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## Glyco-Forum section

# Maintenance of cell surface glycan density by lectin–glycan interactions: A homeostatic and innate immune regulatory mechanism

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It has been recently proposed that lectins such as galectins, C-type lectins and siglecs in innate immunity bind to foreign pathogens by density-dependent recognition of surface glycans (Dam and Brewer 2010). In many cases, foreign pathogens including viruses and bacteria possess glycan epitopes such as Man and LacNAc residues that are also found on host cells (Vasta 2009). Host lectins appear to bind to the pathogens due to their high density and number of glycan epitopes relative to those on host cells (Dam and Brewer 2010). Unique "weak" glycan epitopes on foreign pathogens appear to be strong epitopes when presented in high-density presentations such as polysaccharides and lipopolysaccharides (Dam and Brewer 2010). Thus, the concept of lectins as pattern recognition receptors in innate immunity (Medzhitov and Janeway 2000; Vasta 2009) has been replaced with lectins as density-dependent glycan binding receptors. However, in order for host lectins to bind to density-dependent expression of glycans on foreign pathogens, the glycan density of host cells must be established and controlled.

Evidence that metazoans do regulate their surface glycan density comes from a new study on the effects of mutations in the pathway for the formation of a specific branch chain in N-linked glycoproteins in mouse cells that results in apparent global compensation of glycan epitope number (density) in the remaining branch chains of the mutant's N-linked carbohydrates (Takamatsu et al. 2010). *N*-acetylglucosaminyltransferase-IV (GnT-IV) exists as two isoenzymes, a and b, that initiate the synthesis of the GlcNAcβ1-4 branch on the core Manα1-3 arm of *N*-glycans. One effect of the GlcNAcβ1-4 branch chain is to increase the glycan epitope density per N-linked carbohydrate chain. Takamatsu et al. (2010) engineered and characterized GnT-IVb-deficient mice and double-IVa/IVb-deficient mice. Wild-type mice have GnT-IVa expression restricted to gastrointestinal tissue, while GnT-IVb is broadly expressed among organs. GnT-IVb-deficient mice show aberrant GnT-IVa expression corresponding to the GnT-IVb distribution pattern, and hence, the GnT-IVb-deficient mice show mild phenotypic alterations in hematopoietic cells and hemostasis. Importantly, GnT-IVa/IVb double-deficient mice have completely abolished GnT-IV activity, and thus there is a complete disappearance of the GlcNAcβ1-4 branch on the Manα1-3 arm of the N-linked glycans that was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MS) and gas chromatography-MS analyses. The absence of the GlcNAcβ1-4 branch, however, was shown by MS analyses as additional glycan epitope extensions on the remaining branches in the tissues of the N-linked glycans of the GnT-IVa/IVb double-deficient mice. For example, the $Le^x$ epitope is found in greater number in the N-linked glycans from mouse kidney cells, and biosynthetic compensation is observed in bi- and triantennary *N*-glycans of the double mutant relative to the tetra-antennary *N*-glycans of the wild-type cells (Figure 1) (Takamatsu et al. 2010). Similar biosynthetic compensation was observed for other glycan epitopes such as polylactosamine in the pancreas. Analysis shows that these additional glycan epitopes in the GnT-IVa/IVb double-deficient mice are due to elevated expression of glycosyltransferases that are normally involved in their biosynthesis. Remarkably, the phenotype of the GnT-IVa/IVb double-deficient mice was similar to that of the GnT-IVa single-deficient mice, which is relatively mild compared to the wild-type mice.

The ability of the GnT-IVa/IVb double-deficient mice to effectively restore glycan epitope density in the remaining N-linked glycans of the different organs of the mice suggests that maintenance of this density is critical to the homeostatic function of the cells and organs of the mouse. The authors describe these findings as the first example of induced glycomic compensation of glycosyltranserase activities of cells from different organs of the mice (Takamatsu et al. 2010). They also suggest that maintenance of the overall expression of glycan ligands for endogenous lectins in the double-mutant mice appears to prevent cellular dysfunctions, but no mechanism was suggested for these effects.

We suggest that a possible mechanism for regulating cell surface glycan density in both the wild-type and GnT-IVa/

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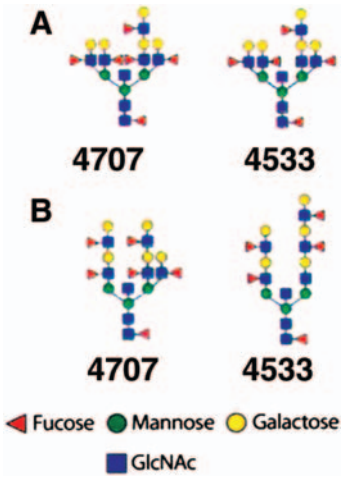


**Fig. 1.** Comparison of the structures of (**A**) two wild-type *N*-glycans with $[M + Na]^+$ molecular ions of 4707 and 4533 from mouse kidney cells and (**B**) two GnT-IVa/IVb double-mutant *N*-glycans with $[M + Na]^+$ molecular ions of 4707 and 4533 from mouse kidney cells (Takamatsu et al. 2010). The *N*-glycan with $[M + Na]^+$ molecular ion of 4707 from the wild-type mouse possesses four $Le^x$ epitopes, and the *N*-glycan with $[M + Na]^+$ molecular ion of 4533 possesses five $Le^x$ epitopes. The *N*-glycan with $[M + Na]^+$ molecular ion of 4707 from the GnT-IVa/IVb double mutant possesses four $Le^x$ epitopes, and the *N*-glycans with $[M + Na]^+$ molecular ion of 4533 possesses five $Le^x$ epitopes. Structures are based on composition, tandem mass spectrometry and the literature.

IVb double-deficient mice is binding and cross-linking by endogenous lectins of the global array of glycoproteins on the surface of the cells. The cross-linking activities of lectins correlate with their biological signaling effects including cell growth, arrest and death (apoptosis) (Perillo et al. 1998; Demetriou et al. 2001; Lau et al. 2007) as well as the regulation of glycoprotein transporter activities including the Glut-2 and Glut-4 glucose transporters (Ohtsubo et al. 2005; Lau et al. 2007). Importantly, transmembrane signaling that occurs from these interactions is known to affect glycosyltransferase gene activity (Lau et al. 2007), possibly at the mRNA level (Chen et al. 2009). Such a feedback mechanism between lectin binding and cross-linking of specific glycoprotein receptors, signaling, gene expression and cell surface glycan density for specific glycoprotein receptors including the T-cell receptor with galectin-3 has been recently demonstrated by Dennis and coworkers (Lau et al. 2007; Grigorian et al. 2009). The level of certain cell surface glycoprotein receptors in mouse tumor cells are regulated by the metabolic flux of *N*-glycan biosynthesis (Lau et al. 2007). Experiments indicate that the Golgi pathway is sensitive to hexosamine flux (UDP-GlcNAc) for production of tri- and tetra-antennary *N*-glycans on glycoprotein receptors, which bind to galectins and form cross-linked complexes (lattices) that oppose glycoprotein endocytosis on the cell surface (Lau et al. 2007). Glycoproteins with few *N*-glycans, such as transforming growth factor-β receptor II, cytotoxic T-lymphocyte-associated antigen-4 and glucose transporter-4 (Glut-4), exhibit enhanced cell surface expression with rapid responses to increasing hexosamine concentration, whereas glycoproteins with high numbers of *N*-glycans like epidermal growth factor receptor, insulin growth factor receptor, fibroblast growth factor receptor and platelet-derived growth factor receptor exhibit hyperbolic responses (Lau et al. 2007). The enhanced branching of *N*-glycans and LacNAc expression on specific cytokine receptors and transport proteins appear to regulate their surface retention by lattice formation with galectin-3, which shows increasing affinity for increased branching of N-linked carbohydrates (Hirabayashi et al. 2002). Hence, the density of LacNAc epitopes on the *N*-glycans of the cell surface glycoprotein receptors determines their binding and cross-linking activity with galectin-3, which regulates the lifetime and activity of the receptors. The intracellular signals of the cross-linked glycoprotein receptors, in turn, regulate gene expression of the glycosyltransferases responsible for the enhanced branching of the N-linked glycans. Thus, the model provides a feedback mechanism between metabolism (UDP-GlcNAc levels), N-linked glycan epitope density, lectin binding and cross-linking of multiple cell surface glycoprotein receptors, and gene expression of the glycosyltransferases involved in the biosynthesis of N-linked glycan epitopes. Compensation by gene expression independent of metabolic supply of UDP-GlcNAc is also evident from these studies. Notably, the up-regulation of Mgat4 and Mgat5 by oncogenic activation results in increased N-glycan branching activity in tumor cells (Lau and Dennis 2008).

A general model for the regulation of glycan density on the surface of cells can be made by combining the study of the maintenance of glycan density on the surface of cells by the GnT-IVa/IVb double-deficient mice (Takamatsu et al. 2010); binding of lectins to density-dependent glycan epitopes on the surface of cells (Dam and Brewer 2010); and the feedback mechanism between cell surface N-linked glycan density, lectin binding and cross-linking of cell surface glycoprotein receptors, gene regulation and expression of glycosyltransferase activity by the Dennis laboratory (Figure 2) (Lau et al. 2007). The general model suggests that glycan density on the surface of cells in the mouse is regulated by endogenous lectin binding to the global array of cell surface glycoprotein receptors, which, in turn, results in cross-linking and intracellular

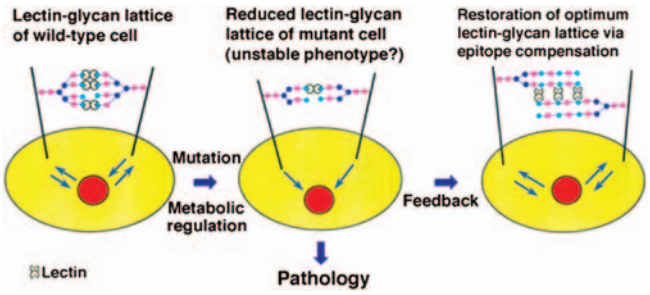


**Fig. 2.** Schematic of lectin–glycan lattice(s) of wild-type cells (left), reduced lectin–glycan lattice(s) of mutant cell (center) and restoration of optimum lectin–glycan lattice(s) via cellular epitope compensation (right). The model suggests that glycan density on the surface of cells is regulated by lectin binding to cell surface glycoprotein receptors, which, in turn, results in cross-linking and intracellular signaling of the receptors that are integrated at the level of gene regulation of glycosyltransferase activities. The model suggests that the *N*-glycans of GnT-IVa/IVb double-deficient mice are compensated by lectin–glycan cross-linking interactions that regulate the glycosyltransferase activities responsible for maintaining overall cell surface glycan density (i.e., restoration of epitope density in the elongated biantennary *N*-glycans in the upper right cell of the schematic).

signaling of the receptors that is integrated at the level of gene regulation of glycosyltransferase activities. The latter must be specific for different glycan epitopes of the different organs of the mouse such as the Lewis$^x$ epitope in mouse kidney cells (Takamatsu et al. 2010). Importantly, the level of glycosylation must allow for metabolic control of the degree of branching of N-linked glycans of specific glycoprotein receptors (Lau et al. 2007; Chen et al. 2009). This fine control of the degree of branching of N-linked glycans on specific glycoprotein receptors such as cytokine receptors and T-cell receptor in response to UDP-GlcNAc levels (Lau et al. 2007, 2008; Chen et al. 2009) suggests that there is a range of glycosylation of specific receptors on cells that is actively maintained for the regulation of cellular functions including metabolism.

Importantly, the level of glycan density on the surface of cells must be matched to the secreted lectin concentration in the extracellular environment (galectins) or density of membrane-bound lectins (C-type lectins, siglecs and selectins) for glycoprotein–lectin cross-linking to occur. Studies of multivalent carbohydrate and glycoprotein cross-linking interactions with lectins in solution typically show bell-shaped profiles for the insoluble complexes that form (Bhattacharyya and Brewer 1989; Mandal and Brewer 1992; Brewer 1996; Dam and Brewer 2003). Excess lectin or carbohydrate/glycoprotein concentrations lead to soluble complexes, while specific stoichiometric ratios of the molecules lead to cross-linking and precipitation of the complexes. These results suggest that both the density of cell surface glycans and concentration or density of host lectins are tightly controlled in order to facilitate lectin–glycan cross-linking and signaling in cells (Figure 2). Indeed, Dennis and coworkers (Lau et al. 2007) have reported that the level of extracellular galectin-3 is regulated in relation to the degree of branching and LacNAc epitope density in the N-linked glycans of cells.

The regulation of lectin levels and glycan density has not only important implications for homeostatic functions of host cells (Grigorian et al. 2009) but also the innate immune response of host lectins to foreign pathogens (Vasta 2009). Pathogens presenting similar glycans as the host but at lower cell surface densities would not initiate binding of host lectins, while pathogens presenting glycans at similar or greater density would bind to host lectins (Dam and Brewer 2010). The interactions of other innate immune receptors of the host including Toll-like receptors together with host lectins would determine the overall innate immune response of the host toward a pathogen (Iwasaki and Medzhitov 2010).

The emerging model also suggests that alteration of glycan density and/or lectin levels beyond the "normal" range of expression may lead to pathological effects (Figure 2) (Grigorian et al. 2009). In this regard, the expression level of glycosyltransferases such as β-1,6-*N*-acetylglucosaminyltransferase V (Mgat5) (Lau and Dennis 2008) and carbohydrate cancer antigens including the Le$^x$ antigen, Tn-antigen, T-antigen and sialyl T-antigen are increased in certain cancers (Byrd and Bresalier 2004). The level of host lectins including galectin-3 has also been observed to increase in specific cancers (Yu et al. 2007). Many carbohydrate cancer antigens including Le$^x$, Tn and T appear to be stage-specific embryonic antigens that are reduced in adult tissue (Glinksky 1992). This raises the possibility that many cancers are recapitulating the glycan density of embryonic cells and hence cell surface receptor density for programmed self-maintenance. Indeed, the presence of different glycan epitopes and densities such as LacNAc and Le$^x$ epitopes in the organs of mice (Takamatsu et al. 2010) indicate that specific lectin–glycan interactions must regulate receptor density and activity in different tissues of metazoans. The present model of metazoan cell surface glycan density and lectin levels raises many questions about how these interactions impact normal and pathological processes including autoimmunity, cancer and metabolic and many other chronic diseases (Grigorian et al. 2009).

The current model does not preclude other factors such as carbohydrate–carbohydrate interactions from playing a significant role in glycan density maintenance in cells. Indeed, Hakomori and others have provided substantial evidence that such interactions are important in cellular signaling and recognition mechanisms (Tromas et al. 2001; Hakomori 2004).

## Abbreviations

GnT-IV, *N*-acetylglucosaminyltransferase-IV; MS, mass spectrometry.

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