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 of the role of glycosyltransferases, specifically α2,6 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 α2,6 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 anticancer therapeutics. Cancer Res; 75(16); 1–8. ©2015 AACR.

Review

Galectin-Binding O-Glycosylations as Regulators of Malignancy

Charles J. Dimitroff1,2

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 toward understanding cancer glyconomics—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 glycemic signatures and microenvironmental glycome features. Cancer cell glycans, in particular, represent glycomic features related to a distinct malignant behavior. Cancer cell glycans are variably produced and related to a distinct malignant behavior. Cancer cell glycans are frequently associated with metastatic mechanisms, binding activity related to a distinct malignant behavior. 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 of the role of glycosyltransferases, specifically α2,6 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 α2,6 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 anticancer therapeutics. Cancer Res; 75(16); 1–8. ©2015 AACR.

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 toward understanding cancer glyconomics—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 glycemic signatures and microenvironmental glycome features. Cancer cell glycans, in particular, represent glycomic features related to a distinct malignant behavior. Cancer cell glycans are variably produced and related to a distinct malignant behavior. Cancer cell glycans are frequently associated with metastatic mechanisms, binding activity related to a distinct malignant behavior. 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 of the role of glycosyltransferases, specifically α2,6 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 α2,6 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 anticancer therapeutics. Cancer Res; 75(16); 1–8. ©2015 AACR.
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 proapoptotic activity in T cells, Gal-3 acts on an antiapoptotic factor (4, 10, 18–20). Considering observations of Gal-1 binding to ligands on cancer cells causing proapoptotic activity (21, 22), most studies demonstrate that both Gal-1 and -3 elicit protumorigenic 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; refs. 5, 6, 9, 23–26). On these glycoproteins, long repeating N-acetyllactosamine chains, known as poly-N-acetyllactosamines, 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 β1,3 N-acetyllactosamine) 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-acetyllactosamines or T antigen, Gal-3’s binding preference is likely dependent on the glycanic gene signature of the respective cancer model. In this perspective, the recent reports on protumorigenic roles of cancer cell Gal-1–/3–binding O-glycans and how they are regulated by O-glycan–modifying N-acetyllactosaminogalactosyltransferases (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 anticancer therapy.

α2,6 Sialylation of O-Glycans and Its Impact on Cancer Progression

Membrane proteins on cancer cells are commonly decorated with posttranslational glycan modifications on asparagine (N) or serine/threonine (OH) residues. Although N-glycans are covalently linked via N-acetylgalactosamine (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 β1,3 galactosyltransferase 1 (C1GaT1) and chaperone Cosmc; core 3 β1,3 N-acetylgalactosaminyltransferases; ST6GalNAcβ; β-galactose: α2,3 sialyltransferase 1 (ST3Gal-1); core 2 β1,6 N-acetylgalactosaminyltransferases 1 and 2 (GCNT1 or 3); and N-acetyllactosamine-forming β1,4 galactosyltransferases and/or β1,3 N-acetylgalactosaminyltransferases (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 α2,3 sialylation and/or α2,6 sialylation or be branched into a core 2 structure by β1,6 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). ST6GalNA1 and ST6GalNA2 generate sialyl-Tn antigen, sialyl-6T antigen, and disialyl-T antigen from Tn antigen, T antigen, and sialyl-T antigen, respectively (Table 1; refs. 33, 34). 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 - G2lys from sialyl-lactotetraosyl-ceramide - GM1b (Table 1; refs. 35, 36). Both ST6GalNAc5 and ST6GalNAc6 show restricted specificity toward GM1b to synthesize G2Dis (Table 1; refs. 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 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 α2,3 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/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). Although 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 antitumorigenic 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 are likely in different species could also theoretically regulate glycolipid binding determinant (14), the potential for ST6GalNAc–modifying enzyme families, 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-acetyllactosaminogalactosyltransferases on core 2 O-glycans or Gal-3–binding core 1 T antigens. Interestingly, due to the ability of ST6GalNAc3–6 to synthesize G2Dis from G2M1b and the fact that G2M1b can serve as a Gal-1–binding determinant (14), the potential for ST6GalNAc3–6 converting Gal-1–binding G2M1b to non–Gal-1–binding G2F1c species could also theoretically regulate glycolipid...
Gal-1 ligands on cancer cells, particularly brain cancers (49, 50).

### ST6GalNAc2 and 4 as regulators of cancer metastasis

In a recent landmark article by Murugaesu and colleagues, studies demonstrate that ST6GalNAc2 functions as a negative regulator of breast cancer metastasis (48). Initial experiments reveal that interfering RNA against ST6GalNAc2 boosts 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)– versus 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 α2,6 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 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 and colleagues 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.

### Table 1. ST6GalNAc family members and their enzymatic acceptor specificity

<table>
<thead>
<tr>
<th>Members</th>
<th>Preferred glycan acceptor(s)</th>
<th>Sialo-glycan product(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST6GalNAc1</td>
<td>GalNAc</td>
<td>Gal &gt; α6 &gt; GlcNAc</td>
<td>33, 34</td>
</tr>
<tr>
<td>ST6GalNAc2</td>
<td>GalNAc</td>
<td>Gal &gt; α6 &gt; GlcNAc</td>
<td>33, 34</td>
</tr>
<tr>
<td>ST6GalNAc3</td>
<td>Cer</td>
<td>Cer</td>
<td>35, 36</td>
</tr>
<tr>
<td>ST6GalNAc4</td>
<td>Cer</td>
<td>Cer</td>
<td>35, 36</td>
</tr>
<tr>
<td>ST6GalNAc5</td>
<td>Cer</td>
<td>Cer</td>
<td>37, 38</td>
</tr>
<tr>
<td>ST6GalNAc6</td>
<td>Cer</td>
<td>Cer</td>
<td>37, 38</td>
</tr>
</tbody>
</table>

© 2015 American Association for Cancer Research

Sialo-Glycans as Regulators of Malignancy

www.aacrjournals.org

Cancer Res; 75(16) August 15, 2015

Published OnlineFirst July 29, 2015; DOI: 10.1158/0008-5472.CAN-15-0834
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 prometastatic 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 IL6 (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 correspond 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 ligands is importantly addressed in this study (47). Gal-3 lung cancer metastasis (47). This inquiry establishes a relation-
vations to investigate the role of lung cancer cell Gal-3 ligands on establishing the innate ability of metastatic lung cancer cells to formation on lung cancer cells. Following pioneering efforts

Dimitroff

OF4 Cancer Res; 75(16) August 15, 2015

Cancer Research

hallmark Gal-3 binding activity data directly correspond with expression of the cells. In an attempt to discern the putative glycosyltransferase ligands is importantly addressed in this study (47). Gal-3

Cancer Research

OF4 Cancer Res; 75(16) August 15, 2015

Cancer Research

While the majority of Gal-1 ligand activity on melanoma cells is contributed by poly-N-acetyllactosaminyl N-glycans, poly-N-acetyllactosamine–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 β1,6 GlcNAc branching activity, decreased formation of poly-N-acetyllactosamine on core 2 O-glycans is hypothesized to lower Gal-1 ligand activity. Real-time RT-PCR of normal and malignant melanocytes, indeed, shows 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-acetyllactosamine-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-acetyllactosaminyl O-glycan–dependent Gal-1 ligand activity. Further exploration is under way to identify other key glycosyltransferase regulators of N-glycan–dependent melanoma cell Gal-1 ligand activity.

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 protumorigenic 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-acetyllactosamines 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 of the roles of ST6GalNAc2 in controlling the formation of Gal-1– and Gal-3–binding

Owing to the importance of this glyco-regulatory perspective opposes other studies purporting that Gal-3 ligand activities are dependent on N-glycans (9, 25, 53). However, capping and/or extension of O-glycans 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 upregulation of ST6GalNAc4.

This glyco-regulatory perspective opposes other studies purporting that Gal-3 ligand activities are dependent on N-glycans (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 and colleagues (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 ligandhi 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.

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 upregulated 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-acetyllactosamines 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-acetyllactosaminyl N-glycans, poly-N-acetyllactosamine–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 β1,6 GlcNAc branching activity, decreased formation of poly-N-acetyllactosamine on core 2 O-glycans is hypothesized to lower Gal-1 ligand activity. Real-time RT-PCR of normal and malignant melanocytes, indeed, shows 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-acetyllactosamine-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-acetyllactosaminyl O-glycan–dependent Gal-1 ligand activity. Further exploration is under way to identify other key glycosyltransferase regulators of N-glycan–dependent melanoma cell Gal-1 ligand activity.

Membrane glycoproteins on cancer cells are well-established effectors of cell adhesion, impacting cancer cell proliferation, survival, migration, production of soluble protumorigenic 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-acetyllactosamines 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 of the roles of ST6GalNAc2 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 Fig. 1. Overall, these findings provide a new perspective 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 (summarized in Fig. 2; refs. 26, 47, 48).

Importantly, these studies reinforce the diversified nature of Gal-3 ligands expressed by cancer cells. Whereas Gal-3 preferentially binds poly-N-acetyllactosamines 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-acetyllactosamine 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-acetyllactosamines, 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

Figure 1.
target these factors as anticancer 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 anticancer glyco-therapeutics, particularly those biologics that target galectins, is 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 protumorigenic 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 anticancer immunity (60) warrants efforts to develop humanized versions of monospecific anti–Gal-1 antibodies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Grant Support
This study was supported by NIH/NCI grant (R01CA173610 to C.J. Dimitroff).

Received March 26, 2015; revised April 28, 2015; accepted April 28, 2015; published OnlineFirst July 29, 2015.

References


Galectin-Binding O-Glycosylations as Regulators of Malignancy

Charles J. Dimitroff

_Cancer Res_ Published OnlineFirst July 29, 2015.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-15-0834

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.