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Review

Sialic acids in cancer biology and immunity

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Abstract

During malignant transformation, glycosylation is heavily altered compared with healthy tissue due to differential expression of glycosyltransferases, glycosidases and monosaccharide transporters within the cancer microenvironment. One key change of malignant tissue glycosylation is the alteration of sialic acid processing that leads to a general upregulation of sialylated glycans (hyper-sialylation) on cell surfaces and an increased introduction of the non-human sialic acid *N*-glycolyl-neuraminic acid (Neu5Gc) instead of *N*-acetyl-neuraminic acid into cell surface glycans. These changes have been shown to be the result of altered sialyltransferase and sialidase expression. Functionally, cancer-associated hypersialylation appears to directly impact tumor cell interaction with the microenvironment, in particular the modulation of sialic acid-binding lectins on immune cells. Moreover, Neu5Gc expression in human tissues enhances inflammation due to an anti-Neu5Gc immune response, which can potentially influence inflammation-induced cancer and cancer-associated inflammation. In this review, we summarize the changes of sialic acid biology within the malignant microenvironment and the resulting effect on cancer immunity.

Key words: *N*-gycolyl-neuraminic acid, selectin, sialidase, sialyltransferase, Siglec, tumor immunology

Overview of changes in glycans during cancer progression

Changes in glycosylation are a classic hallmark of malignant transformation (for a broader overview of glycosylation in cancer, see the following recent review articles Boligan et al. 2015; Pinho and Reis 2015). The mechanisms that produce these aberrant glycosylation patterns are broad, because glycosylation is not template driven, but dependent on multiple interactions resulting from gene expression (processing enzymes discussed later), substrate availability (Tachibana et al. 1994), the cellular environment (Borys et al. 1993) and the underlying protein structure (Berger et al. 1969; Doores et al. 2010; Clark and Baum 2012). Glycans are attached to both proteins and lipids to make glycoproteins and glycolipids, respectively. Classical types of glycosylation are N- and O-linked (on proteins), glycosphin-golipids (on cell-membrane sphingosine), glycosaminoglycans (GAGs) (protein-bound and free) and glycosylphosphatidylinositol anchors (plasma-membrane glycolipid and a protein attached through

glycans) (Varki and Lowe 2009). The type of glycosylation that is finally presented at a given glycosylation site is heterogeneous, giving rise in some cases to many "glycoforms". During organism development, the differentially expressed glycoforms cue signalling for tissue modelling in a rapid and dynamic manner. In some ways, the unusual glycosylation seen in cancer is adapted from roles in development (Haltiwanger and Lowe 2004). For example, the early discovered antigenic structures found on tumor cells were glycans that had already been described in foetal development (Kannagi et al. 1983; Gottschling et al. 2013). N-Glycans on tumor cells are often increasingly branched. This has been explained by upregulation of the enzyme GlcNAcT-V, which adds a second GlcNAc monomer to the core pentasaccharide structure producing dominantly tri-antennary glycan structures that enhance metastasis in animal models (Seberger and Chaney 1999). Malignant transformation of epithelial cells is associated with secretion of both membrane and secreted O-glycans, which often carry altered glycosylation patterns systemically. These cancer mucins can be detected in blood, and have prognostic value.

Classic examples include episialin (MUC1) (Brockhausen et al. 1995) expressed by many carcinomas including breast and ovarian, and CA125 (MUC16), which is overexpressed by ovarian cancer cells and is used in clinical routine (Ricardo et al. 2015). Besides mediating interactions with sialic acid-binding receptors, mucins have a high negative charge associated with their structure that inhibits adhesion of tumor cells within the tissue matrix, and advances metastasis. They may also provide a physical block for blood-borne tumor cells against immune cell interaction (Hollingsworth and Swanson 2004). O-Linked Tn and T antigens are produced by incomplete glycosylation of mucins. These structures occur infrequently in adult organisms and as such are targets for an adaptive immune response that leads to the generation of antibodies. These glycan-targeted antibodies have potential prognostic value, and studies are underway to investigate enhancing the immune response against these tumor-specific ligands (Ju et al. 2014).

Regardless of the change in the underlying glycan structure, the upregulation and alteration of terminal sialic acid structures (Sia) is a hallmark of cancer (Amon et al. 2014). This is summarized in Figure 1. Classically, this results in reduced adhesion of the tumor cell to the ECM allowing in some cases for an increased plasticity of the tumor cells within the tissue matrix, and may also serve to mask detection by the alternative pathway of complement activation. In recent years, however, the upregulation of sialylation has been identified to have another role as ligands for Sia-binding lectins including immune cell expressed Siglec receptors and selectins. In this review, we discuss the known alteration in Sia structure and enzymatic processing in cancer, and recent advances in our understanding of the relationship between cell surface sialylation in cancer progression, concluding with the clinical prospects for targeting Sia-based immune cell interactions as a potential pathway to boost anti-tumor immunity.

Cancer-associated Sia modifications

The Sia family of sugars share a characteristic 9-carbon chain backbone with a carboxylic acid residue attached to carbon-2, the anomeric center. In Figure 2, we summarize and update from (Manzi, Dell, et al. 1990; Schauer 2000) the types of mammalian Sia, including the known cancer antigens. Sia structure differs from the hexoses in having only two hydroxyl residues attached directly to the hexose ring. The amine at position C-5 is modified predominantly as N-acetyl, and N-glycoyl, but can also be a hydroxyl or amine. The aliphatic side-chain of three hydroxyl residues at C-6 can be modified with acetyl, methyl, sulfate, or phosphate residues. The 4-OH can also be acetylated (Schauer 1970a, 1970b); however, very little is known about the function of this group. Overall, the structural flexibility innate within the Sia backbone increases the potential chemical information that can be stored within these molecules when compared with the hexoses and pentoses. The amount of information in the form of chemical complexity that each glycan monomer contains has led to
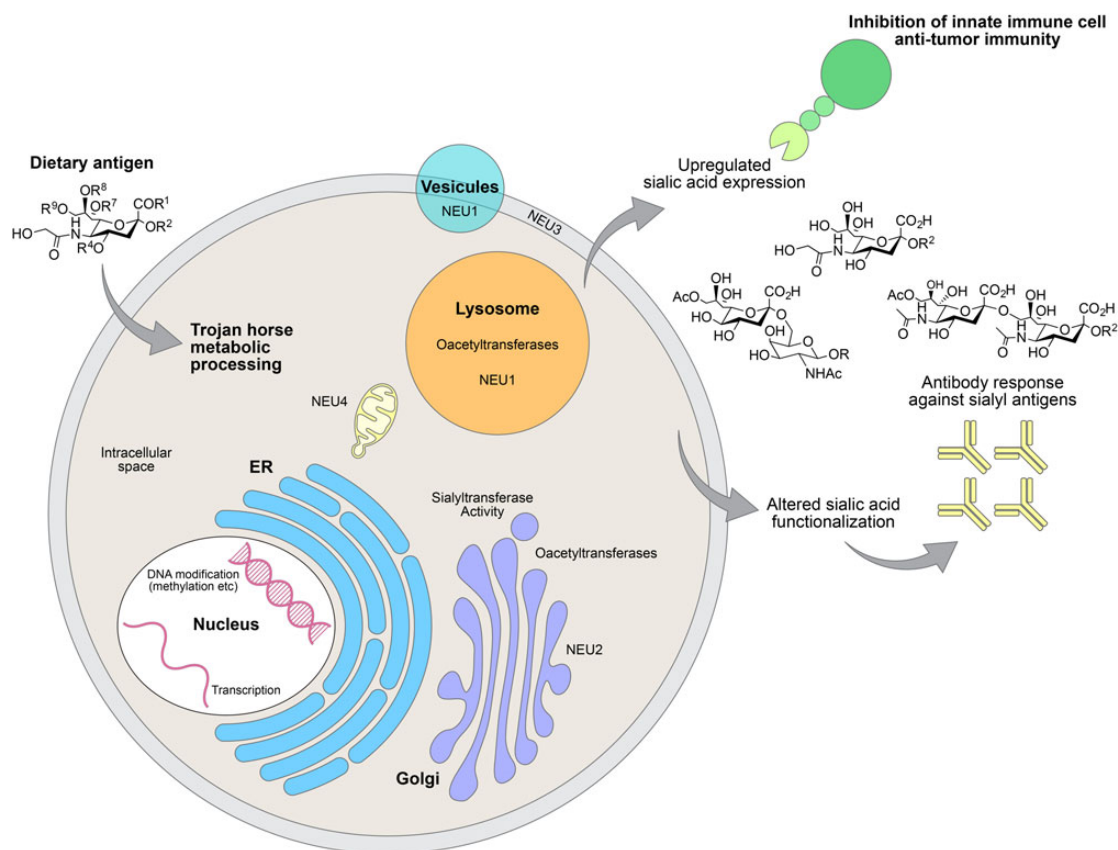
Fig. 1. Overview of sialic acid changes and their role in tumorigenesis and cancer progression. Intracellular changes in sialyltransferase expression alters glycan processing within the Golgi. Changes in sialidase expression effects the sialic acid status of glycans during processing, transport in lysosomes, and at the cell membrane. Protein bound Neu5Gc from dietary sources is metabolically processed and incorporated into endogenous glycans, which in combination with an anti-Neu5Gc immune response generates xenosialitis, a source of cancer-associated inflammation. This figure is available in black and white in print and in color at *Glycobiology* online.
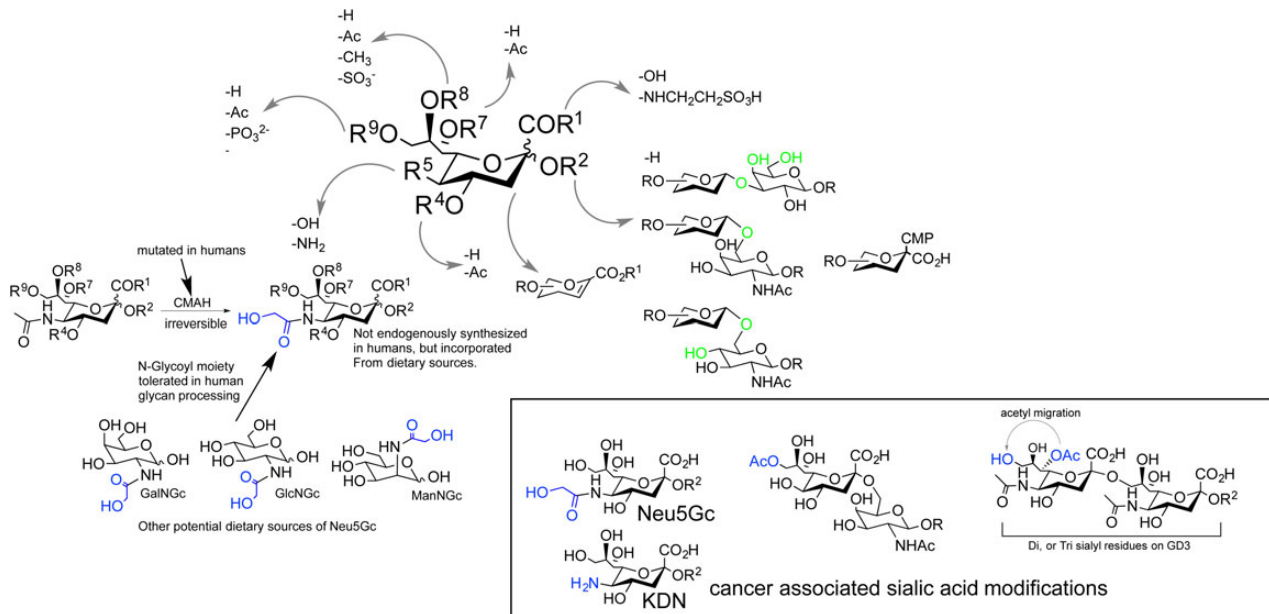
Fig. 2. Structural features of the sialic acid family. Top panel, versatility of the sialic acid backbone for the introduction of functional groups and other sugar residues. Bottom panel, specific modifications overexpressed in cancer. This figure is available in black and white in print and in color at *Glycobiology* online.

them being described as a sugar code (Gabius 2015). Sias are therefore potentially the most versatile of the glycan platforms. Below we discuss cancer-associated O-acetylation (OAc) and the C-5 amine modification in more detail. For more information on the variety of naturally occurring Sia modifications not discussed here see the following articles (Manzi, Dell, et al. 1990; Schauer 2000; Angata and Varki 2002).

C5 *N*-glycolyl modification

The *N*-glycolyl modification is biosynthesized from *N*-acetyl-neuraminic acid (Neu5Ac), to make *N*-glycolyl-neuraminic acid (Neu5Gc) (Figure 2) via the enzyme CMP-Neu5Ac hydroxylase (CMAH) encoded by the gene *CMAH*. Approximately 2–3 million years ago, the human *CMAH* gene was mutated and its product was no longer able to hydroxylate Neu5Ac to Neu5Gc (Varki 2001). This mutation also apparently led to other major evolutionary changes associated with Sias, in particular with Siglecs, in a process referred to as the "sialoquake" (Crocker et al. 2007). Interestingly, while other mammals are able to endogenously synthesize the *N*-glycoyl moiety, it is always absent in brain, which may be the result of *N*-glycolyl significantly altering the conformational shape of polysialic acids on neural cell adhesion molecule, which significantly inhibits sialidase activity (Davies et al. 2012). While humans do not endogenously synthesize Neu5Gc, it can still be acquired from dietary sources, metabolically processed and expressed on epithelial cell surfaces (Bardor et al. 2005; Banda et al. 2012; Bergfeld, Pearce, Diaz, Lawrence, et al. 2012; Bergfeld, Pearce, Diaz, Pham, et al. 2012). The role of Neu5Gc in tumorigenesis and cancer progression is discussed later in this review.

C5 hydroxyl modification: 2-keto-3-deoxy-D-glycerol-D-galacto-nononic acid, KDN

KDN (Figure 2) was first discovered in humans, where it was found overexpressed in ovarian carcinomas in its free form (Inoue et al. 1998, 2006). More recently, free KDN has been shown to be associated with head and neck cancers (Wang et al. 2015). In this case, the level of free KDN relative to Neu5Ac and Neu5Gc was predictive of metastatic potential, and therefore is potentially useful for the prognosis, and detection of early stage cancer from biopsy. KDN has also been associated with pancreatic cancers (Yabu et al. 2013). One potential mechanism for KDN overexpression is the hypoxic microenvironment found in carcinomas (Go et al. 2007). Hypoxia alters the expression of KDN processing enzymes (Angata et al. 1999) and leads to an increased uptake of KDN precursors from the extracellular environment (Go et al. 2006).

O-Acetylation, OAc

Both upregulation and downregulation of OAc on Sias has been shown to be associated with specific carcinomas. In colorectal cancer (CRC), general OAc on colon mucins has been shown to be reduced (Corfield et al. 1999). In this study, colonic adenomas and carcinoma cell lines exhibited this same reduction in OAc, which suggests that this is an early stage event in malignant transformation. These observations were confirmed in human tissue samples of CRC. It was initially thought that these OAc changes specifically occur on di- and tri-sialyl gangliosides, and not on the monomer sialylated structures, whose levels of OAc seem to remain fairly constant (Cheresh, Reisfeld, et al. 1984). However, it was later shown that this effect does occur in mono-sialylated structures such as sialyl-Lewis^x (sLe^x) (Mann et al. 1997; Shen et al. 2004). Interestingly, in the same work, it was found that adjacent normal tissue from resected stage IV CRC also showed this reduction in OAc, specifically 9-OAc, which is suggestive of a local secondary effect. The enzyme responsible for these changes was shown to be sialate-O-acetyltransferase (Corfield et al. 1999; Shen et al. 2004).

In contrast, in melanoma and acute lymphoblastic leukemia (ALL), antigens contain a 9-OAc modification. In ALL circulating antibodies against 9-OAc sialyl residues with an underlying α-2,6-GalNAc have been detected, and serve as a diagnostic marker of disease (Pal et al.

2004). However, this has not been further established for clinical use. The disialyl-ganglioside GD3 is a major glycosphingolipid expressed on melanoma cells, and the 9-O-acetyl sialyloside has long been recognized as an oncofetal antigen, with 9-O-acetyl targeted immunotherapies trialed in the 1980s (Houghton et al. 1985). Interestingly, of all the human tissues and tumors studied the expression of the 9-O-acetyl on the outer sialyl residue of GD3 is quite unique to melanomas (Cheresh, Varki, et al. 1984; Thurin et al. 1985). Later, Manzi, Sjoberg, et al. (1990) further identified a second OAc modification on the 7-hydroxyl of the outer sialyl residue, adding a further structure to these specific melanoma oncofetal antigens.

Enzyme regulation of Sias in cancer

The changes in Sia structure are the culmination of many factors, which come together to generate an unusual sialylated pattern. At the simplest level, changes in the transcript expression of Sia processing enzymes, the sialyltransferases and sialidases, have been associated with malignant disease (Table I). This next section reviews what we currently know about sialyltransferase and sialidase activities in cancer.

Cancer-associated sialyltransferase activity

Mammalian sialyltransferases are a family of 20 conserved enzymes that can be subdivided into four families based on the resulting Sia linkage in the product, and general underlying structure of the substrate (Dall'Olio et al. 2014). These are ST3Gal (6 members, α2–3-linked Sia to an underlying galactose (Gal) residue), ST6Gal (2 members, α2–6-linked Sia to an underlying Gal residue), ST6GalNAc (6 members, α2–6-linked Sia to an underlying GalNAc residue) and ST8SIA (which attach Sia residues to a terminal Sia residue via an α2–8 linkage), of which two are polysialyltransferases (Table I). Sialyltransferase activity has been correlated with nine different carcinoma types (Table I), and in some cases a functional role in disease progression has been elucidated. Of the 20 sialyltransferases, 10 have been associated with malignant disease progression. These studies have mostly focused on epithelial carcinomas.

Breast and colorectal carcinomas have received the most attention, with 5 and 4 sialyltransferases being identified as associated with disease, respectively. In breast cancer, overexpression of ST3GAL1 (Recchi et al. 1998; Burchell et al. 1999) and ST6GALNAC1 (Julien et al. 2001, 2006) is associated with increased sialylation of the abberant O-glycan Tn to Sialyl-Tn and associated with MUC1 expression (Burchell et al. 1999). MUC1 expression correlates with increased tumor invasiveness (Julien et al. 2006). These structures also generate an adaptive immune response against the developing cancer. Clinically, the presence of circulating antibodies at the time of diagnosis correlates with a favorable outcome (Blixt et al. 2011). ST3GAL6 overexpression in breast cancer has been correlated with overexpression of sLe^x structures (Julien et al. 2011). In this study, overexpression of sLe^x was associated with increased metastasis to the bone marrow. This was linked to an interaction of sLe^x with E-selectin that was shown to be consistently expressed in the bone marrow (also see later paragraph on selectins and cancer metastasis). This same ST3GAL6-mediated mechanism of tumor cell homing to bone has been found in multiple myeloma (Glavey et al. 2014). Breast cancer has also been demonstrated to metastasize to the brain via overexpression of ST6GALNAC5, which promotes binding of blood-borne tumor cells to the endothelium of the blood–brain barrier, and in conjunction with two other genes aids infiltration across the blood–brain barrier (Bos et al. 2009).

Hypermethylation of the promoter of ST3GAL6 has been seen in a study comparing normal adjacent mucosa and CRC patient samples (Chen et al. 2013); however, it is not clear whether this modification plays a direct role in CRC biology, other than the expected transcriptional inactivation of ST3GAL6. ST6GAL1 correlates with increased metastasis and poor survival. In one study, ST6GAL1 hypersialylated the N-glycans on the surface of integrin β1 (Seales et al. 2005). This hypersialylation increased tumor cell migration and attachment to collagen I, a matrix protein often upregulated in diseased tissue, and also correlated with some signatures that are associated with increased integrin activation. In a different study, ST6GAL1 expression was enhanced after radiation therapy and therefore sialylation on the β1 integrin (Lee et al. 2008). β1 integrin sialylation was associated with resistance to radiation therapy and tumor survival.

In gastric cancer, both ST3GAL3 and ST3GAL4 are differentially expressed (Gretschel et al. 2003; Gomes et al. 2013). ST3GAL3 expression correlated with the incidence of secondary local tumor occurrence in a cohort of patients; however, there was no independent prognostic value (Gretschel et al. 2003). ST3GAL4 has been implicated in the upregulation of sLe^x structures, which as a result show increased invasive potential via c-MET activation in cell line studies (Gomes et al. 2013).
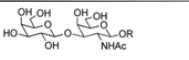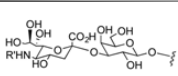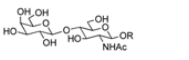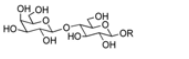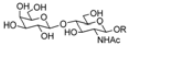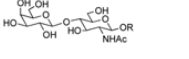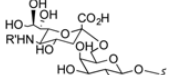
While the above studies are associated with upregulation of sialyltransferase activity, one study of renal cancer cell line has shown that ST6GALNAC3 is downregulated, leading to reduced GD3/GD2 sialylation (Senda et al. 2007), although the biological role and clinical impact are unknown.

Finally, upregulation of sialyltransferases that transfer Sia to terminal Sia residues on O- and N-linked glycans, to generate homopolymer presentations of Sias plays a role in neuroblastoma (Cheung et al. 2006) and non-small cell lung cancer (NSCLC) (Tanaka et al. 2000) progression. The polysialyltransferases ST8SIA2 and ST8SIA4 have been found to be upregulated, which increases the presentation of polysialic acid on the tumor cell surface. In normal development and homeostatic function, polysialic acid has a large volume of hydration that 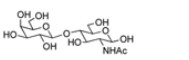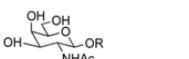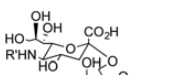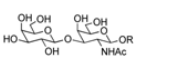reduces the cell–cell contact, and as a result enhances tissue plasticity, and the motility of cells through it (Rutishauser 2008). In the same way, the overexpression of polysialic acid in these cancers provides a potential advantage for cell motility, invasion, and metastasis.

Cancer-associated sialidase activity
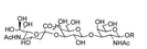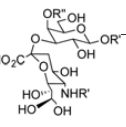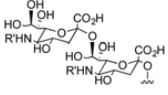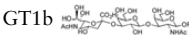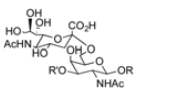
With the exception of ST6GALNAC3, cancer-associated sialyltransferase activity is generally upregulated, contributing towards a hypersialylated cancer glycocalyx. In contrast the regulation of sialidases does not have a clear pattern, with expression levels largely dependent on the cancer type, and the role of the sialidase. There are four mammalian sialidase (neuraminidases) who are split into two groups based on whether they cleave exo (2–3, 2–6 or 2–8 glycosidic linkages) or endo (2–8-linked sialosyl-linkages) Sia residues (Cabezas 1991). Each neuraminidase has a different cellular location: NEU1 lysosomal (Bonten et al. 1996), NEU2 cytoplasmic (Monti et al. 1999), NEU3 plasma-membrane bound (Miyagi et al. 1999; Wada et al. 1999) and NEU4 mitochondrial (Comelli et al. 2003; Monti et al. 2004; Bigi et al. 2010). More recently, NEU1 has been shown to travel in exovesicles to the cell surface and perform on-site hydrolysis of Sia residues (Sumida et al. 2015). The substrate specificity of the neuraminidases has not been very well investigated, although a recent publication addressed the 2–3 and 2–6 specificity of the four
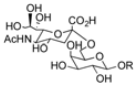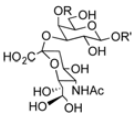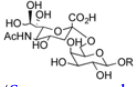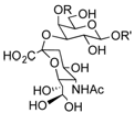
Table I. Enzymatic activities of sialyltransferases and sialidases, and there associations/roles in cancer

Enzyme	Prefered substrate(s)	Sialylated product	Cancers identified[b]	Changes	Biological function	Clinical implication
Sialyltransferases						
ST3GAL1	(Yeh and Cummings 1997)		Breast, CRC	Sialylation of T-antigen	Generation of adaptive host anticancer response	Presence of anti-STn antibodies correlates with a good prognosis
ST3GAL2			Not identified	n/a	n/a	n/a
ST3GAL3			Gastric	Unknown	Unknown	Correlated with secondary local tumor occurance
ST3GAL4	(Basu et al. 1996)		Gastric	Upregulation of slx structures	Increased invasion via c-met activation	Unknown
ST3GAL5	(Ishii et al. 1998)		Not identified	n/a	n/a	n/a
ST3GAL6	(Okajima et al. 1999)		Multiple myeloma, CRC, breast	Hypermethylation (CRC), upregulation of slex structures (breast)	Tumor cell homing (MM), increased metstasis (breast)	High expression associated with poor prognosis (MM)
ST6GAL1 (broad specificity for glycoproteins/ glycolipids)	(Paulson et al. 1977)		CRC, cervical, squamous, breast	Sialylation of B1 integrin (CRC)	Effects cell preference for matrix proteins (CRC), infers resistance to radiation (CRC)	Positive correlation with poor prognosis (colon and breast)
ST6GAL2 (highly specific for the disaccharide form only)	(Takashima, Tsuji, et al. 2002)		Not identified	n/a	n/a	n/a
ST6GALNAC1	(Ikehara et al. 1999)		Breast, CRC	Sialyl-Tn antigen expression (breast)	Reduced adhesion increased motility (breast)	Unknown
ST6GALNAC2	(Samyn-Petit et al. 2000)		Not identified	n/a	n/a	n/a
			Renal	Reduced ST6GalNacIII expression, reduced GD3/2 expression	Unknown	Unknown

Continued

Table I. Continued

Enzyme	Prefered substrate(s)	Sialylated product	Cancers identified[b]	Changes	Biological function	Clinical implication
ST6GALNAC3 (prefers the complete ganglioside GM1b)	(Lee et al. 1999)					
ST6GALNAC4 (prefers the trisaccharide)			Not identified	n/a	n/a	n/a
ST6GALNAC5	Ganglioside GM1b (Tsuchida et al. 2003)		Breast	Unknown	Binding of breast cancer cells to brain endothelium	Allows specific metastasis through the blood–brian barrier and invasion into the brain tissue
ST6GALNAC6 (also has specificity for GD1a and GT1b)			Not identified	n/a	n/a	n/a
ST8SIA1	GM3 (Sasaki et al. 1994)		Not identified	n/a	n/a	n/a
ST8SIA2[a]	Complex-type N-glycans (Scheidegger et al. 1995)		Neuroblastoma, NSCLC	Increased polysialylation	Enhances motility and metastasis	Molecular marker and prognostic for early detection
ST8SIA3	GM3 and GD3 gangliosides (Lee et al. 1998; Angata et al. 2000)		Not identified	n/a	n/a	n/a
ST8SIA4[a]	Complex-type N-glycans (Angata et al. 2000)		NSCLC	Increased polysialylation	Enhances motility and metastasis	Correlates with metastatic potential of the primary tumour
ST8SIA5	GT1b (Kim et al. 1997)		Not identified	n/a	n/a	n/a
ST8SIA6 (only ST8SIA to show specificity for 2–6-linked sia residues)	and Neu5Ac(α2,3) Gal (β1,4) Glc. (Takashima, Ishida, et al. 2002)		Not identified	n/a	n/a	n/a

Sialidases	Prefered substrate(s)	Sialylated product	Cancers identified[b]	Changes	Biological function	Clinical implication
NEU1	(Smutova et al. 2014)		Pancreatic, CRC	Inhibit EGFR signalling using tamiflu which blocks NEU1 activity (pancreatic), inhibits tumour metastasis (CRC)	Inhibits EMT transition, restores chemotherapy sensitivity	Unknown
NEU2	(Smutova et al. 2014)		Prostate, leukemia	Upregulated in prostate, downregulated in leukemia	Silencing of gene results in reduced cell survival and motility (Prostate), inhibits the BCL protein and signalling and increases susceptability to apoptosis (leukemia)	Unknown
NEU3	(Smutova et al. 2014)		CRC, prostate, melanoma, renal, neuroblastoma, ovarian clear cell	Upregulated in CRC and prostate	Modulator of AKT phosphorylation blocks apoptosis	Potential serum biomarker
NEU4	(Smutova et al. 2014)		Neuroblastoma, CRC	Upregulated in neuroblastoma, downregulated in CRC	Enhanced proliferation (neuroblastoma), enhanced cell motility and metastasis (CRC)	Unknown

Enzymes with shared substrate specificities are dependent on the underlying glycan structure not shown here, but further details can be found in the associated reference.

[a] Polysialyltransferase.

[b] For references see text.

neuraminidases using BODIPY-labelled substrates, which for the first time demonstrated some characteristic substrate preferences (Smutova et al. 2014). NEU1 has been shown to be involved in the MMP9-EGFR signalling that facilitates epithelial to mesenchymal transition (EMT) in pancreatic cancer, which facilitates cancer progression and metastasis (Gilmour et al. 2013). In this context, NEU1 activity is required to remove Sia moiety from EGFR removing a steric hindrance that allows access and activation of the receptor, which in turn signals EMT. The same researchers have shown later that blocking neuraminidase with Tamiflu, a small molecule NEU1 inhibitor more commonly used as an anti-influenza drug, blocked EMT in pancreatic cancer cells, and restored their sensitivity to chemotherapy (O'Shea et al. 2014). In CRC, NEU1 expression has a negative correlation with metastasis (Uemura et al. 2009). Here, hydrolysis of sialosides on integrins reduces metastatic potential, which complements the observations regarding sialyltransferase activity in the same cancer (Seales et al. 2005). Two studies on NEU2 activity in cancer cell lines demonstrate a cell-type-specific impact (Tringali et al. 2007; Koseki et al. 2012). In a prostate cancer cell line, NEU2 overexpression correlated with cell survival and motility (Koseki et al. 2012), whereas in a leukemia cell line, induced NEU2 expression sensitized the cell line to apoptosis by blocking BCL2 expression and signaling (Tringali et al. 2007).

Of the four mammalian neuraminidases, the role of NEU3 in disease progression has received most attention. NEU3 activity is most active on ganglioside structures. Overexpression of NEU3 has been found in CRC (Kakugawa et al. 2002; Mozzi et al. 2015; Takahashi et al. 2015; Yamamoto et al. 2015), prostate cancer (Li et al. 2011; Kawamura et al. 2012; Hata et al. 2015), melanoma (Miyata et al. 2011; Tringali et al. 2014), renal cell cancer (Ueno et al. 2006; Tringali, Lupo, et al. 2012), neuroblastoma (Proshin et al. 2002; Mandal et al. 2010) and ovarian clear cell cancer (Nomura et al. 2006). NEU3 has also been shown to desialylate GM3 generating a ligand for the EGRF receptor (Mozzi et al. 2015). In another study, NEU3 action on GM3 and GD1a gangliosides increased ERK and AKT phosphorylation (Bonardi et al. 2014). Separately, NEU3 action has been shown to block apoptotic signals in cell lines (Wada et al. 2007). In this study, blocking NEU3 expression with siRNA induced apoptosis through reduction in BCL-xL. Overall, there was a marked reduction in proliferation. Interestingly, this effect was not seen in a non-cancerous cell line. More recently, a separate study with CRC cells has provided an additional mechanism by which NEU3 activity can increase neoplastic potential (Takahashi et al. 2015). Here, NEU3 activity indirectly activated the Wnt/β-catenin pathway through enhanced LRP6 phosphorylation. NEU3 currently has no clinical value; however, a recent study points towards it being a useful cancer biomarker (Hata et al. 2015).

NEU4 can be expressed as either the short form (NEU4S) or the long form (NEU4L) which differ by one amino acid (Tringali, Cirillo, et al. 2012). In one study, NEU4L was overexpressed in a neuroblastoma cell line leading to increased cell proliferation and activation of Wnt/β-catenin pathway (Tringali, Cirillo, et al. 2012). However, a clear mechanism of NEU4L activity is not understood. Conversely, in a separate study NEU4 is downregulated in CRC cell lines (Shiozaki
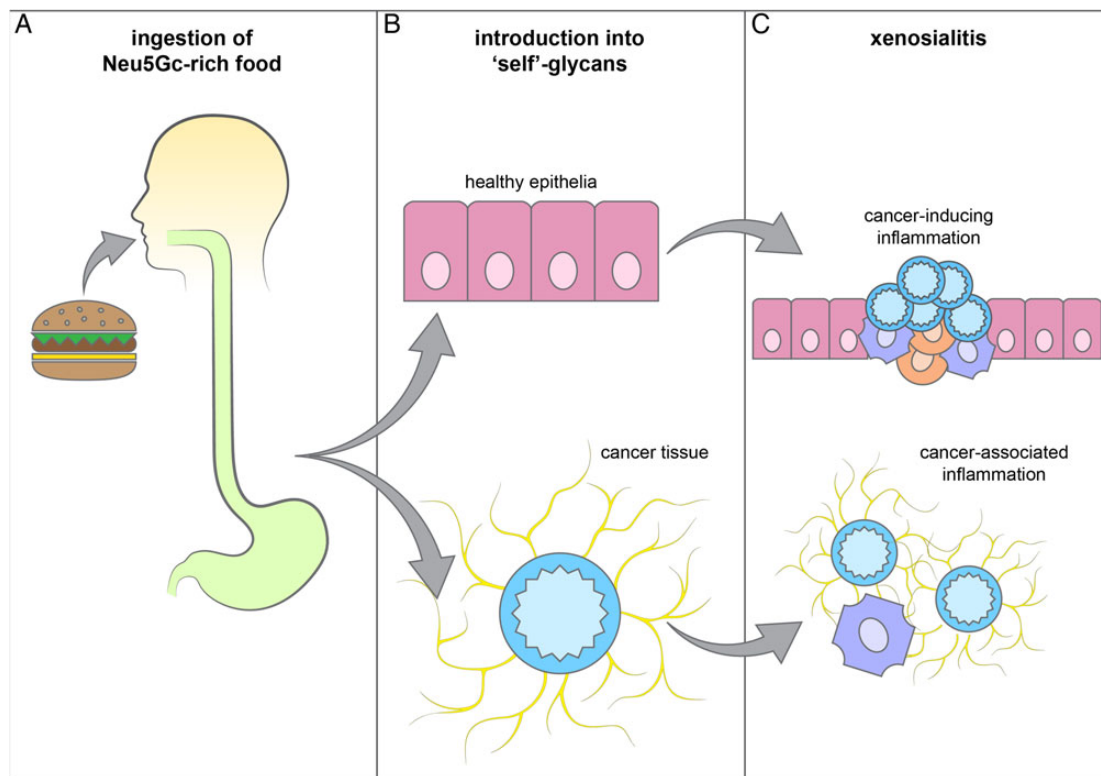
Fig. 3. Consequences of anti-Neu5Gc immune response in cancer initiation and progression: The xenosialitis hypothesis in cancer. (**A**) Neu5Gc, a sugar no longer endogenously synthesized in humans, is still highly expressed in mammals and can be integrated into human tissue by eating Neu5Gc-rich foods such as red meat. (**B**) Neu5Gc in red meat is incorporated from dietary sources into tissues through normal endogenous metabolic pathways. (**C**) Anti-Neu5Gc antibodies that bind to "self"-glycans can induce an inflammation (xenosialitis) and influence thereby tumorigenesis (cancer-inducing inflammation) and cancer progression (cancer-associated inflammation). The combination of Neu5Gc and circulating Neu5Gc antibodies is a new mechanism that could in part explain the increased incident of carcinoma generation in populations who eat a lot of red meat. This figure is available in black and white in print and in color at *Glycobiology* online.

et al. 2011), which was associated with increased expression of sialyl-Lewis[a] (sLe[a]) structures, which have been shown to stimulate E-selectin activity, supporting tumor motility and metastasis (Laubli and Borsig 2010b).

Neu5Gc in cancer initiation and progression

So far we have discussed specific changes in Sia regulation and structure and how this might impact cancer progression. In this section, we now focus on Neu5Gc, one of the three major mammalian sialic acids (Neu5Ac, and KDN being the other two), which is unique in being a non-human Sia, yet found to have a significant role in human cancer immunity, and tumorigenesis, with the potential for clinical impact in the future.

Neu5Gc in tumorigenesis

Although humans are not able to synthetize Neu5Gc due to the loss of function of CMAH, Neu5Gc can still be integrated into glycans expressed by human cells (Tangvoranuntakul et al. 2003; Varki and Varki 2007; Banda et al. 2012; Samraj et al. 2014), a unique feature not shared by the other well-known non-human glycan antigen, α-Gal. Neu5Gc can be taken up via glycoproteins from food prepared from mammals (Banda et al. 2012). Neu5Gc can be detected by an affinity-purified chicken IgY on healthy tissues including epithelia

and also endothelial cells and is also found enriched on cancer-glycans (Tangvoranuntakul et al. 2003; Hedlund et al. 2008). In vitro analysis demonstrated that Neu5Gc-containing glycoproteins can be taken up by cultured epithelial cells via pinocytosis and lysosomes where Neu5Gc is released by sialidases and then actively transported to the cytosol (Bardor et al. 2005). Similarly, extracellular free Neu5Gc can be taken up and cytosolic Neu5Gc is available for activation to CMP-Neu5Gc, which can be integrated in glycans (Bardor et al. 2005), although in vivo it appears free Neu5Gc does not get incorporated within detectable levels into tissues. In addition, anti-Neu5Gc antibodies can be detected during early childhood and can be found in pooled IgG preparations from healthy donors (Taylor et al. 2010; Padler-Karavani et al. 2011), whereby the immunogenicity of Neu5Gc glycans seems to depend on the exact chemical structure (Schneider et al. 2015). Binding of anti-Neu5Gc to Neu5Gc-containing glycans can induce an inflammation termed xenosialitis (Figure 3) since the glycan is not endogenously synthesized in humans (Taylor et al. 2010; Samraj et al. 2014). Therefore, Neu5Gc-containing glycans can be regarded as xeno-autoantigens and anti-Neu5Gc antibodies as xeno-autoantibodies. It was hypothesized that xenosialitis is in part responsible for chronic inflammatory diseases including atherosclerosis (Pham et al. 2009). Chronic inflammation is also a driver of tumorigenesis and cancer progression (Grivennikov et al. 2010; Bondar and Medzhitov 2013; Elinav et al. 2013). Thus, xenosialitis could at least
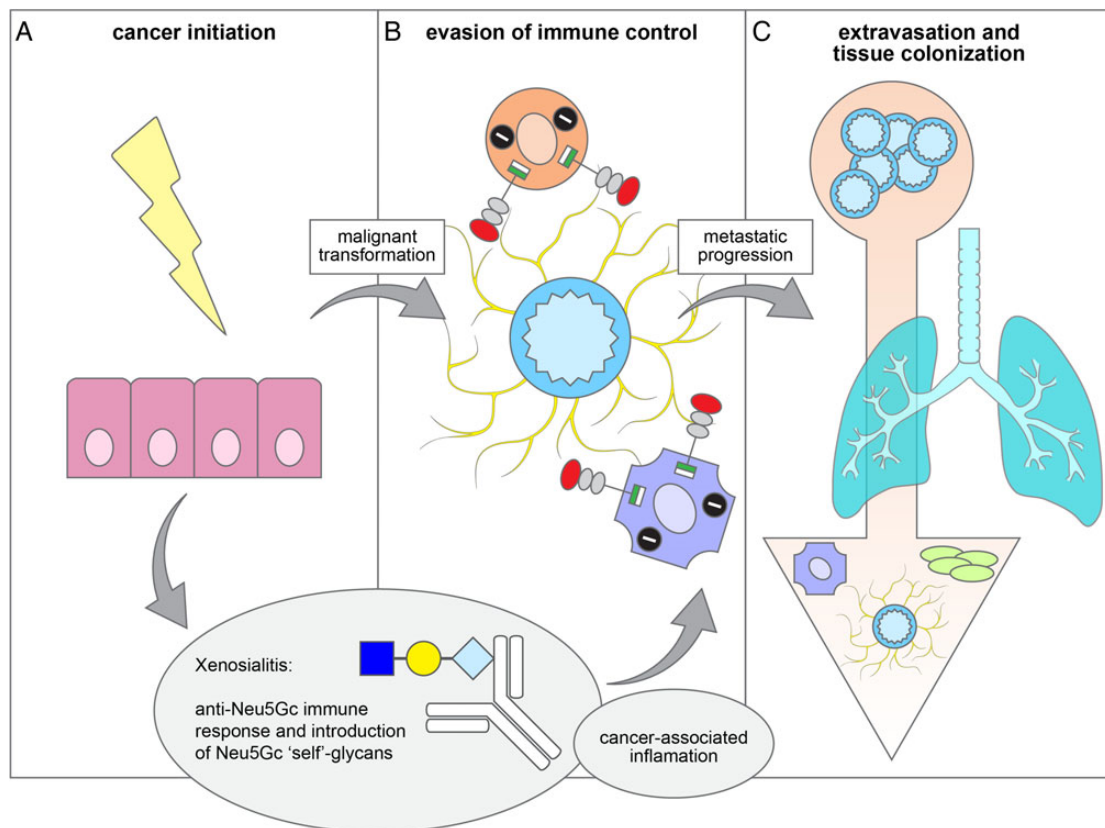
Fig. 4. Sialylation plays a role in all stages of cancer, from initiation to metastasis. (**A**) Xenosialitis (anti-Neu5Gc immune response) can influence tumorigenesis by promoting cancer-inducing inflammation. (**B**) Hypersialylation can mediate interactions with Siglecs and thereby inhibit immunosurveillance and mediate immune evasion of tumor cells. On the other hand, analyses in Siglec deficient mice have demonstrated that Siglecs can inhibit cancer-associated inflammation and thereby reduce tumor growth. Xenosialitis can influence cancer-associated inflammation by fueling tumor-promoting inflammation. (**C**) Interactions between hypersialylated tumor cells and selectins on platelets, endothelial cells and leukocytes (in particular inflammatory monocytes) support metastasis, extravasation of tumor cells and organ colonization. This figure is available in black and white in print and in color at *Glycobiology* online.

partially explain the association between cancer and red meat intake observed epidemiologically as red meat is very rich in Neu5Gc (Pan et al. 2012). The recent generation of a human-like mouse model of Neu5Gc deficiency has helped gain insights in the role of inflammation due to intake of Neu5Gc-rich food and tumorigenesis (Hedlund et al. 2007). The *Cmah* deficient (Cmah−/−) mouse lacks the hydroxylase responsible for the generation of Neu5Gc from Neu5Ac (Hedlund et al. 2007). While feeding of Cmah−/− mice with Neu5Gc-rich mucins leads to the incorporation of Neu5Gc into epithelia of different organs (Banda et al. 2012), vaccination with Neu5Gc-containing glycans (Neu5Gc-containing red blood cell ghosts from chimpanzees) leads to the induction of an anti-Neu5Gc IgG immune response in Cmah−/− mice (Hedlund et al. 2008). Cmah−/− mice in the C57Bl6 background that are fed with Neu5Gc-containing mucins and vaccinated with Neu5Gc-containing glycans have an increased incidence of invasive liver cancer (hepatocellular carcinoma, HCC) than control mice (Samraj et al. 2015). Of note, C57Bl6 mice are prone to develop liver adenoma and it seems xenosialitis is a tumor promoter in this model. Inflammation was previously described as an important promoter in HCC tumorigenesis with IL-6 as mediator (Grivennikov et al. 2010). The Neu5Gc–anti-Neu5Gc response was also associated with an increase of circulating IL-6 in the described xenosialitis model (Samraj et al. 2015). Although these results are interesting, further experimental and epidemiological studies are certainly needed to tighten the link between red meat consumption, Neu5Gc and cancer risk.

Neu5Gc in cancer progression

In the previous section, we discussed how expression of dietary Neu5Gc on the surface of tumors can potentially influence tumorigenesis. Recent studies have also demonstrated that anti-Neu5Gc antibodies can stimulate or inhibit cancer progression of already established tumors (Hedlund et al. 2008; Padler-Karavani et al. 2011; Pearce, Laubli, et al. 2014). In the latest of these studies, it was shown that both stimulation and inhibition by anti-Neu5Gc antibodies was related to the quantity of the antibody used, an effect termed "hormesis". Within the same study, it was shown that hormesis was a general effect that could occur with other tumor-directed antibodies including a clinically used monoclonal anti-CD20 antibody, rituximab, which is used as a therapy for B-cell lymphoma. The mechanism(s) by which this occurs have so far been shown to be due to a change in macrophage phenotype from tumor-promoting M2, and activation of the PI3K/AKT survival pathway (low doses) to tumor-inhibiting M1 and direct lysis of tumor cells via complement activation (at high doses) (Wu et al. 2013). For an overview of the hormesis mechanism, see Pearce, Laubli, Bui, et al. (2014).

Siglecs in cancer immune evasion and cancer-associated inflammation

Sia patterns are recognized through recognition receptors called Siglecs. Siglecs are Sia-binding immuno-modulatory, often inhibitory receptors expressed preferentially on immune cells (Macauley et al. 2014; Schwarz et al. 2015). In recent years, several experimental models have provided evidence that Siglecs are implicated in cancer progression and immune evasion (Hudak et al. 2014; Jandus et al. 2014; Laubli, Alisson-Silva, et al. 2014; Laubli, Pearce, et al. 2014) (Figure 4). These experimental models also suggest that Siglecs could be targeted to improve anti-tumor immunity.

Siglecs can be divided into two distinct groups with regard to their evolutionary conservation and sequence similarity (Crocker et al. 2007; Macauley et al. 2014; Schwarz et al. 2015). Conserved Siglecs such as human Siglec-1 (sialoadhesin), Siglec-2 (CD22), Siglec-4 (myelin-associated glycoprotein) and Siglec-15 have orthologs in different mammalian species and a low-sequence similarity is found between the different conserved Siglecs (Schwarz et al. 2015). On the other hand, CD33 (Siglec-3) and related Siglecs (CD33rSiglecs) belong to a large subfamily of Siglecs that underwent rapid evolutionary changes by duplications within the cluster of Siglec genes (chromosome 19 in humans) (Angata 2006). Siglecs are cell surface receptors and most CD33rSiglecs transmit inhibitory intracellular signals via immunoreceptor tyrosin-based inhibitory motifs (ITIMs) or ITIM-like motifs that are phosphorylated upon activation by Src kinases, which leads to the recruitment of SHP-1 or SHP-2 (Pillai et al. 2012). Some Siglecs lack ITIM or ITIM-like motifs, but have a positively charged amino acid within the transmembrane domain and are able to bind to DNAX-activation protein of 12 kDa(DAP12) that transfers activating intracellular signals via its immunoreceptor tyrosin-based activating motif (Angata 2006; Kameda et al. 2013; Takamiya et al. 2013). Such activating Siglecs probably evolved as a pair to inhibitory Siglecs. In this regard, Siglec-5/14 and Siglec-11/16 have been described in humans that undergo concerted evolution (Angata et al. 2006; Wang et al. 2012; Schwarz et al. 2015). There is phylogenetic and experimental evidence that Siglec-14 evolved from a duplication of Siglec-5 and underwent concerted evolution (Angata et al. 2006). Similarly, inhibitory Siglec-11 and activating Siglec-16 have undergone complex gene conversion events and are considered to be paired receptors on microglial cells (Wang et al. 2012). The hypothesis is that activating receptors are counteracting the co-option of inhibitory Siglecs by pathogens such as group B streptococci that bind to Siglec-5 on myeloid cells and thereby evade immune control (Angata et al. 2006; Ali et al. 2014).

While the physiological role of inhibitory Siglecs is the protection of an over-reactive immune system by the recognition of self-associated patterns, i.e. Sia-containing glycans that are regularly found on mammalian cells, but not on most pathogens (Chen et al. 2009; Varki 2011; Bochner and Zimmermann 2015), some pathogens can evade immune control by decorating themselves with Sia and engagement of inhibitory Siglecs (Carlin, Chang, et al. 2009; Carlin, Uchiyama, et al. 2009), which not only led to the generation of paired receptors, but is also believed to be the main reason for the rapid evolution of CD33rSiglecs (Crocker et al. 2007; Chang and Nizet 2014; Schwarz et al. 2015). This phenomenon can also be described as the "Red Queen" effect, which refers to the "race" of Siglecs between the host-beneficial immune suppression and the host-damaging abuse by some pathogens that drive rapid evolution (the Red Queen of the novel by C.S. Lewis) (Crocker et al. 2007).

Similar to pathogens that bind to inhibitory CD33rSiglecs and thereby evade immune control by inhibiting immune cell activation, recent evidence suggests that cancer-associated, hypersialylated ligands can engage inhibitory CD33rSiglecs and thereby facilitate immune evasion (Hudak et al. 2014; Jandus et al. 2014; Laubli, Alisson-Silva, et al. 2014; Laubli, Pearce, et al. 2014). Studies on tumor cell lines and sections have found an increase of Siglec-9 binding compared with non-malignant cells or sections from healthy tissues (Jandus et al. 2014; Laubli, Pearce, et al. 2014). In addition, an enhanced binding of Siglec-7 to tumor cells was demonstrated (Jandus et al. 2014). Analyses of hypersialylated ligands on tumor cells and secreted proteins within the tumor microenvironment have further identified mucins and also secreted N-glycosylated proteins such as LGALS3BP to be high-affinity ligands for CD33rSiglecs (Laubli, Alisson-Silva, et al. 2014). Engagement of inhibitory Siglec-7 and

Siglec-9 by sialoglycans was further demonstrated to inhibit NK cell-mediated tumor cell killing in vitro (Hudak et al. 2014; Jandus et al. 2014). The introduction of synthetic glycopolymers as Siglec ligands into the cell surface membrane of tumor cells inhibited the NK cell-mediated killing, and blocking with anti-Siglec antibodies reversed this effect in vitro (Hudak et al. 2014). Similarly, enzymatic de-sialylation increased NK cell-mediated killing in cell culture assays (Jandus et al. 2014). In accordance with these findings, the use of ag-onistic antibodies against Siglec-7 and Siglec-9 inhibited NK cell acti-vation and tumor cell killing. Enzymatic removal of sialic acid on tumor cells was also tested in a humanized NOD-SCID-γ_c –/– (NSG) mouse model (Jandus et al. 2014). The desialylated tumor cells were killed more readily after intraperitoneal injection, although other receptors than Siglecs could also be involved.

Siglec-9 and its functional homolog in mice, Siglec-E, were shown to be involved in a dual response of myelomonocytic cells to cancer-associated, hypersialylated ligands (Laubli, Alisson-Silva, et al. 2014; Laubli, Pearce, et al. 2014). While engagement of Siglec-9 or Siglec-E on neutrophils inhibited anti-tumor activity during metastatic organ colonization, engagement of inhibitory CD33rSiglecs on tumor-associated macrophages (TAMs) inhibited tumor-associated inflam-mation and cancer progression (Laubli, Pearce, et al. 2014). Earlier analyses have also implicated a function of CD33rSiglecs on TAMs (Miyazaki et al. 2012). Interestingly, a polymorphism of Siglec-9 (K131Q, A391C) that leads to a reduced binding of Siglec-9 to its li-gands showed an improved early survival of patients with NSCLC (Laubli, Pearce, et al. 2014). This finding is also suggestive that Siglec-9 is a potential immuno-modulatory target that could be exploited to enhance anti-tumor activity in patients. However, it re-mains to be determined which type of Siglec-9-positive immune cell is involved in mediating the survival benefit observed in the population with the A391C minor allele. Above-mentioned studies implicated mainly cells of the innate immune system. While NK cells can be reac-tivated in hematological malignancies, key players in anti-tumor im-munity are cytotoxic T cells (Mittal et al. 2014; Romero and Coukos 2014; Topalian et al. 2015). In contrast to other hominids, humans have very low levels of Siglec receptors on their surface of rest-ing, peripheral T cells (Nguyen et al. 2006). It remains to determined, if Siglecs play a role on cytotoxic T cells and this is a field of ongoing investigations. Taken together, experimental evidence supports the hy-pothesis that Siglecs and in particular inhibitory CD33rSiglecs might be targeted to enhance anti-tumor immune function. Further investi-gations are certainly needed to determine what Siglec is the optimal target and which patient population might benefit from an immuno-modulatory therapy by targeting Siglecs.

Selectin-mediated interactions during metastatic progression

Selectins are another important family of Sia-binding receptors that were experimentally implicated in cancer progression (Frenette and Wagner 1996; Kansas 1996; McEver 2002; Ley 2003; Laubli and Bor-sig 2010b). Selectins are type I membrane proteins with a C-type lectin domain (Kansas 1996; McEver 2002; Ley 2003). L-selectin (CD62L) is expressed on myeloid cells, naïve T cells and some populations of memory T cells (Kansas 1996; Sallusto et al. 1999). E-selectin (CD62E) is expressed upon activation on the surface of endothelial cells and P-selectin is stored in granules of endothelial cells and plate-lets and presented rapidly on the surface upon endothelial or platelet activation (Kansas 1996). Ligands for selectins need proper post-translational modifications including glycosylation with the minimal

binding motif sLe^x (Kansas 1996; Varki 1997). Moreover, sulfation is required for efficient binding of PSGL-1—the best-studied ligand for P- and L-selectin (Kansas 1996; Varki 1997). The study of the function of selectins was significantly helped by the analysis of genetic mouse models (Frenette and Wagner 1997). Physiologically, these vas-cular cell adhesion molecules mediate the early steps of extravasation of leukocytes during inflammation and recirculation to secondary lymphoid organ (Ley et al. 2007). Moreover, P-selectin is involved in platelet-mediated thrombus formation, which was demonstrated by altered coagulation in P-selectin deficient mice (Subramaniam et al. 1996; Polgar et al. 2005). Selectins not only mediate cell adhe-sion but also transmit intracellular signals and thereby influence leukocyte activation (McEver 2015).

Selectins have been experimentally implicated in different steps of cancer progression (Laubli and Borsig 2010b; Coupland and Parish 2014). In particular, various steps of metastatic dissemination and organ colonization were associated with selectin-mediated interac-tions in various experimental models. Cancer-associated, hypersialy-lated ligands, in particular mucins can bind and interact with selectins (Mannori et al. 1995; Kim et al. 1999). Also other selectin ligands have been associated with cancer progression such as properly glyco-sylated CD24, CD44, E-selectin ligand-1, podocalyxin-like protein and more (Aigner et al. 1997; Dimitroff et al. 2005; Hanley et al. 2005; Gout et al. 2006; Thomas et al. 2009).

E-selectin has been involved in metastasis in several experimental models. E-selectin-mediated arrest of circulating tumor cells have been described by mediating adhesion of E-selectin ligands on tumor cells (Mannori et al. 1997; Fukuda et al. 2000; Burdick et al. 2001, 2003; Gout et al. 2006; Li et al. 2013; Yasmin-Karim et al. 2014; Shir-ure et al. 2015). E-selectin facilitated metastasis to the liver in a mouse model and inhibition of E-selectin is associated with less liver metas-tasis (Brodt et al. 1997; Khatib et al. 2002). Constitutive expression of E-selectin on hepatic endothelial cells led to the redirection of metas-tasis from the lung to the liver in another model, a finding that suggests an important role for E-selectin in liver metastasis (Biancone et al. 1996). Interestingly, E-selectin was not needed for experimental me-tastasis to the lungs where tumor cells are injected intravenously into the tail vein (Laubli and Borsig 2010a), but it was shown to sup-port spontaneous lung metastasis from a xenograft (Stubke et al. 2012). Such observations indicate a model-dependent role of E-selectin in metastatic progression and also implicate a potential ac-tivation of some endothelia by the primary tumor.

P-selectin deficient mice show strongly reduced colonization of the lung after intravenous injection (Kim et al. 1998; Borsig et al. 2002). This finding was associated a reduction of platelet-tumor cell interac-tions that led to reduction of tumor embolus formation (Borsig et al. 2002; Laubli et al. 2009). Tumor embolus formation was associated with metastasis in various experimental models (Gay and Felding-Habermann 2011). While the relation between platelets was noted already in the 19th century by Trousseau who observed an in-creased frequency of thrombo-embolism in cancer patients (Varki 2007), recent evidence of genetic models that involve molecules im-portant for their function clearly demonstrated a metastasis support-ive function of platelets (Bakewell et al. 2003; Boucharaba et al. 2004; Camerer et al. 2004; Palumbo et al. 2005; Jain et al. 2007, 2009; Laubli and Borsig 2010b). As a side note, the hypercoagulability first described by Trousseau is also to some part attributed to selectin-mediated interactions between cancer mucins, platelet P-selectin and L-selectin on leukocytes (Wahrenbrock et al. 2003; Shao et al. 2011). Further functional analysis of P-selectin-mediated metastasis promotion demonstrated that the activation of microvascular

endothelial cells is involved after arrest of tumor cells, which leads to the generation of a permissive metastatic microenvironment or a metastatic niche (Laubli et al. 2009). In particular, the upregulation of chemokines including CCL5 in endothelial cells and subsequent recruitment of inflammatory monocytes was demonstrated to support extravasation and lung colonization (Laubli et al. 2009).

Similar as observed with P-selectin deficiency, L-selectin deficient mice are significantly less prone to develop metastasis in the lungs after intravenous injection of tumor cells (Borsig et al. 2001, 2002). L-selectin ligands were clearly upregulated around metastasizing tumor cells in the same model (Laubli et al. 2006). Also the reduction of L-selectin ligands within the metastatic microenvironment led to a significant reduction of metastasis in lungs of mice injected intravenously with tumor cells (Laubli et al. 2006; Hoos et al. 2014). L-selectin seems to be relevant for the recruitment of inflammatory monocytes to the metastatic niche, which subsequently support the extravasation of tumor cells (Laubli et al. 2006; Hoos et al. 2014). Other analyses have also demonstrated that the recruitment of monocytes support metastatic progression (Qian et al. 2011; Wolf et al. 2012). Taken together, selectin-mediated interactions between tumor cells, platelets, endothelial cells and leukocytes are involved in various steps of metastasis and can mediate the generation of metastatic niche in experimental metastasis models. Interestingly, GAGs such as heparin and heparin sulfates bind to selectins (Koenig et al. 1998). Interactions of cancer-associated ligands with selectins can be inhibited by heparin and derivatives and can be therefore used to interfere with selectin-mediated promotion of metastasis (Borsig et al. 2001; Laubli and Borsig 2009).

Therapeutic opportunities and outlook

The role of Neu5Gc in cancer initiation and progression has potential implications for cancer prevention and treatment. Further corroboration of the link between red meat consumption, xenosialitis and cancer risk could help advise our dietary intake of Neu5Gc-containing food. Moreover, strategies to reduce the uptake of Neu5Gc or its inhibition into our own glycans could potentially lead to a decrease of xenosialitis and prevention of cancer. For example, the reduction of Neu5Gc consumption could lead to a reduction of cancer risk. On the other hand, Neu5Gc-containing glycans may also be a target for immunotherapy (Padler-Karavani et al. 2011; Samraj et al. 2014). Anti-Neu5Gc antibodies isolated from human IVIG preparations were able to inhibit subcutaneous tumor growth in a mouse model (Padler-Karavani et al. 2011). Increased uptake of Neu5Gc into tumor tissue due to cancer-associated hypersialylation leads to the enhanced expression of Neu5Gc-sialyl-Tn antigen (Padler-Karavani et al. 2011). This epitope is relatively tumor specific and could be potentially targeted. Moreover, the anti-idiotype antibody 1E10 (racotumomab) that induced an anti (Neu5Gc)GM3 immune response targeting the ganglioside GM3 showed promising efficacy in patients with NSCLC (Hernandez et al. 2008). In a recent study, racotumomab was used as maintenance after initial chemotherapy in patients with NSCLC, which improved progression-free survival from 3.9 to 5.3 months (Alfonso et al. 2014). Another approach is the vaccination with Neu5Gc-sialyl-Tn containing MUC1 (von Mensdorff-Pouilly et al. 2000; Huang et al. 2012). Recently, a synthetic approach that uses a multicomponent strategy to elicit an immune response against either Tn or Sialyl-Tn has been successfully used (Thompson et al. 2015). This vaccine candidate was shown to elicit ADCC-dependent tumor cell killing, and facilitate the expansion of cytotoxic T cells via the Sialyl-Tn or Tn antigens.

Antibodies to Neu5Gc-containing glycans, in particular Neu5Gc-containing GM3 are upregulated in patients with cancer (Samraj et al. 2014). In fact, (Neu5Gc)GM3 is the target of the antibodies described by Hanganutziu and Deicher (H-D antibodies) nearly 100 years ago (Samraj et al. 2014). Thus, anti-Neu5Gc antibodies could be used as tumor markers. Sera from patients with cancer were analyzed by sialo-glycan array and four targets of Neu5Gc-containing glycans could be identified (Padler-Karavani et al. 2011). Finally, Neu5Gc on therapeutically used antibodies such as cetuximab (anti-EGFR1 antibody, mainly used in colorectal cancer or head and neck cancers) could lead to enhanced clearance of the antibody due to immune complex formation with anti-Neu5Gc IgG (Ghaderi et al. 2010).

While Siglec-2 (CD22, e.g. inotuzumab ozogamicin) and Siglec-3 (CD33, gemtuzumab ozogamicin, Mylotarg®) are directly targeted with antibodies to treat hematological malignancies (Hills et al. 2014; Jabbour et al. 2015), previously discussed pre-clinical evidence suggests that Siglecs might be a target to enhance anti-tumor immunity (Hudak et al. 2014; Jandus et al. 2014; Laubli, Alisson-Silva, et al. 2014; Laubli, Pearce, et al. 2014). Recently, Siglec-1 on antigen-presenting cells (APCs) was targeted with high-affinity ligands on liposomes to deliver antigens in a mouse model (Kawasaki et al. 2013). Siglec-1 could therefore be used to deliver antigens to APCs in cancer patients. Further investigations are needed to determine which Siglec and in which situation Siglecs could be targeted. In particular, potential redundancy of inhibitory CD33rSiglecs, not yet completely determined cell populations that express Siglecs and unclear effects on intracellular signaling need to be considered for the next steps of investigations. In addition, more studies are required to understand what, where and when a specific Siglec binding structure (or pattern of structures) are expressed to further elucidate how tumour cells evade the immune response. In one study using synthetic sialosides differences in structure specificity across 10 of the 11 human Siglecs has been investigated (Blixt et al. 2003).

Selectins are implicated experimentally in metastatic progression of cancer (Laubli and Borsig 2010b). Since heparins and derivatives bind to selectins (Koenig et al. 1998; Laubli and Borsig 2009), heparin and also fractionated heparin products can interfere with metastatic lung colonization by binding to selectins (Borsig et al. 2001; Stevenson et al. 2005, 2007; Laubli et al. 2006). Also the generation of heparin derivatives or other ligands that bind to selectins can inhibit experimental metastasis in mice (Borsig 2007; Borsig et al. 2007, 2011; Hostettler et al. 2007; Kozlowski et al. 2011; Gomes et al. 2015). It is important to note that heparin and fractionated heparins can interfere with metastatic not only by binding to selectins but has a more pleiotropic effect on different molecules including heparanase inhibition (Laubli and Borsig 2009). Interestingly, the use of heparins in cancer patients is associated with an improved prognosis when compared with other anticoagulants (Lee et al. 2003; Kakkar et al. 2004; Klerk et al. 2005). This effect might be partially due to the anti-metastatic effect of heparin by inhibiting selectin-mediated interactions.

The discussed topics on the implications of Sia in cancer biology and immunology demonstrate that the understanding of glycans during cancer progression provide ample opportunities for novel approaches to target tumors. In particular, blocking interactions with sialic acid-binding lectins including inhibitory CD33rSiglecs is promising approach and warrants further investigations. Furthermore, investigations should be made to develop strategies to elicit immune responses against aberrant sialylation.

Funding

Abbreviations

ALL, acute lymphoblastic leukemia; APCs, antigen-presenting cells; CMAH, CMP-Neu5Ac hydroxylase; CRC, colorectal cancer; GAGs, glycosaminoglycans; Gal, galactose; ITAM, immunoreceptor tyrosin-based activating motif; ITIMs, immunoreceptor tyrosin-based inhibitory motifs; Neu5Ac, *N*-acetyl-neuraminic acid; Neu5Gc, *N*-glycolyl-neuraminic acid; OAc, O-acetylation; sLe[x], sialyl-Lewis[x]; TAMs, tumor-associated macrophages.

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