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The bisecting GlcNAc in cell growth control and tumor progression

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Abstract The bisecting GlcNAc is transferred to the core mannose residue of complex or hybrid N-glycans on glycoproteins by the β1,4-*N*-acetylglucosaminyltransferase III (GlcNAcT-III) or MGAT3. The addition of the bisecting GlcNAc confers unique lectin recognition properties to N-glycans. Thus, LEC10 gain-of-function Chinese hamster ovary (CHO) cells selected for the acquisition of ricin resistance, carry N-glycans with a bisecting GlcNAc, which enhances the binding of the erythroagglutinin E-PHA, but reduces the binding of ricin and galectins-1, -3 and -8. The altered interaction with galactose-binding lectins suggests that the bisecting GlcNAc affects N-glycan conformation. LEC10 mutants expressing polyoma middle T antigen (PyMT) exhibit reduced growth factor signaling. Furthermore, PyMT-induced mammary tumors lacking MGAT3, progress more rapidly than tumors with the bisecting GlcNAc on N-glycans of cell surface glycoproteins. In recent years, evidence for a new paradigm of cell growth control has emerged involving regulation of cell surface residency of growth factor and cytokine receptors via interactions and cross-linking of their branched N-glycans with a lattice of galectin(s). Specific cross-linking of glycoprotein receptors in the lattice regulates their endocytosis, leading to effects on growth factor-induced signaling. This review will describe evidence that the bisecting GlcNAc of N-glycans regulates cellular signaling and tumor progression, apparently through modulating N-glycan/galectin interactions.

Keywords Glycosylation · Bisecting GlcNAc · Complex N-glycans · Galectins · Mgat3

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Introduction

Glycosylation is the most prevalent post-translational modification of membrane-bound and secreted glycoproteins that traverse the conventional secretory pathway. Most protein glycosylation is either Asn-linked or initiated by O-linked GalNAc added to Ser or Thr. The consensus site for N-glycans has recently been expanded to Phe–Yyy–Asn–Xxx–Thr in a Type I β-bulge and Phe–Yyy–Zzz–Asn–Xxx–Thr in a reverse turn (where Yyy can likely be any amino acid and Xxx is any amino acid but Pro) [1, 2]. The ability to predict O-GalNAc addition to Ser or Thr is improving based on *in silico* predictions [3, 4] and experimental determinations [5]. While all N-glycans have a common core consisting of $Man_3GlcNAc_2Asn$, there is currently no way of predicting the structures of the final complement of N-glycans on the many glycoforms of a glycoprotein. However, N-glycan structures may be

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determined by enzymatic release of N-glycans followed by glycomics analyses using mass spectrometry (MS) including matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS, gas chromatography (GC)/MS and tandem MS techniques [6]. Glycoproteomics is used to identify glycans at specific sites in a glycoprotein [7].

The bisecting GlcNAc is a unique modification of hybrid or complex N-glycans whose addition is catalyzed by β1,4-*N*-acetylglucosaminyltransferase III (GlcNAcT-III) or MGAT3 (E.C. 2.4.1.144) (Fig. 1a), an activity originally identified in hen oviduct [8]. *In vitro* glycosyltransferase assays indicated that the presence of a bisecting GlcNAc on a biantennary N-glycan terminating in GlcNAc prevents the subsequent action of N-glycan branching glycosyltransferases including GlcNAcT-II, GlcNAcT-IV, and GlcNAcT-V and the core fucosyltransferase FUT8 [9, 10]. Interestingly, however, glycomics profiling of the N-glycans from glycoproteins of LEC10 CHO cells that express the *Mgat3* gene [11], revealed that many bisected N-glycans carry a core Fuc, and that N-glycans with up to 17 LacNAc units have a bisecting GlcNAc [12]. These LacNAc units must be extensions of branched N-glycans because LEC10 glycoproteins bind the lectins E-PHA and L-PHA much better than glycoproteins from parent CHO cells that do not express *Mgat3* [13, 14]. Therefore, the inhibition of branching GlcNAc-transferases and FUT8 by the bisecting GlcNAc observed *in vitro*, does not occur in a CHO cell Golgi environment. Nevertheless, the bisecting GlcNAc profoundly affects the interaction of LEC10 cells with galactose-binding plant lectins including ricin [15, 16]. Thus, LEC10 CHO cells are highly resistant to ricin compared to parent CHO cells (Fig. 1b). By contrast, they are hypersensitive to the cytotoxicity of E-PHA and L-PHA, and bind more of these lectins than parent CHO cells. These data suggest that the bisecting GlcNAc has a major impact on the conformation of Gal residues in a bisected N-glycan. Models of N-glycans with and without a bisecting GlcNAc are consistent with this proposal [17, 18]. Thus, it is clear that the presence of the bisecting GlcNAc on the N-glycans of cell surface glycoproteins may modulate their interactions with galectins, siglecs or other glycan binding proteins. These effects may, however, vary with cell type because overexpression of the *Mgat3* gene has been shown to reduce N-glycan branching or core fucosylation in some cell types (reviewed in [19, 20]).

The *Mgat3* gene has a unique tissue specific expression pattern with particularly high levels of transcripts in mouse brain and kidney, and a moderate level in intestine, based on Northern blot analyses [21, 22]. Kidney extracts are abundant in E-PHA-binding glycoproteins, consistent with the presence of the bisecting GlcNAc on N-glycans [14, 21]. Physiological functions of the bisecting GlcNAc have been proposed for the maintenance of kidney homeostasis [23]. However, mice with targeted inactivation of the *Mgat3* gene, are viable and fertile with no gross anatomical or significant physiological abnormalities [22, 24] (http://www.functionalglycomics.org), suggesting that modification of N-glycans by the bisecting GlcNAc is dispensable for normal growth and development. Nevertheless, stress may reveal requirements that reflect predictions for functions of the bisecting GlcNAc. Interestingly, truncated, inactive MGAT3 produced by disruption of the *Mgat3* gene by a neomycin cassette, causes a mild neurological phenotype in mice [13], suggesting that certain MGAT3

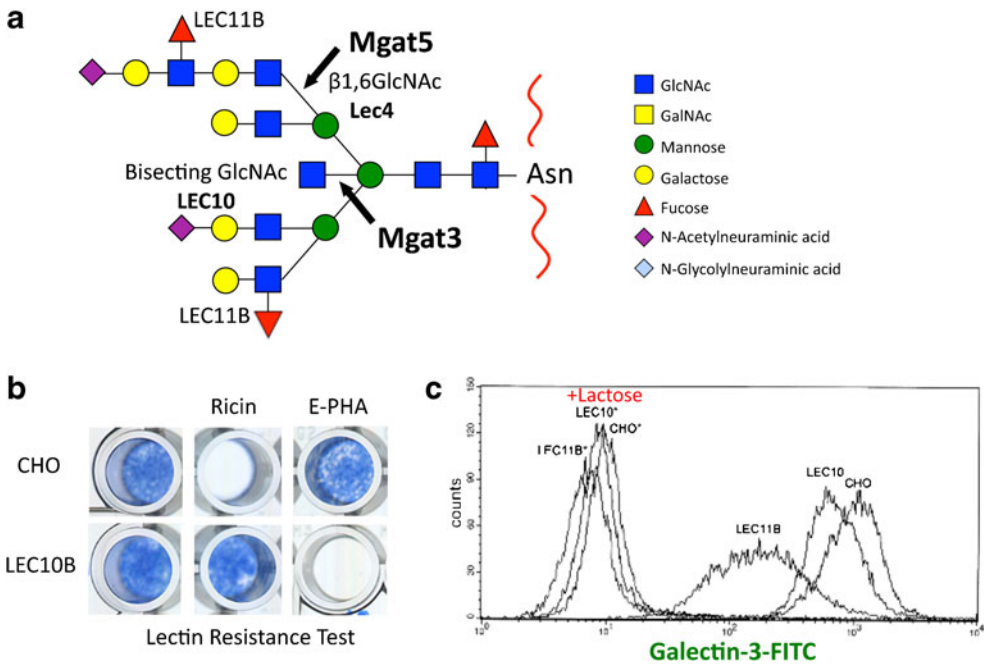


Fig. 1 The bisecting GlcNAc and lectin binding. a A proposed complex N-glycan containing the bisecting GlcNAc added by MGAT3 expressed in LEC10 cells, and the β1,6GlcNAc branch initiated by MGAT5 and absent from Lec4 mutant cells. b Lectin resistance test of CHO wild type and LEC10B cells expressing MGAT3 using the lectins ricin and E-PHA (adapted from [14]). c Flow cytometry of FITC-labeled galectin-3 binding to CHO, LEC10 or LEC11 cells in the presence or absence of lactose (courtesy of Santosh Patnaik [36]). LEC11 cells express *Fut6* and add Fuc to LacNAc to generate the LeX and SLeX epitopes (Fig. 1a [76])

mutations in humans may have neurological or psychological effects.

In the past two decades, numerous studies have been directed towards understanding functions of the bisecting GlcNAc in modulating cell-cell and cell-matrix interactions, as well as cell growth control (reviewed in [19, 20, 25]). In this review, we focus on roles of the bisecting GlcNAc in galectin binding, growth factor signal transduction and tumor progression.

The bisecting GlcNAc and galectins

While the altered binding of bisected N-glycans to plant lectins is useful, an important question is whether the bisecting GlcNAc affects interactions of cell surface glycoproteins and endogenous animal lectins. Galectins belong to a large family of animal lectins that binds to β-galactosides. At least 15 galectins have been identified, although not all are found in every species [26, 27]. Galectins are expressed in the cytoplasm and nucleus and have been shown to play roles in intracellular regulation of pre-mRNA splicing [28]. However, many of their physiological functions in cell proliferation, survival, adhesion, migration and apoptosis have been attributed to their actions outside of the cell via glycan binding to cell surface glycoconjugates on cells, viruses or bacteria [29]. Lacking a signaling peptide, galectins are secreted via unconventional mechanism(s), which are poorly understood. However, some evidence suggests that both the glycan binding activity of a galectin and binding to ligand on the cell surface are required for efficient secretion [30, 31].

Galectins are categorized into three subtypes (proto, chimera, and tandem repeat) based on their sequence. All galectins contain at least one carbohydrate recognition domain (CRD) of ~130 amino acids, which interacts with glycans. Prototype galectins contain one CRD, and are normally present as a divalent homodimer. They include galectin-1, -2, -5, -7, -10, -11, -13, -14, and -15. Galectin-3 is the only chimera-type, with a CRD at the C-terminus and a long flexible N-terminus, which mediates oligomerization to form a pentameric structure that cross-links bound ligands [32]. Tandem-repeat type galectins include galectin-4, -6, -8, -9, -12. They are divalent with two distinct CRDs - one at the N-terminus and the other at the C-terminus connected by a linker peptide. Alternative splicing allows this linker region to be variable in length and may influence cross-linking potency [7, 32–34]. Furthermore, the presence of two CRDs with different glycan specificities may allow cross-linking of structurally distinct subsets of glycans [35, 36].

Complex N-glycans often carry repeating units of *N*-acetyl-lactosamine (LacNAc) Galβ1-4GlcNAc that bind several members of the galectin family [37]. The importance of LacNAc repeats for galectin binding to cell surfaces was shown using a series of CHO glycosylation mutants [36, 38]. Lec1 CHO mutants that lack hybrid and complex N-glycans on cell surface glycoproteins do not bind galectin-1, -3 or -8. Lec8 CHO mutants, that have no LacNAc units on N-glycans but carry terminal GalNAc on Ser/Thr residues, also bind very low amounts of these three galectins. Therefore, complex N-glycans are the major ligands for galectin-1, -3 and -8 on CHO cells. The effect of the bisecting GlcNAc on galectin binding was tested in LEC10 CHO cells in which most complex N-glycans carry the bisecting GlcNAc [12]. Binding of galectin-1, -3 and -8 was reduced to LEC10 compared to parent CHO cells [39]. The inhibitory effect of the bisecting GlcNAc for galectin binding was observed using fluorescinated galectins and flow cytometry (Fig. 1c; galectin-3) or using an array-based assay with glycans attached to a solid surface (Fig. 2; galectin-1). The effects of the bisecting GlcNAc on galectin binding have also been tested on chemoenzymatically synthesized bi-antennary N-glycans attached to BSA [18, 40]. Interestingly, the bisecting GlcNAc had no effect on the binding of galectin-4 to BSA-N-glycans, while the binding of galectin-1 was slightly enhanced and was further potentiated by the presence of core α1-6-linked fucose with the bisecting GlcNAc [18, 40]. By contrast, the binding of galectin-3 was reduced by the bisecting GlcNAc, consistent with its reduced binding to LEC10 CHO cells [39] (Fig. 1c). In another study, the promotion of laminin 332-dependent cell migration by galectin-3 in keratinocytes was inhibited when laminin 332 was modified with a bisected N-glycan [41], further supporting a negative effect of the bisecting GlcNAc on galectin-3 binding. It is thus clear, that the bisecting GlcNAc modulates galectin binding to complex N-glycans, and that it may therefore have functional consequences. It is also clear that care must be taken in extrapolating *in vitro* binding specificities to artificial substrates to specificities predicted for cell surface glycoproteins that may cluster and are mobile in the membrane [33].

Complex N-glycans in signal transduction and growth control

Dennis and colleagues first reported that β1-6GlcNAc-branched complex N-glycans play important roles in growth control [42]. β1-6GlcNAc branching is initiated by the addition of GlcNAc to complex N-glycans by the enzyme GlcNAcT-V, encoded by the *Mgat5* gene (Fig. 1a). When *Mgat5* was ablated in transgenic mice overexpressing the polyoma middle T (PyMT) oncogene under the control of the mouse mammary tumor virus (MMTV) promoter, mammary tumor development and progression to lung metastasis were markedly reduced [42]. While the increase in β1-6GlcNAc-branched N-glycans had previously been

noted in tumor formation [43], the report in 2000 was the first to present direct evidence suggesting that reducing the degree of N-glycan branching retards tumor progression. Importantly, a mechanism was subsequently proposed whereby branched N-glycans promote the interaction of growth factor/cytokine receptors (*e.g.*, epidermal growth factor receptor (EGFR) and transforming growth factor-β (TGF-β) receptor) with galectin-3, thereby enhancing their cell surface residency time by preventing their loss due to constitutive endocytosis [44]. This in turn will increase the number of growth factor receptors on the cell surface that are available to respond to their respective ligands, leading to increased and prolonged ligand-induced signaling. This mechanism is supported by modeling studies [45] and investigations of autoimmunity modulated by surface residency of cytotoxic T-lymphocyte antigen 4 (CTLA4) [46, 47]. The retention of cell surface proteins by a galectin lattice has also been implicated in glucose homeostasis. For example, galectin-9 stabilizes glucose transporter 2 (GLUT2) on the cell surface increasing its half-life life in pancreatic β cells [48]. Reduced N-glycan branching due to the loss of MGAT4a or GLUT2 results in the development of diabetes [49]. Furthermore, the integrity of corneal epithelium that relies on cell surface signaling by vascular endothelial growth factor receptor 2 (VEGFR2) is dependent on interactions of branched N-glycans on VEGFR2 with galectin-3 [50]. In each of these cases, modification of a cell surface glycoprotein by highly branched N-glycans is essential to assure its optimal interactions with galectin(s). It should be noted, however, that roles for galectins in growth factor signaling may vary with cell context. Thus, the EGFR in human cancer cell lines with reduced MGAT5 activity and reduced N-glycan branching, does not exhibit enhanced ligand-induced endocytosis, but nevertheless shows impaired signal transduction that occurs primarily from endosomes in these cells [51]. The effect of branched N-glycans on growth factor signaling in this case appears to be galectin independent.

Galectin interactions with branched N-glycans may also function in regulating integrin-mediated cell motility. For example, galectin-3 stimulates $\alpha_5\beta_1$ integrin-mediated activation of focal adhesion kinase (FAK) and phosphoinositide-3-kinase (PI3K) through its interactions with branched N-glycans of glycoproteins. This leads to increased fibronectin fibrillogenesis and fibronectin-dependent tumor cell spreading and motility, which may explain metastasis in MGAT5-expressing tumor cells [52]. $\alpha_3\beta_1$ integrin is also activated by the interaction of galectin-3 with complex N-glycans to promote lamellipodia formation in corneal epithelial cells [53]. In general, cells with integrins carrying bisected branched N-glycans exhibit reduced migratory activity (reviewed in [19, 25, 54, 55]). In these cells, the addition of a bisecting GlcNAc concomitantly reduces β1-6GlcNAc branching, as indicated by reduced L-PHA binding to integrin subunits. This presumably reflects the fact that MGAT5 and other N-glycan branching glycosyltransferases may not utilize bisected N-glycans as acceptor substrates in certain cells [9]. Together, these studies suggest that the bisecting GlcNAc of complex or hybrid N-glycans may regulate biological functions of glycoproteins by altering N-glycan conformation, branching or composition, leading to reduced galectin binding.

The bisecting GlcNAc in growth factor signaling

Overexpression of *Mgat3* in various cell lines has yielded valuable information on potential functions of MGAT3 in cell growth control and growth factor signaling. Bisected N-glycans are found on EGFR, which is often deregulated in cancer and plays key roles in the control of cell proliferation. In human U373 MG glioma cells, transfection of an *Mgat3* cDNA causes reduced EGF binding and decreased EGFR autophosphorylation, but stimulates cell proliferation [56]. Overexpression of *Mgat3* in Hela S3 cells increases signaling as shown by increased phosphorylation of extracellular signal-regulated kinase (ERK), and this correlates with reduced EGF binding, but increased EGFR endocytosis [57]. However, there is no change in EGFR dimerization or autophosphorylation in *Mgat3*-transfected Hela S3 cells. In PC2 neuronal cells, when *Mgat3* is overexpressed, there is again a significant decrease in EGF binding and EGFR autophosphorylation, but this is accompanied by a decrease in ERK activation required for EGFR- and integrin-mediated neurite outgrowth [58]. Together, these results suggest that, while the bisecting GlcNAc of complex N-glycans affects EGFR-mediated signaling, the consequences vary with cell type, indicating that different mechanisms may underlie the effects of the bisecting GlcNAc on EGFR and other growth factor receptor signaling in different cellular environments.

One caveat of experiments with transfected *Mgat3* is that the overexpression of *Mgat3* under a strong promoter may cause non-physiological effects. Therefore, LEC10 CHO mutants, that express *Mgat3* from the endogenous CHO gene [11], provide a platform to study more physiological effects of the bisecting GlcNAc. Functions of the bisecting GlcNAc in cell growth control and growth factor signaling were compared between LEC10 CHO cells, wild type CHO cells that lack MGAT3 and bisected N-glycans, Lec4 CHO mutant cells lacking MGAT5 and β1-6GlcNAc-branched, as well as, bisected N-glycans, and Lec8 CHO mutant cells lacking LacNAc extensions of all N-glycan branches [39]. These cells were shown to bind galectins in the following order: CHO>LEC10>Lec4>Lec8. While these lines proliferate at a similar rate in medium containing 10% serum, each mutant grew slower than wild type in 7.5% serum [14]. To mimic conditions in PyMT-induced tumors, the CHO

cells were transfected with PyMT. The growth rate of LEC10B/PyMT remained slower that that of CHO/PyMT cells, but slightly faster than Lec4/PyMT and Lec8/PyMT. To investigate growth factor signaling, the PyMT-expressing CHO lines were stimulated by platele-derived growth factor AB (PDGF-AB), since CHO cells express PDGFR but not EGFR. Responsiveness to PDGF-AB was significantly reduced in LEC10B/PyMT and Lec4/PyMT cells based on reduced ERK1/2 phosphorylation. For Lec8/PyMT cells, that do not bind galectins, there was no detectable response to PDGF-AB. When growth factor responsiveness was compared after treating the cells with lactose to remove surface-bound galectins, or sucrose as control, cells treated with lactose showed markedly reduced ERK1/2 activation, while sucrose treatment had no effect. Together, these results provide evidence for essential roles of growth factor receptor/galectin-lattice interactions in growth factor signaling in the CHO/PyMT lines. Therefore, it appears that the bisecting GlcNAc on N-glycans reduces growth factor signaling in a galectin-dependent manner (Fig. 3).

The bisecting GlcNAc in tumor progression

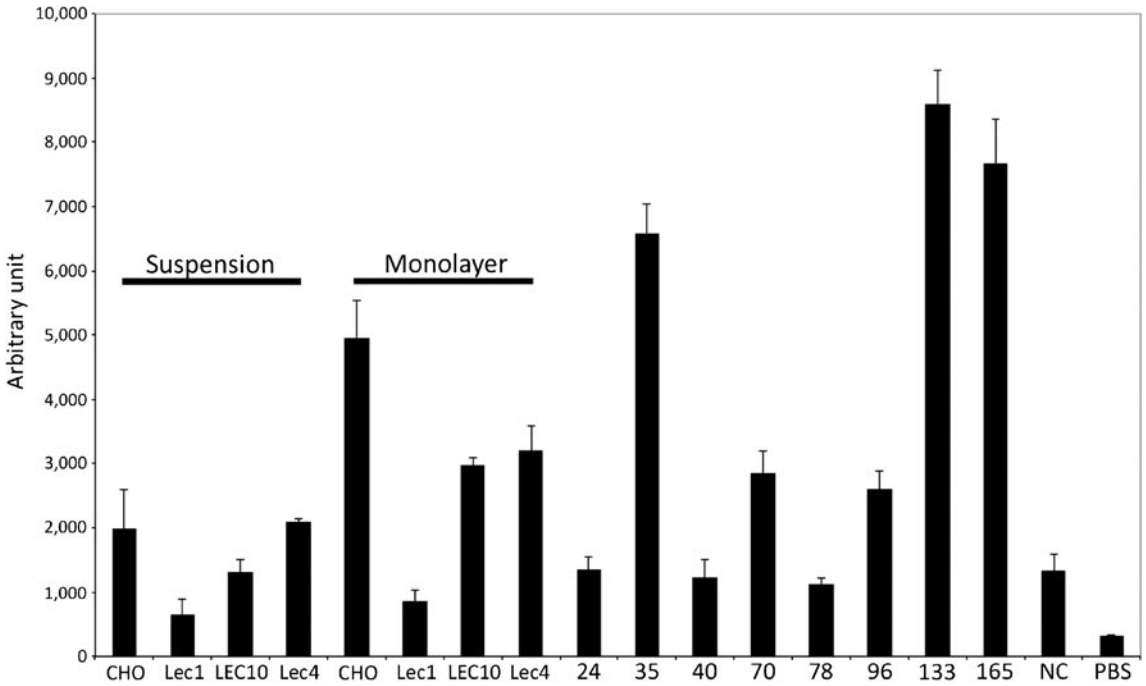
Evidence from cell-based assays and *in vivo* studies has shown that the bisecting GlcNAc may affect tumor progression and metastasis. In initial studies using tumor cell lines, overexpression of *Mgat3* in a highly metastatic subclone of B16 melanoma cells resulted in significant suppression of lung colonization, which correlated with a decrease in β1-6GlcNAc branching [59]. By contrast, tumor growth and metastasis to spleen was increased in the same cell model, mediated in part by CD44-hyaluronan interactions enhanced by the bisecting GlcNAc on CD44 [60]. Furthermore, a K562 leukemia cell line overexpressing *Mgat3* was resistant to natural killer cell cytotoxicity and showed increased spleen colonization [61]. Studies using transgenic mouse models expressing *Mgat3* in liver also gave variable results.

In rat liver, *Mgat3* expression is upregulated during chemically-induced hepatocarcinogenesis [62, 63], prompting an investigation into the function of *Mgat3* in tumorigenesis induced by diethylnitrosamine (DEN). In mice overexpressing *Mgat3* in liver under the control of the serum amyloid P component gene promoter, DEN-induced tumor incidence was significantly reduced [64]. On the other hand, no significant change in tumor incidence was observed in mice overexpressing *Mgat3* under the control of the major urinary protein (MUP) promoter following treatment with DEN and phenobarbitol (PB)[65]. Additionally, tumor progression was retarded in mice with a targeted *Mgat3* mutation after DEN alone or DEN and PB treatments [65, 66]. This appeared to be due to a non cell-autonomous mechanism since overexpression of *Mgat3* in hepatocytes did not restore tumor progression to the levels obtained in wild type mice [65].

A direct role for the promotion of tumor progression by complex N-glycans has been established in mammary tumors induced by PyMT expressed from the MMTV/PyMT transgene [67]. In *Mgat5* null mice that lack the β1-6GlcNAc branch of complex N-glycans (Fig. 1a), mammary tumor progression is greatly inhibited due to reduced growth factor signaling that can be restored by introduction of an *Mgat5* cDNA into *Mgat5* null tumor epithelial cells [44]. The consequence of MGAT5 deficiency in HER2-induced mammary tumorigenesis has also been investigated. As observed in the MMTV/PyMT transgenic model, mammary tumor cells lacking *Mgat5* have reduced ERK1/2 and Akt/protein kinase B (PKB) activation [68]. Furthermore, in this case MGAT5 was implicated in tumor initiation and tumor onset. In humans, increased expression of complex N-glycans with β1-6GlcNAc branching is observed in breast and colorectal carcinomas, and the degree of expression correlates with the stage of progression of the cancer [69].

Because LEC10 CHO cells with the bisecting GlcNAc to N-glycans exhibit a reduced growth rate that persists when they overexpress the PyMT oncogene [14] and this correlates with reduced galectin-lattice dependent growth factor signaling, it was hypothesized that PyMT-induced mammary tumor progression might be enhanced in mice null for the *Mgat3* gene. *Mgat3* is not expressed in virgin mammary glands, however, its expression is upregulated during lactation and this is reflected by enhanced E-PHA binding to glycoproteins from mammary gland tissue lysates.

To investigate the effects of MGAT3 and the bisecting GlcNAc in tumor progression, MMTV/PyMT transgenic mice lacking MGAT3 were analyzed [14]. *Mgat3* gene expression is observed in MMTV/PyMT mammary glands by around 4–5 weeks. Tumor onset and tumor sizes at 17 weeks are significantly enhanced in *Mgat3* null mice. The first palpable tumor appears ~7 days earlier in mice lacking MGAT3. In addition, lung metastasis evaluated by the presence of PyMT transcripts in the lung, is significantly higher in *Mgat3* null mice in the early stages of tumorigenesis, suggesting accelerated lung metastasis in the absence of MGAT3. Moreover, tumor cells isolated from *Mgat3* null mice are more responsive to EGF- and PDGF-AB-induced growth factor signaling. Finally, an *in vivo* cell migration assay demonstrated that mammary tumor cells lacking MGAT3 have a higher migratory activity, with or without stimulation by EGF. Rescue of the *Mgat3* null phenotype was evaluated by overexpressing *Mgat3* in mammary gland under the control of MMTV promoter. Early-stage tumor development was delayed in the *Mgat3* transgenic mice and they also exhibited reduced cell migratory activity in the *in*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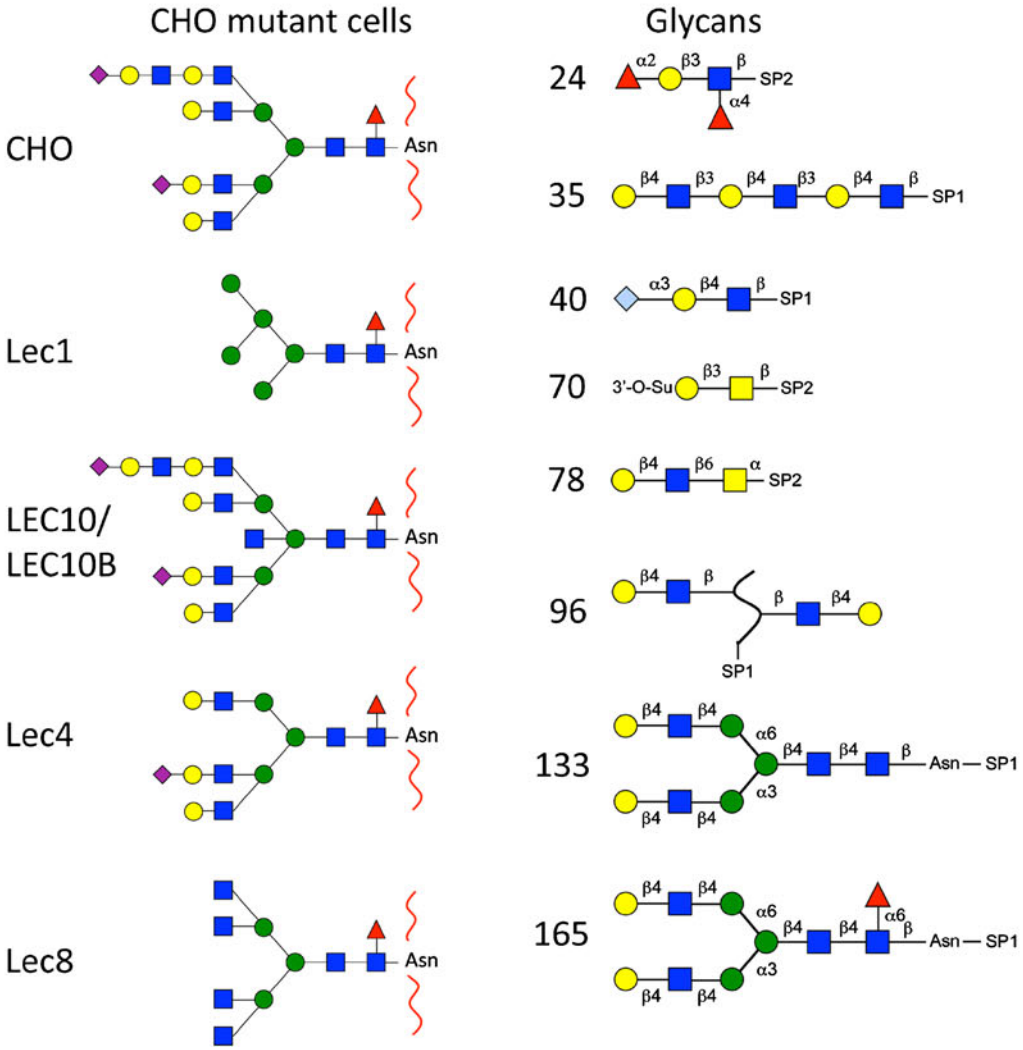
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◀ **Fig. 2** Galectin-1 binding to CHO and glycosylation mutants. CHO cell lines (CHO, Lec1, LEC10, and Lec4) grown in monolayer or suspension culture were washed, biotinylated, harvested and fixed. The biotinylated cells (50,000 cells/well) were arrayed on neutravidin-coated black ELISA plates. A subset of biotinylated glycans from the Consortium for Functional Glycomics (CFG) Glycan Array version 2.3, including known binders and non-binders to galectin-1, were also arrayed on the same plate at a concentration of 60 pmol/well. Alexa-488 labeled human galectin-1 (30 μg/ml) was applied to each well in binding buffer (20 mm Tris–HCl, pH 7.4, 150 mm NaCl, 2 mm $CaCl_2$, 2 mm $MgCl_2$, and 0.05% Tween 20 with 1% bovine serum albumin (BSA)) and incubated for 1 h at room temperature. After galectin-1 removal, plates were washed three times with binding buffer lacking BSA and relative fluorescence units measured. Structures of complex N-glycans typical of CHO mutant cells and a subset of glycans on the array are shown. CHO cells contain β1,6GlcNAc branched complex N-glycans that lack the bisecting GlcNAc; Lec1 cells have no complex N-glycans; LEC10/LEC10B cells contain complex N-glycans modified with the bisecting GlcNAc; Lec4 cells lack both β1,6GlcNAc branched N-glycans and the bisecting GlcNAc. A typical Lec8 mutant complex N-glycan is shown as it is included in the model in Fig. 3. See Fig. 1a for glycan symbols. NC, no cells or compound; PBS, phosphate buffered saline (no galectin-1)

vivo cell migration assay. Thus, MGAT3 and the presence of the bisecting GlcNAc on mammary glycoproteins, reduces mammary tumor progression in a cell-autonomous manner. Based on the fact that the bisecting GlcNAc reduces galectin binding (Figs. 1 and 2), we propose that MGAT3 and the bisecting GlcNAc reduce galectin-lattice dependent growth factor signaling leading to retarded mammary tumor progression as depicted in the model in Fig. 3.

The galectin lattice

While galectins have been widely implicated as regulators of cell signaling [70], it remains unclear how galectin specificity is determined. The simple CHO cell synthesizes N-glycans with up to 26 LacNAc units [12], and all 15 galectins bind to LacNAc. The loss of one N-glycan branch and its associated LacNAc units, or the addition of the bisecting GlcNAc, reduces the binding of galectins, as discussed above. But how does this affect the nature of the predicted galectin lattice, which may include multiple galectins, depending on cell type? Thus, while the interaction of galectin-3 with growth factor receptors is proposed as part of the mechanism by which MGAT5 regulates growth factor signaling [44], genetic ablation of galectin-3 has no effect on mammary tumor progression [71]. If the enhancement of tumor growth and metastasis by MGAT5 were mediated solely by galectin-3, one would expect galectin-3 null mice to exhibit reduced tumor progression. It seems likely that one or more other galectins play a role in galectin-dependent cellular signaling, compensating for the absence of galectin-3. We have observed that eight galectin genes (galectin-1, -2, -3, -4, (−6), -7, -8, -9, and, -12) are expressed in mouse mammary tumor tissue (unpublished), and thus any combination, or all eight, could be involved in forming galectin lattices on the cell surface. *In vitro* titration experiments using concanavalin A have shown that structurally distinct

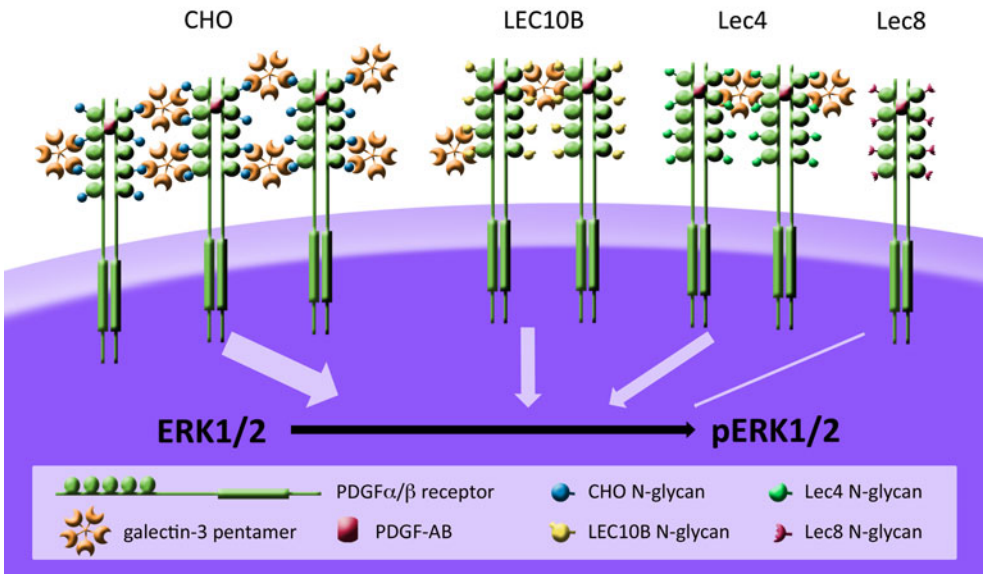

Fig. 3 Model of galectin-dependent PDGFR signaling in CHO cells. Higher order clustering of PDGFRs on the CHO cell surface may be achieved through galectin-N-glycan interactions, which are thought to form a lattice that restrains endocytosis and promotes optimal ERK1/2 activation. Complex N-glycans on wild type CHO cells have the most binding sites for galectins and the greatest response to PDGF-AB. ERK1/2 activation is reduced in LEC10B cells that add the bisecting GlcNAc to complex N-glycans, and in Lec4 cells that lack a branch of complex N-glycans, and occurs at background levels in Lec8 cells that have few if any LacNAc units on complex N-glycans. Signaling strength correlates with the degree of interaction with the galectin lattice. Binding of N-glycans to galectin-3 pentamers is shown as an example, although other galectins are likely to participate in CHO galectin lattice(s). See Fig. 2 for complex N-glycan structures typical of CHO mutant cells

and separable lattices are formed by Con A and $Man_5GlcNAc_2$ *versus* Con A and $Man_6GlcNAc_2$ [72]. Therefore, a range of intricate cross-linked lattices of different structure may potentially be formed by galectins and N-glycans, depending on fine glycan binding specificity, concentration and many other factors. Imaging such galectin lattices and determining the nature and specificity of their cross-linked structures is a key challenge for the future. Only then will it be possible to understand how the complement of galectins at the cell surface may function, alone or in concert, to control growth factor signaling, and how this control is altered by the presence of the bisecting GlcNAc on complex N-glycans.

Conclusions

In recent years, it has become apparent that complex N-glycans play pivotal roles in growth factor signaling and tumor progression. At the cell surface, the LacNAc units of N-glycans are cross-linked by galectins. We and others have shown that the bisecting GlcNAc on complex N-glycans modulates galectin interactions and thereby presumably affects galectin-lattice structure, the turnover of growth factor receptors and downstream signaling. Interestingly, the human *MGAT3* gene is located on chromosome 22q13.2, in a region for which loss of heterozygosity has been associated with breast and colorectal cancer [73–75]. Thus, understanding the mechanisms by which MGAT3 and the bisecting GlcNAc alter growth factor signaling, tumor growth and metastasis may lead to prognostic or diagnostic assays for human cancers.

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