Aiming at the sweet side of cancer: Aberrant glycosylation as possible target for personalized-medicine

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Abstract

One of the frontiers in cancer personalized-medicine aims at glycosylation. Cells are covered with a dense sugar coat of glycolipids, glycoproteins and free glycans. In cancer, the characteristic cell surface glycosylation is frequently transformed due to altered expression of glycan-modifying enzymes. This often leads to aberrant expression of sialic acids (Sia) that cap glycan-chains. Additionally, dietary intake of the non-human Sia N-glycolylneuraminic acid (Neu5Gc) leads to natural metabolic-glycoengineering of human carcinomas that accumulate and express Neu5Gc. This Sia provokes a polyclonal anti-Neu5Gc xeno-autoantibodies response that can exacerbate cancer. This review highlights cancer-associated changes in Sia expression and their potential for personalized-theranostics.

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1. Introduction

Cancer is a complex heterogeneous disease and the leading cause of death worldwide. Selective biological therapies have emerged to effectively treat certain types of cancers or to target specific determinants that are expressed by many different tumor types [1,2]. Yet patients do not respond uniformly to a given therapy reflecting their inherent heterogeneity. Technological advances in genomics paved the way towards matching a subset of patients with unique genetic signatures that are most likely to benefit from a certain therapy [3–6], thus leading to individually tailored treatments [6–8]. Other high-throughput technologies have also emerged to expand personalized-medicine beyond genomics including proteomics, pharmacogenomics, nutrigenomics [6,8,9] and more recently glycomics [10,11].

Carbohydrates play a major role in cancer and circulating or cell surface tumor-associated carbohydrate antigens (TACA) serve as diagnostics markers [12,13]. TACA represent a modified version of the carbohydrates normal expression, which frequently involve altered expression of sialic acids. While this has been extensively described in the literature, recent research suggests a novel group of TACA that arise from the consumption of the dietary non-human sialic acid, N-glycolylneuraminic acid (Neu5Gc). This sugar metabolically incorporates into cells like a 'Trojan horse' replacing the human sialic acid thereby generating neo-TACA that become immunogenic. Since Neu5Gc is a consumed dietary sugar it may provide a unique opportunity for personalized-medicine that likely relates to the individual's dietary habits. To fully appreciate its potential, this essay will first review current literature of glycosylation and its changes in cancer focusing on aberrant sialic acid expression (Sections 2–4). Subsequently, current knowledge on the role of Neu5Gc and the related anti-Neu5Gc antibodies in cancer will be described (Section 5), followed by discussion of their potential as novel personalized-cancer theranostics (Section 6).

2. Cell-surface glycosylation

Cell surface glycosylation is universal to all living cells reflecting their physiological state, and perfectly positioned to mediate adhesion and motility, as well as intracellular signaling events [14,15]. Monosaccharide units serve as building blocks that are synthesized via covalent glycosidic-linkages into chains termed glycans (oligosaccharides and polysaccharides) [16]. Each monosaccharide is transferred from activated sugar-nucleotide donor to the acceptor molecule in a stepwise fashion. The combined action of various enzymes (i.e., glycosyltransferases and glycosidases) leads to diverse glycan structures [17]. These can exist either as free forms or conjugated to proteins and lipids (Fig. 1) and include: (1) glycoproteins with complex and branched N-linked glycans (conjugated to Asparagine) and/or with O-linked glycans (conjugated to Serine...
or Threonine) that are abundant on mucins; (2) glycosylphosphatidylinositol (GPI)-anchored proteins; (3) glycosaminoglycans (GAGs) either as linear free polysaccharides (such as hyaluronan) or attached to Serine residues of proteoglycans (such as heparan sulphate and chondroitin sulphate); and (4) glycolipids, which consist of glycans linked to ceramide. In addition to cell surface glycans, nuclear and cytoplasmic proteins can be modified with O-glycans linked to underlying sugars via their second carbon to either galactose (α2−3Gal or α2−6Gal), N-acetylgalactosamine (α2−6GalNAc) or to another Sia (α2−8Sia) [49] (but in some cases also to N-acetylgalactosamine (α2−6GlcNAc) [50]). Changes in Sia level, linkage and distribution are associated with various aspects of malignant transformation [44,51–54].

General increase in cell surface Sia was shown to promote metastatic potential [19,51,52,55–57] and result from various routes [44]. Changes to the core structures of N-glycans are some of the most common aberrant glycosylation in cancer. Increased activity of the β1,6GlcNAc branching enzyme, N-acetylgalactosaminyltransferase V (GlcNAc-TV or MGAT5), lead to larger and more branched N-glycans thus providing additional acceptors for terminal sialylation (Fig. 2) [38,44,58]. Together with increased expression of sialyltransferases [59,60], these changes contribute to increased cell surface sialylation and metastatic potential [38,38]. Similarly, aberrant O-linked glycosylation can lead to increased sialylation. Carcinomas (tumors of epithelial origin) overproduce mucins that are heavily glycosylated high molecular weight glycoproteins (e.g., MUC1, MUC4, MAC6, MAC5AC) characterized by dense clusters of O-glycans, although N-glycans can also be present [61–64]. Cancer-associated mucin-type O-glycans tend to be truncated due to a shift in the normal enzymatic machinery and are usually highly sialylated and less sulphated (Fig. 2) [61–63,65,66].

Increased sialylation is also evident by expression of polysialic acid (polySia or PSA) that is an oncofetal antigen associated with various types of cancers (e.g., neuroblastoma, non-small cell lung carcinoma, breast cancer) [12,67], as well as other diseases [67]. PSA is synthesized in the Golgi by the polysialyltransferases STBSiall and STBSialV, generating N-glycans with a linear homopol-

3. Aberrant cell-surface glycosylation in cancer

Glycosylation is remarkably dynamic and commonly modified in cancer leading to the expression of cancer-associated antigens [19], sometimes referred to as “onco-fetal antigens” that recapitulate expression normally limited to embryonic tissues [19,20]. The two basic principles that phenotypically guide these changes are incomplete synthesis and neo-synthesis of cancer-associated cell surface glycans [21–23]. These changes commonly apply to early-stage cancers and to advanced-stage cancers, respectively [24]. In general, a shift from the normal glycosylation pathway leads to altered glycan expression due to one or more of the following changes: (1) under- or overexpression of glycosyltransferases deregulated at the level of epigenetics [25,26], transcription [27–31], post-transcription [32] and/or chaperone [33]; (2) altered glycosidase activity [34–36,36,37]; (3) altered expression of glycoconjugate acceptor together with availability and abundance of the sugar nucleotide donors [38]; (4) altered sugar nucleotide transporter activity [39]; and (5) improper function of the Golgi structure [40] where many of the glycosyltransferases are harbored [41]. Evidence is accumulating that aberrant glycosylation contributes to various aspects of cancer development and progression, including proliferation, invasion, angiogenesis, metastasis and immunity [12,16,42]. Yet, oncogenic glycosylation is not random but rather is limited to a distinct subset of glycans that become modified, enriched or decreased on the tumor cell surface and mediate either promotion or inhibition of tumor progression [12,43,44].

4. Altered sialic acids expression

As early as 1960s there was considerable evidence that the surface properties of cancer cells are different than those of normal cells [45] and that sialic acids largely contribute to that phenotypic change [46–48]. Sialic acids (Sia) are nine-carbon backbone α-keto acidic sugars with their carboxylate group normally negatively charged at physiological pH. They are capping vertebrate glycans and found α-linked to underlying sugars via their second carbon to either galactose (α2−3Gal or α2−6Gal), N-acetylgalactosamine (α2−6GalNAc) or to another Sia (α2−8Sia) [49] (but in some cases also to N-acetylgalactosamine (α2−6GlcNAc) [50]). Changes in Sia level, linkage and distribution are associated with various aspects of malignant transformation [44,51–54].

Fig. 1. Common glycans on human cells. Examples of the major glycoconjugates classes are depicted, as described in the main text.
Changes in the general expression of Sia, increased activity of sialyltransferases also leads to the overexpression of certain terminal glycan structures in various malignant tissues. Rather than a single change in glycosylation, each type of malignant tissue is characterized by a set of changes in glycan expression that can be broadly expressed on various cancers [94–96].

In addition to changes in the general expression of Sia, increased activity of sialyltransferases also leads to the overexpression of certain terminal glycan structures in various malignant tissues. Rather than a single change in glycosylation, each type of malignant tissue is characterized by a set of changes in glycan expression that can be broadly expressed on various cancers [94–96]. Fig. 2 summarizes some of the most common aberrant glycan motifs in cancer including the aforementioned β1-6GlcNAc branching in N-linked structures (competing with β1-4GlcNAc) and polysialic acid (PSA) in N-linked structures; as well as Thomsen–Friedrich (T or TF), Thomsen–Nouvelle (Tn) and sialyl-Tn (STn or CD175s) in O-linked structures; sialyl Lewis (SL) in various tissues. Rather than a single change in high-grade tumors than in low-grade tumors [72], and progression is associated with higher expression level of PSA and its biosynthesizing enzymes (mainly ST8SiaII) [73,74]. In addition, PSA has an anti-adhesive effect on cell-cell interactions thus facilitating the detachment of cells from the primary tumor, and thereby promoting cancer invasion and metastasis [67,70,75,76].

Changes in expression of α2–3/6-linked Sia have also been described in cancer. Mass spectrometry analysis of human serum sialo-glycoproteins revealed that many sialylated glycan featured increased levels of α2–6 sialylation in breast cancer [77] and lung cancer samples [78], while α2–3 sialylation was decreased in lung cancer [78] but increased in prostate cancer samples [79], in malignant brain tumors [80] and in ovarian serous carcinoma [81]. In addition, tumor cells often express increased levels of α2–6Sia, mainly due to upregulation of the ST6Gal-I [82–86] or ST6GalNAc sialyltransferases [87–89] that respectively conjugate terminal α2,6-sialic acids at their tips, and those sialoglycans can be conjugated to either protein- or lipid-carriers (Fig. 2). Following is an overview of the expression of specific sialoglycans in malignancy and their biological effects that are commonly facilitated by interactions with lectins.

4.1. Expression of sialyl-Lewis structures

The histoblood group Lewis (Le) antigens are found in most human epithelial tissues at the tips of various glycolipids and glycoproteins [97]. The biosynthetic acceptors for Le antigens are two unique disaccharides to which activated fucose and sialic acid residues are added by specific fucosyltransferases and sialyltransferases, respectively. Type-1 disaccharide (Galβ1-3GlcNAc) serves as the precursor for Le a, Le b and Le x, while the Type-2 (Galβ1-4GlcNAc) serves as the precursor for Le a, Le b and Le x (Fig. 3). Normally, Le b and Le x (Type-1 based) are widely expressed, while Le a and Le b (Type-2 based) are found at relatively low levels. However, the expression of the sialylated antigens, SL and Le x, is significantly enhanced in cancer (Fig. 2) [24,57]. The expression of these cancer-associated antigens results mainly from incomplete synthesis (e.g., increased expression of ST3Gal IV [57] and impairment of subsequent α2–6 GlcNAc sialylation lead to expression of SL x instead of the normal Disialyl-Le x [50; Fig. 3], but in some cases represent neo-synthesis (e.g., induction of SL x expression on adult T-cell leukemia) [24,48]. Abnormal expression of such cancer-associated carbohydrate antigens can be intimately associated with genetic transformation within the cells [99]. For example, it has been shown that in adult T-cell leukemia, the strong and constitutive expression of SL x is due to the transcriptional activation of the fucosyltransferase, Fuc-T VII (encoded by FUT7), which is the rate-limiting enzyme in SL x synthesis in these cells [99]. On the other hand, in advanced stage cancers of epithelial origin (carcino-

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<td>STn</td>
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Fig. 2. Common cancer-associated glycans. Some of the most common aberrant glycan motifs in cancer are described. Sialylated glycoconjugates are shaded in gray.
enhanced expression of SLeα or UDP-galactose transporters) was implicated in the increased expression of the genes for some sugar transporters (i.e., SLeα complex glycans have also been implicated in the expression of glycosylation or histone modification) that are involved in synthesis of Lewis antigens. Lewis antigens derive from the substitution of Type-1 (Galβ1-4GlcNAc) by Type-2 (Galβ1-3GlcNAc) disaccharide sequences by subsequent addition of fucose and sialic acid residues [24,37,112]. Main enzymes involved are indicated (red or green). Cancer-associated carbohydrate antigens (Leα, SLeα, SLeβ) are in bold-dashed boxes, with the sialylated antigens in gray shading. ST6GalNAc VI and FucT III (green) compete for their common substrate Leα and can lead to expression of SLeα instead of the normal 6S-SLeα (L-selectin ligand; the suggested normal biochemical pathway in green [183,184]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Biosynthesis of Lewis antigens. Lewis antigens derive from the substitution of Type-1 (Galβ1-3GlcNAc) and Type-2 (Galβ1-4GlcNAc) disaccharide sequences by subsequent addition of fucose and sialic acid residues [24,37,112]. Main enzymes involved are indicated (red or green). Cancer-associated carbohydrate antigens (Leα, SLeα, SLeβ) are in bold-dashed boxes, with the sialylated antigens in gray shading. ST6GalNAc VI and FucT III (green) compete for their common substrate Leα and can lead to expression of SLeα instead of the normal 6S-SLeα (L-selectin ligand; the suggested normal biochemical pathway in green [183,184]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mas) other fucosyltransferase isoenzymes are involved, mainly Fuc-T III and VI (which are not expressed by leukemic cells) [100]. Epigenetic silencing of some glycogenes (e.g., by DNA methylation or histone modification) that are involved in synthesis of complex glycans have also been implicated in the expression of SLeα/SLeα in the early stage cancers (representing incomplete synthesis), while in advanced stages transcription of other glycogenes are accelerated under hypoxic conditions [25]. In addition, increased expression of the genes for some sugar transporters (i.e., GLUT-1 or UDP-galactose transporters) was implicated in the enhanced expression of SLeα/SLeα [101].

Both SLeα and SLeα contribute to hematogenous metastasis, in which blood-invading cancer cells adhere to blood vessels endothelial cells in a process that requires the presence of carbohydrate ligands on cancer cells and at the same time E-selectin receptors on endothelial cells [24,102]. SLeα mediates adhesion of cancer cells derived from the lower digestive organs (colon and rectum), pancreas and biliary tract, while the SLeα mediates adhesion of breast, ovarian and pulmonary cancer cells [102,103].

E-Selectin is not constitutively expressed on endothelial cells, but once induced by an inflammatory stimulus (i.e., TNF, IL-1α, IL-1β) can be vigorously shed from the endothelial cell surface. Elevated levels of serum E-selectin in patients bearing SLeα/SLeα-positive tumors predicts a high risk for developing metastasis [102,104,105]. In addition, under hypoxic tumor microenvironment there is a significant induction of SLeα and SLeα expression with concomitant increase in E-selectin binding activity, together promoting tumor vascularization and growth [108]. Two other members of the selectin family include P- and L-selectin; The P-selectin expressed within endothelial cells or platelets is stored in specific granules but translocated to the surface upon stimulation, while L-selectin is known to be constitutively expressed on leukocytes. It was recently demonstrated that ST3Gal-IV and ST3Gal-VI are the major sialyltransferases involved in the generation of functional selectin ligands in vivo [107]. Each selectin member has a distinct ligand specificity: E-selectin binds SLeα, while L-selectin requires SLeα carrying 6-O-sulfation at the GlcNAc moiety (Neu5Acα2-3Galβ1-4(Fucα1-3)SO3α2-6GlcNAcβ-R; 6S-SLeα), and P-selectin reacts only when the carbohydrate determinant is carried by the specific core protein PSGL-1, which has sulfated tyrosine residues at its N-terminal region (in some cases replaced by other core proteins e.g., CD24) [24,108,109]. Importantly, all selectins cross-react with 6S-SLeα making it a ‘universal selectin ligand’ and potential potent selectin inhibitor [24]. However, 6S-SLeα is preferentially expressed on non-malignant epithelial cells, and tends to decrease upon malignant transformation. Finally, because most cancer cells express the non-sulfated version of SLeα they are likely more responsive to the E-selectins [110].

4.2. Expression of Sialyl-Tn

Incomplete synthesis of O-glycans lead to generation of the low molecular weight sugar antigens Tn, T and STn that become enriched in cancer, especially on mucins (Figs. 2 and 4) [111]. STn (Neu5Acα2-6GalNAcα-O-R) can be detected in almost all carcinomas (e.g., pancreas, ovarian, colorectal, stomach, liver, and breast) at variable frequencies (5–100%) but correlating with invasive and aggressive potential [112–115]. Conversely, it is rare in normal healthy tissues mostly limited to the upper digestive tract [114,116,117], or covered with O-acetylation [115]. The STn-expressing glycoproteins in human carcinoma include MUC1, CD44 and MUC2 [87,116,118,119]. Two major alterations in the normal O-glycan synthesis pathway lead to overexpression of STn: (i) mutations in the T-synthase chaperone COSMC that blocks
O-glycan elongation and shift the pathway towards generation of GalNAc (Tn) [120,121], and/or (ii) overexpression of ST6GalNAc-I that combines sialic acid onto GalNAc to make the STn [88,89] (Fig. 4). Likewise, transfection of ST6GalNAc-I cDNA can induce STn expression in various cancer cell lines expressing Core-1 and Core-2 glycan-competent active T-synthase (Core-1 β1-3-galactosyltransferase) [57,88,122]. Expression of STn leads to decreased adhesion and increased tumor growth, mobility, migration and invasion [87,123,124].

Altogether, these characteristics prompted the design of the STn cancer vaccine ‘Theratope’, in which STn is conjugated to the immunogenic protein carrier keyhole limpet haemocyanin (KLH) [125]. However, despite initial encouraging results [126–128] a large scale Phase III trial on women with metastatic breast cancer failed to demonstrate that Theratope improved median time to disease progression or overall patient survival [129,130]. However, it is worth noting that the vaccine was given to all enrolled patients without prior screening for the expression of STn, thus it is possible that if the treatment was ‘personalized’ for the potential responsive patients the outcome of the trial may have been different [57]. In addition, it was demonstrated in mice that Theratope-induced tumor protection was dependent on the quantity of anti-STn antibodies raised by immunization [131].

4.3. Altered gangliosides expression

Gangliosides are sialic acid-containing glycosphingolipids. Biosynthesis of gangliosides (Fig. 5) involves the sequential addition of sialic acids to the precursor lactosylceramide (LacCer) by ST3Gal V (GM3 synthase), ST8Sia I (GD3 synthase) and ST8Sia V (GT3 synthase) that leads to the biosynthesis of the precursor of a-series, b-series and c-series gangliosides, respectively representing the mono-, di- and tri-sialylated gangliosides (Fig. 5) [57]. These are normally arranged in glycosynapses, with their hydrophobic cera-
mide moiety anchored in the outer leaflet of the plasma membrane and the hydrophilic sialglycans extending outward from the cell surface [132,133]. Gangliosides function as regulatory elements either as receptors in cell–cell trans recognition or interact laterally in cis to regulate downstream signaling of various proteins (e.g., insulin, epidermal growth factor, and vascular endothelial growth factor receptors) [134,135].

Some gangliosides are frequently overexpressed in cancer (Fig. 2; [94]) and play a key role in the induction of invasion and metastasis [136,137]: GD2 in neuroblastoma [138] and small cell lung cancer (SCLC) [139], GD3 and its modified version 9-O-acet-
yl-GD3 in melanoma [94,140–142], and GM2 in SCLC [143], (all showing some expression in normal tissues). Fucosylated GM1 (Fucosyl-GM1) is more tumor-restricted and expressed in SCLC with limited expression in sensory nerves [94,144–146]. Cancer-associated gangliosides are expressed either on the cell surface or shed by tumors, exerting an immunosuppressive effect by sensitizing T lymphocytes to apoptosis [147]. Consequently, there have been several efforts to generate cancer vaccines to these ganglio-
sides [148]. Recent studies in human breast cancer stem cells (CSC) indicated a functional role for the overexpressed GD2 and GD3. Reduction in their expression caused a phenotypic change from CSC to a non-CSC suggesting a possible novel approach in targeting human breast CSCs to interfere with cancer recurrence [149]. Another novel approach to improve on cancer specificity concerns the type of terminal sialic acid in the sialylated-cancer associated antigen.

5. Expression of dietary immunogenic Neu5Gc in cancer and related anti-Neu5Gc xeno-autoantibodies

N-glycolyneuraminic acid (Neu5Gc) is a non-human Sia that accumulates on human carcinomas [150,151]. Neu5Gc is an excellent candidate for personalized-theranostics due to its unique
characteristic properties: it originates from the diet, it combines with various glycans generating neo-sialylated-antigens, and it is immunogenic in humans.

The two major Sias in mammals are N-acetylneuraminic acid (Neu5Ac) and Neu5Gc that differ by a single oxygen atom. Hydroxylation of nucleotide-activated Neu5Ac by CMP-Neu5Ac hydroxylase (CMAH) leads to generation of Neu5Gc. However, this enzyme is specifically inactivated only in humans, but not in all other mammals studied to date [152], with no alternative Neu5Gc-synthesis pathway [153]. Despite that, Neu5Gc is compatible with human cells’ biochemical pathways and is metabolically incorporated from dietary mammalian foods [154–157] (Neu5Gc is enriched in red meat and milk products, but low or undetected in chicken and fish) [154,158]. In vitro studies showed that Neu5Gc is incorporated into tumor cells mainly by macrophagocytosis then delivered into the cytosol by a lysosomal transporter [155] that can be up-regulated under hypoxic conditions [159]. It was also shown that Neu5Gc metabolism is enhanced by high cell growth rates [160]. Once in the cytosol Neu5Gc is activated and incorporated into cell surface glycans replacing the native Neu5Ac [155]. Consequently, Neu5Gc appears at low levels on the cell surface of human epithelia and endothelia but is especially enriched on carcinomas [150,154,161]. Conversely, Neu5Gc is recognized as foreign by the human immune system that promotes a polyclonal highly diverse antibody response in all humans [154,160,162,163], recognizing multiple Neu5Gc-containing antigens [162]. Anti-Neu5Gc IgM/IgG arise in infants at 6 month old coinciding with dietary-Neu5Gc, and are likely induced via dietary-Neu5Gc uptake by commensal bacteria [164]. Thus, Neu5Gc-glycans are termed ‘xeno-autoantigens’ and the antibodies against it are termed ‘xeno-autoantibodies’, because Neu5Gc is presented as self (auto) on the cell surface but at the same time recognized as foreign (xeno) [162]. Consequently, such antibodies are potential barrier for xenotransplantation [165], and it was shown that burn patients transiently exposed to xeno-pig-skin had high serum anti-Neu5Gc IgG even 10 years (and up to 30 years) after the actual procedure [166]. Furthermore, human anti-Neu5Gc antibodies could be affinity-purified from human sera and were shown to bind human carcinomas [162,167].

5.1. Role of anti-Neu5Gc IgG in cancer promotion and diagnostics

The effect of anti-Neu5Gc antibodies on tumor growth was tested in a relevant mouse model. Neu5Gc-deficient mice (Cmah<sup>−/−</sup>) were injected with Neu5Gc-positive tumors, and once established were treated with low dose anti-Neu5Gc antibodies. This resulted in chronic inflammation-mediated tumor growth stimulation that was suppressed by an anti-inflammatory treatment [168]. These results supported the notion that the combination of Neu5Gc with circulating anti-Neu5Gc antibodies might contribute to the high frequency of diet-related human carcinomas [169–173]. These findings established a novel paradigm of chronic inflammation-mediated disease induced by metabolized dietary-sugar that also spurs an immune response [168]. It was also later supported by follow up studies in humans [174]; Subsequent development of a specialized sialoglycan-microarray with multiple Neu5Gc-/Neu5Ac-glycans allowed testing anti-Neu5Gc IgG responses in sera samples of cancer versus non-cancer patients. This led to discovery of the novel antibody carcinoma biomarker, anti-Neu5Gc-Sialyl-Tn IgG [174]. Neu5Gc-Sialyl-Tn resembles the well-known cancer-associated carbohydrate antigen STn (Fig. 2), only that Neu5Gc replaces Neu5Ac [174]. To further establish anti-Neu5Gc antibodies as potential cancer biomarkers for early detection of cancer, larger and longitudinal population studies are needed. Recently, a novel method for detection of the total polyclonal anti-Neu5Gc antibodies response was developed that may facilitate high-throughput screening of human sera samples for such further studies [175].

Similar to Neu5Gc-Sialyl-Tn, other sialylated-cancer-associated antigens (Fig. 2) can occupy the sialic acid Neu5Gc instead of Neu5Ac and thus may result in enhanced theranostic potential. It was shown that expression of GM2 ganglioside is induced in can-
cancers, but in addition its Neu5Gc content increases by hypoxia-induced transcription of a sialic acid transporter gene resulting in (Neu5Gc)GM2 that has high cancer specificity [25]. Similarly, (Neu5Gc)GM3 was described as a cancer-associated antigen and potential therapeutic target [176,177].

5.2. Role of anti-Neu5Gc IgG in immunotherapy

Since anti-Neu5Gc antibodies recognize a unique cell surface marker on cancer cells they are also potential immunotherapeutics. Early evidence suggested that human sera with high anti-Neu5Gc humoral response could promote complement-dependent killing of leukemic cells [160]. Likewise, human serum or affinity-purified antibodies rich with high anti-Neu5Gc-Sialyl-Tn IgG reactivity could promote killing of human cancer cells expressing this unique Neu5Gc-antigen by both complement- or antibody-dependent cellular cytotoxicity (CDC or ADCC) [175]. Further studies in the human-like Neu5Gc-deficient mice model (Cmah−/−) suggested that the impact of the anti-Neu5Gc immune response may be dose-dependent: while a low dose of affinity-purified human IgG supported tumor growth [168], a higher dose inhibited tumor growth [174]. Anti-Neu5Gc antibodies can bind to Neu5Gc-positive tumors, but they can also recognize acellular circulating Neu5Gc-antigens such as Neu5Gc-sialoglycoproteins.

5.3. Effects of Neu5Gc-antigens on glycosylated therapeutic antibodies

Varying amounts of Neu5Gc are found on many clinically-used biotechnological glycoproteins (e.g., antibodies, inhibitors, cytokines etc.), likely originating from the production process within non-human mammalian cell lines and/or the addition of animal-derived tissue culture supplements [178,179]. Circulating anti-Neu5Gc antibodies can capture such Neu5Gc-containing biotechnologies (e.g., anti-cancer drug Erbitux (Cetuximab)) and generate immune complexes that can mediate rapid drug clearance. This leads to decreased effective concentration of the administered drug [178] that eventually can reduce its efficacy. Thus, individuals with high anti-Neu5Gc antibodies response that are treated with such Neu5Gc-glycosylated biotechnologies would likely have a diminished response to the therapy. Such detrimental outcome may be avoided if patients take initial personalized-screening for presence of circulating anti-Neu5Gc antibodies.

6. Conclusions and perspectives

It is now well accepted that nutrition is likely the most important environmental factor that modulates expression of genes involved in metabolic pathways leading to various diseases [180]. It was also suggested that consumption of micronutrients can even have long-lasting effects on the health of adult descendants [181]. Thus, identification of the mechanisms that lead to glycosylation changes in cancer is crucial for designing better therapeutic interventions. A unique cancer-associated-glycan generally reflects severe changes in cancer is crucial for designing better therapeutic interventions. Such detrimental outcome may be avoided if patients take initial personalized-screening for presence of circulating anti-Neu5Gc antibodies.

Conflict of Interest

None declared.

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