artemenko KA, Bergquist J, Lityńska A (2013). L1CAM from human melanoma carries a novel type of *N*-glycan with Galβ1-4Galβ1- motif. Involvement of *N*-linked glycans in migratory and invasive behaviour of melanoma cells. Glycoconj J 30: 205–225.

Hollenstein K, Dawson RJ, Locher KP (2007). Structure and mechanism of ABC transporter proteins. Curr Opin Struct Biol 17: 412–418.

Hua D, Qin F, Shen L, Jiang Z, Zou ST, Xu L, Cheng ZH, Wu SL (2012). β3GnT8 regulates laryngeal carcinoma cell proliferation via targeting MMPs/TIMPs and TGF-β1. Asian Pac J Cancer Prev 13: 2087–2093.

Huang B, Sun L, Cao J, Zhang Y, Wu Q, Zhang J, Ge Y, Fu L, Wang Z (2013). Downregulation of the GnT-V gene inhibits metastasis and invasion of BGC823 gastric cancer cells. Oncol Rep 29: 2392–2400.

Huang B, Wu Q, Ge Y, Zhang J, Sun L, Zhang Y, Fu L, Fan J, Wang Z (2014). Expression of *N*-acetylglucosaminyltransferase V in gastric cancer correlates with metastasis and prognosis. Int J Oncol 44: 849–857.

Huang W, Luo WJ, Zhu P, Tang J, Yu XL, Cui HY, Wang B, Zhang Y, Jiang JL, Chen ZN (2013). Modulation of CD147-induced matrix metalloproteinase activity: role of CD147 *N*-glycosylation. Biochem J 449: 437–448.

Ide Y, Miyoshi E, Nakagawa T, Gu J, Tanemura M, Nishida T, Ito T, Yamamoto H, Kozutsumi Y, Taniguchi N (2006). Aberrant expression of *N*-acetylglucosaminyltransferase-IVa and IVb (GnT-IVa and b) in pancreatic cancer. Biochem Biophys Res Commun 341: 478–482.

Ihara S, Miyoshi E, Ko JH, Murata K, Nakahara S, Honke K, Dickson RB, Lin CY, Taniguchi N (2002). Prometastatic effect of *N*-acetylglucosaminyltransferase V is due to modification and stabilization of active matriptase by adding β1-6 GlcNAc branching. J Biol Chem 277: 16960–16967.

Ihara S, Miyoshi E, Nakahara S, Sakiyama H, Ihara H, Akinaga A, Honke K, Dickson RB, Lin CY, Taniguchi N (2004). Addition of β1-6 GlcNAc branching to the oligosaccharide attached to Asn 772 in the serine protease domain of matriptase plays a pivotal role in its stability and resistance against trypsin. Glycobiology 14: 139–146.

Inamori K, Endo T, Ide Y, Fujii S, Gu J, Honke K, Taniguchi N (2003). Molecular cloning and characterization of human GnT-IX, a novel beta1,6-*N*-acetylglucosaminyltransferase that is specifically expressed in the brain. J. Biol Chem 278: 43102–43109.

Irollo E, Pirozzi G (2013). CD133: to be or not to be, is this the real question? Am J Transl Res 5: 563–581.

Isaji T, Kariya Y, Xu Q, Fukuda T, Taniguchi N, Gu J (2010). Functional roles of the bisecting GlcNAc in integrin-*N*-mediated cell adhesion. Methods Enzymol 480: 445–459.

Ishida H, Togayachi A, Sakai T, Iwai T, Hiruma T, Sato T, Okubo R, Inaba N, Kudo T, Gotoh M et al. (2005). A novel β1,3-*N*-acetylglucosaminyltransferase (β3Gn-T8), which synthesizes poly-*N*-acetyllactosamine, is dramatically upregulated in colon cancer. FEBS Lett 579: 71–78.

Ito Y, Akinaga A, Yamanaka K, Nakagawa T, Kondo A, Dickson RB, Lin CY, Miyauchi A, Taniguchi N, Miyoshi E (2006). Co-expression of matriptase and *N*-acetylglucosaminyltransferase V in thyroid cancer tissues--its possible role in prolonged stability in vivo by aberrant glycosylation. Glycobiology 16: 368–374.

Jaeken J (2013). Congenital disorders of glycosylation. Handb Clin Neurol 113: 1737–1743.

Kanekiyo K, Inamori K, Kitazume S, Sato K, Maeda J, Higuchi M, Kizuka Y, Korekane H, Matsuo I, Honke K et al. (2013). Loss of branched O-mannosyl glycans in astrocytes accelerates remyelination. J Neurosci 33: 10037–10047.

Kaneko M, Alvarez-Manilla G, Kamar M, Lee I, Lee JK, Troupe K, Zhang WJ, Osawa M, Pierce M (2003). A novel β(1,6)-*N*-acetylglucosaminyltransferase V (GnT-VB). FEBS Lett 554: 515–519.

Kang JG, Ko JH, Kim YS (2011). Pros and cons of using aberrant glycosylation as companion biomarkers for therapeutics in cancer. BMB Rep 44: 765–771.

Karaçalı S (2003). Glikobiyoloji, Güncel moleküler biyoloji. Turk J Vet Anim 27: 489–495 (in Turkish).

Karaçalı S, Deveci R, Pehlivan S, Özcan A (2000). Adhesion of hemocytes to desialylated prothoracic glands of *Galleria mellonella* (Lepidoptera) in larval stage. Invertebr Reprod Dev 37: 167–170.

Karaçalı S, İzzetoğlu S, Deveci R (2011). Kanserde glikozilasyon değişiklikleri. In: Haydaroğlu A, Vatansever S, Kitapçıoğlu G, editors. Meme Kanserinde Moleküler ve Genetik Yaklaşım. 1st ed. İzmir, Turkey: Ege Üniversitesi Yayınları, pp. 45–59.

Kariya Y, Kawamura C, Tabei T, Gu J (2010). Bisecting GlcNAc residues on laminin-332 down-regulate galectin-3-dependent keratinocyte motility. J Biol Chem 285: 3330–3340.

Katsuda TL, Kosaka N, Ochiya T (2014). The roles of extracellular vesicles in cancer biology: toward the development of novel cancer biomarkers. Proteomics 14: 412–425.

Kilpatrick LM, Harris RL, Owen KA, Bass R, Ghorayeb C, Bar-Or A, Ellis V (2006). Initiation of plasminogen activation on the surface of monocytes expressing the type II transmembrane serine protease matriptase. Blood 108: 2616–2623.

Kim JI, Lee I, Park S, Park MS (2012). Surface glycoproteins determine the feature of the pandemic H1N1 virus. BMB Rep 45: 653–658.

Kim YS, Hwang SY, Kang HY, Sohn H, Oh S, Kim JY, Yoo JS, Kim YH, Kim CH, Jeon JH et al. (2008). Functional proteomics study reveals that *N*-acetylglucosaminyltransferase V reinforces the invasive/metastatic potential of colon cancer through aberrant glycosylation on tissue inhibitor of metalloproteinase-1. Mol Cell Proteomics 7: 1–14.

Kim YW, Park J, Lee HJ, Lee SY, Kim SJ (2012). TGF-β sensitivity is determined by *N*-linked glycosylation of the type II TGF-β receptor. Biochem J 445: 403–411.

Kimura A, Terao M, Kato A, Hanafusa T, Murota H, Katayama I, Miyoshi E (2012). Upregulation of *N*-acetylglucosaminyltransferase-V by heparin-binding EGF-like growth factor induces keratinocyte proliferation and epidermal hyperplasia. Exp Dermatol 21: 515–519.

Kinoshita M, Mitsui Y, Kakoi N, Yamada K, Hayakawa T, Kakehi K (2014). Common glycoproteins expressing polylactosamine-type glycans on matched patient primary and metastatic melanoma cells show different glycan profiles. J Proteome Res 13: 1021–1033.

Kizuka Y, Kitazume S, Okahara K (2014). Epigenetic regulation of a brain-specific glycosyltransferase *N*-acetylglucosaminyltransferase-IX (GnT-IX) by specific chromatin modifiers. J Biol Chem 289: 11253–11261.

Kizuka Y, Kitazume S, Yoshida M, Taniguchi N (2011). Brain-specific expression of *N*-acetylglucosaminyltransferase IX (GnT-IX) is regulated by epigenetic histone modifications. J Biol Chem 286: 31875–31884.

Kok JW, Sietsma H (2004). Sphingolipid metabolism enzymes as targets for anticancer therapy. Curr Drug Targets 5: 375–382.

Korekane H, Park JY, Matsumoto A, Nakajima K, Takamatsu S, Ohtsubo K, Miyamoto Y, Hanashima S, Kanekiyo K, Kitazume S et al. (2013). Identification of ectonucleotide phosphatase/phosphodiesterase 3 (ENPP3) as a regulator of *N*-acetylglucoseaminyltransferase GnT-IX (GnT-Vb). J Biol Chem 288: 27912–27926.

Kotthaus J, Steinmetzer T, Kotthaus J, Schade D, van de Locht A, Clement B (2010). Metabolism and distribution of two highly potent and selective peptidomimetic inhibitors of matriptase. Xenobiotica 40: 93–101.

Kumar R, Stanley P (1989). Transfection of a human gene that corrects the Lec1 glycosylation defect: evidence for transfer of the structural gene for *N*-acetylglucosaminyltransferase I. Mol Cell Biol 9: 5713–5717.

Kurimoto A, Kitazume S, Kizuka Y, Nakajima K, Oka R, Fujinawa R, Korekane H, Yamaguchi Y, Wada Y, Taniguchi N (2014). The absence of core fucose up-regulates GnT-III and Wnt target genes: a possible mechanism for an adaptive response in terms of glycan function. Biol Chem 289: 11704–11714.

Lagana A, Goetz JG, Cheung P, Raz A, Dennis JW, Nabi IR (2006). Galectin binding to Mgat5-modified *N*-glycans regulates fibronectin matrix remodeling in tumor cells. Mol Cell Biol 26: 3181–3193.

Lajoie P, Partridge EA, Guay G, Goetz JG, Pawling J, Lagana A, Joshi B, Dennis JW, Nabi IR (2007). Plasma membrane domain organization regulates EGFR signaling in tumor cells. J Cell Biol 179: 341–356.

Lee JH, Kang JG, Song KJ, Jeon SK, Oh S, Kim YS, Ko JH (2013). *N*-Acetylglucosaminyltransferase V triggers overexpression of MT1-MMP and reinforces the invasive/metastatic potential of cancer cells. Biochem Biophys Res Commun 431: 658–663.

Lee SL, Huang PY, Roller P, Cho EG, Park D, Dickson RB (2010). Matriptase/epithin participates in mammary epithelial cell growth and morphogenesis through HGF activation. Mech Dev 127: 82–95.

Li M, Song L, Qin X (2010). Glycan changes: cancer metastasis and anti-cancer vaccines. J Biosci 35: 665–673.

Li S, Mo C, Peng Q, Kang X, Sun C, Jiang K, Huang L, Lu Y, Sui J, Qin X et al. (2013). Cell surface glycan alterations in epithelial mesenchymal transition process of Huh7 hepatocellular carcinoma cell. PLoS One 8: e71273.

List K (2009). Matriptase: a culprit in cancer. Future Oncol 5: 97–104.

List K, Bugge TH, Szabo R (2006). Matriptase: potent proteolysis on the cell surface. Mol Med 12: 1–7.

Liu J, Shen L, Yang L, Hu S, Xu L, Wu S (2014). High expression of β3GnT8 is associated with the metastatic potential of human glioma. Int J Mol Med 33: 1459–1468.

Liu L, Yan B, Huang J, Gu Q, Wang L, Fang M, Jiao J, Yue X (2013). The identification and characterization of novel *N*-glycan-based biomarkers in gastric cancer. PLoS One 8: e77821.

Liu YC, Yen HY, Chen CY, Chen CH, Cheng PF, Juan YH, Chen CH, Khoo KH, Yu CJ, Yang PC et al. (2011). Sialylation and fucosylation of epidermal growth factor receptor suppress its dimerization and activation in lung cancer cells. P Natl Acad Sci USA 108: 11332–11337.

Loffe E, Stanley P (1994). Mice lacking *N*-acetylglucosaminyltransferase I activity die at mid-gestation revealing an essential role for complex or hybrid *N*-linked carbohydrates. P Natl Acad Sci USA 91: 728–732.

Lombardi A, Andreozzi C, Pavone V, Triglione V, Angiolini L, Caccia P (2013). Evaluation of the oligosaccharide composition of commercial follicle stimulating hormone preparations. Electrophoresis 34: 2394–2406.

Lu CH, Wu WY, Lai YJ, Yang CM, Yu LC (2014). Suppression of B3GNT7 gene expression in colon adenocarcinoma and its potential effect in the metastasis of colon cancer cells. Glycobiology 24: 359–367.

Luo W, Xia T, Xu L, Chen YG, Fang X (2013). Visualization of the post-Golgi vesicle-mediated transportation of TGF-β receptor II by quasi-TIRFM. J Biophotonics (in press).

Matsumoto K, Yokote H, Arao T, Maegawa M, Tanaka K, Fujita Y, Shimizu C, Hanafusa T, Fujiwara Y, Nishio K (2008). *N*-Glycan fucosylation of epidermal growth factor receptor modulates receptor activity and sensitivity to epidermal growth factor receptor tyrosine kinase inhibitor. Cancer 99: 1611–1617.

Meany DL, Chan DW (2011). Aberrant glycosylation associated with enzymes as cancer biomarkers. Clin Proteomics 8: 7.

Mehta A, Norton P, Liang P, Comunale MA, Wang M, Rodemich-Betesh L, Koszycki A, Noda K, Miyoshi E, Block T (2012). Increased levels of tetra-antennary *N*-Linked glycan but not core fucosylation are associated with hepatocellular carcinoma tissue. Cancer Epidemiol Biomarkers Prev 21: 925–933.

Mi Y, Lin A, Fiete D, Steirer L, Baenziger JU (2014). Modulation of mannose and asialoglycoprotein receptor expression determines glycoprotein hormone half-life at critical points in the reproductive. Cycle J Biol Chem 289: 12157–12167.

Mitsui Y, Yamada K, Hara S, Kinoshita M, Hayakawa T, Kakehi K (2012). Comparative studies on glycoproteins expressing polylactosamine-type *N*-glycans in cancer cells. J Pharm Biomed Anal 70: 718–726.

Miwa HE, Song Y, Alvarez R, Cummings RD, Stanley P (2012). The bisecting GlcNAc in cell growth control and tumor progression. Glycoconj J 29: 609–618.

Muinelo-Romay L, Vázquez-Martín C, Villar-Portela S, Cuevas E, Gil-Martín E, Fernández-Briera A (2008). Expression and enzyme activity of α(1,6)fucosyltransferase in human colorectal cancer. Int J Cancer 123: 641–646.

Nabi IR, Dennis JW (1998). The extent of polylactosamine glycosylation of MDCK LAMP-2 is determined by its Golgi residence time. Glycobiology 8: 947–953.

Nairn AV, Aoki K, dela Rosa M, Porterfield M, Lim JM, Kulik M, Pierce JM, Wells L, Dalton S, Tiemeyer M et al. (2012). Regulation of glycan structures in murine embryonic stem cells: combined transcript profiling of glycan-related genes and glycan structural analysis. J Biol Chem 287: 37835–37856.

Nakahara S, Saito T, Kondo N, Moriwaki K, Noda K, Ihara S, Takahashi M, Ide Y, Gu J, Inohara H et al. (2006). A secreted type of β1,6 *N*-acetylglucosaminyltransferase V (GnT-V), a novel angiogenesis inducer, is regulated by γ-secretase. FASEB J 20: 2451–2459.

Nangia-Makker P, Balan V, Raz A (2008). Regulation of tumor progression by extracellular galectin-3. Cancer Microenviron 1: 43–51.

Ni J, Jiang Z, Shen L, Gao L, Yu M, Xu X, Zou S, Hua D, Wu S (2014). β3GnT8 regulates the metastatic potential of colorectal carcinoma cells by altering the glycosylation of CD147. Oncol Rep 31: 1795–801.

Niimi K, Yamamoto E, Fujiwara S, Shinjo K, Kotani T, Umezu T, Kajiyama H, Shibata K, Ino K, Kikkawa F (2012). High expression of *N*-acetylglucosaminyltransferase IVa promotes invasion of choriocarcinoma. Br J Cancer 107: 1969–1077.

North SJ, Huang, HH, Sundaram S, Jang-Lee J, Etienne AT, Trollope A, Chalabi S, Dell A, Stanley P, Haslam SM (2010). Glycomics profiling of Chinese hamster ovary cell glycosylation mutants reveals *N*-glycans of a novel size and complexity. J Biol Chem 285: 5759–5775.

Ochwat D, Hoja-Lukowicz D, Litynska A (2004). *N*-glycoproteins bearing β1–6 branched oligosaccharides from the A375 human melanoma cell line analysed by tandem mass spectrometry. Melanoma Res 14: 479–485.

Oguri S, Yoshida A, Minowa MT, Takeuchi M (2006). Kinetic properties and substrate specificities of two recombinant human *N*-acetylglucosaminyltransferase-IV isozymes. Glycoconj J 23: 473–80.

Ohtsubo K, Takamatsu S, Minowa MT, Yoshida A, Takeuchi M, Marth JD (2005). Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes. Cell 123: 1307–1321.

Osumi D, Takahashi M, Miyoshi E, Yokoe S, Lee SH, Noda K, Nakamori S, Gu J, Ikeda Y, Kuroki Y et al. (2009). Core fucosylation of E-cadherin enhances cell-cell adhesion in human colon carcinoma WiDr cells. Cancer 100: 888–895.

Owen KA, Qiu D, Alves J, Schumacher AM, Kilpatrick LM, Li J, Harris JL, Ellis V (2010). Pericellular activation of hepatocyte growth factor by the transmembrane serine proteases matriptase and hepsin, but not by the membrane-associated protease uPA. Biochem J 426: 219–228.

Özkan M, Karaçalı S (2006). *Galleria mellonella* (Lepidoptera)'da metamorfoz geçiren sinir sisteminde sialik asidin rolü. İzmir, Turkey: Ege Üniversitesi Fen Bilimleri Araştırma Projesi (in Turkish).

Padler-Karavani V (2014). Aiming at the sweet side of cancer: aberrant glycosylation as possible target for personalized-medicine. Cancer Lett 352: 102–112.

Partridge EA, Le Roy C, Di Guglielmo GM, Pawling J, Cheung P, Granovsky M, Nabi IR, Wrana JL, Dennis JW (2004). Regulation of cytokine receptors by Golgi *N*-glycan processing and endocytosis. Science 306: 120–124.

Peng W, Pranskevich J, Nycholat C, Gilbert M, Wakarchuk W, Paulson JC, Razi N (2012). *Helicobacter pylori* β1,3-*N*-acetylglucosaminyltransferase for versatile synthesis of type 1 and type 2 poly-LacNAcs on *N*-linked, O-linked and I-antigen glycans. Glycobiology 22: 1453–1464.

Pinho SS, Figueiredo J, Cabral J, Carvalho S, Dourado J, Magalhães A, Gärtner F, Mendonfa AM, Isaji T, Gu J et al. (2013). E-cadherin and adherens-junctions stability in gastric carcinoma: functional implications of glycosyltransferases involving *N*-glycan branching biosynthesis, *N*-acetylglucosaminyltransferases III and V. Biochim Biophys Acta 1830: 2690–2700.

Pinho SS, Seruca R, Gärtner F, Yamaguchi Y, Gu J, Taniguchi N, Reis CA (2011). Modulation of E-cadherin function and dysfunction by *N*-glycosylation. Cell Mol Life 68: 1011–1020.

Pocheć E, Janik M, Hoja-Łukowicz D, Link-Lenczowski P, Przybyło M, Lityńska A (2013). Expression of integrins α3β1 and α5β1 and GlcNAc β1,6 glycan branching influences metastatic melanoma cell migration on fibronectin. Eur J Cell Biol 92: 355–362.

Priglinger CS, Szober CM, Priglinger SG, Mer J, Euler KN, Kernt M, Gondi G, Behler J, Geerlof A, Kampik A et al. (2013). Galectin-3 induces clustering of CD147 and integrin-β1 transmembrane glycoprotein receptors on the RPE cell surface. PLoS One 8: e70011.

Qiu D, Owen K, Gray K, Bass R, Ellis V (2007). Roles and regulation of membrane-associated serine proteases. Biochem Soc Trans 35: 583–587.

Rabinovich GA, Toscano MA, Jackson SS, Vasta GR (2007). Functions of cell surface galectin-glycoprotein lattices. Curr Opin Struct Biol 17: 513–520.

Redzic JS, Kendrick AA, Bahmed K, Dahl KD, Pearson CG, Robinson WA, Robinson SE, Graner MW, Eisenmesser EZ (2013). Extracellular vesicles secreted from cancer cell lines stimulate secretion of MMP-9, IL-6, TGF-β1 and EMMPRIN. PLoS One 8: e71225.

Reticker-Flynn NE, Malta DF, Winslow MM, Lamar JM, Xu MJ, Underhill GH, Hynes RO, Jacks TE, Bhatia SN (2012). A combinatorial extracellular matrix platform identifies cell-extracellular matrix interactions that correlate with metastasis. Nat Commun 3: 1122.

Ries C (2014). Cytokine functions of TIMP-1. Cell Mol Life 71: 659–672.

Rosnoblet C, Peanne R, Legrand D, Foulquier F (2013). Glycosylation disorders of membrane trafficking. Glycoconj J 30: 23–31.

Ruhaak LR, Uh HW, Beekman M, Hokke CH, Westendorp RG, Houwing-Duistermaat J, Wuhrer M, Deelder AM, Slagboom PE (2011). Plasma protein *N*-glycan profiles are associated with calendar age, familial longevity and health. J Proteome Res 10: 1667–1674.

Saito T, Miyoshi E, Sasai K, Nakano N, Eguchi H, Honke K, Taniguchi N (2002). A secreted type of beta 1,6-*N*-acetylglucosaminyltransferase V (GnT-V) induces tumor angiogenesis without mediation of glycosylation: a novel function of GnT-V distinct from the original glycosyltransferase activity. J Biol Chem 277: 17002–17008.

Saldova R, Fan Y, Fitzpatrick JM, Watson RW, Rudd PM (2011). Core fucosylation and α2-3 sialylation in serum *N*-glycome is significantly increased in prostate cancer comparing to benign prostate hyperplasia. Glycobiology 21: 195–205.

Saldova R, Piccard H, Pérez-Garay M, Harvey DJ, Struwe WB, Galligan MC, Berghmans N, Madden SF, Peracaula R, Opdenakker G et al. (2013a). Increase in sialylation and branching in the mouse serum *N*-glycome correlates with inflammation and ovarian tumour progression. PLoS One 8: e71159.

Saldova R, Struwe WB, Wynne K, Elia G, Duffy MJ, Rudd PM (2013b). Exploring the glycosylation of serum CA125. Int J Mol 14: 15636–15654.

Sauerzapfe B, Krenek K, Schmiedel J, Wakarchuk WW, Pelantová H, Kren V, Elling L (2009). Chemo-enzymatic synthesis of poly-*N*-acetyllactosamine (poly-LacNAc) structures and their characterization for CGL2-galectin-mediated binding of ECM glycoproteins to biomaterial surfaces. Glycoconj J 26: 141–159.

Seberger PJ, Chaney WG (1999). Control of metastasis by Asn-linked, β1–6 branched oligosaccharides in mouse mammary cancer cells. Glycobiology 9: 235–241.

Seko A, Yamashita K (2005 Oct). Characterization of a novel galactose β1,3-N-acetylglucosaminyltransferase (β3Gn-T8): the complex formation of β3Gn-T2 and β3Gn-T8 enhances enzymatic activity. Glycobiology 15: 943–951.

Seto K, Uchida F, Baba O, Yamatoji M, Karube R, Warabi E, Sakai S, Hasegawa S, Yamagata K, Yanagawa T et al. (2013). Negative expression of *N*-acetylglucosaminyltransferase V in oral squamous cell carcinoma correlates with poor prognosis. Springerplus 2: 657.

Shen L, Liu Z, Tu Y, Xu L, Sun X, Wu S (2011). Regulation of MMP-2 expression and activity by β-1,3-N-acetylglucosaminyltransferase-8 in AGS gastric cancer cells. Mol Biol Rep 38: 1541–1550.

Siddiqui SF, Pawelek J, Handerson T, Lin CY, Dickson RB, Rimm DL, Camp RL (2005). Coexpression of β1,6-*N*-acetylglucosaminyltransferase V glycoprotein substrates defines aggressive breast cancers with poor outcome. Cancer Epidemiol Biomarkers Prev 14: 2517–2523.

Siu A, Chang J, Lee C, Lee S, Lee C, Ramos DM (2013). Expression of EMMPRIN modulates mediators of tumor invasion in oral squamous cell carcinoma. J Calif Dent Assoc 41: 831–838.

Srinivasan N, Bane SM, Ahire SD, Ingle AD, Kalraiya RD (2009). Poly *N*-acetyllactosamine substitutions on *N*- and not *O*-oligosaccharides or Thomsen-Friedenreich antigen facilitate lung specific metastasis of melanoma cells via galectin-3. Glycoconj J 26: 445–456.

Stetler-Stevenson WG, Gavil NV (2014). Normalization of the tumor microenvironment: evidence for tissue inhibitor of metalloproteinase-2 as a cancer therapeutic. Connect Tissue Res 55: 13–19.

Stowell SR, Arthur CM, Mehta P, Slanina KA, Blixt O, Leffler H, Smith DF, Cummings RD (2008). Galectin-1, -2, and -3 exhibit differential recognition of sialylated glycans and blood group antigens. J Biol Chem 283: 10109–10123.

Sumer-Bayraktar Z, Nguyen-Khuong T, Jayo R, Chen DD, Ali S, Packer NH, Thaysen-Andersen M (2012). Micro- and macroheterogeneity of *N*-glycosylation yields size and charge isoforms of human sex hormone binding globulin circulating in serum. Proteomics 12: 3315–3327.

Suzuki M, Kobayashi H, Kanayama N, Saga Y, Suzuki M, Lin CY, Dickson RB, Terao T (2004). Inhibition of tumor invasion by genomic down-regulation of matriptase through suppression of activation of receptor-bound pro-urokinase. J Biol Chem 279: 14899–14908.

Takahashi M, Tsuda T, Ikeda Y, Honke K, Taniguchi N (2004). Role of *N*-glycans in growth factor signaling. Glycoconj J 20: 207–212.

Takamatsu S, Antonopoulos A, Ohtsubo K, Ditto D, Chiba Y, Le DT, Morris HR, Haslam SM, Dell A, Marth JD et al. (2010). Physiological and glycomic characterization of *N*-acetylglucosaminyltransferase-IVa and -IVb double deficient mice. Glycobiology 20: 485–497.

Takamatsu S, Korekane H, Ohtsubo K, Oguri S, Park JY, Matsumoto A, Taniguchi N (2013). *N*-Acetylglucosaminyltransferase (GnT) assays using fluorescent oligosaccharide acceptor substrates: GnT-III, IV, V, and IX (GnT-Vb). In: Brockhausen I, editor. Glycosyltransferases: Methods and Protocols. 1st ed. New York, NY, USA: Springer Science Business Media, pp. 283–298.

Takeuchi H, Haltiwanger RS (2014). Significance of glycosylation in Notch signaling. Biochem Biophys Res Commun (in press).

Takeuchi T, Harris JL, Huang W, Yan KW, Coughlin SR, Craik CS (2000). Cellular localization of membrane-type serine protease 1 and identification of protease-activated receptor-2 and single-chain urokinase-type plasminogen activator as substrates. J Biol Chem 275: 26333–26342.

Tan Z, Lu W, Li X, Yang G, Guo J, Yu H, Li Z, Guan F (2014). Altered *N*-glycan expression profile in epithelial-to-mesenchymal transition of NMuMG cells revealed by an integrated strategy using mass spectrometry and glycogene and lectin microarray analysis. J Proteome Res 13: 2783–2795.

Tanaka K, Moriwaki K, Yokoi S, Koyama K, Miyoshi E, Fukase K (2013). Whole-body imaging of tumor cells by azaelectrocyclization: visualization of metastasis dependence on glycan structure. Bioorg Med Chem 21: 1074–1047.

Taniguchi N, Korekane H (2011). Branched *N*-glycans and their implications for cell adhesion, signaling and clinical applications for cancer biomarkers and in therapeutics. BMB Rep 44: 772–781.

Taylor ME, Drickamer K (2011). Introduction to Glycobiology. 3rd ed. New York, NY, USA: Oxford University Press.

Tian Y, Zhang H (2013). Characterization of disease-associated *N*-linked glycoproteins. Proteomics 13: 504–511.

Togayachi A, Kozono Y, Kuno A, Ohkura T, Sato T, Hirabayashi J, Ikehara Y, Narimatsu H (2010). β3GnT2 (B3GNT2), a major polylactosamine synthase: analysis of B3GNT2-deficient mice. Methods Enzymol 479: 185–204.

Tokhtaeva E, Sachs G, Vagin O (2010). Diverse pathways for maturation of the Na, K-ATPase β1 and β2 subunits in the endoplasmic reticulum of Madin-Darby canine kidney cells. J Biol Chem 285: 39289–39302.

Tsui KH, Chang PL, Feng TH, Chung LC, Sung HC, Juang HH (2008). Evaluating the function of matriptase and *N*-acetylglucosaminyltransferase V in prostate cancer metastasis. Anticancer Res 28: 1993–1999.

Tuccillo FM, de Laurentiis A, Palmieri C, Fiume G, Bonelli P, Borrelli A, Tassone P, Scala I, Buonaguro FM, Quinto I et al. (2014). Aberrant glycosylation as biomarker for cancer: focus on CD43. Biomed Res Int 2014: 742831.

Twu YC, Hsieh CY, Lin M, Tzeng CH, Sun CF, Yu LC (2010). Phosphorylation status of transcription factor C/EBPα determines cell-surface poly-LacNAc branching (I antigen) formation in erythropoiesis and granulopoiesis. Blood 115: 2491–2499.

Uhland K (2006). Matriptase and its putative role in cancer. Cell Mol Life 63: 2968–2978.

Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J (2009). Essentials of Glycobiology. 2nd ed. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Venkatachalam MA, Weinberg JM (2013). New wrinkles in old receptors: core fucosylation is yet another target to inhibit TGF-β signaling. Kidney Int 84: 11–14.

Wang C, Yang Y, Yang Z, Liu M, Li Z, Sun L, Mei C, Chen H, Chen L, Wang L et al. (2009). EGF-mediated migration signaling activated by *N*-acetylglucosaminyltransferase-V via receptor protein tyrosine phosphatase kappa. Arch Biochem Biophys 486: 64–72.

Wang X, Gu J, Ihara H, Miyoshi E, Honke K, Taniguchi N (2006). Core fucosylation regulates epidermal growth factor receptor-mediated intracellular signaling. J Biol Chem 281: 2572–2577.

Wang X, Inoue S, Gu J, Miyoshi E, Noda K, Li W, Mizuno-Horikawa Y, Nakano M, Asahi M, Takahashi M et al. (2005). Dysregulation of TGF-β1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. P Natl Acad Sci USA 102: 15791–15796.

Wang Y, Tan J, Smith M, Ditto D, Panico M, Campbell R, Varki N, Long J, Jaeken J, Levinson S et al. (2001). Modeling human congenital disorder of glycosylation type IIa in the mouse: conservation of asparagine-linked glycan-dependent functions in mammalian physiology and insights into disease pathogenesis. Glycobiology 11: 1050–1070.

Weidle UH, Scheuer W, Eggle D, Klostermann S, Stockinger H (2010). Cancer-related issues of CD147. Cancer Genomics Proteomics 7: 157–169.

Wu SL, Taylor AD, Lu Q, Hanash SM, Im H, Snyder M, Hancock WS (2013). Identification of potential glycan cancer markers with sialic acid attached to sialic acid and up-regulated fucosylated galactose structures in epidermal growth factor receptor secreted from A431 cell line. Mol Cell Proteomics 12: 1239–1249.

Xu O, Isaji T, Lu Y, Gu W, Kondo M, Fukuda T, Du Y, Gu J (2012). Roles of *N*-acetylglucosaminyltransferase III in epithelial-to-mesenchymal transition induced by transforming growth factor β1 (TGF-β1) in epithelial cell lines. J Biol Chem 287: 16563–16574.

Yamamoto H, Swoger J, Greene S, Saito T, Hurh J, Sweeley C, Leestma J, Mkrdichian E, Cerullo L, Nishikawa A et al. (2000). β1,6-*N*-Acetylglucosamine-bearing *N*-glycans in human gliomas implication for role in regulating invasivity. Cancer Res 60: 134–142.

Yang HM, Yu C, Yang Z (2012). *N*-acetylglucosaminyltransferase V negatively regulates integrin α5β1-mediated monocyte adhesion and transmigration through vascular endothelium. Int J Oncol 41: 589–598.

Yao M, Zhou DP, Jiang SM, Wang QH, Zhou XD, Tang ZY, Cu JX (1999). Elevated activity of *N*-acetylglucosaminyltransferase V in human hepatocellular carcinoma. J Cancer Res Clin Oncol 124: 27–30.

Ye Z, Marth JD (2004). *N*-glycan branching requirement in neuronal and postnatal viability. Glycobiology 14: 547–58.

Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW (1997). AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 90: 5002–5012.

Yin H, Lin Z, Nie S, Wu J, Tan Z, Zhu J, Dai J, Feng Z, Marrero J, Lubman DM (2014). Mass-selected site-specific core-fucosylation of ceruloplasmin in alcohol-related hepatocellular carcinoma. J Proteome Res 13: 2887–2896.

Yip B, Chen SH, Mulder H, Höppener JW, Schachter H (1997). Organization of the human beta-1,2-*N*-acetylglucosaminyltransferase I gene (MGAT1), which controls complex and hybrid N-glycan synthesis. Biochem J 321: 465–474.

Zhang WL, Revers L, Pierce M, Schachter H (2000). Regulation of expression of the human beta-1,2-*N*-acetylglucosaminyltransferase II gene (MGAT2) by Ets transcription factors. Biochem J 47: 511–518.

Zhang X, Wang Y, Qian Y, Wu X, Zhang Z, Liu X, Zhao R, Zhou L, Ruan Y, Xu J et al. (2014). Discovery of specific metastasis-related *N*-glycan alterations in epithelial ovarian cancer based on quantitative glycomics. PLoS One 9: e87978.

Zhang Z, Sun J, Hao L, Liu C, Ma H, Jia L (2013). Modification of glycosylation mediates the invasive properties of murine hepatocarcinoma cell lines to lymph nodes. PLoS One 8: e65218.

Zhao Y, Sato Y, Isaji T, Fukuda T, Matsumoto A, Miyoshi E, Gu J, Taniguchi N (2008). Branched *N*-glycans regulate the biological functions of integrins and cadherins. FEBS J 275: 1939–1948.

Zhao Y, Chen S, Gou WF, Niu ZF, Zhao S, Xiao LJ, Takano Y, Zheng HC (2013). The role of EMMPRIN expression in ovarian epithelial carcinomas. Cell Cycle 12: 2899–2913.

Zhao YP, Xu XY, Fang M, Wang H, You Q, Yi CH, Ji J, Gu X, Zhou PT, Cheng C et al. (2014). Decreased core-fucosylation contributes to malignancy in gastric cancer. PLoS One 9: e94536.

Zheng HC, Takahashi H, Murai Y, Cui ZG, Nomoto K, Miwa S, Tsuneyama K, Takano Y (2006). Upregulated EMMPRIN/CD147 might contribute to growth and angiogenesis of gastric carcinoma: a good marker for local invasion and prognosis. Br J Cancer 95: 1371–1378.