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Galectin-binding O-glycosylations as Regulators of Malignancy*

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Abstract

Cancer cells commonly display aberrant surface glycans and related glycoconjugate scaffolds. Compared with their normal counterparts, cancer cell glycans are variably produced and often structurally distinct, serving as biomarkers of cancer progression or as functional entities to malignancy. The glycan signature of a cancer cell is created by the collaborative activities of glycosyltransferases, glycosidases, nucleotide-sugar transporters, sulfotransferases and glycan-bearing protein/lipid scaffolds. In a coordinated fashion, these factors regulate the synthesis of cancer cell glycans and thus are considered correlates of cancer cell behavior. Functionally, cancer cell glycans can serve as binding targets for endogenous lectin effectors, such as C-type selectins and S-type galectins. There has been a recent surge of important observations on the role of glycosyltransferases, specifically α 2,6 sialyltransferases, in regulating the length and lectin-binding features of serine/threonine (O)-glycans found on cancer cells. The capping activity of O-glycan-specific α 2,6 sialyltransferases, in particular, has been found to regulate cancer growth and metastasis in a galectin-dependent manner. These findings highlight the functional importance of cancer cell O-glycans and related galectin-binding features in the virulent activity of cancer and raise the prospect of targeting cancer cell glycans as effective anti-cancer therapeutics.

Keywords

α 2; 6 sialyltransferase; O-glycosylations; galectin-1; galectin-3; metastasis

A. CANCER CELL GLYCANS, LECTIN-BINDING AND MALIGNANCY

The glycobiology of malignant transformation and pathways relating to cancer progression is now considered a defining feature of cancer. There has been an increasing trend towards understanding cancer glycomics – the impact of glycans, glycoconjugates and glycosyltransferases and glycosidases on cancer progression. The role of these glycomic factors on cancer is appreciated through both intrinsic cancer glycomic signatures as well as microenvironmental glycome features. Cancer cell glycans, in particular, represent glycomic features by their relative uniqueness or level of expression compared with the normal fully-

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differentiated cell counterparts. These glycan features often serve as biomarkers of cancer stage or metastatic potential and/or confer a particular lectin-binding activity related to a distinct malignant behavior. Cancer cell glycans are frequently associated with metastatic mechanisms, such as intravascular trafficking, seeding and growth of cancer cells in a non-orthotopic tissue.

There is strong data showing that certain cancer cell membrane proteins bear carbohydrate sialyl Lewis^X and ^A antigens that serve as ligands for C-type lectins, endothelial (E)-, platelet (P)- or leukocyte (L)-selectin (1). Selectin – selectin ligand interactions are critical for initiating intravascular adhesion of circulating cancer cells and increasing metastatic potential (1, 2). To this end, there is supportive evidence showing the importance of glycosyltransferases, α 1,3 fucosyltransferases, α 2,3 sialyltransferases and β 1,6 N-acetylglucosaminyltransferases that are necessary for synthesis of sialyl Lewis antigens and related malignant activity (3).

While the control of cancer cell selectin ligand expression continues to be studied, investigating other cancer cell glycans and related membrane glycoprotein scaffolds capable of binding a family of S-type lectins, known as galectins, has intensified (4). Of the 15 mammalian galectins, galectin (Gal)-1, -2, -3, -4, -7, -8, -9, -10, -12 and -13 have been identified in humans. Galectins are widely-distributed in a variety of cells and tissues though often differentially-expressed in cancer cells. That is, compared with their normal tissue counterpart, they are either overexpressed or downregulated depending on the galectin and the cancer type. By secretion via non-classical transport pathway, galectins are deposited on cell surface glycoprotein ligands or on extracellular matrices (ECM). They bind β -galactoside-containing glycan moieties characteristically found in asparagine (N)- and serine/threonine (O)-linked glycans on membrane proteins, in ECM glycoconjugates and in membrane glycolipids. Glycan-binding specificity for each galectin is governed by sulfation, sialylation, fucosylation, repeating N-acetylglucosamine units and β 1,6 GlcNAc branching on the glycan core of a select protein/lipid scaffolds. In nature, an authentic galectin ligand is a galectin-binding carbohydrate moiety displayed by a discrete membrane protein/lipid or ECM component. Examples of galectin ligands are CD4, CD7, CD43, CD29, CD45, 90k/ MAC-2BP, CD146, carcinoembryonic antigen (CEA), lysosomal-associated membrane proteins-1 and -2 (LAMP-1/2), MUC-1, laminin, α 3 β 1, vascular endothelial growth factor receptor-2, T-cell immunoglobulin mucin-3 and glycolipid GM1 (5–14).

Several galectins and their ligands have been found to play a critical role in cancer progression. Galectin – galectin ligand engagement has been shown to induce tumor angiogenesis, immunoregulation, homotypic aggregation and/or heterotypic adhesion (4, 10, 15, 16). Gal-1 and Gal-3, in particular, have historically received much attention as regulators of cancer cell behavior (3, 4, 15, 17). Interestingly, when binding ligands on immune cells, Gal-1 and Gal-3 often have opposing effects. Whereas Gal-1 induces pro-apoptotic activity in T cells, Gal-3 acts an anti-apoptotic factor (4, 10, 18–20). Considering observations on Gal-1-binding to ligands on cancer cells causing pro-apoptotic activity (21, 22), most studies demonstrate that both Gal-1 and -3 elicit pro-tumorigenic activity upon binding cancer cell ligands. Some major Gal-1 and -3 ligands on cancer cells are represented by membrane proteins, LAMP-1/2, 90k/MAC-2BP, CEA and melanoma cell adhesion

molecule (MCAM) (5, 6, 9, 23–26). On these glycoproteins, long repeating N-acetylglucosamine chains, known as poly-N-acetylglucosamines, displayed on N- and O-glycans provide adequate presentation of β -galactoside-determinants for Gal-1- and Gal-3-binding activity (27, 28). Gal-3 can also bind a short core 1 O-glycan (Galactose β 1,3 N-acetylglucosamine) structure, known as T antigen, which is heavily displayed on cancer cell MUC-1 (29–31). Based on the strong evidence for Gal-3-binding to either poly-N-acetylglucosamines or T antigen, Gal-3's binding preference is likely dependent on the glycomic gene signature of the respective cancer model.

In this perspective, the recent reports on pro-tumorigenic roles of cancer cell Gal-1/-3-binding O-glycans and how they are regulated by O-glycan-modifying N-acetylglucosamine: α 2,6 sialyltransferases (ST6GalNAc) are examined. These new published findings from a subset of glycobiological reports provide supportive evidence that Gal-1- and Gal-3-binding to cancer cell O-glycans is regulated, in part, through the activity of ST6GalNAcs. The O-glycan capping activities of these enzymes also highlight their functional importance in cancer cell malignancy, representing potential targets for anti-cancer therapy.

B. α 2,6 SIALYLATION OF O-GLYCANS AND ITS IMPACT ON CANCER PROGRESSION

Membrane proteins on cancer cells are commonly decorated with post-translational glycan modifications on asparagine (-N) or serine/threonine (-OH) residues. While N-glycans are covalently linked via N-acetylglucosamine (GlcNAc) to asparagine by an N-glycosidic bond, O-glycans are covalently linked via GalNAc to serine/threonine by an O-glycosidic bond. N-glycans are large in size with a common penta-saccharide core with the potential for multiple chain extensions and terminal diversification by sialylation and/or fucosylation. O-glycans, on the other hand, are relatively small with a simple GalNAc-O (Tn antigen) structure that can be used as template to generate 8 highly-diverse core structures. Notably, core 1 and core 2 O-glycans, and variations thereof, have been associated, in part, with binding of cancer cells to galectins (32).

What helps dictate the synthesis of modified core 1 and/or core 2 O-glycans are: core 1 β 1,3 galactosyltransferase 1 (C1GalT1) and chaperone Cosmc; core 3 β 1,3 N-acetylglucosaminyltransferases; ST6GalNAcs; β -galactose: α 2,3 sialyltransferase 1 (ST3Gal-1); core 2 β 1,6 N-acetylglucosaminyltransferases 1 and 2 (GCNT1 or 3); and N-acetylglucosamine-forming β 1,4 galactosyltransferases and/or β 1,3 N-acetylglucosaminyltransferases (32). These enzymes function sequentially and often, in competition, for the same glycan acceptor to yield structurally diverse O-glycan species. For example, the GalNAc in the core 1 O-glycan, can either be capped by α 2,3 sialylation and/or α 2,6 sialylation or be branched into a core 2 structure by β 1,6 GCNT1 or 3 branching activity.

Of the core 1/core 2-modifying enzyme families, ST6GalNAcs have recently received attention for their ability to control Gal-1- and Gal-3-binding moieties on O-glycans and significantly impact the ferocity of cancer growth and metastasis. There are six

ST6GalNAcs that have been identified to date (17). ST6GalNAc1 and ST6GalNAc2 generate sialyl-Tn antigen, sialyl-6T antigen and disialyl-T antigen from Tn antigen, T antigen and sialyl-T antigen, respectively (33, 34) (Table 1). While ST6GalNAc1 prefers Tn antigen as an acceptor, ST6GalNAc2 favors T antigen and sialyl-T antigen (Table 1). ST6GalNAc3 and ST6GalNAc4 both synthesize disialyl-T antigen from sialyl-T antigen and disialyl-lactotetraosyl-ceramide - $G_{D1\alpha}$ from sialyl-lactotetraosyl-ceramide - G_{M1b} (Table 1) (35, 36). Both ST6GalNAc5 and ST6GalNAc6 show restricted specificity towards G_{M1b} to synthesize $G_{D1\alpha}$ (Table 1) (37, 38). Regarding O-glycan-modifying capabilities, because ST6GalNAc1-4 enzymatically compete with core 2 (GCNT1/3)-branching activity, they could theoretically compromise core 1 T antigen expression while inhibiting the formation of core 2 O-glycans. It should be noted that ST6GalNAc3 and 4 can only generate disialyl-T antigen after first synthesis of $\alpha 2,3$ sialyl-T antigen by ST3Gal-1 (21, 39, 40). This precursor biosynthetic step emphasizes the key role of ST3Gal-1 for ST6GalNAc3/4 enzymatic activity on O-glycans.

The influence of ST6GalNAcs on cancer progression is most widely-recognized through their regulation of Tn and sTn antigens, which are considered biomarkers of cancer (3, 17, 41–43). Either elevated ST6GalNAc1 levels or compromised synthesis of T antigen via mutant Cosmic can raise sTn levels on cancer cells – a direct correlate of progression and poor prognosis (42–44). Malignant activities associated with altered Tn/sTn levels include cell-cell/cell-ECM adhesion, cell migration, cell invasion, and immunoregulation (3, 17, 32, 45). A direct interaction with sTn on cancer cell has recently been established with sialic acid-binding lectin Siglec-15 on macrophages that appears to encourage an immune tolerant cancer microenvironment (46). While all lectin – carbohydrate interactions are likely influenced by truncated O-glycans (i.e. sTn) on cancer cells, concomitant decreases in extended core 2 O-glycan structures and T antigen could, paradoxically, diminish malignant activities associated with Gal-1- and Gal-3-binding. The pro- or anti-tumorigenic role of truncated O-glycans through ST6GalNAc regulation, as highlighted in the review, is perhaps related to the cancerous tissue of origin and its relative reliance on distinct lectin-carbohydrate interactions for malignant potential. Defining which and how endogenous lectin(s) engage with cancer cell Tn/sTn structures or whether elevations in these truncated O-glycans may indirectly potentiate/inhibit lectin-binding are still poorly understood.

Recent reports now provide convincing evidence that O-glycan-modifying ST6GalNAcs regulate synthesis of Gal-1- and Gal-3-binding activity on cancer cells and related malignant activities conferred by interactions with host Gal-1 and Gal-3 (26, 47, 48). Heightened ST6GalNAc1-4 activities could result in a lower level of Gal-1-binding poly-N-acetyllactosamines on core 2 O-glycans or Gal-3-binding core 1 T antigens. Interestingly, due to the ability of ST6GalNAc3-6 to synthesize $G_{D1\alpha}$ from G_{M1b} and the fact that G_{M1} can serve as a Gal-1-binding determinant (14), the potential for ST6GalNAc3-6 converting Gal-1-binding G_{M1b} to non-Gal-1-binding $G_{D1\alpha}$ species could also theoretically regulate glycolipid Gal-1 ligands on cancer cells, particularly brain cancers (49, 50).

1. ST6GalNAc2 and 4 as Regulators of Cancer Metastasis

In a recent landmark paper by Murugaesu et al., studies demonstrate that ST6GalNAc2 functions as a negative regulator of breast cancer metastasis (48). Initial experiments reveal that interfering RNA against ST6GalNAc2 boost lung colonization in an experimental breast cancer metastasis model. Subsequent supportive data in experimental and spontaneous metastasis assays using ST6GalNAc2-silenced mammary cancer cells show that ST6GalNAc2 downregulation augments the frequency and burden of metastasis.

Alternatively, human breast cancer cells expressing high levels of ST6GalNAc2 exhibit significantly reduced metastases formation. Clinical data on estrogen receptor (ER)⁻ vs. ER⁺ breast cancer specimens in patients (and cell lines) show that ST6GalNAc2 expression is directly correlated with improved survival in patients with ER⁻ breast cancer. Mechanistic assessments reveal ST6GalNAc2's ability to α 2,6 sialylate GalNAc in breast cancer cell O-glycans and indeed lower T antigen levels, while increasing disialyl T antigen (48). As T antigen is known as a Gal-3-binding moiety (30), results, in fact, support this concept in that Gal-3-binding activity is heightened in ST6GalNAc2-silenced breast cancer cells. Gal-3's critical role in the context of ST6GalNAc2-silencing is further validated in experimental metastasis assays, in which robust metastatic activity of ST6GalNAc2-silenced breast cancer cells is reversed when cells are similarly silenced for Gal-3 expression or treated with Gal-3 inhibitor, GCS-100 (51). Additional experiments addressing Gal-3's positive role in metastasis formation in conjunction with downregulated ST6GalNAc2 expression indicate that these molecules help regulate adherence to ECs and homotypic cell aggregation.

In all, studies by Murugaesu et al. provide a novel glycobiological mechanism by which breast cancer cell O-glycans modified by ST6GalNAc2 help control interactions with Gal-3 to encourage metastasis (48). The critical collaborative roles of ST6GalNAc2 and Gal-3 in breast cancer metastasis suggest that assaying for ST6GalNAc2 levels in ER⁻ breast cancer patients could help justify initiation of Gal-3 antagonism treatments to effectively blunt metastasis.

In a related study on ST6GalNAc and influence on metastasis, Reticker-Flynn et al. report on the importance Gal-3-binding O-glycans expressed by lung cancer cells and how they interact with myeloid cell-derived Gal-3 to promote lung cancer metastasis (47). This study provides novel data on the role of ST6GalNAc4 in conjunction with core 2 β 1,6 N-acetylglucosaminyltransferase, GCNT3, as key regulators of Gal-3 ligand formation on lung cancer cells. Following pioneering efforts establishing the innate ability of metastatic lung cancer cells to bind Gal-3 (52), the current study expands on these prior observations to investigate the role of lung cancer cell Gal-3 ligands on lung cancer metastasis (47). This inquiry establishes a relationship between Gal-3 expression in the host as a pro-metastatic niche in the seeding of lung cancer cells. Data indicate that Gal-3 is expressed a high level on F4/80⁺ liver macrophages and that circulating Gal-3⁺/CD11b⁺ leukocytes in lung cancer-bearing mice are elevated – a mobilization mechanism triggered by lung cancer-derived IL-6 (47).

How leukocytic Gal-3 interacts with lung cancer cell Gal-3 ligands is importantly addressed in this study (47). Gal-3-binding activity data directly corresponds with expression of the hallmark Gal-3-binding glycan, T antigen, and that this functional activity enhances the

metastatic potential of lung cancer cells. In an attempt to discern the putative glycosyltransferase modifiers of T antigen expression, expression array data reveal that the core 2 β 1,6 GlcNAc-branching enzyme GCNT3 is notably downregulated in metastatic lung cancer cells. Furthermore, ST6GalNAc4 is overexpressed in metastatic cells, indicating that ST6GalNAc4's ability to α 2,6 sialylate GalNAc on sialyl-T antigen (or capping) may be associated with increased Gal-3 ligand/T antigen levels. These expression results are corroborated in ligand binding assays, in which GCNT3-overexpressing or ST6GalNAc4-silenced metastatic lung cancer cells exhibit reduced Gal-3 ligand activity. *In vivo* data further strengthen these findings by showing that ST6GalNAc4-silenced cells with lower Gal-3 ligand activity produce significantly less metastases. These observations advance the hypothesis that cancer cell Gal-3-binding O-glycans, namely T antigen, can be regulated by preventing core 2 branching via downregulation of GCNT3 and capping sialyl-T antigen via up-regulation of ST6GalNAc4.

This glyco-regulatory perspective opposes other studies purporting that Gal-3 ligand activities are dependent on N-glycan poly-N-acetylglucosamines, namely on melanoma cell models (9, 25, 53). If T antigen is the preferred Gal-3-binding moiety on lung cancer cells, then GCNT3's role in preventing T antigen seems logical through its ability to form core 2 structures. The interpretation of ST6GalNAc4's role, on the other hand, is less intuitive. The ability of ST6GalNAc4 to generate disialyl-T antigen from sialyl-T antigen does not directly result in higher T antigen expression and Gal-3-binding activity per se. Rather, as argued by Reticker-Flynn et al. (47), the observed increase in Gal-3-binding activity in metastatic lung cancer cells is driven by both high ST6GalNAc4 and low GCNT3 levels. This results in overall fewer core 2 O-glycans and actually the same Gal-3 ligand^{hi} phenotype as on highly-metastatic breast cancer cells expressing low ST6GalNAc2 levels (48). It should also be noted that lower core 2 O-glycans levels could potentially augment exposure of T antigen on protein scaffold(s). Increases in disialyl-T antigen moieties in the absence of core 2 O-glycans may result in a cell surface devoid of extended O-glycan steric hindrance, thereby enhancing access for Gal-3 to residual T antigen.

2. ST6GalNAc2 as a Regulator of Malignant Potential

In recent work by Yazawa and colleagues, a novel role for ST6GalNAc2 in blocking the synthesis of melanoma-associated O-glycans capable of binding Gal-1 and mediating malignant activity is demonstrated (26). After establishing that Gal-1 ligands are up-regulated on primary and metastatic melanoma cells compared with epidermal melanocytes in normal skin or benign nevi, biochemical assessments reveal that Gal-1-binding moieties and protein scaffold identities ('Gal-1 ligand') are principally represented by poly-N-acetylglucosamines on N-glycans on MCAM as well as on 90k/MAC-2B, CEA and LAMP-1/2.

While the majority of Gal-1 ligand activity on melanoma cells is contributed by poly-N-acetylglucosaminyl N-glycans, poly-N-acetylglucosamine-containing O-glycans, also provide a significant level of Gal-1 ligand activity (26). Considering the purported role of ST6GalNAc2 in preventing core 2 O-glycans and lowering Gal-3-binding T antigen on breast cancer cells (48), this study also focuses on ST6GalNAc2 as a putative regulator of

Gal-1-binding activity (26). Since ST6GalNAc2 activity could compete with core 2 β 1,6 GlcNAc branching activity, decreased formation of poly-N-acetylglucosamine on core 2 O-glycans are hypothesized to lower Gal-1 ligand activity. Real-time RT-PCR of normal and malignant melanocytes, indeed, show that ST6GalNAc2 expression is significantly downregulated in Gal-1 ligand⁺ malignant melanoma cells compared with Gal-1 ligand⁻ normal epidermal melanocytes. Subsequent lectin-binding experiments address ST6GalNAc2's putative role as a negative regulator of Gal-1 ligand expression and show that Gal-1- and *Lycopersicon esculentum* agglutinin (poly-N-acetylglucosamine-specific)-binding to O-glycans is inhibited on melanoma cells overexpressing ST6GalNAc2. In a cell migration assay leveraging the presence of native Gal-1 in Matrigel, ST6GalNAc2-overexpressing melanoma cells exhibit attenuated Gal-1-dependent cell migration. Furthermore, in an *in vivo* syngeneic tumor model, ST6GalNAc2-overexpressing melanoma cells form tumors at a significantly lower rate than control cells in Gal-1-deficient mice.

Altogether, while melanoma cell Gal-1 ligand activity is predominantly conferred by N-glycans, the critical influence of ST6GalNAc2 in regulating O-glycan-dependent Gal-1 ligand activity and related growth *in vivo* indicates that only partial impairment in melanoma cell Gal-1 ligand expression can significantly impact melanoma progression. These findings support the hypothesis that ST6GalNAc2 interferes, in part, with Gal-1 ligand-mediated melanoma malignancy by preventing poly-N-acetylglucosaminyl O-glycan-dependent Gal-1 ligand activity. Further exploration is underway to identify other key glycosyltransferase regulators of N-glycan-dependent melanoma cell Gal-1 ligand activity.

C. CONCLUSIONS AND FUTURE PERSPECTIVES

Membrane glycoproteins on cancer cells are well-established effectors of cell adhesion, impacting cancer cell proliferation, survival, migration, production of soluble pro-tumorigenic factors and vascular seeding. N- and O-glycans on these cancer cell membrane proteins, specifically, play crucial roles in how and what these membrane proteins bind and are often considered key drivers of malignant behavior. Regarding galectin-related activities, poly-N-acetylglucosamines on N-glycans contribute significantly to Gal-1 and Gal-3 ligand activity, particularly on melanoma cells (9, 25, 26, 53). However, capping and/or extension of O-glycans can also provide a considerable level of ligand activity, depending on the cancer type. Recent observations on the roles of ST6GalNAcs in controlling the formation of Gal-1- and Gal-3-binding O-glycans suggest that they indeed significantly impact cancer growth and metastasis (26, 47, 48). Mechanistic data from these studies support the postulate that subtle modifications (or lack thereof) by O-glycan-modifying enzymes can shape malignancy traits governed by host Gal-1 and/or Gal-3. A model depicting enzymatic glycan products of ST6GalNAcs conferring O-glycan-dependent Gal-1- and Gal-3-binding activity on cancer cells is provided in Figure 1. Overall, these findings provide a new perspectives on how Gal-1/-3-binding can be regulated by O-glycan-modifying ST6GalNAcs to support malignant behaviors, including seeding of metastatic breast cancer cells, myeloregulation of lung cancer cells in the liver-metastatic niche and intrinsic melanoma growth activity (26, 47, 48) (Summarized in Figure 2).

Importantly, these studies reinforce the diversified nature of Gal-3 ligands expressed by cancer cells. Whereas Gal-3 preferentially binds poly-N-acetylglucosamines on N-glycans on melanoma cells (25), data highlighted here reveal the key role of T antigen on O-glycans as Gal-3-binding moieties on breast and lung cancer cells. The relative abundance of each respective glycan species is often governed by the type of protein scaffold(s) and/or by the level of poly-N-acetylglucosamine enzymatic machinery that can dictate the preferred Gal-3 ligand(s) on a given cancer cell type. Mucinous adenocarcinomas of the lung, breast and colon, contain a preponderance of O-glycan-bearing scaffold MUC-1, which provides a distinct Gal-3-binding glycan repertoire. Melanoma cells, on the other hand, express an abundance of N-glycan poly-N-acetylglucosamines, such as those on LAMPs-1/2, providing an alternative source of Gal-3-binding moieties.

Discovering identity, function and enzymatic regulation of glycosylations on cancer cells continues to invigorate efforts to target these factors as anti-cancer therapies. Importantly, glycan-synthetic inhibitors (1, 54), glyco-mimetic antagonists (55–57) and neutralizing antibodies (11, 58) designed to therapeutically block the function of malignant-associated glycans are now mainstream and imminent for evaluation in humans. The emerging emphasis on precision medicine through genomic screening to help predict progression of disease and/or guide treatment decisions will undoubtedly heighten efforts to develop such agents. Cancer patients presenting with a glycomic gene signature suggestive of a particular glyco-phenotype, such as high Gal-1/-3 ligand expression, may be ideal candidates to effectively treat the virulent behavior of cancer with biologics antagonizing ligand binding functions of Gal-1 and/or Gal-3.

The main challenge in developing anti-cancer glyco-therapeutics, particularly those biologics that target galectins, are the overlapping glycan-binding specificities. Identifying ligand blocking reagents to either Gal-1, -3, -8 or -9, as examples, should be carefully considered, due to some sharing of glycan-binding repertoires and potential alternative roles in cancer development and immunoprotection. Whether using competitive inhibitors of carbohydrate-recognition domains or using metabolic inhibitors of glycan biosynthesis, there will need to be assurance of galectin specificity to develop clinically-useful agents. Recent efforts using neutralizing monoclonal antibodies to individual galectins, notably Gal-1, have shown promise (11, 58, 59). In these reports, the implied therapeutic targets are Gal-1 interactions with Gal-1 ligands on T cells and/or ECs. As reviewed here, the pro-tumorigenic roles of melanoma cell Gal-1 ligands (26), as an example, raise the possibility for even more effective clinical utility in melanoma patients with late stage disease. The promise of humanized antibodies against immune checkpoint molecules PD-1 and CTLA-4 to stimulate anti-cancer immunity (60) warrants efforts to develop humanized versions of mono-specific anti-Gal-1 antibodies.

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ST6GalNAc Regulators of Gal-1- & Gal-3-binding O-glycans

Figure 1. ST6GalNAc1-4 enzyme activities help regulate Gal-1- and Gal-3-binding moieties on O-glycans

Data indicate that the virulent behavior of breast and lung cancer cells is regulated, in part, by ST6GalNAc2 and ST6GalNAc4 and related synthesis of Gal-3-binding moieties (47, 48). Results from other work indicate that O-glycan-bearing poly-N-acetylactosamines are regulated by ST6GalNAc2 and help mediate Gal-1 ligand activity and related tumorigenic potential of melanoma cells (26).

A. Seeding of Metastatic Breast Cancer Cells in Lung

B. Liver Metastatic Niche

C. Melanoma Growth Potential

Figure 2. Novel insights on ST6GalNAcs and Gal-1/Gal-3-binding O-glycans in cancer growth and metastasis

Results from recent studies indicate that: **(A)** Gal-3 facilitates intravascular aggregation and vascular adhesion of breast cancer cells expressing low levels of ST6GalNAc2 expression and related α2,6 sialyl-O-glycan moieties, increasing lung metastatic potential (48); **(B)** Gal-3 on liver-resident macrophages encourages binding activity to metastatic lung cancer cells expressing elevated levels of ST6GalNAc4 and low levels of GCNT3 (47); and **(C)** host Gal-1 encourages melanoma cell migration and *in vivo* growth, in part, by binding poly-N-acetylglucosaminyl O-glycans inhibited by ST6GalNAc2 (26).

Table 1
ST6GalNAc Family Members and Their Enzymatic Acceptor Specificity

Members	Preferred Glycan Acceptor(s)	Sialo-glycan Product(s)	References
ST6GalNAc1			33, 34
ST6GalNAc2			33, 34
ST6GalNAc3			35, 36
ST6GalNAc4			35, 36
ST6GalNAc5			37, 38
ST6GalNAc6			37, 38

GalNAc

GlcNAc

Gal

Neu5Ac

Glucose

Key

Tn antigen

Sialyl-Tn antigen

T antigen

Sialyl-6T antigen

Sialyl-T antigen

Disialyl-T antigen

Serine/Threonine (O)-Linked Glycoprotein

Sialyl-Lactotetraosyl-Ceramide (G_{M1b})

Disialyl-Lactotetraosyl-Ceramide (G_{D1α})