Published in final edited form as:

Cancer Metastasis Rev. 2012 December ; 31(0): 501–518. doi:10.1007/s10555-012-9359-7.

Regulation of the metastatic cell phenotype by sialylated glycans

Matthew J. Schultz, Amanda F. Swindall, and Susan L. Bellis

Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, MCLM 982A 1918 University Boulevard, Birmingham, AL 35294-0005, USA

Susan L. Bellis: bellis@uab.edu

Abstract

Tumor cells exhibit striking changes in cell surface glycosylation as a consequence of dysregulated glycosyltransferases and glycosidases. In particular, an increase in the expression of certain sialylated glycans is a prominent feature of many transformed cells. Altered sialylation has long been associated with metastatic cell behaviors including invasion and enhanced cell survival; however, there is limited information regarding the molecular details of how distinct sialylated structures or sialylated carrier proteins regulate cell signaling to control responses such as adhesion/migration or resistance to specific apoptotic pathways. The goal of this review is to highlight selected examples of sialylated glycans for which there is some knowledge of molecular mechanisms linking aberrant sialylation to critical processes involved in metastasis.

Keywords

Sialylation; Metastatic cell phenotype; Sialylated glycoproteins; Invasion

1 Introduction

It has been known for decades that glycoconjugates play an important role in cancer development and progression. An alteration in the profile of cell surface glycans was one of the earliest-identified hallmarks of a tumor cell, and many of the anti-tumor antibodies produced by patients are specific for carbohydrate antigens [1–4]. Cancer-associated glycoconjugates in serum and tissue have been used as important biomarkers for disease progression [5–7]. Notably, the changes in glycan structure following tumorigenic transformation are not random. There is a specific subset of oligosaccharides that becomes enriched on the tumor cell surface, implicating a functional contribution to the tumor phenotype, and many of the glycosyltransferases that synthesize these oligosaccharides are upregulated in response to oncogenes such as Ras [3, 8].

Despite these long-standing observations, our understanding of the molecular mechanisms linking altered glycosylation to tumor cell behavior has lagged behind most other areas of

cancer research. This is unfortunate in that this dearth of knowledge has left largely unexplored an important category of potential biomarkers or targets for drug discovery and vaccine development. So why has the field of cancer glycobiology progressed so slowly? While many factors are likely involved, one of the challenges encountered is that, unlike the template-driven synthesis of oligo-nucleotides and proteins, the synthesis of glycans elaborating cell surface molecules is complex and not readily predictable. Technologies for defining glycoconjugate structure are still evolving, and there are limited methods that can be used to determine the position of an oligosaccharide within the three-dimensional structure of a glycan carrier. For example, X-ray crystallography is difficult to perform with glycosylated proteins, therefore the glycans are typically enzymatically removed prior to crystallization. As a consequence, current literature describing conformational analyses of proteins, or identification of protein–protein interaction domains, often excludes information regarding how glycans might alter protein conformational features or peptide-binding interfaces. It is generally assumed that glycans extend into the extracellular space with high mobility (and many do), however there is evidence that at least some glycans are relatively fixed within the larger glycoprotein tertiary structure [9, 10], and glycans can also form direct bonds with primary amino acid sequence [11, 12]. Another important factor not always appreciated is that some monosaccharides, such as sialic acid, are negatively charged at physiologic pH, thus the addition of such a sugar (comparable to a phosphate group) has potential to alter protein conformation and/or oligomerization. As well, sialylation is emerging as a major regulator of cell surface retention of various receptors.

In addition to modulating the conformation, clustering and/or surface retention of an individual glycoprotein, glycoconjugates are ligands for numerous glycan-binding proteins such as lectins [13, 14]. While studies of protein–protein interactions have predominated in cancer research, there is increasing recognition that associations between tumor glycans and lectins are of great importance in regulating many aspects of tumor cell behavior. Lectins exist as intracellular, cell surface, or secreted molecules, depending upon the species, and many secreted lectins are incorporated into the extracellular matrix. Hence, glycan-lectin binding partners represent another fundamental class of molecular regulators of cell–cell and cell–matrix interactions. Elucidating the biochemical details of these interactions has proven challenging, however mounting evidence points to a high degree of specificity, comparable to the role of distinct amino acid motifs that drive protein–protein interactions. Indeed, glycans have been referred to as the “third alphabet of molecular biology” (the other two being proteins and nucleic acids) [15]. This capacity to control cell–cell and cell–matrix interactions, in combination with the known effects of glycans on the structure/function of individual glycoproteins, underlies the presumed role of glycans in most metastatic cell behaviors including migration/invasion through matrix, dissemination through the vasculature or lymphatics, evasion from immune surveillance, and resistance to apoptosis.

Given the multiplicity of carbohydrate modifications associated with human cancer, this review will have a restricted focus on one type of glycan modification, protein sialylation. Experimental results gleaned from patient tissue samples, animal cancer models, and cell culture studies, suggest that altered sialylation is a major contributor to the metastatic cell phenotype [16, 17]. The term “sialic acid” refers to a group of approximately 50 different

chemical derivatives of neuraminic acid, with the most common variant represented by *N*-acetylneuraminic acid (Neu5Ac, Fig. 1a) [18]. Sialic acids are added onto the termini of either *N*- or *O*-linked glycans of glycoproteins and can also be added onto glycolipids (Fig. 1b). The overall level of cell surface sialylation is regulated by numerous enzymes including: (1) enzymes that control synthesis and availability of the activated sialic acid substrate, CMP-sialic acid, (2) the sialyltransferase family, which adds sialic acid during glycoprotein biosynthesis, and (3) the sialidase (also called neuraminidase) family, which cleaves sialic acid during glycoprotein degradation. These enzymes typically reside within subcellular compartments, with most sialyltransferases localized to the Golgi, and many of the sialidases localized to lysosomes or endosomes. Aberrant activity of both sialyltransferases and sialidases has been observed in cancer; however, the literature overwhelmingly suggests that sialylation levels are higher on tumor cells [3, 19]. Elevated sialylation is thought to be a relatively static tumor cell characteristic, given that sialic acid is added during glycoprotein biosynthesis, but recent studies indicate that some enzymes involved in sialylation can be expressed on the cell surface, or secreted as active soluble enzymes into the extracellular milieu [20–23]. While not a focus of this review, this opens up the fascinating possibility that the sialylation of certain glycoproteins may be dynamically regulated at the cell surface, providing a unique mechanism for transient control of individual glycoprotein structure or glycan/lectin interactions. One of the major barriers in our understanding of tumor cell sialylation is that much of the prior research was directed at correlating total surface sialylation levels with cell responses, with limited regard for the specific type of sialic acid chemical structure or linkage, or the specific glycoprotein carrier of the sialic acid. This lack of mechanistic knowledge has hindered investigative pursuit of glycans as clinical targets. The goal of this report is to highlight some of the more prevalent tumor-associated changes in specific types of sialylation, and discuss potential molecular mechanisms by which these modifications influence metastatic progression.

2 Regulation of tumor cell surface sialylation

An upregulation in the expression of selected sialyltransferases is a common event in tumorigenic transformation [16]. Sialyltransferases comprise a family of at least 20 different enzymes that differ in tissue distribution as well as the type of sialic acid linkage elaborated [16, 24]. Some sialyltransferases add sialic acid in an α 2-3 linkage to galactose (Gal); whereas others add sialic acid in an α 2-6 linkage to either Gal (e.g., the ST6Gal-I and ST6Gal-II sialyltransferases) or *N*-acetylgalactosamine (GalNAc, added by multiple ST6GalNAc sialyltransferases) (Fig. 2). The third type of sialic acid linkage is directed by the polysialyltransferase family, which adds an α 2-8 linked sialic acid onto another sialic acid. Cancer-associated dysregulation has been observed for selected members of all three of these sialyltransferase categories. In conjunction with aberrant sialyltransferase expression, certain sialidases are also disrupted in human cancer, although far less is known about the tumorigenic role of this enzyme family. One example is the Neu1 sialidase, which is downregulated in cancer cells, leading to higher levels of cell surface sialylation (due to diminished sialic acid cleavage) [25].

An increase in α 2-6-linked sialylation is frequently observed in tumor cells, and is usually attributed to an upregulation in either the ST6Gal-I sialyltransferase [16, 17, 26–28], which

primarily sialylates *N*-linked glycans, or members of the ST6GalNAc family, which sialylate either *O*-linked glycans or glycolipids [24, 29]. The selective enrichment of α 2-6 sialic acids on tumor cells is significant in that α 2-6 sialylation can elicit very distinct biologic outcomes as compared with α 2-3 sialylation. One striking example is the effect of α 2-6 sialylation on galectin-dependent cell behaviors. Galectins are lectins that bind galactose-containing oligosaccharides [30–32]. Depending upon the galectin species, galectins can be expressed either intracellularly or extracellularly; in the latter case, some galectins are found associated with the extracellular matrix [33–35]. Extensive evidence suggests that α 2-6 sialylation of galactose serves as a generic inhibitor of galectin binding [36, 37], unlike α 2-3 sialic acids, which have variable effects on binding depending upon the individual galectin (Fig. 3). Accordingly, α 2-6 sialylation serves as a key negative regulator of many critical galectin functions. One important activity of cell surface α 2-6 sialylation is to block the binding of pro-apoptotic galectins, thereby promoting tumor cell survival [38].

Galectins are not the only glycan-binding proteins influenced by the type of sialylation present on cognate glycoconjugate ligands. Sialic acid linkage, as well as chemical structure (e.g., acetylation), are major determinants in ligand recognition by sialic acid-binding immunoglobulin superfamily lectins (siglecs) [39–41]. Siglecs are mainly expressed by immune cells, and the potential function of siglecs in tumor biology has received minimal attention. One envisions that changes in tumor cell sialylation could affect the activity of siglec-expressing immune cells, and consequently modulate the anti-tumor immune response.

Beyond their pivotal role in regulating interactions with glycan-binding proteins, sialic acids can have direct effects on specific glycoproteins that carry the sialic acid, which is not surprising given the large size and negative charge of this sugar moiety. The effect of sialylation on the structure/function of a given glycoprotein will depend upon the localization of the sialic acid within the larger glycoprotein tertiary structure, which is difficult to determine due to technical challenges. However, it is becoming clear that sialylation can affect glycoprotein activity through many different mechanisms. As examples, sialylation alters: (1) conformation of the β 1 integrin [42, 43]; (2) clustering of CD45 [44], EGFR [45], and PECAM [46]; and (3) cell surface retention of PECAM [46] and the Fas death receptor [47]. There is also evidence that sialylation modulates heterotypic associations between two distinct cell surface glycoproteins, as reported for the noncognate interaction between CD8 and MHC class I proteins [48–50]. In addition, many membrane receptors are anchored on the cell surface through a galectin-dependent mechanism that is sensitive to α 2-6 sialylation. Extracellular galectins form a multimeric lattice-type structure that binds galactose-containing receptor glycans, and stabilizes glycoprotein surface localization [51–54]. Receptor α 2-6 sialylation causes release from the galectin lattice, leading to receptor internalization [20]. Conversely, α 2-6 sialylation can facilitate the surface retention of other types of receptors, albeit through pathways that are not generally well-defined. Taken together, the literature indicates that sialylation holds potential to influence tumor cell behavior at many different levels including: regulation of individual glycoprotein conformation, clustering or surface retention, modulation of *cis* or *trans* interactions between two distinct surface receptors, and formation of ligands for glycan-

binding proteins that correspondingly control cell–cell and cell–matrix interactions. In the remaining sections of this review, several specific examples of tumor-associated sialoglycans are discussed. These were selected because of the greater knowledge of molecular mechanisms linking these modifications to tumor cell behavior as compared with many of the other prevalent changes in tumor glycosylation.

3 Role of integrin sialylation in tumor cell migration through extracellular matrix

Numerous studies suggest that increased cell surface sialylation contributes to metastasis by stimulating tumor cell movement through the extracellular matrix (ECM). *In vitro* assays performed with many different cancer cell lines indicate a strong positive correlation between migration/invasion and high levels of surface sialylation [55–58]. Likewise, subclones of cell lines selected for enhanced invasiveness often display elevated surface sialylation, including clonal variants from lung [45], colon [59], melanoma [60], and T lymphoma [61] cells. The functional importance of hypersialylation is supported by animal models of metastasis [62, 63]. Intrasplenic injections of two differing populations of 51B colon cells; a heavily $\alpha 2$ -6 sialylated population and a poorly sialylated population, resulted in hepatic tumors formed almost exclusively by the highly sialylated cells, indicating selective metastasis [63]. Many other studies have shown that metastasis in murine models can be blocked by pharmacologic inhibitors of sialyltransferase activity [64–66] or sialic acid incorporation [62] or alternately, by pre-treatment of tumor cells with sialidases [63]. Interestingly, certain types of sialic acid linkages may regulate metastatic targeting of selected organs; a breast cancer cell line selected for targeting to bone had higher levels of $\alpha 2$ -6-linked sialic acid [67], whereas upregulation of ST6GalNAcV, which adds $\alpha 2$ -6 sialic acid to gangliosides, directs breast cancer metastasis to brain [68].

Though the circumstantial evidence linking sialylation to metastasis is extensive, data regarding the specific molecular events driving invasive tumor cell behavior are lacking. The prior experimental use of sialylation inhibitors and sialidases, most of which have low specificity, generally produced widespread ablation of cell surface sialylation, affecting a multitude of glycoproteins and glycolipids. Compounding this issue, such generic approaches are not typically representative of the physiologic changes that occur during metastatic progression, which involve alterations in the expression of specific enzymes. More recent studies applying RNAi technology, or forced overexpression models that better recapitulate the tumor phenotype, are beginning to reveal a more defined view of the role of sialylation in metastasis. Several sialylation-related enzymes have been targeted using this strategy, and such studies are yielding new insight into how distinct types of sialylation regulate specific receptors to promote tumorigenic cell responses.

Some of the more compelling results implicating a specific sialoprotein in tumor cell migration and invasion have been provided by studies of the integrin family of cell adhesion receptors [69]. Integrin activity is involved in many aspects of tumor metastasis including tumor cell detachment from basement membrane, migration through the stromal matrix, anchorage-independent cell survival in the vasculature, adhesion to endothelium during extravasation, and establishment of metastatic foci in novel ECMs. Glycosylation of

integrins has long been known to be required for integrin function [69, 70], and integrins are regulated by several different types of glycan structures [71–73]. Among these, α 2-6 sialylation of *N*-glycans is an important modulator of a specific subset of integrins. The β 1 integrin subunit (but not β 3 or β 5 [74]) has been identified as a substrate for the ST6Gal-I sialyltransferase in multiple established cancer cell lines [56, 57, 75, 76]. Furthermore, β 1 integrins in human colon cancer tissues display elevated α 2-6 sialylation [56], which corresponds to the well-documented overexpression of ST6Gal-I in many different cancers, including colon carcinoma [16, 36]. ST6Gal-I is upregulated in cancer as a consequence of signaling by oncogenic Ras [74, 77–79].

The addition of α 2-6-linked sialic acid to the β 1 integrin subunit alters the binding activity of several β 1-containing heterodimers including receptors for fibronectin (α 5 β 1 [43, 80, 81]), VCAM-1 (α 4 β 1 [42]), laminin (α 3 β 1 [82]), and collagen (α 1 β 1 [74] and α 2 β 1 [75]). Regulation of integrin function by α 2-6 sialylation has been confirmed by studies using engineered cell lines, as well as ligand binding assays performed with purified integrin receptors that have been manipulated to express varying levels of sialylation. It has also been shown that, of the ten *N*-linked glycans on the β 1 integrin subunit [80], the three *N*-glycans within the β 1 I-like domain, a region involved in ligand binding, are essential for heterodimerization of the α and β subunits, and also for ligand-induced cell spreading [83]. These recent results confirm studies performed approximately 20 years ago showing that *N*-glycosylation was indispensable for β 1 integrin function [84, 85].

Mechanistic studies of sialylation-related β 1 integrin activity are few in number, however experiments using activation-state reporter antibodies suggest that sialylation alters β 1 integrin conformation [42, 43], a finding supported by molecular modeling approaches [86]. α 2-6 sialylation of collagen-selective integrins increases adhesion to collagen I, enhances coupling of talin to the integrin cytosolic tail, and stimulates cell migration (Fig. 4) [56, 57, 75]. *In vivo* support for sialylation-dependent effects on integrin signaling has been provided by Varki's group, who used the polyomavirus middle T antigen model of spontaneous breast cancer to study ST6Gal-I [87]. Results from this study showed that mammary tumors from ST6Gal-I null mice exhibited a selective alteration in genes associated with focal adhesion signaling, as well as diminished activation of Focal Adhesion Kinase (FAK), a known downstream target of integrin signaling. Tumors from mice lacking ST6Gal-I were also more differentiated, suggesting that the overexpression of ST6Gal-I that occurs in human carcinoma may contribute to a poorly differentiated tumor phenotype.

ST6Gal-I-directed α 2-6 sialylation of the β 1 integrin stimulates tumor cell migration and invasion through reconstituted ECM (e.g., Matrigel) [56, 57, 75]. Cells that are null for the β 1 integrin do not exhibit differential invasion upon forced ST6Gal-I expression [75], supporting the hypothesis that the effect of upregulated ST6Gal-I is mediated specifically by the β 1 integrin. The interaction between integrins and collagen I is thought to be important in metastasis. Microarray studies performed on diverse tumor types identified collagen I as part of a 17-gene signature associated with increased metastasis [88]. The deposition of collagen I in the metastatic microenvironment induces dormant tumor cells to form proliferative metastatic lesions, and this transition is dependent upon β 1-integrin signaling [89]. In addition, collagen reorganization at the tumor-stromal interface facilitates local

invasion [90] and collagen I fibers provide tracks along which tumor cells migrate during transit to blood vessels [91]. The $\alpha 2\beta 1$ collagen-selective integrin has been suggested as a principal player in metastatic progression; comparative analyses of primary colorectal cancers with corresponding liver and lung metastases suggest that the $\alpha 2$ integrin subunit contributes to liver targeting [92].

Recently a second integrin family member, $\alpha 6\beta 4$ (a laminin-binding receptor), has been reported to be affected by sialylation [25]. In this instance, elevated sialylation of the $\beta 4$ integrin subunit resulted from decreased expression of a sialidase rather than increased expression of a sialyltransferase. Downregulation of the Neu1 sialidase, which localizes in part to the plasma membrane [25, 93, 94], was associated with enhanced cell invasiveness and metastatic potential in rat and murine cancer cells [95, 96]. To elucidate the effects of Neu 1 on integrin activity, Neu1 expression was forced in human colon cancer cell lines [25], which led to an accompanying gain in sialylation on the *O*-linked glycans of the $\beta 4$ subunit. While the effect of sialylation on $\alpha 6\beta 4$ structure has yet to be determined, sialylation clearly influenced $\alpha 6\beta 4$ signaling because the $\beta 4$ subunit displayed reduced phosphorylation, and $\alpha 6\beta 4$ -induced FAK activation was diminished. Importantly, forced Neu1 expression inhibited experimental metastasis to liver [25]. These studies of $\alpha 6\beta 4$, along with $\beta 1$, point to specific integrin sialoforms (and other glycoforms not discussed herein) as critical mediators of tumor cell migratory and invasive behavior.

4 Sialyl Tn antigen and tumor invasion

The sialyl Thomsen-nouvelle antigen (sialyl Tn) and its unsialylated form, Tn, are well-known tumor-associated carbohydrate antigens, and are highly correlated with cancer invasion and metastasis [97–99]. Sialyl Tn is formed by the sialylation of the Tn antigen: GalNAc linked to serine or threonine (Fig. 5). GalNAc is the first sugar added during *O*-linked glycan synthesis, and this basic unit can be extended to form multiple glycan structures. The sialylation of Gal-NAc prevents further sugar additions, and effectively truncates the *O*-linked glycan extension [100, 101]. Sialyl Tn is detected in a wide range of cancers including gastric, colorectal, pancreatic, endometrial, breast, and ovarian [102–108], yet sialyl Tn expression is low or absent in normal epithelial cells [109–112]. Sialyl Tn expression is associated with metastatic disease, recurrence, and reduced survival rates in breast cancer [98, 113], and a negative association between sialyl Tn and survival has been confirmed by many other studies [98, 114–120]; although this is not a universal finding [121, 122]. Approximately 30 % of breast cancers are sialyl Tn positive [123, 124] and over 80 % of all carcinomas express either Tn or sialyl Tn structures [125]. Given that antibodies against sialyl Tn antigen are cancer specific [97, 109], serum sialyl Tn levels are used as a prognostic indicator for cancer aggressiveness and metastatic potential [126]. The relationship between sialyl Tn and cancer progression has been demonstrated experimentally by the forced expression of sialyl Tn structures in cancer cells. Forced sialyl Tn in gastric cancer lines resulted in increased metastasis and decreased survival in nude mice after intraperitoneal injection of tumor cells [127]. This enhanced metastatic capability of sialyl Tn-expressing cells was abrogated by pretreatment with anti-sialyl Tn antibodies. In related studies, various cancer cell lines with forced sialyl Tn expression gained metastatic

characteristics such as altered adherence to matrix molecules and increased motility and invasiveness [128–130].

The generation of sialyl Tn is primarily associated with a single sialyltransferase, ST6GalNAc-I, which adds sialic acid in an α 2-6 linkage to the Tn antigen [131–133]. Forced expression of ST6GalNAc-I results in sialyl Tn expression in breast and gastric cancer cell lines [127, 129]. Other ST6GalNAc family members may be able to synthesize sialyl Tn; however, while ST6GalNAc-II can create the sialyl Tn structure *in vitro* on peptide-GalNAc substrates [133, 134], to date no other ST6GalNAc has been shown to generate sialyl Tn expression *in vivo*. ST6GalNAc family members have been linked to carcinogenesis: ST6GalNAc-I is upregulated in intestinal metaplasia [132]; ST6GalNAc-II is elevated in colon cancer and is prognostic for patient survival [135], and ST6GalNAc-V is one of four genes upregulated in breast cancer cells with increased metastatic potential to the brain [68]. As an alternate mechanism to ST6GalNAc overexpression, accumulation of sialyl Tn can result from dysregulation of other glycosyltransferases that regulate formation or availability of the Tn substrate. *Cosmc* is a molecular chaperone protein necessary for the activity of T-synthase, an enzyme that adds galactose to GalNAc and therefore competes with ST6GalNAc-I for GalNAc-modified *O*-linked glycan precursors [136, 137]. Cummings' group reported that *Cosmc* disruptions in colon and melanoma cell lines contribute to sialyl Tn expression (due to downregulated T-synthase activity), and further documented two cervical cancer cases with mutations at the *Cosmc* locus, and elevated sialyl Tn expression [125].

Even though there is a strong association between sialyl Tn expression and cancer progression, the specific effects of sialyl Tn on tumor cell behavior remain obscure. Not many studies have been aimed at identifying the carriers of sialyl Tn, or determining the corresponding influence of sialyl Tn on carrier function. There is also a lack of knowledge regarding physiologically relevant glycan binding proteins that might interact with sialyl Tn in the tumor milieu. However, a few glycoproteins have been determined as carriers of sialyl Tn. CD44 and the mucin, Muc 1, are elaborated with sialyl Tn in breast and gastric cancer cells that have been forced to express ST6GalNAc-I [127, 129]. Mucins are large, densely *O*-glycosylated proteins that act in cell adhesion and signaling [138]. They are upregulated in a variety of cancers and characteristically display truncated *O*-linked glycans as compared with mucins in normal tissue [101, 138, 139]. A general increase in sialylation influences Muc1's role in cell–cell adhesion [140, 141] and elevated levels of Muc 1 with sialyl Tn have been observed in multiple carcinomas [128, 142]. The other sialyl Tn carrier, CD44, is another well-known adhesion protein; this molecule displays a binding specificity for hyaluronan. Certain CD44 splice variants, such as CD44v6, have been associated with breast, lung, colon, and pancreatic carcinomas, among others [143–145]. Two other glycoproteins, β 1 integrin [146] and osteopontin [112], are reportedly modified with sialyl Tn in murine cancer cells, although this has not been confirmed in human cells. The β 1 integrin was shown to be the primary carrier of sialyl Tn antigen in the non-mucin expressing TS/A murine breast cancer cell line after ST6GalNAc-I forced expression [146]. In this investigation, ST6GalNAc-I expressing cells had reduced mobility and proliferation, suggesting a possible inhibitory effect on the metastatic process. However, in another study

which identified Muc1 and CD44 as sialyl Tn carriers in human breast cancer cells, β 1 integrin was not found to carry the sialyl Tn antigen [129]. These conflicting reports may be due to differences in mucin expression and competing substrates for ST6GalNAc in the Golgi. Recently, osteopontin was found to carry the sialyl Tn epitope in murine breast cancer cell lines [112]. Osteopontin levels in serum are elevated in a number of human cancers, and increased expression is associated with a negative prognosis [145]. Interestingly, osteopontin serves as a ligand for both CD44 and β 1 integrins [145], and it is worth noting that all four proteins identified as sialyl Tn carriers (Muc1, osteopontin, CD44, and β 1) are involved in cell adhesion and migration. It is tempting to speculate that the expression of sialyl Tn may subvert the normal function of these proteins to promote an invasive tumor phenotype (Fig. 6).

Sialyl Tn expression may also play an immunologic role in tumor progression. Natural killer cells pre-treated with Muc1 bearing the sialyl Tn antigen, but not Muc1 without sialyl Tn, exhibited diminished capacity for cell-mediated cytotoxicity against K562 leukemia cells [147]. Although mechanistic information is lacking, sialyl Tn structures are capable of binding to siglecs, which are important mediators in immune recognition [148]. The sialyl Tn antigen is the preferred substrate for siglec 6 [149] while some additional siglecs may bind sialyl Tn along with other sialoglycans [148]. It is possible that altered sialyl Tn expression on tumor cells may shift the immune response in ways that promote tumor development through interactions with different siglecs. As well, sialyl Tn-antibody complexes formed from soluble sialyl Tn and antisera stimulated VEGF release from macrophages and granulocytes, leading to increased tumor angiogenesis and invasion [150, 151]. Enhanced blood vessel formation was similarly observed in SCID mice with subcutaneously injected, sialyl Tn expressing breast cancer cells following the introduction of anti-sialyl Tn antibodies into the systemic circulation. It is still unclear whether this effect was separate from the general immune response to tumor antigens or specific for sialyl Tn.

Theratope® is a cancer vaccine against sialyl Tn conjugated to keyhole limpet hemocyanin, and was initially designed for use in metastatic breast cancer. In a phase II clinical study, patients receiving the vaccine showed a sialyl Tn-specific humoral response and improved overall survival [123]; however, a phase III study concluded that the vaccine did not increase survival in patients with metastatic disease [152]. Independent studies of Theratope in murine models reported detection of sialyl Tn-specific antibodies, and significantly delayed tumor growth [112]. A potential pitfall of the phase III clinical trial is that the patient population was not evaluated for sialyl Tn expression prior to enrollment, possibly masking any benefit from the vaccine due to heterogeneous sialyl Tn expression between patients. While additional investigation is needed to clarify the discrepant results concerning sialyl Tn, the pursuit of tumor-associated carbohydrate antigens as candidates for vaccine development remains an active area of investigation.

5 Sialyl Lewis structures in tumor dissemination

After entering the systemic circulation, tumor cells must be able to survive within, and then exit, the vasculature in order to metastasize to distant organs. The upregulation of sialyl Lewis (sLe) structures on the tumor cell surface serves as a key mechanism for directing

tumor cell adhesion to the endothelium by providing ligands for endothelial selectins [153] (Fig. 7). sLe/selectin interactions also promote the formation of aggregates comprised of tumor cells, platelets and leukocytes, which shields tumor cells from immune attack [154]. sLe glycans are normally present on leukocytes and are critical in leukocyte adhesion and extravasation during an inflammatory response. Conversely, sLe expression is typically low in non-cancerous epithelial cells [19, 155]. sLe structures are tetrasaccharides composed of a GlcNAc-Gal backbone with an α 2-3-linked sialic acid attached to Gal, and fucose linked to GlcNAc (Fig. 8). sLe is primarily found on glycolipids or O-glycans of glycoproteins [19], and the expression of sLe is elevated in many different types of cancer [156–158]. sLe occurs in two isomers, sLe^a and sLe^x (Fig. 8), and cancer cells arising from different organs tend to adhere to endothelium more strongly through one isomer over the other. Colon and pancreatic cancer cells adhere to E-selectin via sLe^a while lung and liver cancer cells adhere through sLe^x [159]. Expression of sLe is increased in patients with metastatic disease and is negatively correlated with patient survival [160–169] although this has been refuted [170]. This association with metastasis is the basis for clinical monitoring of sLe^a structures in cancers of the digestive tract. Screening of serum sLe^a (CA19-9) is part of the standard treatment regimen for colorectal cancer [171–173] and higher pre-operative serum CA19-9 levels predict colon cancer recurrence [174]. The CA19-9 antibody is specific for sLe^a and does not recognize the unsialylated Le^a structure [175]. In a cohort of 94 advanced colorectal cancer patients, greater sLe^a expression was positively correlated to hepatic metastasis [176], although this study failed to find a significant association in a similarly sized gastric cancer cohort. A retrospective examination of more than 300 colorectal cancers and their associated metastases found significantly higher sLe^a expression in metastases compared with the primary tumor [171], and a similar finding was reported for breast cancer [162]. In addition to sLe^a, sLe^x may serve as a prognostic indicator; sLe^x expression predicts outcome in prostate cancer cases after orchiectomy [177] and is also being investigated for use in breast cancer monitoring [178, 179].

A clear clinical correlation continues to prompt investigation of sLe antigens, however the regulation of these structures is far from understood. O-linked glycan synthesis requires the coordinated activity of multiple glycosyltransferases, and many of these are altered in carcinogenesis [19, 100]. Glycans are often truncated in cancer cells, due, in part, to incomplete synthesis, contributing to the expression of sLe [100, 173]. Evidence now suggests that the disruption or activation of a single glycosyltransferase may be sufficient to upregulate sLe structures. For instance, epigenetic silencing of ST6GalNAc-VI, as well as experimental reduction of this gene's expression, leads to the accumulation of sLe [173, 180]. ST6GalNAc-VI adds an α 2-6-linked sialic acid to GlcNAc, creating the di-sLe (a) structure, which predominates in normal epithelial cells. On the other hand, forced expression of a β 1-4 GalNAc transferase reduces sLe expression, and restores a more normal carbohydrate profile [181]. Cells with forced β 1-4 GalNAc transferase expression have reduced adhesion to human umbilical cord endothelial cells, and decreased metastasis *in vivo*. Additional factors within the tumor microenvironment likely play a part in sLe expression. Hypoxic conditions stimulate sLe upregulation through HIF-1 α signaling [182], while the hormone receptor status of certain cancers appears to influence sLe-E-selectin interactions [183].

Fucosyltransferase activity may also contribute to tumor-associated sLe expression [184–187], although the relative importance of this pathway is debated [173]. Dimitroff's group reported that expression of the Fut-3, Fut-6, or Fut-7 fucosyltransferases in prostate cancer cells was sufficient to stimulate sLe^x production and promote prostate cancer metastasis to bone and liver [185]. Several carriers of sLe structures were identified in this study including CD44, carcinoembryonic antigen, podocalyxin-like protein, and melanoma cell adhesion molecule [185]. Mucins, including Muc1, are also modified with sLe [188, 189]. Antisense strategies directed at Fut-3 reduce sLe expression as well as the number of hepatic metastases observed in mice [190, 191]. sLe structures mediate adhesion to the endothelium through their interactions with selectins on activated endothelial cells [192–195]. The physiologic relevance of tumor cell sLe structures in cancer progression has been confirmed by studies in which sLe/selectin interactions were perturbed. Experimental interventions that blocked sialylation of Lewis structures were effective in inhibiting tumor cell adhesion to both E-selectin-coated plates and endothelial monolayers [196, 197]. Pretreatment of mice with E-selectin peptide agonists decreased the number of metastases in a lung metastasis model [198], and forced liver-specific expression of E-selectin redirected melanoma cell metastasis from the lung to the liver [199]. Finally, E-selectin-deficient SCID mice developed fewer lung metastases in a xenograft colon cancer model [200]. This study also observed a higher number of circulating tumor cells in E-selectin-deficient mice, suggesting metastasis was inhibited at the endothelial binding step of the metastatic cascade.

Despite the established value of sLe as a cancer-associated biomarker, therapeutics targeting these structures have been relatively slow to develop. Cancer vaccines against sLe^a have yielded mixed results [201], even though experimental results support a role for sLe overexpression in stimulating natural killer cell responses [202]. Work by Esko's group demonstrated the utility of disaccharide decoy molecules in reducing overall sialylation and the expression of sLe antigens. Treatment of cells with decoy disaccharides inhibited: sLe^x expression; adhesion to selectin-coated plates; and metastasis to the lung in murine models [203–205], suggesting a possible therapeutic benefit. Additionally, several antibodies against sLe have been shown to be cancer specific and cytotoxic *in vivo*. For example, two monoclonal antibodies developed against sLe^a have demonstrated substantial antitumor effects in an *in vivo* colon cancer model [206]. Although further research is needed, these collective studies highlight the potential for targeting sLe structures in clinical treatment.

6 Tumor cell α 2-6 sialylation confers resistance to cell death

Much of the literature regarding tumor cell sialylation has centered on its role in cell adhesion, migration and invasion, but some studies also implicate sialylation in regulating cell death pathways. In particular, α 2-6-linked sialic acids may confer an apoptosis-resistant phenotype by modulating the activity of selected receptors and signaling mechanisms. One of the better-characterized functions for α 2-6-sialylation is an inhibitory effect on galectin-dependent apoptosis [36]. Many galectins, including gal-1, gal-3, and gal-9, bind to cell surface galactosides and induce cell death [207]. Each galectin exhibits specificity for certain galactosyl structures, and there is evidence that galectins may selectively bind to distinct glycoproteins. The mechanisms underlying galectin selectivity are still under investigation, although some of the documented binding partners for galectins include

integrins [38, 208, 209], EGFR [210], CD45 [211], and TRPV5 [20]. Galectins are secreted by immune (and other) cells, therefore α 2-6 sialylation on the tumor cell surface may protect tumor cells from the actions of infiltrating immune cells. However, the relationship between galectins and tumor cell α 2-6 sialylation is complex. Many tumor cells overexpress galectins [32], which raises the paradox of why a cancer cell would upregulate an apoptosis-inducing molecule. In fact, galectins have many different tumor-promoting activities; for example, intracellular forms of galectins have anti-apoptotic functions that are independent of cell glycosylation status, and some galectins amplify signaling by the ras oncogene [32, 212]. Thus, tumor cells that coordinately upregulate galectins and α 2-6 sialyltransferases would benefit from the pro-tumorigenic activities of intracellular galectins (that are carbohydrate-independent), while simultaneously acquiring resistance to the pro-apoptotic features of secreted galectins (that are carbohydrate-dependent and blocked by α 2-6 sialylation).

In tandem with inhibiting galectin-mediated apoptosis, α 2-6 sialylation enhances tumor cell survival by regulating the function of individual cell surface receptors. Lee *et al.* reported that treatment of colon tumor cells with ionizing radiation induced increased α 2-6 sialylation of the β 1 integrin as a secondary consequence of ST6Gal-I upregulation [76, 213]. In this system, α 2-6 sialylation of the β 1 integrin promoted cell adhesion to fibronectin and contributed to cell survival through the activation of paxillin and AKT [76]. Recently, two members of the TNFR death receptor family, Fas and TNFR1, were also identified as ST6Gal-I substrates, and it was shown that α 2-6 sialylation blocked apoptotic signaling by these receptors [47, 214]. Reduced Fas-mediated apoptosis is a well-established factor in tumor cell survival, and Fas expression is downregulated in many different tumor types [215–217]. However, in addition to decreased expression, Fas signaling cascades are disrupted in tumor cells, with Fas activation triggering pro-survival, rather than apoptotic, pathways [218]. These non-apoptotic functions of Fas contribute to tumor-promoting phenotypes [219–222]. Complete knockout of Fas in tumor cell xenografts prevented tumor growth, supporting the hypothesis that Fas is essential for some aspect of tumor cell survival or proliferation [223]. This newer concept is in agreement with many reports that some cancer cells express high levels of Fas, but are yet resistant to Fas-induced apoptosis [224–227].

Several studies have suggested that Fas apoptotic activity is inhibited by sialylation [228–230], although most of these did not define which type of sialic acid linkage is functionally important. In more recent work, forced overexpression or knockdown of ST6Gal-I caused altered α 2-6 sialylation of Fas (without affecting α 2-3 sialylation) [47]. Elevated α 2-6 sialylation of Fas by ST6Gal-I prevented apoptosis stimulated by both Fas-activating antibodies and FasL (the native ligand for Fas). Fas α 2-6 sialylation did not interfere with binding of the agonist, but rather inhibited formation of the death-inducing signaling complex, and also restrained Fas receptor internalization (Fig. 9). Intriguingly, there is evidence that plasma membrane-localized Fas receptors may send a pro-survival signal, whereas receptor internalization is important for induction of apoptosis [231]. Hence, the α 2-6 sialylation-dependent retention of Fas at the cell surface could serve as a switching mechanism responsible for diverting signaling away from apoptosis and toward survival. It isn't currently known why α 2-6 sialylation prevents Fas internalization, but it can be

speculated, based on information from other sialylated receptors, that Fas sialylation could regulate: (1) Fas receptor homotrimerization or higher order clustering, (2) tertiary conformation of the receptor, and/or (3) localization of the receptor to lipid raft microdomains.

Similar to Fas, ST6Gal-I-mediated α 2-6 sialylation of the TNFR1 death receptor inhibits apoptosis directed by the TNFR1 ligand, TNF α , although at present TNFR1 sialylation has only been evaluated in macrophages [214]. TNFR1 is expressed in epithelial cells, however neither the glycan composition, nor function, of TNFR1 glycans have been characterized in epithelial tumor cells. Nonetheless, the finding that ST6Gal-I-mediated sialylation blocks apoptotic signaling through three major pathways (galectins, TNFR1 and Fas), suggests that upregulated ST6Gal-I may facilitate tumor cell escape from immune surveillance. The ligands for TNFR1 and Fas (TNF α and FasL, respectively) are primarily expressed by immune cells, and immune cells are also a rich source of galectins. Furthermore, as noted previously, changes in sialylation likely affect tumor cell interactions with siglec-expressing immune cells. These findings underscore the need for elucidating the molecular mechanisms by which distinct tumor-associated sialoglycans or sialoproteins influence the host immune response. The paucity of studies on this topic is noteworthy, particularly given that it has long been believed that surface sialylation shields cancer cells from immune attack. Sialic acids mask antigenic sites on cells, thus weakening immunoreactivity, and sialic acids also protect cells against complement-mediated cell lysis [232]. In addition, loss of sialylation from the cell surface serves as an “eat-me” signal for phagocytes [233] suggesting that high sialylation levels on cancer cells may inhibit phagocytotic targeting by immune cells. These combined observations offer provocative clues that alterations in the profile of tumor cell sialoglycans may be a driving factor in immune escape.

7 Summary

A role for tumor cell sialylation in cancer progression has been presumed for many years, however mechanistic studies of this cell surface modification have been limited when compared with other areas of cancer cell biology. Particularly lacking are studies of: (1) signaling mechanisms that alter transcription or translation of sialylation-related enzymes, (2) sialyltransferase specificity for selected glycoprotein targets, (3) sialylation-dependent changes in glycoprotein structure (e.g., conformation and clustering), and (4) the effects of variant sialylation on the actions of glycan binding proteins. A better understanding of these molecular events is necessary for defining causal relationships between elevated sialylation and metastatic cell behaviors such as invasiveness, hematogenous dissemination, and apoptosis-resistance. The goal of this review was not to comprehensively overview the many reported changes in tumor sialoglycans but rather focus on a select number of examples for which there is substantive information regarding molecular mechanism. The elucidation of sialylation-dependent pathways that control distinct tumor cell responses holds promise for identifying important new diagnostic or prognostic markers, as well as targets for vaccine and drug development.

Acknowledgments

The authors are grateful for support from NIH grant R01CA84248, American Heart Association grant 7710013, and a grant from the Department of Defense CDMRP program, OC100141. Mr. Schultz is an NSF Predoctoral Fellow, and Ms. Swindall is an American Heart Association Predoctoral Fellow. Mr. Schultz and Ms. Swindall are members of the Howard Hughes Med-into-Grad predoctoral program.

References

1. Lloyd KO, Old LJ. Human monoclonal antibodies to glycolipids and other carbohydrate antigens: dissection of the humoral immune response in cancer patients. *Cancer Research*. 1989; 49:3445–3451. [PubMed: 2471585]
2. Sell S. Cancer-associated carbohydrates identified by monoclonal antibodies. *Human Pathology*. 1990; 21:1003–1019. [PubMed: 2210723]
3. Varki, A.; Kannagi, R.; Toole, BP. Glycosylation changes in cancer. In: Varki, A.; Cummings, RD.; Esko, JD.; Freeze, HH.; Stanley, P.; Bertozzi, CR.; Hart, GW.; Etzler, ME., editors. *Essentials of Glycobiology*. 2. New York: Cold Spring Harbor Laboratory Press; 2009. p. 580-670.
4. Hakomori S. Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. *Advances in Cancer Research*. 1989; 52:257–331. [PubMed: 2662714]
5. Zhou D, Levery SB, Hsu FF, Wang PG, Teneberg S, Almeida IC, Li Y, Xu H, Wang LX, Xia C, Ibrahim NK, Michael K. Immunologic mapping of glycomes: implications for cancer diagnosis and therapy. *Frontiers in Bioscience (Scholar Edition)*. 2011; 3:1520–1532. [PubMed: 21622287]
6. Reis CA, Osorio H, Silva L, Gomes C, David L. Alterations in glycosylation as biomarkers for cancer detection. *Journal of Clinical Pathology*. 2010; 63:322–329. [PubMed: 20354203]
7. Drake PM, Cho W, Li B, Prakobphol A, Johansen E, Anderson NL, et al. Sweetening the pot: adding glycosylation to the biomarker discovery equation. *Clinical Chemistry*. 2010; 56:223–236. [PubMed: 19959616]
8. Fuster MM, Esko JD. The sweet and sour of cancer: glycans as novel therapeutic targets. *Nature Reviews Cancer*. 2005; 5:526–542.
9. Petrescu AJ, Milac AL, Petrescu SM, Dwek RA, Wormald MR. Statistical analysis of the protein environment of N-glycosylation sites: implications for occupancy, structure, and folding. *Glycobiology*. 2004; 14:103–114. [PubMed: 14514716]
10. Sutton BJ, Phillips DC. The three-dimensional structure of the carbohydrate within the Fc fragment of immunoglobulin G. *Biochemical Society Transactions*. 1983; 11:130–132.
11. Mer G, Hietter H, Lefevre JF. Stabilization of proteins by glycosylation examined by NMR analysis of a fucosylated proteinase inhibitor. *Nature Structural Biology*. 1996; 3:45–53.
12. van Zuylen CW, de Beer T, Leeftang BR, Boelens R, Kaptein R, Kamerling JP, Vliegthart JF. Mobilities of the inner three core residues and the Man(alpha 1–6) branch of the glycan at Asn78 of the alpha-subunit of human chorionic gonadotropin are restricted by the protein. *Biochemistry*. 1998; 37:1933–1940. [PubMed: 9485320]
13. Lotan R, Raz A. Lectins in cancer cells. *Annals of the New York Academy of Sciences*. 1988; 551:385–396. [PubMed: 3072905]
14. Drickamer K, Taylor ME. Biology of animal lectins. *Annual Review of Cell Biology*. 1993; 9:237–264.
15. Perkel, J. Glycoproteomics: The sweet smell of we're-getting there. *Science Magazine*. 2011. Available at: http://www.sciencemag.org/site/products/1st_20110107.xhtml
16. Dall'Olio F, Chiricolo M. Sialyltransferases in cancer. *Glycoconjugate Journal*. 2001; 18:841–850. [PubMed: 12820717]
17. Varki NM, Varki A. Diversity in cell surface sialic acid presentations: implications for biology and disease. *Laboratory Investigation*. 2007; 87:851–857. [PubMed: 17632542]
18. Schauer R. Sialic acids as regulators of molecular and cellular interactions. *Current Opinion in Structural Biology*. 2009; 19:507–514. [PubMed: 19699080]
19. Cazet A, Julien S, Bobowski M, Burchell J, Delannoy P. Tumour-associated carbohydrate antigens in breast cancer. *Breast Cancer Research*. 2010; 12:204. [PubMed: 20550729]

20. Cha SK, Ortega B, Kurosu H, Rosenblatt KP, Kuro OM, Huang CL. Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:9805–9810. [PubMed: 18606998]
21. Isaev D, Isaeva E, Shatskih T, Zhao Q, Smits NC, Shworak NW, Khazipov R, Holmes GL. Role of extracellular sialic acid in regulation of neuronal and network excitability in the rat hippocampus. *Journal of Neuroscience*. 2007; 27:11587–11594. [PubMed: 17959801]
22. Rifat S, Kang TJ, Mann D, Zhang L, Puche AC, Stamatou NM, Goldblum SE, Brossmer R, Cross AS. Expression of sialyltransferase activity on intact human neutrophils. *Journal of Leukocyte Biology*. 2008; 84:1075–1081. [PubMed: 18664529]
23. Jones MB, Nasirikenari M, Lugade AA, Thanavala Y, Lau JT. Anti-inflammatory IgG production requires a functional P1 promoter in the ST6Gal-1 sialyltransferase gene. *Journal of Biological Chemistry*. 2012; 287:15365–15370. [PubMed: 22427662]
24. Harduin-Lepers A, Vallejo-Ruiz V, Krzewinski-Recchi MA, Samyn-Petit B, Julien S, Delannoy P. The human sialyltransferase family. *Biochimie*. 2001; 83:727–737. [PubMed: 11530204]
25. Uemura T, Shiozaki K, Yamaguchi K, Miyazaki S, Satomi S, Kato K, Sakuraba H, Miyagi T. Contribution of sialidase NEU1 to suppression of metastasis of human colon cancer cells through desialylation of integrin beta4. *Oncogene*. 2009; 28:1218–1229. [PubMed: 19151752]
26. Gangopadhyay A, Perera SP, Thomas P. Differential expression of alpha2,6-sialyltransferase in colon tumors recognized by a monoclonal antibody. *Hybridoma*. 1998; 17:117–123. [PubMed: 9627051]
27. Gessner P, Riedl S, Quentmaier A, Kemmner W. Enhanced activity of CMP-neuAc:Gal beta 1-4GlcNAc:alpha 2,6-sialyltransferase in metastasizing human colorectal tumor tissue and serum of tumor patients. *Cancer Letters*. 1993; 75:143–149. [PubMed: 8313349]
28. Dall'Olio F, Malagolini N, di Stefano G, Minni F, Marrano D, Serafini-Cessi F. Increased CMP-NeuAc:Gal beta 1,4GlcNAc-R alpha 2,6 sialyltransferase activity in human colorectal cancer tissues. *International Journal of Cancer*. 1989; 44:434–439.
29. Patel RY, Balaji PV. Identification of linkage-specific sequence motifs in sialyltransferases. *Glycobiology*. 2006; 16:108–116. [PubMed: 16207893]
30. Danguy A, Camby I, Kiss R. Galectins and cancer. *Biochimica et Biophysica Acta*. 2002; 1572:285–293. [PubMed: 12223276]
31. Nakahara S, Raz A. Biological modulation by lectins and their ligands in tumor progression and metastasis. *Anti-Cancer Agents in Medicinal Chemistry*. 2008; 8:22–36. [PubMed: 18220503]
32. Liu FT, Rabinovich GA. Galectins as modulators of tumour progression. *Nature Reviews Cancer*. 2005; 5:29–41.
33. He J, Baum LG. Galectin interactions with extracellular matrix and effects on cellular function. *Methods in Enzymology*. 2006; 417:247–256. [PubMed: 17132509]
34. Ochieng J, Furtak V, Lukyanov P. Extracellular functions of galectin-3. *Glycoconjugate Journal*. 2004; 19:527–535. [PubMed: 14758076]
35. Elola MT, Wolfenstein-Todel C, Troncoso MF, Vasta GR, Rabinovich GA. Galectins: matricellular glycan-binding proteins linking cell adhesion, migration, and survival. *Cellular and Molecular Life Sciences*. 2007; 64:1679–1700. [PubMed: 17497244]
36. Zhuo Y, Bellis SL. Emerging role of alpha2,6-sialic acid as a negative regulator of galectin binding and function. *Journal of Biological Chemistry*. 2010; 286:5935–5941. [PubMed: 21173156]
37. Hirabayashi J, Hashidate T, Arata Y, Nishi N, Nakamura T, Hirashima M, Urashima T, Oka T, Futai M, Muller WE, Yagi F, Kasai K. Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. *Biochimica et Biophysica Acta*. 2002; 1572:232–254. [PubMed: 12223272]
38. Zhuo Y, Chammas R, Bellis SL. Sialylation of beta1 integrins blocks cell adhesion to galectin-3 and protects cells against galectin-3-induced apoptosis. *Journal of Biological Chemistry*. 2008; 283:22177–22185. [PubMed: 18676377]
39. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. *Nature Reviews Immunology*. 2007; 7:255–266.

40. Varki A, Angata T. Siglecs—the major subfamily of I-type lectins. *Glycobiology*. 2006; 16:1R–27R. [PubMed: 16118287]
41. Jandus C, Simon HU, von Gunten S. Targeting siglecs—a novel pharmacological strategy for immuno- and glycotherapy. *Biochemical Pharmacology*. 2011; 82:323–332. [PubMed: 21658374]
42. Woodard-Grice AV, McBrayer AC, Wakefield J, Zhuo Y, Bellis SL. Proteolytic shedding of ST6Gal-I by BACE1 regulates the glycosylation and function of alpha4beta1 integrins. *Journal of Biological Chemistry*. 2008; 283:26364–26373. [PubMed: 18650447]
43. Pretzlaff RK, Xue VW, Rowin ME. Sialidase treatment exposes the beta1-integrin active ligand binding site on HL60 cells and increases binding to fibronectin. *Cell Adhesion and Communication*. 2000; 7:491–500. [PubMed: 11051459]
44. Amano M, Galvan M, He J, Baum LG. The ST6Gal I sialyltransferase selectively modifies *N*-glycans on CD45 to negatively regulate galectin-1-induced CD45 clustering, phosphatase modulation, and T cell death. *Journal of Biological Chemistry*. 2003; 278:7469–7475. [PubMed: 12499376]
45. Liu YC, Yen HY, Chen CY, Chen CH, Cheng PF, Juan YH, Khoo KH, Yu CJ, Yang PC, Hsu TL, Wong CH. Sialylation and fucosylation of epidermal growth factor receptor suppress its dimerization and activation in lung cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:11332–11337. [PubMed: 21709263]
46. Kitazume S, Imamaki R, Ogawa K, Komi Y, Futakawa S, Kojima S, Hashimoto Y, Marth JD, Paulson JC, Taniguchi N. Alpha2,6-sialic acid on platelet endothelial cell adhesion molecule (PECAM) regulates its homophilic interactions and downstream antiapoptotic signaling. *Journal of Biological Chemistry*. 2010; 285:6515–6521. [PubMed: 20048157]
47. Swindall AF, Bellis SL. Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. *Journal of Biological Chemistry*. 2011; 286:22982–22990. [PubMed: 21550977]
48. Gascoigne NR. T-cell differentiation: MHC class I's sweet tooth lost on maturity. *Current Biology*. 2002; 12:R99–R101. [PubMed: 11839290]
49. Daniels MA, Devine L, Miller JD, Moser JM, Lukacher AE, Altman JD, Kavathas P, Hogquist KA, Jameson SC. CD8 binding to MHC class I molecules is influenced by T cell maturation and glycosylation. *Immunity*. 2001; 15:1051–1061. [PubMed: 11754824]
50. Moody AM, Chui D, Reche PA, Priatel JJ, Marth JD, Reinherz EL. Developmentally regulated glycosylation of the CD8alphabeta coreceptor stalk modulates ligand binding. *Cell*. 2001; 107:501–512. [PubMed: 11719190]
51. Boscher C, Dennis JW, Nabi IR. Glycosylation, galectins and cellular signaling. *Current Opinion in Cell Biology*. 2011; 23:383–392. [PubMed: 21616652]
52. Grigorian A, Torossian S, Demetriou M. T-cell growth, cell surface organization, and the galectin-glycoprotein lattice. *Immunological Reviews*. 2009; 230:232–246. [PubMed: 19594640]
53. Garner OB, Baum LG. Galectin-glycan lattices regulate cell-surface glycoprotein organization and signalling. *Biochemical Society Transactions*. 2008; 36:1472–1477. [PubMed: 19021578]
54. Lau KS, Partridge EA, Grigorian A, Silvescu CI, Reinhold VN, Demetriou M, Dennis JW. Complex *N*-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. *Cell*. 2007; 129:123–134. [PubMed: 17418791]
55. Lin S, Kemmner W, Grigull S, Schlag PM. Cell surface alpha 2,6 sialylation affects adhesion of breast carcinoma cells. *Experimental Cell Research*. 2002; 276:101–110. [PubMed: 11978012]
56. Seales EC, Jurado GA, Brunson BA, Wakefield JK, Frost AR, Bellis SL. Hypersialylation of beta1 integrins, observed in colon adenocarcinoma, may contribute to cancer progression by up-regulating cell motility. *Cancer Research*. 2005; 65:4645–4652. [PubMed: 15930282]
57. Christie DR, Shaikh FM, Lucas JA IV, Lucas JA III, Bellis SL. ST6Gal-1 expression in ovarian cancer cells promotes an invasive phenotype by altering integrin glycosylation and function. *Journal of Ovarian Research*. 2008; 1:3. [PubMed: 19014651]
58. Zhu Y, Srivatana U, Ullah A, Gagneja H, Berenson CS, Lance P. Suppression of a sialyltransferase by antisense DNA reduces invasiveness of human colon cancer cells *in vitro*. *Biochimica et Biophysica Acta*. 2001; 1536:148–160. [PubMed: 11406350]

59. Morgenthaler J, Kemmner W, Brossmer R. Sialic acid dependent cell adhesion to collagen IV correlates with *in vivo* tumorigenicity of the human colon carcinoma sublines HCT116, HCT116a and HCT116b. *Biochemical and Biophysical Research Communications*. 1990; 171:860–866. [PubMed: 2119581]
60. Reddy BV, Kalraiya RD. Sialylated beta1,6 branched N-oligosaccharides modulate adhesion, chemotaxis and motility of melanoma cells: Effect on invasion and spontaneous metastasis properties. *Biochimica et Biophysica Acta*. 2006; 1760:1393–1402. [PubMed: 16806716]
61. Collard JG, Schijven JF, Bikker A, La Riviere G, Bolscher JG, Roos E. Cell surface sialic acid and the invasive and metastatic potential of T-cell hybridomas. *Cancer Research*. 1986; 46:3521–3527. [PubMed: 3486712]
62. Harvey BE, Toth CA, Wagner HE, Steele GD Jr, Thomas P. Sialyltransferase activity and hepatic tumor growth in a nude mouse model of colorectal cancer metastases. *Cancer Research*. 1992; 52:1775–1779. [PubMed: 1312899]
63. Bresalier RS, Rockwell RW, Dahiya R, Duh QY, Kim YS. Cell surface sialoprotein alterations in metastatic murine colon cancer cell lines selected in an animal model for colon cancer metastasis. *Cancer Research*. 1990; 50:1299–1307. [PubMed: 2297775]
64. Chiang CH, Wang CH, Chang HC, More SV, Li WS, Hung WC. A novel sialyltransferase inhibitor AL10 suppresses invasion and metastasis of lung cancer cells by inhibiting integrin-mediated signaling. *Journal of Cellular Physiology*. 2010; 223:492–499. [PubMed: 20112294]
65. Chen JY, Tang YA, Huang SM, Juan HF, Wu LW, Sun YC, Wang SC, Wu KW, Balraj G, Chang TT, Li WS, Cheng HC, Wang YC. A novel sialyltransferase inhibitor suppresses FAK/paxillin signaling and cancer angiogenesis and metastasis pathways. *Cancer Research*. 2011; 71:473–483. [PubMed: 21224350]
66. Drinnan NB, Halliday J, Ramsdale T. Inhibitors of sialyltransferases: potential roles in tumor growth and metastasis. *Mini Reviews in Medicinal Chemistry*. 2003; 3:501–517. [PubMed: 12871154]
67. Carcel-Trullols J, Stanley JS, Saha R, Shaaf S, Bendre MS, Monzavi-Karbassi B, Suva LJ, Kieber-Emmons T. Characterization of the glycosylation profile of the human breast cancer cell line, MDA-231, and a bone colonizing variant. *International Journal of Oncology*. 2006; 28:1173–1183. [PubMed: 16596233]
68. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, Minn AJ, van de Vijver MJ, Gerald WL, Foekens JA, Massague J. Genes that mediate breast cancer metastasis to the brain. *Nature*. 2009; 459:1005–1009. [PubMed: 19421193]
69. Bellis SL. Variant glycosylation: an underappreciated regulatory mechanism for beta1 integrins. *Biochimica et Biophysica Acta*. 2004; 1663:52–60. [PubMed: 15157607]
70. Gu J, Taniguchi N. Regulation of integrin functions by N-glycans. *Glycoconjugate Journal*. 2004; 21:9–15. [PubMed: 15467392]
71. Isaji T, Kariya Y, Xu Q, Fukuda T, Taniguchi N, Gu J. Functional roles of the bisecting GlcNAc in integrin-mediated cell adhesion. *Methods in Enzymology*. 2010; 480:445–459. [PubMed: 20816221]
72. Janik ME, Litynska A, Vereecken P. Cell migration —the role of integrin glycosylation. *Biochimica et Biophysica Acta*. 2010; 1800:545–555. [PubMed: 20332015]
73. Guo HB, Lee I, Bryan BT, Pierce M. Deletion of mouse embryo fibroblast N-acetylglucosaminyltransferase V stimulates alpha5beta1 integrin expression mediated by the protein kinase C signaling pathway. *Journal of Biological Chemistry*. 2005; 280:8332–8342. [PubMed: 15615721]
74. Seales EC, Jurado GA, Singhal A, Bellis SL. Ras oncogene directs expression of a differentially sialylated, functionally altered beta1 integrin. *Oncogene*. 2003; 22:7137–7145. [PubMed: 14562042]
75. Shaikh FM, Seales EC, Clem WC, Hennessy KM, Zhuo Y, Bellis SL. Tumor cell migration and invasion are regulated by expression of variant integrin glycoforms. *Experimental Cell Research*. 2008; 314:2941–2950. [PubMed: 18703050]

76. Lee M, Park JJ, Lee YS. Adhesion of ST6Gal I-mediated human colon cancer cells to fibronectin contributes to cell survival by integrin beta1-mediated paxillin and AKT activation. *Oncology Reports*. 2010; 23:757–761. [PubMed: 20127017]
77. Dalziel M, Dall'Olio F, Mungul A, Piller V, Piller F. Ras oncogene induces beta-galactoside alpha2,6-sialyl-transferase (ST6Gal I) via a RalGEF-mediated signal to its housekeeping promoter. *European Journal of Biochemistry*. 2004; 271:3623–3634. [PubMed: 15355339]
78. Easton EW, Bolscher JG, van den Eijnden DH. Enzymatic amplification involving glycosyltransferases forms the basis for the increased size of asparagine-linked glycans at the surface of NIH 3T3 cells expressing the *N*-Ras proto-oncogene. *Journal of Biological Chemistry*. 1991; 266:21674–21680. [PubMed: 1657976]
79. Le Marer N, Laudet V, Svensson EC, Cazlaris H, Van Hille B, Lagrou C, Stehelin D, Montreuil J, Verbert A, Delannoy P. The *c*-Ha-Ras oncogene induces increased expression of beta-galactoside alpha-2, 6-sialyltransferase in rat fibroblast (FR3T3) cells. *Glycobiology*. 1992; 2:49–56. [PubMed: 1550989]
80. Seales EC, Shaikh FM, Woodard-Grice AV, Aggarwal P, McBrayer AC, Hennessy KM, Bellis SL. A protein kinase C/Ras/ERK signaling pathway activates myeloid fibronectin receptors by altering beta1 integrin sialylation. *Journal of Biological Chemistry*. 2005; 280:37610–37615. [PubMed: 16157583]
81. Semel AC, Seales EC, Singhal A, Eklund EA, Colley KJ, Bellis SL. Hyposialylation of integrins stimulates the activity of myeloid fibronectin receptors. *Journal of Biological Chemistry*. 2002; 277:32830–32836. [PubMed: 12091385]
82. Pohech E, Litynska A, Amoresano A, Casbarra A. Glycosylation profile of integrin alpha 3 beta 1 changes with melanoma progression. *Biochimica et Biophysica Acta*. 2003; 1643:113–123. [PubMed: 14654234]
83. Isaji T, Sato Y, Fukuda T, Gu J. *N*-glycosylation of the I-like domain of beta1 integrin is essential for beta1 integrin expression and biological function: identification of the minimal *N*-glycosylation requirement for alpha5beta1. *Journal of Biological Chemistry*. 2009; 284:12207–12216. [PubMed: 19261610]
84. Akiyama SK, Yamada SS, Yamada KM. Analysis of the role of glycosylation of the human fibronectin receptor. *Journal of Biological Chemistry*. 1989; 264:18011–18018. [PubMed: 2530217]
85. Zheng M, Fang H, Hakomori S. Functional role of *N*-glycosylation in alpha 5 beta 1 integrin receptor. De-*N*-glycosylation induces dissociation or altered association of alpha 5 and beta 1 subunits and concomitant loss of fibronectin binding activity. *Journal of Biological Chemistry*. 1994; 269:12325–12331. [PubMed: 7512965]
86. Liu Y, Pan D, Bellis SL, Song Y. Effect of altered glycosylation on the structure of the I-like domain of beta1 integrin: a molecular dynamics study. *Proteins*. 2008; 73:989–1000. [PubMed: 18536010]
87. Hedlund M, Ng E, Varki A, Varki NM. Alpha 2-6-linked sialic acids on *N*-glycans modulate carcinoma differentiation *in vivo*. *Cancer Research*. 2008; 68:388–394. [PubMed: 18199532]
88. Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. *Nature Genetics*. 2003; 33:49–54. [PubMed: 12469122]
89. Barkan D, El Touny LH, Michalowski AM, Smith JA, Chu I, Davis AS, Webster JD, Hoover S, Simpson RM, Gauldie J, Green JE. Metastatic growth from dormant cells induced by a col-I-enriched fibrotic environment. *Cancer Research*. 2010; 70:5706–5716. [PubMed: 20570886]
90. Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Medicine*. 2006; 4:38. [PubMed: 17190588]
91. Sidani M, Wyckoff J, Xue C, Segall JE, Condeelis J. Probing the microenvironment of mammary tumors using multiphoton microscopy. *Journal of Mammary Gland Biology and Neoplasia*. 2006; 11:151–163. [PubMed: 17106644]
92. Yoshimura K, Meckel KF, Laird LS, Chia CY, Park JJ, Olino KL, Tsunedomi R, Harada T, Iizuka N, Hazama S, Kato Y, Keller JW, Thompson JM, Chang F, Romer LH, Jain A, Iacobuzio-

- Donahue C, Oka M, Pardoll DM, Schulick RD. Integrin alpha2 mediates selective metastasis to the liver. *Cancer Research*. 2009; 69:7320–7328. [PubMed: 19738067]
93. Liang F, Seyrantepe V, Landry K, Ahmad R, Ahmad A, Stamos NM, Pshezhetsky AV. Monocyte differentiation up-regulates the expression of the lysosomal sialidase, Neu1, and triggers its targeting to the plasma membrane via major histocompatibility complex class II-positive compartments. *Journal of Biological Chemistry*. 2006; 281:27526–27538. [PubMed: 16835219]
 94. Hinek A, Pshezhetsky AV, von Itzstein M, Starcher B. Lysosomal sialidase (neuraminidase-1) is targeted to the cell surface in a multiprotein complex that facilitates elastic fiber assembly. *Journal of Biological Chemistry*. 2006; 281:3698–3710. [PubMed: 16314420]
 95. Sawada M, Moriya S, Saito S, Shineha R, Satomi S, Yamori T, Tsuruo T, Kannagi R, Miyagi T. Reduced sialidase expression in highly metastatic variants of mouse colon adenocarcinoma 26 and retardation of their metastatic ability by sialidase overexpression. *International Journal of Cancer*. 2002; 97:180–185.
 96. Miyagi T, Sato K, Hata K, Taniguchi S. Metastatic potential of transformed rat 3Y1 cell lines is inversely correlated with lysosomal-type sialidase activity. *FEBS Letters*. 1994; 349:255–259. [PubMed: 8050577]
 97. Springer GF. T and Tn, general carcinoma autoantigens. *Science*. 1984; 224:1198–1206. [PubMed: 6729450]
 98. Miles DW, Happerfield LC, Smith P, Gillibrand R, Bobrow LG, Gregory WM, Rubens RD. Expression of sialyl-Tn predicts the effect of adjuvant chemotherapy in node-positive breast cancer. *British Journal of Cancer*. 1994; 70:1272–1275. [PubMed: 7981088]
 99. Davidson B, Gotlieb WH, Ben-Baruch G, Kopolovic J, Goldberg I, Nesland JM, Berner A, Bjamer A, Bryne M. Expression of carbohydrate antigens in advanced-stage ovarian carcinomas and their metastases—a clinicopathologic study. *Gynecologic Oncology*. 2000; 77:35–43. [PubMed: 10739688]
 100. Brockhausen I. Pathways of *O*-glycan biosynthesis in cancer cells. *Biochimica et Biophysica Acta*. 1999; 1473:67–95. [PubMed: 10580130]
 101. Taylor-Papadimitriou J, Burchell JM, Plunkett T, Graham R, Correa I, Miles D, Smith M. MUC1 and the immunobiology of cancer. *Journal of Mammary Gland Biology and Neoplasia*. 2002; 7:209–221. [PubMed: 12463741]
 102. Ikeda Y, Kuwano H, Baba K, Ikebe M, Matsushima T, Adachi Y, Mori M, Sugimachi K. Expression of Sialyl-Tn antigens in normal squamous epithelium, dysplasia, and squamous cell carcinoma in the esophagus. *Cancer Research*. 1993; 53:1706–1708. [PubMed: 8453646]
 103. Itzkowitz S, Kjeldsen T, Frieri A, Hakomori S, Yang US, Kim YS. Expression of Tn, sialosyl Tn, and T antigens in human pancreas. *Gastroenterology*. 1991; 100:1691–1700. [PubMed: 1850375]
 104. Itzkowitz SH, Yuan M, Montgomery CK, Kjeldsen T, Takahashi HK, Bigbee WL, Kim YS. Expression of Tn, sialosyl-Tn, and T antigens in human colon cancer. *Cancer Research*. 1989; 49:197–204. [PubMed: 2908846]
 105. Schuessler MH, Pintado S, Welt S, Real FX, Xu M, Melamed MR, Lloyd KO, Oettgen HF. Blood group and blood-group-related antigens in normal pancreas and pancreas cancer: enhanced expression of precursor type 1, Tn and sialyl-Tn in pancreas cancer. *International Journal of Cancer*. 1991; 47:180–187.
 106. Inoue M, Ogawa H, Tanizawa O, Kobayashi Y, Tsujimoto M, Tsujimura T. Immunodetection of sialyl-Tn antigen in normal, hyperplastic and cancerous tissues of the uterine endometrium. *Virchows Archiv A, Pathological Anatomy and Histopathology*. 1991; 418:157–162.
 107. Inoue M, Ton SM, Ogawa H, Tanizawa O. Expression of Tn and sialyl-Tn antigens in tumor tissues of the ovary. *American Journal of Clinical Pathology*. 1991; 96:711–716. [PubMed: 1720922]
 108. Werther JL, Rivera-MacMurray S, Bruckner H, Tatematsu M, Itzkowitz SH. Mucin-associated sialosyl-Tn antigen expression in gastric cancer correlates with an adverse outcome. *British Journal of Cancer*. 1994; 69:613–616. [PubMed: 8123499]
 109. Kjeldsen T, Clausen H, Hirohashi S, Ogawa T, Iijima H, Hakomori S. Preparation and characterization of monoclonal antibodies directed to the tumor-associated *O*-linked sialosyl-2-6

- alpha-*N*-acetylgalactosaminyl (sialosyl-Tn) epitope. *Cancer Research*. 1988; 48:2214–2220. [PubMed: 2450649]
110. Zhang S, Zhang HS, Reuter VE, Slovin SF, Scher HI, Livingston PO. Expression of potential target antigens for immunotherapy on primary and metastatic prostate cancers. *Clinical Cancer Research*. 1998; 4:295–302. [PubMed: 9516914]
 111. Gilewski TA, Ragupathi G, Dickler M, Powell S, Bhuta S, Panageas K, Koganty RR, Chin-Eng J, Hudis C, Norton L, Houghton AN, Livingston PO. Immunization of high-risk breast cancer patients with clustered sTn-KLH conjugate plus the immunologic adjuvant QS-21. *Clinical Cancer Research*. 2007; 13:2977–2985. [PubMed: 17504999]
 112. Julien S, Picco G, Sewell R, Vercoutter-Edouart AS, Tarp M, Miles D, Clausen H, Taylor-Papadimitriou J, Burchell JM. Sialyl-Tn vaccine induces antibody-mediated tumour protection in a relevant murine model. *British Journal of Cancer*. 2009; 100:1746–1754. [PubMed: 19436292]
 113. Ibrahim NK, Murray JL. Clinical development of the STn-KLH vaccine (Theratope). *Clinical Breast Cancer*. 2003; 3(Suppl 4):S139–S143. [PubMed: 12620151]
 114. Kobayashi H, Terao T, Kawashima Y. Circulating serum STN antigen as a prognostic marker in epithelial ovarian cancer. *Gan to Kagaku Ryoho*. 1991; 18:1651–1655. [PubMed: 1678592]
 115. Takahashi I, Maehara Y, Kusumoto T, Kohnoe S, Kakeji Y, Baba H, Sugimachi K. Combined evaluation of preoperative serum sialyl-Tn antigen and carcinoembryonic antigen levels is prognostic for gastric cancer patients. *British Journal of Cancer*. 1994; 69:163–166. [PubMed: 8286201]
 116. Kinney AY, Sahin A, Vernon SW, Frankowski RF, Annegers JF, Hortobagyi GN, Buzdar AU, Frye DK, Dhingra K. The prognostic significance of sialyl-Tn antigen in women treated with breast carcinoma treated with adjuvant chemotherapy. *Cancer*. 1997; 80:2240–2249. [PubMed: 9404700]
 117. Imai J, Ghazizadeh M, Naito Z, Asano G. Immunohistochemical expression of T, Tn and sialyl-Tn antigens and clinical outcome in human breast carcinoma. *Anticancer Research*. 2001; 21:1327–1334. [PubMed: 11396208]
 118. Leivonen M, Nordling S, Lundin J, von Boguslawski K, Haglund C. STn and prognosis in breast cancer. *Oncology*. 2001; 61:299–305. [PubMed: 11721177]
 119. Itzkowitz SH, Bloom EJ, Kokal WA, Modin G, Hakomori S, Kim YS. Sialosyl-Tn. A novel mucin antigen associated with prognosis in colorectal cancer patients. *Cancer*. 1990; 66:1960–1966. [PubMed: 2224793]
 120. Ma XC, Terata N, Kodama M, Jancic S, Hosokawa Y, Hattori T. Expression of sialyl-Tn antigen is correlated with survival time of patients with gastric carcinomas. *European Journal of Cancer*. 1993; 29A:1820–1823. [PubMed: 8260233]
 121. Therkildsen MH, Andersen LJ, Christensen M, Hansen HS, Schiodt T, Dabelsteen E. Salivary gland carcinomas: prognostic significance of simple mucin-type carbohydrate antigens. *Oral Oncology*. 1998; 34:44–51. [PubMed: 9659519]
 122. Lundin M, Nordling S, Roberts PJ, Lundin J, Carpelan-Holmstrom M, von Boguslawsky K, Haglund C. Sialyl Tn is a frequently expressed antigen in colorectal cancer: no correlation with patient prognosis. *Oncology*. 1999; 57:70–76. [PubMed: 10394128]
 123. Miles D, Papazisis K. Rationale for the clinical development of STn-KLH (Theratope) and anti-MUC-1 vaccines in breast cancer. *Clinical Breast Cancer*. 2003; 3(Suppl 4):S134–S138. [PubMed: 12620150]
 124. Sewell R, Backstrom M, Dalziel M, Gschmeissner S, Karlsson H, Noll T, Gatgens J, Clausen H, Hansson GC, Burchell J, Taylor-Papadimitriou J. The ST6GalNAc-I sialyltransferase localizes throughout the Golgi and is responsible for the synthesis of the tumor-associated sialyl-Tn *O*-glycan in human breast cancer. *Journal of Biological Chemistry*. 2006; 281:3586–3594. [PubMed: 16319059]
 125. Ju T, Lanneau GS, Gautam T, Wang Y, Xia B, Stowell SR, Willard MT, Wang W, Xia JY, Zuna RE, Laszik Z, Benbrook DM, Hanigan MH, Cummings RD. Human tumor antigens Tn and sialyl Tn arise from mutations in *Cosmc*. *Cancer Research*. 2008; 68:1636–1646. [PubMed: 18339842]

126. Kobayashi H, Terao T, Kawashima Y. Clinical evaluation of circulating serum sialyl Tn antigen levels in patients with epithelial ovarian cancer. *Journal of Clinical Oncology*. 1991; 9:983–987. [PubMed: 1851822]
127. Ozaki H, Matsuzaki H, Ando H, Kaji H, Nakanishi H, Ikehara Y, Narimatsu H. Enhancement of metastatic ability by ectopic expression of ST6GalNAcI on a gastric cancer cell line in a mouse model. *Clinical & Experimental Metastasis*. 2012; 29:229–238. [PubMed: 22228572]
128. Julien S, Lagadec C, Krzewinski-Recchi MA, Courtand G, Le Bourhis X, Delannoy P. Stable expression of sialyl-Tn antigen in T47-D cells induces a decrease of cell adhesion and an increase of cell migration. *Breast Cancer Research and Treatment*. 2005; 90:77–84. [PubMed: 15770530]
129. Julien S, Adriaenssens E, Ottenberg K, Furlan A, Courtand G, Vercoutter-Edouart AS, Hanisch FG, Delannoy P, Le Bourhis X. ST6GalNAc I expression in MDA-MB-231 breast cancer cells greatly modifies their *O*-glycosylation pattern and enhances their tumorigenicity. *Glycobiology*. 2006; 16:54–64. [PubMed: 16135558]
130. Pinho S, Marcos NT, Ferreira B, Carvalho AS, Oliveira MJ, Santos-Silva F, Harduin-Lepers A, Reis CA. Biological significance of cancer-associated sialyl-Tn antigen: modulation of malignant phenotype in gastric carcinoma cells. *Cancer Letters*. 2007; 249:157–170. [PubMed: 16965854]
131. Julien S, Krzewinski-Recchi MA, Harduin-Lepers A, Gouyer V, Huet G, Le Bourhis X, Delannoy P. Expression of sialyl-Tn antigen in breast cancer cells transfected with the human CMP-Neu5Ac: GalNAc alpha2,6-sialyltransferase (ST6GalNAc I) cDNA. *Glycoconjugate Journal*. 2001; 18:883–893. [PubMed: 12820722]
132. Marcos NT, Bennett EP, Gomes J, Magalhaes A, Gomes C, David L, Dar I, Jeanneau C, DeFrees S, Krstrup D, Vogel LK, Kure EH, Burchell J, Taylor-Papadimitriou J, Clausen H, Mandel U, Reis CA. ST6GalNAc-I controls expression of sialyl-Tn antigen in gastrointestinal tissues. *Frontiers in Bioscience (Elite Edition)*. 2011; 3:1443–1455. [PubMed: 21622148]
133. Marcos NT, Pinho S, Grandela C, Cruz A, Samyn-Petit B, Harduin-Lepers A, Almeida R, Silva F, Morais V, Costa J, Kihlberg J, Clausen H, Reis CA. Role of the human ST6GalNAc-I and ST6GalNAc-II in the synthesis of the cancer-associated sialyl-Tn antigen. *Cancer Research*. 2004; 64:7050–7057. [PubMed: 15466199]
134. Kono M, Tsuda T, Ogata S, Takashima S, Liu H, Hamamoto T, Itzkowitz SH, Nishimura S, Tsuji S. Redefined substrate specificity of ST6GalNAc II: a second candidate sialyl-Tn synthase. *Biochemical and Biophysical Research Communications*. 2000; 272:94–97. [PubMed: 10872809]
135. Schneider F, Kemmner W, Haensch W, Franke G, Gretscher S, Karsten U, Schlag PM. Overexpression of sialyltransferase CMP-sialic acid:Galbeta1,3GalNAc-R alpha6-sialyltransferase is related to poor patient survival in human colorectal carcinomas. *Cancer Research*. 2001; 61:4605–4611. [PubMed: 11389097]
136. Ju T, Otto VI, Cummings RD. The Tn antigen-structural simplicity and biological complexity. *Angewandte Chemie (International Ed in English)*. 2011; 50:1770–1791. [PubMed: 21259410]
137. Wang Y, Ju T, Ding X, Xia B, Wang W, Xia L, He M, Cummings RD. Cosmc is an essential chaperone for correct protein *O*-glycosylation. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:9228–9233. [PubMed: 20439703]
138. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nature Reviews Cancer*. 2004; 4:45–60.
139. Van Elssen CH, Clausen H, Germeraad WT, Bennet EP, Menheere PP, Bos GM, Vanderlocht J. Flow cytometry-based assay to evaluate human serum MUC1-Tn antibodies. *Journal of Immunological Methods*. 2010; 365:87–94. [PubMed: 21194532]
140. Ligtenberg MJ, Buijs F, Vos HL, Hilken J. Suppression of cellular aggregation by high levels of episialin. *Cancer Research*. 1992; 52:2318–2324. [PubMed: 1559234]
141. Wesseling J, van der Valk SW, Hilken J. A mechanism for inhibition of E-cadherin-mediated cell-cell adhesion by the membrane-associated mucin episialin/MUC1. *Molecular Biology of the Cell*. 1996; 7:565–577. [PubMed: 8730100]

142. Burdick MD, Harris A, Reid CJ, Iwamura T, Hollingsworth MA. Oligosaccharides expressed on MUC1 produced by pancreatic and colon tumor cell lines. *Journal of Biological Chemistry*. 1997; 272:24198–24202. [PubMed: 9305871]
143. Gunthert U, Hofmann M, Rudy W, Reber S, Zoller M, Haussmann I, Matzku S, Wenzel A, Ponta H, Herrlich P. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell*. 1991; 65:13–24. [PubMed: 1707342]
144. Hofmann M, Rudy W, Zoller M, Tolg C, Ponta H, Herrlich P, Gunthert U. CD44 splice variants confer metastatic behavior in rats: homologous sequences are expressed in human tumor cell lines. *Cancer Research*. 1991; 51:5292–5297. [PubMed: 1717145]
145. Wai PY, Kuo PC. The role of osteopontin in tumor metastasis. *Journal of Surgical Research*. 2004; 121:228–241. [PubMed: 15501463]
146. Clement M, Rocher J, Loirand G, Le Pendu J. Expression of sialyl-Tn epitopes on beta1 integrin alters epithelial cell phenotype, proliferation and haptotaxis. *Journal of Cell Science*. 2004; 117:5059–5069. [PubMed: 15383613]
147. Ogata S, Maimonis PJ, Itzkowitz SH. Mucins bearing the cancer-associated sialosyl-Tn antigen mediate inhibition of natural killer cell cytotoxicity. *Cancer Research*. 1992; 52:4741–4746. [PubMed: 1511439]
148. Brinkman-Van der Linden EC, Varki A. New aspects of siglec binding specificities, including the significance of fucosylation and of the sialyl-Tn epitope. Sialic acid-binding immunoglobulin superfamily lectins. *Journal of Biological Chemistry*. 2000; 275:8625–8632. [PubMed: 10722702]
149. Patel N, Brinkman-Van der Linden EC, Altmann SW, Gish K, Balasubramanian S, Timans JC, Peterson D, Bell MP, Bazan JF, Varki A, Kastelein RA. OB-BP1/Siglec-6. A leptin- and sialic acid-binding protein of the immunoglobulin superfamily. *Journal of Biological Chemistry*. 1999; 274:22729–22738. [PubMed: 10428856]
150. Barbera-Guillem E, May KF Jr, Nyhus JK, Nelson MB. Promotion of tumor invasion by cooperation of granulocytes and macrophages activated by anti-tumor antibodies. *Neoplasia*. 1999; 1:453–460. [PubMed: 10933061]
151. Barbera-Guillem E, Nyhus JK, Wolford CC, Friece CR, Sampsel JW. Vascular endothelial growth factor secretion by tumor-infiltrating macrophages essentially supports tumor angiogenesis, and IgG immune complexes potentiate the process. *Cancer Research*. 2002; 62:7042–7049. [PubMed: 12460925]
152. Miles D, Roche H, Martin M, Perren TJ, Cameron DA, Glaspy J, Dodwell D, Parker J, Mayordomo J, Tres A, Murray JL, Ibrahim NK. Phase III multicenter clinical trial of the sialyl-TN (STn)-keyhole limpet hemocyanin (KLH) vaccine for metastatic breast cancer. *The Oncologist*. 2011; 16:1092–1100. [PubMed: 21572124]
153. Geng Y, Marshall JR, King MR. Glycomechanics of the metastatic cascade: tumor cell-endothelial cell interactions in the circulation. *Annals of Biomedical Engineering*. 2011; 40:790–805. [PubMed: 22101756]
154. Laubli H, Borsig L. Selectins promote tumor metastasis. *Seminars in Cancer Biology*. 2010; 20:169–177. [PubMed: 20452433]
155. Croce MV, Isla-Larrain M, Rabassa ME, Demichelis S, Colussi AG, Crespo M, Lacunza E, Segal-Eiras A. Lewis x is highly expressed in normal tissues: a comparative immunohistochemical study and literature revision. *Pathology and Oncology Research*. 2007; 13:130–138. [PubMed: 17607374]
156. Khabaz MN, McClure J, McClure S, Stoddart RW. Glycophenotype of prostatic carcinomas. *Folia Histochemica et Cytobiologica*. 2010; 48:637–645. [PubMed: 21478109]
157. Renkonen J, Paavonen T, Renkonen R. Endothelial and epithelial expression of sialyl Lewis(x) and sialyl Lewis(a) in lesions of breast carcinoma. *International Journal of Cancer*. 1997; 74:296–300.
158. Croce MV, Rabassa ME, Pereyra A, Segal-Eiras A. Differential expression of MUC1 and carbohydrate antigens in primary and secondary head and neck squamous cell carcinoma. *Head & Neck*. 2008; 30:647–657. [PubMed: 18302268]

159. Takada A, Ohmori K, Yoneda T, Tsuyuoka K, Hasegawa A, Kiso M, Kannagi R. Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. *Cancer Research*. 1993; 53:354–361. [PubMed: 7678075]
160. Matsuura N, Narita T, Mitsuoka C, Kimura N, Kannagi R, Imai T, Funahashi H, Takagi H. Increased level of circulating adhesion molecules in the sera of breast cancer patients with distant metastases. *Japanese Journal of Clinical Oncology*. 1997; 27:135–139. [PubMed: 9255266]
161. Nakagoe T, Fukushima K, Itoyanagi N, Ikuta Y, Oka T, Nagayasu T, Ayabe H, Hara S, Ishikawa H, Minami H. Expression of ABH/Lewis-related antigens as prognostic factors in patients with breast cancer. *Journal of Cancer Research and Clinical Oncology*. 2002; 128:257–264. [PubMed: 12029441]
162. Jeschke U, Mylonas I, Shabani N, Kunert-Keil C, Schindlbeck C, Gerber B, Friese K. Expression of sialyl lewis X, sialyl Lewis A, E-cadherin and cathepsin-D in human breast cancer: immunohistochemical analysis in mammary carcinoma *in situ*, invasive carcinomas and their lymph node metastasis. *Anticancer Research*. 2005; 25:1615–1622. [PubMed: 16033070]
163. Fukuoka K, Narita N, Saijo N. Increased expression of sialyl Lewis(x) antigen is associated with distant metastasis in lung cancer patients: immunohistochemical study on bronchofiberscopic biopsy specimens. *Lung Cancer*. 1998; 20:109–116. [PubMed: 9711529]
164. Tatsumi M, Watanabe A, Sawada H, Yamada Y, Shino Y, Nakano H. Immunohistochemical expression of the sialyl Lewis x antigen on gastric cancer cells correlates with the presence of liver metastasis. *Clinical & Experimental Metastasis*. 1998; 16:743–750. [PubMed: 10211987]
165. Baldus SE, Zirbes TK, Monig SP, Engel S, Monaca E, Rafiqpoor K, Hanisch FG, Hanski C, Thiele J, Pichlmaier H, Dienes HP. Histopathological subtypes and prognosis of gastric cancer are correlated with the expression of mucin-associated sialylated antigens: sialosyl-Lewis(a), sialosyl-Lewis(x) and sialosyl-Tn. *Tumour Biology*. 1998; 19:445–453. [PubMed: 9817972]
166. Nakamori S, Kameyama M, Imaoka S, Furukawa H, Ishikawa O, Sasaki Y, Izumi Y, Irimura T. Involvement of carbohydrate antigen sialyl Lewis(x) in colorectal cancer metastasis. *Diseases of the Colon & Rectum*. 1997; 40:420–431. [PubMed: 9106690]
167. Nakamori S, Nishihara S, Ikehara Y, Nagano H, Dono K, Sakon M, Narimatsu H, Monden M. Molecular mechanism involved in increased expression of sialyl Lewis antigens in ductal carcinoma of the pancreas. *Journal of Experimental & Clinical Cancer Research*. 1999; 18:425–432. [PubMed: 10606190]
168. Amado M, Carneiro F, Seixas M, Clausen H, Sobrinho-Simoes M. Dimeric sialyl-Le(x) expression in gastric carcinoma correlates with venous invasion and poor outcome. *Gastroenterology*. 1998; 114:462–470. [PubMed: 9496936]
169. Grabowski P, Mann B, Mansmann U, Lovin N, Foss HD, Berger G, Scherubl H, Riecken EO, Buhr HJ, Hanski C. Expression of SIALYL-Le(x) antigen defined by MAb AM-3 is an independent prognostic marker in colorectal carcinoma patients. *International Journal of Cancer*. 2000; 88:281–286.
170. Sozzani P, Arisio R, Porpiglia M, Benedetto C. Is Sialyl Lewis x antigen expression a prognostic factor in patients with breast cancer? *International Journal of Surgical Pathology*. 2008; 16:365–374. [PubMed: 18977761]
171. Nakayama T, Watanabe M, Katsumata T, Teramoto T, Kitajima M. Expression of sialyl Lewis(a) as a new prognostic factor for patients with advanced colorectal carcinoma. *Cancer*. 1995; 75:2051–2056. [PubMed: 7697593]
172. Ugorski M, Laskowska A. Sialyl Lewis(a): a tumor-associated carbohydrate antigen involved in adhesion and meta-static potential of cancer cells. *Acta Biochimica Polonica*. 2002; 49:303–311. [PubMed: 12362971]
173. Kannagi R. Carbohydrate antigen sialyl Lewis a—its pathophysiological significance and induction mechanism in cancer progression. *Chang Gung Medical Journal*. 2007; 30:189–209. [PubMed: 17760270]
174. Gebauer G, Muller-Ruchholtz W. Tumor marker concentrations in normal and malignant tissues of colorectal cancer patients and their prognostic relevance. *Anticancer Research*. 1997; 17:2731–2734. [PubMed: 9252706]

175. Bechtel B, Wand AJ, Wroblewski K, Koprowski H, Thurin J. Conformational analysis of the tumor-associated carbohydrate antigen 19-9 and its Lea blood group antigen component as related to the specificity of monoclonal antibody CO19-9. *Journal of Biological Chemistry*. 1990; 265:2028–2037. [PubMed: 2298737]
176. Isozaki H, Ohyama T, Mabuchi H. Expression of cell adhesion molecule CD44 and sialyl Lewis A in gastric carcinoma and colorectal carcinoma in association with hepatic metastasis. *International Journal of Oncology*. 1998; 13:935–942. [PubMed: 9772282]
177. Jorgensen T, Berner A, Kaalhus O, Tveter KJ, Danielsen HE, Bryne M. Up-regulation of the oligosaccharide sialyl LewisX: a new prognostic parameter in metastatic prostate cancer. *Cancer Research*. 1995; 55:1817–1819. [PubMed: 7728744]
178. Kurebayashi J, Nomura T, Hirono M, Okubo S, Udagawa K, Shiiki S, Ikeda M, Nakashima K, Tanaka K, Sonoo H. Combined measurement of serum sialyl Lewis X with serum CA15-3 in breast cancer patients. *Japanese Journal of Clinical Oncology*. 2006; 36:150–153. [PubMed: 16520359]
179. Fujita T, Murayama K, Hanamura T, Okada T, Ito T, Harada M, Komatsu A, Koyama H, Kanai T, Maeno K, Mochizuki Y, Hama Y, Ito K, Amano J, Fujimori M. CSLEX (sialyl Lewis X) is a useful tumor marker for monitoring of breast cancer patients. *Japanese Journal of Clinical Oncology*. 2011; 41:394–399. [PubMed: 20956388]
180. Tsuchida A, Okajima T, Furukawa K, Ando T, Ishida H, Yoshida A, Nakamura Y, Kannagi R, Kiso M. Synthesis of disialyl Lewis a (Le(a)) structure in colon cancer cell lines by a sialyltransferase, ST6GalNAc VI, responsible for the synthesis of alpha-series gangliosides. *Journal of Biological Chemistry*. 2003; 278:22787–22794. [PubMed: 12668675]
181. Kawamura YI, Kawashima R, Fukunaga R, Hirai K, Toyama-Sorimachi N, Tokuhara M, Shimizu T, Dohi T. Introduction of Sd(a) carbohydrate antigen in gastrointestinal cancer cells eliminates selectin ligands and inhibits metastasis. *Cancer Research*. 2005; 65:6220–6227. [PubMed: 16024623]
182. Kannagi R, Sakuma K, Miyazaki K, Lim KT, Yusa A, Yin J, Izawa M. Altered expression of glycan genes in cancers induced by epigenetic silencing and tumor hypoxia: clues in the ongoing search for new tumor markers. *Cancer Science*. 2009; 101:586–593. [PubMed: 20085584]
183. Julien S, Ivetic A, Grigoriadis A, QiZe D, Burford B, Sproviero D, Picco G, Gillett C, Papp SL, Schaffer L, Tutt A, Taylor-Papadimitriou J, Pinder SE, Burchell JM. Selectin ligand sialyl-Lewis x antigen drives metastasis of hormone-dependent breast cancers. *Cancer Research*. 2011; 71:7683–7693. [PubMed: 22025563]
184. Yamada N, Chung YS, Takatsuka S, Arimoto Y, Sawada T, Dohi T, Sowa M. Increased sialyl Lewis A expression and fucosyltransferase activity with acquisition of a high metastatic capacity in a colon cancer cell line. *British Journal of Cancer*. 1997; 76:582–587. [PubMed: 9303355]
185. Barthel SR, Wiese GK, Cho J, Opperman MJ, Hays DL, Siddiqui J, Pienta KJ, Furie B, Dimitroff CJ. Alpha 1,3 fucosyltransferases are master regulators of prostate cancer cell trafficking. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:19491–19496. [PubMed: 19889975]
186. Matsuura N, Narita T, Hiraiwa N, Hiraiwa M, Murai H, Iwase T, Funahashi H, Imai T, Takagi H, Kannagi R. Gene expression of fucosyl- and sialyl-transferases which synthesize sialyl Lewisx, the carbohydrate ligands for E-selectin, in human breast cancer. *International Journal of Oncology*. 1998; 12:1157–1164. [PubMed: 9538143]
187. Majuri ML, Niemela R, Tiisala S, Renkonen O, Renkonen R. Expression and function of alpha 2,3-sialyl- and alpha 1,3/1,4-fucosyltransferases in colon adenocarcinoma cell lines: role in synthesis of E-selectin counter-receptors. *International Journal of Cancer*. 1995; 63:551–559.
188. Matsushita Y, Cleary KR, Ota DM, Hoff SD, Irimura T. Sialyl-dimeric Lewis-X antigen expressed on mucin-like glycoproteins in colorectal cancer metastases. *Laboratory Investigation*. 1990; 63:780–791. [PubMed: 1979361]
189. Hanski C, Drechsler K, Hanisch FG, Sheehan J, Manske M, Ogorek D, Klussmann E, Hanski ML, Blank M, Xing PX, et al. Altered glycosylation of the MUC-1 protein core contributes to the colon carcinoma-associated increase of mucin-bound sialyl-Lewis(x) expression. *Cancer Research*. 1993; 53:4082–4088. [PubMed: 7689422]

190. Opolski A, Laskowska A, Madej J, Wietrzyk J, Klopocki A, Radzikowski C, Ugorski M. Metastatic potential of human CX-1 colon adenocarcinoma cells is dependent on the expression of sialosyl Le(a) antigen. *Clinical & Experimental Metastasis*. 1998; 16:673–681. [PubMed: 10211980]
191. Weston BW, Hiller KM, Mayben JP, Manousos GA, Bendt KM, Liu R, Cusack JC Jr. Expression of human alpha(1,3)fucosyltransferase antisense sequences inhibits selectin-mediated adhesion and liver metastasis of colon carcinoma cells. *Cancer Research*. 1999; 59:2127–2135. [PubMed: 10232599]
192. Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B. Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. *Science*. 1990; 250:1132–1135. [PubMed: 1701275]
193. Phillips ML, Nudelman E, Gaeta FC, Perez M, Singhal AK, Hakomori S, Paulson JC. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. *Science*. 1990; 250:1130–1132. [PubMed: 1701274]
194. Ben-David T, Sagi-Assif O, Meshel T, Lifshitz V, Yron I, Witz IP. The involvement of the sLe—a selectin ligand in the extravasation of human colorectal carcinoma cells. *Immunology Letters*. 2008; 116:218–224. [PubMed: 18191232]
195. Takada A, Ohmori K, Takahashi N, Tsuyuoka K, Yago A, Zenita K, Hasegawa A, Kannagi R. Adhesion of human cancer cells to vascular endothelium mediated by a carbohydrate antigen, sialyl Lewis A. *Biochemical and Biophysical Research Communications*. 1991; 179:713–719. [PubMed: 1716885]
196. Hsu CC, Lin TW, Chang WW, Wu CY, Lo WH, Wang PH, Tsai YC. Soyasaponin-I-modified invasive behavior of cancer by changing cell surface sialic acids. *Gynecologic Oncology*. 2005; 96:415–422. [PubMed: 15661230]
197. Shiozaki K, Yamaguchi K, Takahashi K, Moriya S, Miyagi T. Regulation of sialyl Lewis antigen expression in colon cancer cells by sialidase NEU4. *Journal of Biological Chemistry*. 2011; 286:21052–21061. [PubMed: 21521691]
198. OI, Otvos L, Kieber-Emmons T, Blaszczyk-Thurin M. Role of SA-Le(a) and E-selectin in metastasis assessed with peptide antagonist. *Peptides*. 2002; 23:999–1010. [PubMed: 12084533]
199. Biancone L, Araki M, Araki K, Vassalli P, Stamenkovic I. Redirection of tumor metastasis by expression of E-selectin *in vivo*. *The Journal of Experimental Medicine*. 1996; 183:581–587. [PubMed: 8627169]
200. Kohler S, Ullrich S, Richter U, Schumacher U. E-/P-selectins and colon carcinoma metastasis: first *in vivo* evidence for their crucial role in a clinically relevant model of spontaneous metastasis formation in the lung. *British Journal of Cancer*. 2010; 102:602–609. [PubMed: 20010946]
201. Ragupathi G, Damani P, Srivastava G, Srivastava O, Sucheck SJ, Ichikawa Y, Livingston PO. Synthesis of sialyl Lewis(a) (sLe (a), CA19-9) and construction of an immunogenic sLe(a) vaccine. *Cancer Immunology, Immunotherapy*. 2009; 58:1397–1405. [PubMed: 19190907]
202. Ohyama C, Tsuboi S, Fukuda M. Dual roles of sialyl Lewis X oligosaccharides in tumor metastasis and rejection by natural killer cells. *EMBO Journal*. 1999; 18:1516–1525. [PubMed: 10075923]
203. Fuster MM, Brown JR, Wang L, Esko JD. A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. *Cancer Research*. 2003; 63:2775–2781. [PubMed: 12782582]
204. Brown JR, Fuster MM, Whisenant T, Esko JD. Expression patterns of alpha 2,3-sialyltransferases and alpha 1,3-fucosyltransferases determine the mode of sialyl Lewis X inhibition by disaccharide decoys. *Journal of Biological Chemistry*. 2003; 278:23352–23359. [PubMed: 12686549]
205. Brown JR, Fuster MM, Li R, Varki N, Glass CA, Esko JD. A disaccharide-based inhibitor of glycosylation attenuates metastatic tumor cell dissemination. *Clinical Cancer Research*. 2006; 12:2894–2901. [PubMed: 16675586]
206. Sawada R, Sun SM, Wu X, Hong F, Ragupathi G, Livingston PO, Scholz WW. Human monoclonal antibodies to sialyl-Lewis (CA19.9) with potent CDC, ADCC, and antitumor activity. *Clinical Cancer Research*. 2011; 17:1024–1032. [PubMed: 21343375]

207. Hsu DK, Yang RY, Liu FT. Galectins in apoptosis. *Methods in Enzymology*. 2006; 417:256–273. [PubMed: 17132510]
208. Fukumori T, Takenaka Y, Yoshii T, Kim HR, Hogan V, Inohara H, Kagawa S, Raz A. CD29 and CD7 mediate galectin-3-induced type II T-cell apoptosis. *Cancer Research*. 2003; 63:8302–8311. [PubMed: 14678989]
209. Sanchez-Ruderisch H, Detjen KM, Welzel M, Andre S, Fischer C, Gabius HJ, Rosewicz S. Galectin-1 sensitizes carcinoma cells to anoikis via the fibronectin receptor alpha5beta1-integrin. *Cell Death and Differentiation*. 2011; 18:806–816. [PubMed: 21113146]
210. Lajoie P, Partridge EA, Guay G, Goetz JG, Pawling J, Lagana A, Joshi B, Dennis JW, Nabi IR. Plasma membrane domain organization regulates EGFR signaling in tumor cells. *The Journal of Cell Biology*. 2007; 179:341–356. [PubMed: 17938246]
211. Earl LA, Bi S, Baum LG. *N*- and *O*-glycans modulate galectin-1 binding, CD45 signaling, and T cell death. *Journal of Biological Chemistry*. 2010; 285:2232–2244. [PubMed: 19920154]
212. Nangia-Makker P, Nakahara S, Hogan V, Raz A. Galectin-3 in apoptosis, a novel therapeutic target. *Journal of Bioenergetics and Biomembranes*. 2007; 39:79–84. [PubMed: 17318396]
213. Lee M, Lee HJ, Bae S, Lee YS. Protein sialylation by sialyltransferase involves radiation resistance. *Molecular Cancer Research*. 2008; 6:1316–1325. [PubMed: 18708363]
214. Liu Z, Swindall AF, Kesterson RA, Schoeb TR, Bullard DC, Bellis SL. ST6Gal-I regulates macrophage apoptosis via alpha2-6 sialylation of the TNFR1 death receptor. *Journal of Biological Chemistry*. 2011; 286:39654–39662. [PubMed: 21930713]
215. Moller P, Koretz K, Leithauser F, Bruderlein S, Henne C, Quentmeier A, Krammer PH. Expression of APO-1 (CD95), a member of the NGF/TNF receptor superfamily, in normal and neoplastic colon epithelium. *International Journal of Cancer*. 1994; 57:371–377.
216. Lebel M, Bertrand R, Mes-Masson AM. Decreased Fas antigen receptor expression in testicular tumor cell lines derived from polyomavirus large T-antigen transgenic mice. *Oncogene*. 1996; 12:1127–1135. [PubMed: 8649805]
217. Strand S, Hofmann WJ, Hug H, Muller M, Otto G, Strand D, Mariani SM, Stremmel W, Krammer PH, Galle PR. Lymphocyte apoptosis induced by CD95 (APO-1/Fas) ligand-expressing tumor cells—a mechanism of immune evasion? *Nature Medicine*. 1996; 2:1361–1366.
218. Peter ME, Budd RC, Desbarats J, Hedrick SM, Hueber AO, Newell MK, Owen LB, Pope RM, Tschopp J, Wajant H, Wallach D, Wiltout RH, Zornig M, Lynch DH. The CD95 receptor: apoptosis revisited. *Cell*. 2007; 129:447–450. [PubMed: 17482535]
219. Ametller E, Garcia-Recio S, Costamagna D, Mayordomo C, Fernandez-Nogueira P, Carbo N, Pastor-Arroyo EM, Gascon P, Almendro V. Tumor promoting effects of CD95 signaling in chemoresistant cells. *Molecular Cancer*. 2010; 9:161. [PubMed: 20573240]
220. Lai YJ, Lin VT, Zheng Y, Benveniste EN, Lin FT. The adaptor protein TRIP6 antagonizes Fas-induced apoptosis but promotes its effect on cell migration. *Molecular and Cellular Biology*. 2010; 30:5582–5596. [PubMed: 20876301]
221. Nijkamp MW, Hoogwater FJ, Steller EJ, Westendorp BF, van der Meulen TA, Leenders MW, Borel Rinkes IH, Kranenburg O. CD95 is a key mediator of invasion and accelerated outgrowth of mouse colorectal liver metastases following radiofrequency ablation. *Journal of Hepatology*. 2010; 53:1069–1077. [PubMed: 20832890]
222. Yuan K, Jing G, Chen J, Liu H, Zhang K, Li Y, Wu H, McDonald JM, Chen Y. Calmodulin mediates Fas-induced FADD-independent survival signaling in pancreatic cancer cells via activation of Src-extracellular signal-regulated kinase (ERK). *Journal of Biological Chemistry*. 2011; 286:24776–24784. [PubMed: 21613217]
223. Chen L, Park SM, Tumanov AV, Hau A, Sawada K, Feig C, Turner JR, Fu YX, Romero IL, Lengyel E, Peter ME. CD95 promotes tumour growth. *Nature*. 2009; 465:492–496. [PubMed: 20505730]
224. Baldwin RL, Tran H, Karlan BY. Primary ovarian cancer cultures are resistant to Fas-mediated apoptosis. *Gynecologic Oncology*. 1999; 74:265–271. [PubMed: 10419743]
225. Landowski TH, Gleason-Guzman MC, Dalton WS. Selection for drug resistance results in resistance to Fas-mediated apoptosis. *Blood*. 1997; 89:1854–1861. [PubMed: 9058704]

226. Natoli G, Ianni A, Costanzo A, De Petrillo G, Ilari I, Chirillo P, Balsano C, Levrero M. Resistance to Fas-mediated apoptosis in human hepatoma cells. *Oncogene*. 1995; 11:1157–1164. [PubMed: 7566976]
227. O'Connell J, Bennett MW, O'Sullivan GC, Roche D, Kelly J, Collins JK, Shanahan F. Fas ligand expression in primary colon adenocarcinomas: evidence that the Fas counterattack is a prevalent mechanism of immune evasion in human colon cancer. *The Journal of Pathology*. 1998; 186:240–246. [PubMed: 10211111]
228. Keppler OT, Peter ME, Hinderlich S, Moldenhauer G, Stehling P, Schmitz I, Schwartz-Albiez R, Reutter W, Pawlita M. Differential sialylation of cell surface glycoconjugates in a human B lymphoma cell line regulates susceptibility for CD95 (APO-1/Fas)-mediated apoptosis and for infection by a lymphotropic virus. *Glycobiology*. 1999; 9:557–569. [PubMed: 10336988]
229. Peter ME, Hellbardt S, Schwartz-Albiez R, Westendorp MO, Walczak H, Moldenhauer G, Grell M, Krammer PH. Cell surface sialylation plays a role in modulating sensitivity towards APO-1-mediated apoptotic cell death. *Cell Death and Differentiation*. 1995; 2:163–171. [PubMed: 17180039]
230. Suzuki O, Nozawa Y, Abe M. Sialic acids linked to glycoconjugates of Fas regulate the caspase-9-dependent and mitochondria-mediated pathway of Fas-induced apoptosis in Jurkat T cell lymphoma. *International Journal of Oncology*. 2003; 23:769–774. [PubMed: 12888916]
231. Lee KH, Feig C, Tchikov V, Schickel R, Hallas C, Schutze S, Peter ME, Chan AC. The role of receptor internalization in CD95 signaling. *EMBO Journal*. 2006; 25:1009–1023. [PubMed: 16498403]
232. Buhe V, Loisel S, Pers JO, Le Ster K, Berthou C, Youinou P. Updating the physiology, exploration and disease relevance of complement factor H. *International Journal of Immunopathology and Pharmacology*. 2010; 23:397–404. [PubMed: 20646335]
233. Meesmann HM, Fehr EM, Kierschke S, Herrmann M, Bilyy R, Heyder P, Blank N, Krienke S, Lorenz HM, Schiller M. Decrease of sialic acid residues as an eat-me signal on the surface of apoptotic lymphocytes. *Journal of Cell Science*. 2010; 123:3347–3356. [PubMed: 20826457]

Fig. 1.

Types of glycosylation. **a** Structure of the most common sialic acid, Neu5Ac. The negative charge is from the carboxylic acid group on carbon 1. **b** *N*-linked glycans (*left*) are attached to asparagine (N) residues on selected proteins containing the N-X-S/T consensus sequence, while *O*-linked glycans (*center*) are linked to serine (S) or threonine (T) residues. Glycolipids (*right*) are lipids which carry glycan structures

Fig. 2.

Sialic acid linkages. Sialic acids are added to the termini of glycans in an α2-3, α2-6, or α2-8 linkage. In the *top two panels*, sialic acid linkage to galactose is depicted, however other sugars, such as GalNAc, can be modified with sialic acid, depending upon the type of linkage. Note that the structures shown in the figure have been simplified (e.g., hydroxyl and acetyl groups are not represented)

Fig. 3. α2-6 sialylation blocks galectin binding. Galectins require a free hydroxyl group on the 6 carbon of galactose for binding [37], therefore α2-6 sialylation at this site inhibits galectin binding. In contrast, α2-3-linked sialic acids have variable effects on binding, depending upon the specific galectin species

Fig. 4. Regulation of integrins by sialylation. $\alpha 2$ -6 sialylation of *N*-linked glycans on the $\beta 1$ integrin enhances cell adhesion to collagen I and stimulates migration and invasion

Fig. 5.
Sialyl Thomsen-nouvelle antigen. The sialyl Tn antigen is formed by α 2-6 sialylation of GalNAc bound to serine or threonine

Fig. 6.

Sialyl Tn antigen expression correlates to increased invasion. Upregulation of the sialyltransferase, ST6GalNAc-I, or inactivation of the chaperone, *Cosmc*, contributes to an increase in sialyl Tn expression on *O*-linked glycans. Elevated levels of sialyl Tn antigen expressed on tumor cells is correlated to increased invasion; however, the mechanism remains unclear

Fig. 7. Sialyl Lewis structures promote tumor dissemination. Sialyl Lewis structures on tumor cell glycoproteins interact with selectins expressed by activated endothelial cells, thereby facilitating tumor cell extravasation

Fig. 8. Sialyl Lewis antigens. Sialyl Lewis (sLe) antigens are tetrasaccharide structures composed of a GlcNAc-Gal backbone with fucose linked to GlcNAc and sialic acid $\alpha 2-3$ linked to Gal. sLe^x and sLe^a are different isomers, both of which bind to endothelial selectins

Fig. 9. Inhibition of Fas-mediated apoptosis by $\alpha 2-6$ sialylation. $\alpha 2-6$ sialylation of the Fas receptor blocks apoptosis by preventing receptor internalization and formation of the death-inducing signaling complex (*DISC*)