Chapter 3

Monoclonal Antibodies Against Tumour-Associated Carbohydrate Antigens

Jia Xin Chua and Lindy Durrant

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66996

Abstract

Glycomic profiling of tumour tissues consistently shows alterations in N- and O-glycosylation profiles of glycoproteins and glycolipids compared to healthy tissues, with important functional implications for cancer cell biology. The overexpression of tumour-associated carbohydrate antigens (TACAs), as a result of aberrant glycosylation in tumours, is usually correlated with poor prognosis and survival of cancer patients. In tumours, TACAs are associated with worse tumour progression than the deletion and inactivation of tumour suppressor genes. The findings of TACAs acting are not merely tumour markers but also constitute part of the machinery in inducing cancer metastasis and invasiveness further strengthen the scientific rationales for immunotherapy targeting TACAs. Despite the attractiveness of the TACAs, there are very few anti-glycan monoclonal antibodies (mAbs), as glycans usually induce low-affinity IgM responses. This chapter provides an overview of TACAs, direct killing anti-glycan mAbs, and introduces two murine mAbs (FG88 mAbs) that recognise Lewis carbohydrate antigens overexpressed on tumour glycoconjugates with high functional affinity. Although the production of anti-glycan mAbs against cancers is not new, the production of high-affinity IgG anti-glycan mAbs is novel. FG88 mAbs definitely have great potential in cancer therapy and serve as valuable tools in glycobiology research.

Keywords: cancer, Lewis carbohydrate antigen, therapeutic monoclonal antibody, oncosis, antibody drug conjugate

1. Introduction

Cell surface glycosylation is a post-translational modification of proteins and lipids, which is universal to all living cells and plays an important role in cell signalling, immune recognition and cell-cell interactions [1]. Monosaccharide units serve as building blocks of glycans

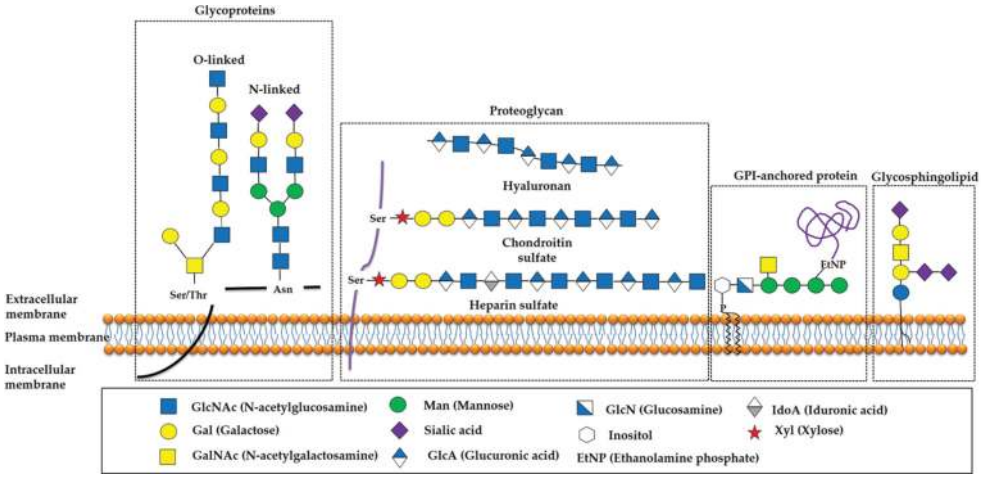

Figure 1. Common glycoconjugates on human cells.

(polysaccharides) that are synthesised by a complex series of post-translational enzymatic steps [1, 2]. There are several main families of glycoconjugates: (1) the Asn-linked (N-linked) and Ser-/Thr-linked (O-linked) oligosaccharides that are present on many glycoproteins, (2) the glycosaminoglycans (GAGs) either as linear-free polysaccharides (such as hyaluronan) or attached to serine residues of proteoglycans (such as heparin sulphate and chondroitin sulphate), (3) the sphingolipids that consist of oligosaccharides linked to ceramide and (4) the glycosylphosphatidylinositol (GPI)-linked proteins, which are proteins that express a glycan chain linked to phosphatidylinositol [2, 3] (**Figure 1**).

2. Glycoproteins

It has been appreciated for some time that protein glycosylation is the most complicated post-translational modification that a protein can undergo [4]. Protein glycosylation is important as it alters the behaviour of proteins, making them more soluble, protecting them from proteolysis, covering antigenic sites and altering the orientation of proteins on cell surfaces [5]. In glycoproteins, the carbohydrate units are linked to the protein backbone by N- and/or O-glycosidic bonds, C-mannosyl bonds, phosphoglycosyl bonds and glypiated linkage (GPI anchor) [4].

N-glycans are covalently attached to the asparagine (Asn) residues of proteins, and the consensus sequence for N-glycosylation is Asn-X-Ser/Thr, where X can be any amino acid except proline. In O-glycosylation, the glycan is attached to the side chains of serine or threonine residues. Unlike N-linked glycosylation, no consensus sequence defining an O-linked glycosylation site has been reported in Ref. [4]. C-mannosylation is a novel type of protein glycosylation, which differs fundamentally from N- and O-glycosylations. It involves covalently

http://dx.doi.org/10.5772/66996

attachment of an α-mannopyranosyl residue to the indole C2 carbon atom of tryptophan (Trp) via a C-C link [6, 7]. The phosphoglycosyl bond is another distinct type of glycopeptide linkage [N-acetylglucosamine (GlcNac), mannose (Man) and fucose (Fuc)] involving an attachment of a carbohydrate to protein via a phosphodiester bond [8]. Another important carbohydrate-protein connection is the GPI anchor. In this connection, mannose is linked to phosphoethanolamine, which in turn is attached to the terminal carboxyl group of the protein [9].

3. Glycolipids

Glycolipids constitute approximately 3% of the outer layer of the plasma membrane, and they are composed of a lipid tail and a carbohydrate head. Glycolipids are classified into three main groups, including glycoglycerolipids, glycosylphosphatidylinositols (GPI) and glycosphingolipids (GSLs), based on the type of lipid component. Of these glycolipids, GSLs are the ones that are most widely overexpressed on tumours [10].

GSLs are ubiquitous membrane constituents, which are embedded in the cell plasma membrane [11]. Ninety percent of mammalian GSL biosynthesis begins with the synthesis of glucosylceramide (GlcCer), which is a key precursor of the glycosphingolipid series [12]. The process takes place on the cytosolic face of the Golgi complex via the action of the Type I transmembrane protein glucosylceramide (GlcCer) synthase [13], which transfers a glucose (Glu) residue to ceramide in the β-glycosidic linkage [14, 15]. Galactose is then added to GlcCer to generate lactosylceramide (LacCer) by β-1,4-galactosyltransferases in the lumen of the Golgi apparatus [10]. Further glycosylation steps are catalysed by different glycosyltransferases

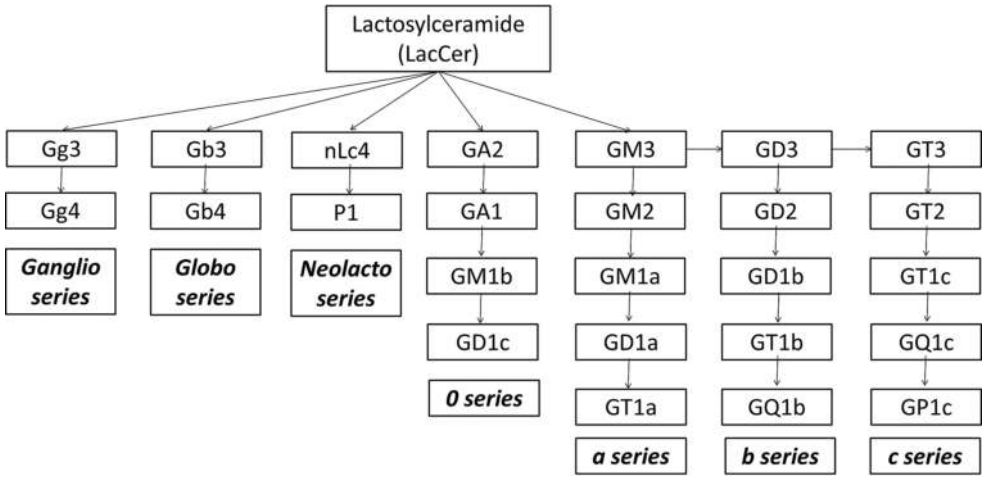

Figure 2. Synthetic pathways for the major GSL species. Lactosylceramide provides the branch point for different GSL series.

with different specificities and result in the generation of more complex GSLs [16]. The major GSL series are defined by their internal core carbohydrate sequence. They are the ganglio-series (galNacβ1-4 gal), globo-series (galα1-4 gal), lacto-series (galβ1-3glcNacβ1-3 gal) and neolacto-series (galβ1-4glcNacβ1-3 gal). LacCer provides the branch point for the synthesis of all these GSL series (**Figure 2**) [12].

4. Aberrant glycosylation in cancers

Aberrant glycosylation has been described as one of the hallmarks of cancer. Aberrant glycosylation of proteins and lipids during malignant transformation leads to the overexpression of tumour-associated carbohydrate antigens (TACAs) [17]. Evidence is accumulating that TACAs have contributed to various aspects of cancer development and progression, including proliferation, invasion, angiogenesis and metastasis [2, 18]. Thus, studying the mechanisms and consequences of variations in glycosylation associated with cancers will provide crucial insight into cancer progression. Importantly, TACAs are overexpressed mostly on tumour cell surface, making them potential diagnostic markers and ideal therapeutic targets [19].

In general, aberrant glycosylation in cancers is due to the following changes: (1) under- or overexpression of glycosyltransferases, (2) altered expression of glycoconjugate acceptor [20], (3) altered sugar nucleotide transporter activity [21] and (4) improper function of the Golgi structure [22].

4.1. Altered glycosylation patterns in cancer

In cancers, the expression of glycosyltransferases is often deregulated. For example, N-acetylglucosaminyltransferase V (GnT-V), which catalyses the formation of β-1,6-GlcNAc branching structures, is expressed only at very low level in normal mammary gland. However, in cancer, the expression of GnT-V has been upregulated, resulting in highly branched N-glycan structures which were found to be associated with cancer growth and metastasis [20, 23–25]. Overexpression of beta-1,3-N-acetylglucosaminyltransferase 8 (β3GnT8) results in increased levels of polylactosamine structures in colorectal carcinoma [26], and upregulation of N-acetylglucosaminyltransferase III (GnT-III) increase bisected N-glycans in liver cancer [27, 28]. Aberrant expression of alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1 (ST6GalNAC-I) in breast cancer resulted in the sialylation of Tn antigen to form the sialyl-Tn (STn) antigen [29]. Altered expression of fucosyltransferases is responsible for the aberrant expression of Lewis carbohydrate antigens such as Lewis a (Le^a), sialyl-Lewis a (SLe^a), Lewis x (Le^x) and Sialyl-Lewis x (SLe^x) in many types of tumours [30–32].

Incomplete glycosylation is another abnormal feature found in human cancer. The expression of truncated O-glycans such as Tn, STn and T antigens had been reported in a wide range of tumours [33–36]. Incomplete glycosylation of these truncated glycans is due to the defects in the secretary pathway organelles (endoplasmic reticulum and Golgi) [33], the absence of glycosyltransferases responsible for the generation of core glycan for chain elongation [37] and the overexpression of sialyltransferases responsible for the addition of terminal sialic acid

http://dx.doi.org/10.5772/66996

(i.e. conversion of Tn to STn antigen) [33, 38, 39]. For example, in neoplastic cells, alterations in glycosylation of O-linked glycans had been shown to affect oligomerisation of cell surface receptors, thus influencing the stimulation of these receptors. Wagner et al. demonstrated the inhibition of complex O-glycan formation (N-acetyl-galactosamine galactose core I structure and its subsequent sialylation), resulted in the impairment of death receptors 4 and 5 (DR4 and DR5), which significantly impact the apoptotic pathway signalling by TNF-related apoptosis-inducing ligand (TRAIL) [40].

In addition, specific changes in O-glycan (GalNAc-Ser/Thr) and N-glycan core structures have been reported to lead to the generation of different core glycans with different degrees of glycan branching [27, 28], which in turn significantly impact the overall glycan structure and function.

4.2. Tumour-associated carbohydrate antigens (TACAs)

4.2.1. Altered sialic acid expression

As early as the 1960s, there was evidence that tumour cells of various origins increased the expression of sialic acids on membrane glycoproteins and glycolipids as well as their secretion into the tumour microenvironment [41–43]. Sialic acids on normal cells are involved in multiple different physiologic processes [44]. However, hypersialylation of tumour cells specifically benefits tumour cell growth, promotes metastases [45, 46] and correlates with a poor prognosis of cancer patients [47].

Sialic acids are nine-carbon backbone α-ketoacidic sugars [3]. In general, sialic acids terminate the outer end of glycans (sialoglycans) via more than 20 distinct Golgi-resident sialyltransferases (ST). This enzymatic process is carried out via their second carbon (C2) to either galactose (α2-3Gal or α2-6Gal), N-acetylgalactosamine (α2-6GalNac) or another sialic acid (α2-8Sia) [48]. Altogether, the different linkages to underlying sugars result in a tremendous diversity of sialoglycans [44]. Sialoglycans are known to participate in cell-cell and cell-extracellular matrix interaction, including adhesion, migration and immune recognition [44]. Sialic acid-binding immunoglobulin-like lectins (Siglecs) are receptor families that specifically recognise sialoglycans. Siglecs can be found on most immune cells, and they can transmit immunosuppressive signals upon binding to sialic acid ligands. Thus, increased expression of siglec ligands by tumour cells could contribute to tumour immune invasion [49].

There are a number of causes of the increase in cell surface sialic acid [50]. Changes to the core structures of N-glycans are one of the most common aberrant glycosylations in cancer. Increased activity of GNT-V (also known as MGAT5) was found to result in larger and more branched N-glycans, thus providing additional acceptors for terminal sialylation [20, 50]. Similarly, carcinomas that overproduce mucins (heavily glycosylated high-molecular-weight glycoproteins, e.g. MUCI and MUC4) which contain aberrant O-linked glycosylation can lead to increased sialylation [51, 52]. Together with increased expression of sialyltransferases [53], these enzymes increase cell surface sialylation and metastatic potential [20]. In addition, tumour cells often overexpress α2-6 sialic acid, mainly due to upregulation of the ST6Gal-I [54–56] or ST6GalNAc sialyltransferases [29, 57] that respectively conjugate terminal sialic

acid to N-glycans or O-glycans and glycolipids [53]. Mass spectrometry analysis of human serum sialo-glycoproteins revealed increased expression level of α2-6 sialylation in breast cancer [58] and lung cancer samples [59], whereas α2-3 sialylation was increased in prostate cancer samples [60], malignant brain tumours [61] and ovarian serous carcinomas [62].

Aberrant expression of sialic acids confers major advantages to tumour cells. Therefore, by targeting these, sialoglycans overexpressed on tumours may be highly beneficial.

4.2.2. Altered Lewis carbohydrate antigen expression

Lewis carbohydrate antigens can be found on various glycoconjugates in most human epithelial tissues [63]. They are formed by the sequential addition of fucose onto oligosaccharide precursor chains on glycoproteins or glycolipids through the action of a set of glycosyltransferases [64]. Le^x was reported to be overexpressed in breast and gastrointestinal carcinomas. Normal expression of Le^x is restricted on certain normal epithelial cells including the oesophagus, stomach, small bowel, ciliated epithelium of trachea, bronchus [65, 66] and normal human polymorphonuclear neutrophils (PMNs) [67]. Le^y was reported to be overexpressed on ovarian, breast, prostate, colon and lung carcinomas. Although Le^y expression can be found on both normal and neoplastic tissues, Le^y distribution differs between the two tissue types. Expression of Le^y on normal epithelial tissues is restricted to the secretory borders of epithelial surfaces, making it less accessible to circulating antibodies. Conversely, Le^y expression on epithelial cancer cells occurs on all surfaces including luminal surfaces [65].

Sialylated Lewis carbohydrate antigens such as SLe^a and SLe^x are significantly enhanced in cancer [68, 69]. The expression of these cancer-associated antigens results mainly from the upregulation of sialyltransferases [68]. SLe^a is normally present on the inner surface of the ductal epithelium of a variety of epithelial tissues, which makes it largely inaccessible to antibodies and immune effector cells [70]. SLe^x can be found on granulocytes, normal oral mucosa and breast tissue [71]. Both SLe^a and SLe^x are found to be aberrantly expressed on the surface of a broad range of carcinomas such as breast, ovarian, melanoma, colon, liver, lung and prostate [72]. Overexpression of SLe^x and SLe^a appears to directly correlate with increased metastatic disease and poorer overall survival in patients with colorectal cancer invasion [73]. Similar results were obtained from analysis the combination of SLe^a, SLe^x and Le^y antigens in non-small-cell lung cancer (NSCLC) patients [74].

4.2.3. Altered ganglioside expression

Gangliosides are acidic glycosphingolipids with the presence of at least one sialic acid linked to their oligosaccharide chain [75]. Biosynthesis of gangliosides involves sequential addition of sialic acids to lactosylceramide (LacCer) by ST3Gal V (GM3 synthase), ST8Sial I (GD3 synthase) and ST8Sia V (GT3 synthase) that leads to the generation of the a-, b-, and c-series ganglioside precursors, respectively, representing the mono-, di-, and tri-sialylated gangliosides (**Figure 3**) [68]. In general, gangliosides are involved in cell-cell recognition or regulation of downstream signalling of various proteins (e.g. insulin, epidermal growth factor and vascular endothelial growth factor receptors) [76, 77].

http://dx.doi.org/10.5772/66996

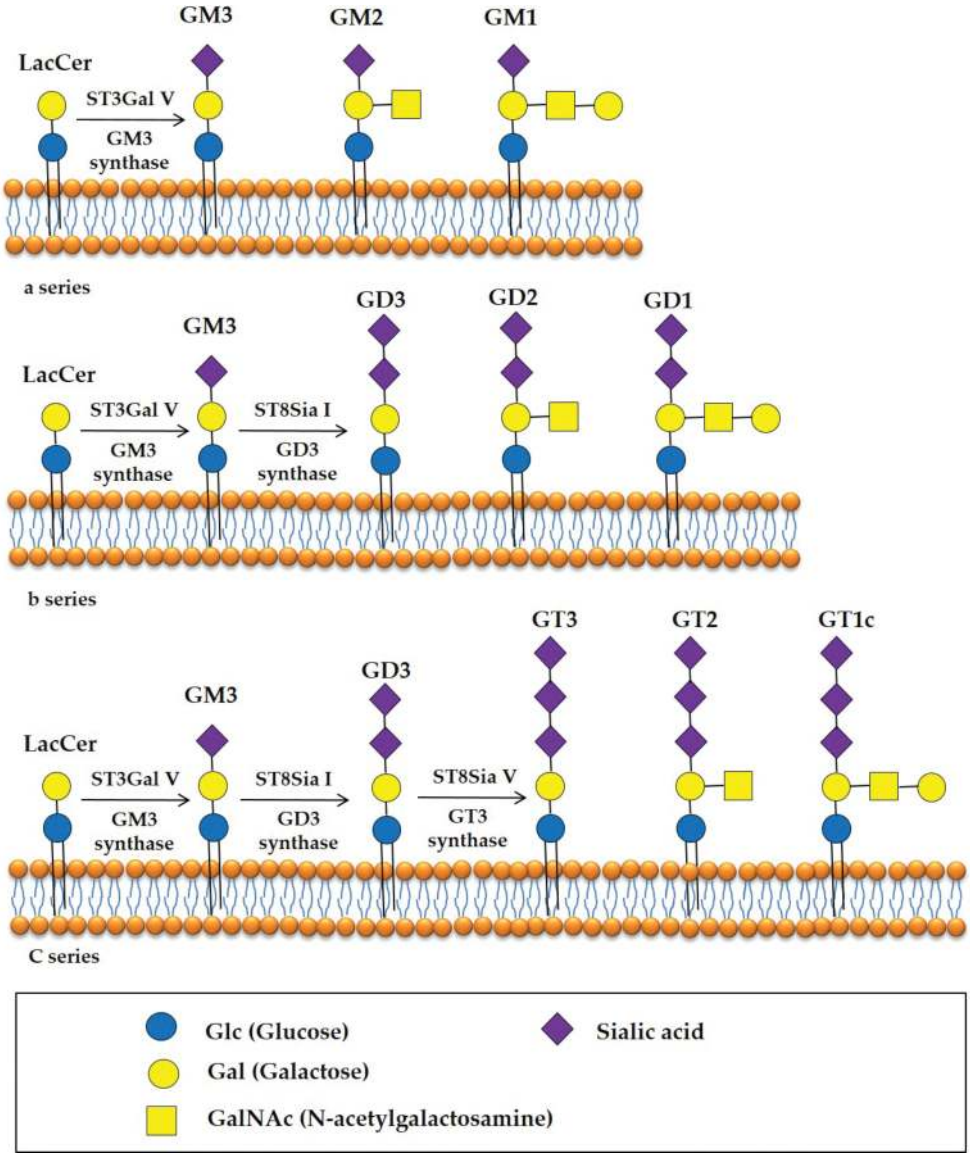

Figure 3. Biosynthesis of gangliosides.

Tumour-associated gangliosides have been suggested as a result of initial oncogenic transformation and play a key role in the induction of invasion and metastasis [75, 78]. Examples of gangliosides that are overexpressed in cancers are GD2 in neuroblastoma [79] and small cell lung cancer (SCLC) [80], GD3 in melanoma [81], GM2 [82] and fucosyl-GM1 in SCLC [81].

Some gangliosides overexpressed in cancers have been identified as adhesion molecules, which promote tumour cell metastasis. Gangliosides such as GD2, GD3 and GT1b form complexes with integrins [83] in melanoma where the terminal sialic acid residues of these gangliosides inhibit cell attachment by abrogating the interaction between integrin $\alpha 5\beta 1$ and fibronectin [84]. Gangliosides on tumour cells also promote metastasis by forming aggregates with peripheral blood mononuclear cells (PBMCs) or platelets. This is due to the interaction between sialic acid moieties on gangliosides with sialic acid-binding proteins named Siglecs (sialic acid/immunoglobulin/lectin) [85], which are expressed on various types of blood cells [86]. These tumour aggregates may induce the blood cells to release factors that activate endothelial cells to elicit cell adhesion molecules (ICAMs, VCAMs, E-selectin and P-selectin), which in turn initiate tumour cell adhesion or invasion [87, 88]. Siglec-7 has been reported to bind preferentially to sialyl-2→6 GalNAc [86]. Interestingly, GD3, GD2 and GT1b share the same sialyl2→6 GalNAc moiety [86], and they are overexpressed on a variety of tumours [89]. Thus, disialo epitopes may promote metastasis by binding to Siglecs expressed on blood cells.

In addition to the cell adhesion function, gangliosides have been reported to act as immune checkpoint molecules to aid in the escape of tumour cells from immune surveillance. Gangliosides released from the active secretion of tumour cells into serum can be taken up by T cells, with ensuing inhibition of T-cell proliferation and activation. The inhibitory action includes the defects in antigen presentation and reduction of cytokine [IFN-gamma (IFN-γ), interleukin-2 (IL-2) and IL-4] production [90–95]. The molecular mechanism of ganglioside-induced T-cell dysfunction was suggested to involve the inhibition of NF-kappa B (NF-κB) activity of T cells via degradation of RelA/p50 dimer and p50/p50 homodimer proteins [96, 97].

4.3. Serum cancer biomarkers

Better survival rates among cancer patients are correlated with earlier detection. The utilisation of serum cancer biomarkers has played a major role in not only early detection of cancer but also prediction of cancer recurrence following initial therapy [98]. However, current clinically approved serum cancer biomarkers are characterised by low sensitivity in detecting cancers [99]. Thus, the development of highly sensitive novel serum cancer biomarkers with better diagnostic and prognostic performance may enhance early detection rates and identification of new targets for anticancer therapy.

4.3.1. α-Fetoprotein (AFP)

α-Fetoprotein (AFP) is a 70 kDa glycoprotein [99], normally only secreted by foetal liver and present in foetal serum [100]. However, under certain pathological conditions, when it is present in adult serum, AFP is associated with cancer. Thus, AFP has been used as a serological marker for the early diagnosis of hepatocellular carcinoma (HCC) [101] and non-seminomatous germ cell tumours (NSGCT) [102].

AFP has a single N-linked oligosaccharide with a biantennary complex-type structure, with altered core fucosylation and terminal sialylation in HCC and NSGCT (**Figure 4**) [102]. Kobayashi et al. reported higher α-1,6-fucosyltransferase (FUT8) expression in HCC tissues than non-cancerous tissues and increased in fucosylation were correlated with HCC

http://dx.doi.org/10.5772/66996

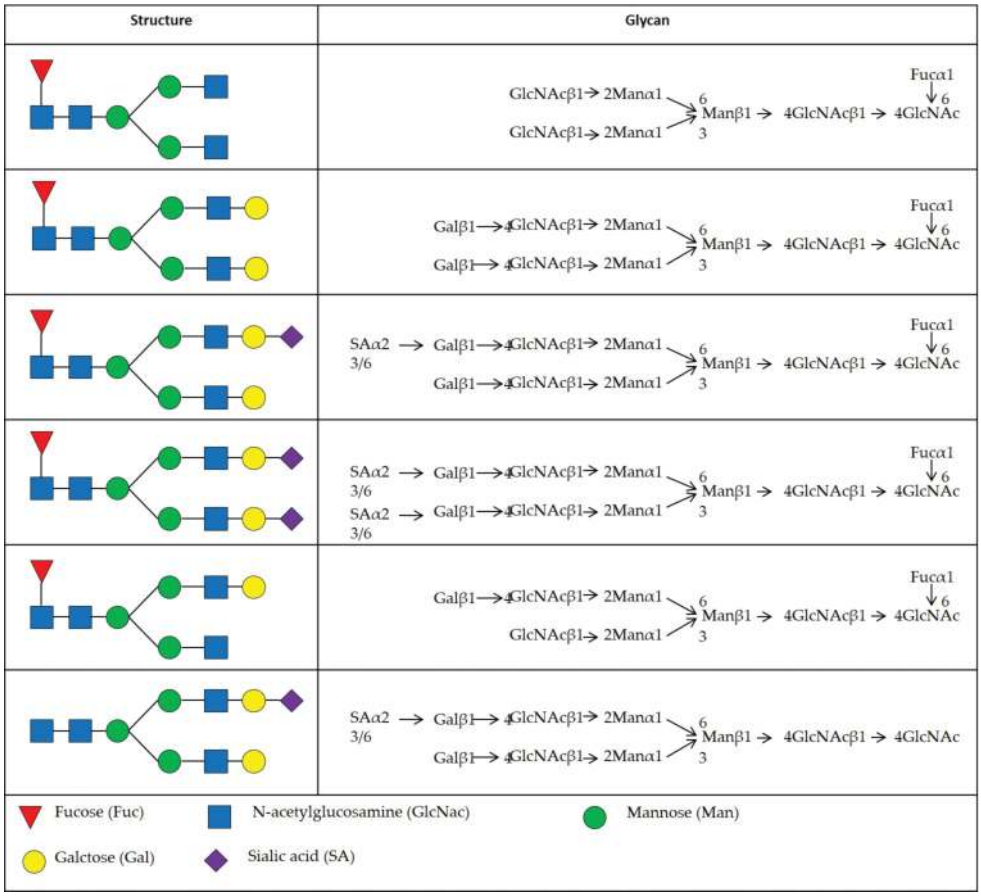

Figure 4. The structures of the N-linked glycans expressed on AFP associated with HCC and NSGCT patients.

progression [101]. In addition to HCC, overexpression of FUT8 in thyroid carcinoma tissue has been linked directly to tumour size and lymph node metastasis [103]. In a study, Osumi and coworkers demonstrated that upregulated of FUT8 expression in cancer cell regulated the expression of E-cadherin. E-cadherin was responsible in the enhancement of cell-cell adhesion, which in turns contributes to the metastatic potential of cancer cells [104].

4.3.2. Prostate-specific antigen (PSA)

Prostate-specific antigen (PSA) is a 28.4 kDa glycoprotein with an N-linked glycosylation site. PSA has been used widely to screen for prostate cancer in men [99]. PSA is normally secreted by the prostate epithelium and periurethral glands. In prostate cancer, disruption of the prostate epithelium leads to the release of PSA into serum [99]. When compared to PSA isolated from healthy individuals, PSA isolated from the serum of prostate cancer patients shown

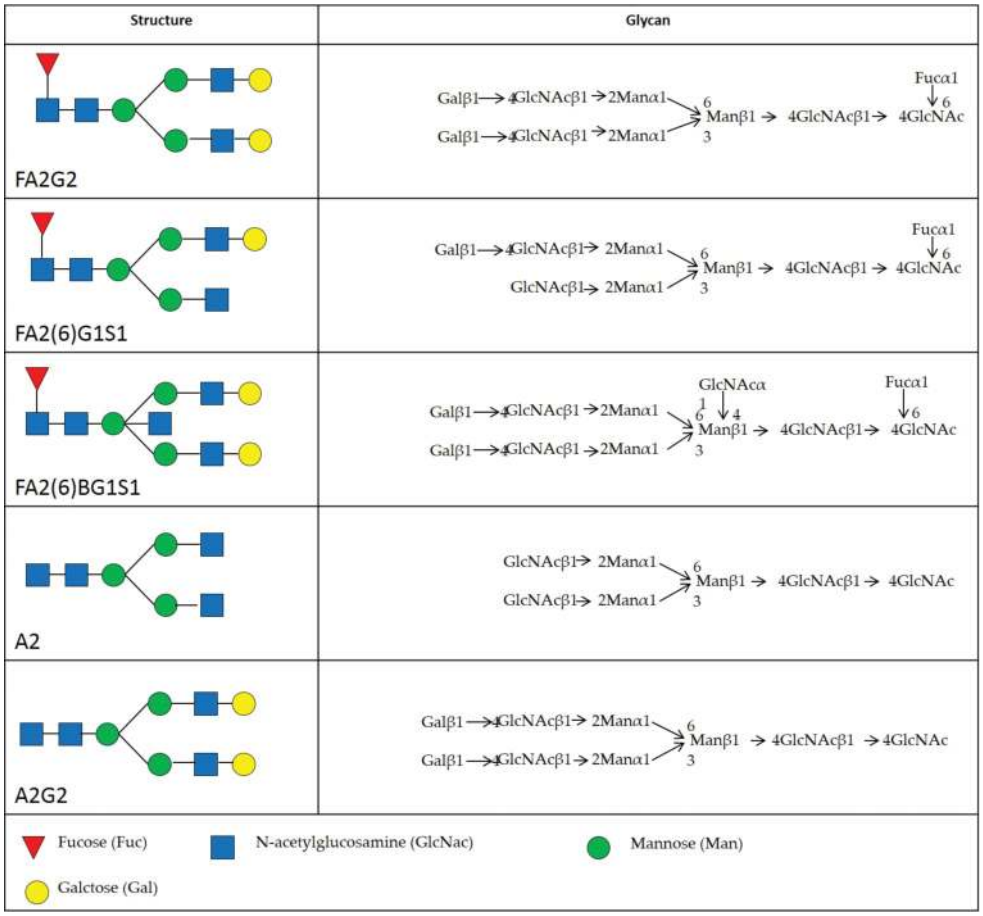

Figure 5. N-linked oligosaccharide structures of PSA elevated in prostate cancer patient serum.

significant higher levels of core-fucosylated biantennary glycans and α-(2,3)-linked sialic acids [105–109]. PSA is composed of several glycoforms [110, 111]. PSA with core-fucosylated biantennary glycans (FA2G2, FA2(6)G1S1 and FA2(6)BG1S1) and terminal α-(2,3)-linked sialic acids (A2 and A2G2) (**Figure 5**) were found elevated in prostate cancer patient serum [60].

4.3.3. Cancer antigen 19-9 (CA19-9)

Cancer antigen 19-9 (CA19-9) corresponds to a carbohydrate structure, sialyl-Lewis a (SLe^a) [99], which is overexpressed on cancer cell surface as a glycolipid and/or as an O-linked glycoprotein [112]. CA19-9 was first characterised by 1116-NS19-9 mAb [113] and has been found primarily in pancreatic and biliary tract cancers [113]. It has been used as a serum biomarker for pancreatic cancer [114]. In neoplastic tissues, epigenetic silencing of the gene for

http://dx.doi.org/10.5772/66996

α-(2,6)-sialyl transferase leads to the abnormal synthesis and accumulation of SLe^a, instead of its normal counterpart disialyl Lewis a (di-SLe^a). SLe^a has been reported to play a crucial role in cancer invasion/metastasis by acting as ligand for endothelial cell E-selectin, which is responsible for cell adhesion [115–118].

It is worth noting that majority of these glycobiomarkers were discovered by generating tumour-specific monoclonal antibodies (mAbs). In addition to aid in the discovery of additional carbohydrate-based biomarkers, these tumour-specific mAbs have great potential in treating neoplastic disease.

4.4. Antibody-based immunotherapy of cancer

Specific recognition and elimination of malignant cells by antibodies were proposed over a century ago [119]. The development of antibody-based therapies for cancer has been the focus of considerable interest for decades. Several criteria have been described for the selection of antitumour mAbs: (1) the mAb binds to cell surface tumour antigen, (2) the mAb binds to tumour antigen at high affinity, (3) the mAb recognises tumour antigen that is overexpressed on tumours but has limited expression on normal tissues, (4) the mAb has potent immune-mediated and non-immune-mediated cytotoxicity effects, (5) the mAb directly kills tumour cells and/or (6) the mAb internalises into target cells so it can delivery toxic payloads. To date, many therapeutic monoclonal antibodies (mAbs) have been developed, mostly against protein antigens, and have proven useful in cancer therapy.

4.5. Anti-glycan monoclonal antibody against cancer

Anti-glycan mAbs have also found usage in clinical applications. Dinutuximab is a chimeric mAb directed against GD2 on neuroblastoma and induced cell lysis via Antibody dependent cellular cytotoxicity (ADCC) and Complement dependent cytotoxicity (CDC) (http://www.fda.gov/). It was approved by FDA in 2015, for use in high-risk neuroblastoma paediatric patients, in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2) and 13-cis-retinoic acid (RA). GD2 is a disialoganglioside overexpressed on neuroblastoma and melanoma [79, 120] with limited expression on normal neurons in the cerebellum, skin melanocytes and peripheral nerves [121], making it well suited as target for cancer therapy. The FDA approval was based on the findings from a phase III trial, focused on 226 children with high-risk neuroblastoma who had responded to initial treatment [122]. Patients were enrolled to receive either Dinutuximab associated with IL-12, GM-CSF and RA or RA alone, after having responded to the first-line treatment. Three years after treatment assignment, patients received Dinutuximab combination showed superior rates of event-free survival and overall survival when compared to standard therapy.

BR96 is an anti-Le^y mAb. It was shown to induce direct tumour cell death to Le^y-positive tumour cell lines in addition to ADCC or CDC [123]. In a phase I trial, BR96-doxorubicin immunoconjugates showed limited clinical antitumour activity with patients experiencing a clinically significant hypersensitivity reaction to the mouse man [124]. BR96 was conjugated to doxorubicin and docetaxel (also known as SGN-15), and 62 advanced non-small-cell lung cancer patients were treated in a randomised Phase II trial. An increased in survival was

reported for patients receiving SGN-15 when compared with patients receiving doxorubicin and docetaxel alone [125]. hu3S193 is a humanised anti-Le^y mab. It contains only 3–5% of murine residues in the antibody variable domain, conferring a low risk of hypersensitivity responses. In a MCF-7 xenograft preventive model, hu3S193 managed to significantly slow tumour growth compared with placebo and isotype-matched control IgG1 antibody [126]. In a phase I trial in 15 cancer patients (six breast, eight colorectal and one non-small-cell lung cancers), hu3S193 showed only minimal toxicity. The biodistribution of indium 111 radiolabeled hu3S193 ((111)In-hu3S193) showed no evidence of normal tissue uptake, but (111) In-hu3S193 uptake was seen in cutaneous, lymph node and hepatic metastases [127].

KM231 is a murine mAb recognising SLe^a. KM231 was observed to react with many human gastrointestinal cancer tissues and could detect shed antigen in the sera of cancer patients. Shitara et al. made KM231-ricin A chain immunotoxin to evaluate the tumoricidal effect of KM231 on ascites and subcutaneous xenograft tumours growing in nude mice. KM231 significantly inhibited the growth of established subcutaneous tumours. This result suggested that it was an effective tumoricidal drug when it was conjugated to cytotoxic reagents [128]. 5B1 (IgG1) and 7E3 (IgM) are other anti-SLe^a mAbs generated by immunising mice with SLe^a-KLH vaccine. Both mAbs are very potent in inducing CDC. Moreover, 5B1 is also highly active in inducing ADCC [129].

NCCT-ST-421 (IgG3) is a murine mAb raised by immunising mice with human gastric cancer xenograft (ST-4). The mAb recognises dimeric Le^a epitope and cross reacts with simple Le^a and extended Le^a epitopes. NCCT-ST-421 induced ADCC and CDC to antigen-positive cells. It was shown to induce direct cell death as well through apoptotic mechanism. Although NCCT-ST-421 showed promising antitumour responses, no further details regarding clinical studies were described.

4.6. Direct killing anti-glycan monoclonal antibody

Oncosis is a progressive cell death process initially involving the impairment of ionic pumps of the cell membrane accompanied by cellular and organelle swelling. Subsequently, a gradually increase in membrane permeability due to an increasing cytosolic calcium concentration as well as rearrangement of cytoskeletal proteins results in pore formation in the cell membrane [130, 131]. It has been well accepted that tumours are able to manipulate the tumour microenvironment by releasing cytokines and other soluble factors, which create an immunosuppressive environment [132]. Release of cellular content into immunosuppressive tumour microenvironment via mAb-induced pore formation during oncosis may help in evoking immune responses via the release of danger-associated molecular patterns ('DAMPs'; [133]).

FG88 (FG88.2 and FG88.7) are internalising murine IgG3 mAbs recognise $Le^{a\text{-}c\text{-}x}$ glycans (**Figure 6**) overexpressed on a wide range of tumour cells and tissues at subnanomolar potency [134]. The FG88.2 mAb showed excellent tumour cell surface antigen binding and good levels of binding to a large percentage of tumours with low level binding to a limited number of normal tissues by immunohistochemistry. The significant association of strong FG88.2 binding with poor outcome in the colorectal sample cohort, independent of stage and vascular invasion, suggests that FG88.2 mAb has great potential targeting the most aggressive

http://dx.doi.org/10.5772/66996

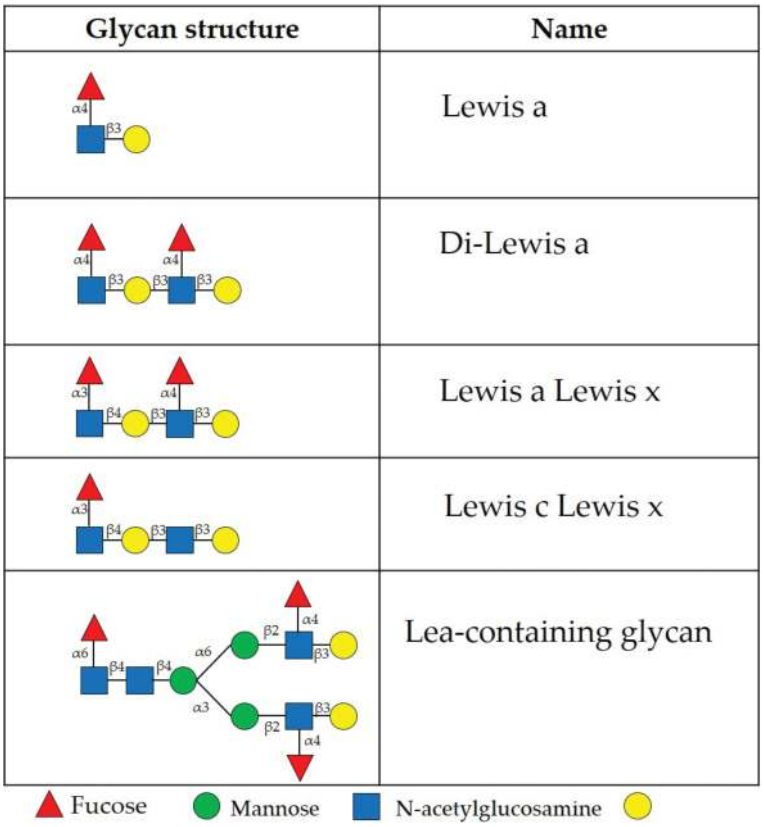

Figure 6. Details of glycan binding by FG88 mAbs.

colorectal cancers. FG88 mAbs induce potent ADCC, CDC and direct killing of tumour cells via oncosis. In the in vivo xenograft study, FG88 mAbs eradicated both primary and metastatic tumours. By releasing tumour cellular contents via mAb-induced pores, the FG88 mAbs might also be able to reverse the immunosuppressive tumour microenvironment leading to effective presentation of multiple epitopes from the lysed tumour cells and amplifying the antitumour immune response.

Several other anti-glycan mAbs can also induce similar direct tumour killing to FG88. MAb 84, when bound to human embryonic stem cells, induced cytoskeletal protein (α-actinin, paxillin and talin) degradation, which in turned increased the mobility of the plasma membrane, resulting in the clustering of antigens on the cell surface. Following antigen clustering, formation of pores through the plasma membrane resulted in oncosis [135]. N-glycolylneuraminic acid (NeuGc) is a sialic acid variant of N-acetylneuraminic acid (NeuAc). Humans cannot synthesise NeuGc because they lack the enzyme cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH), responsible for its biosynthesis.

However, recent evidence suggests that NeuGc can be incorporated into human glycan from dietary sources. More interestingly, although not fully understood, human tumours actively incorporate NeuGc at a much higher rate than normal primary cells. As such, it is highly expressed in several human cancer cells [136], making it an appealing target for immunotherapy. It is noteworthy that natural circulating antibodies to NeuGc can be detected in normal human serum and that these antibodies have been shown to induce complement-dependent cell lysis [137]. Anti-NGcGM3 14 F7 mAb induced rapid cell death which was accompanied by cellular swelling, membrane lesion formation and cytoskeleton activation and cell aggregation. Moreover, no evidences of DNA fragmentation, chromatin modification or caspase activation were found. But 14 F7-treated cells showed large lesions at the plasma membrane, much bigger pores created by complement, perforin or bacterial toxins, suggesting an oncosis-like phenomenon [138]. Currently, the NeuGc-ganglioside anti-idiotypic mAb, Racotumomab (1E10), is under development. By molecular mimicry, a selected anti-idiotypic mAb will behave like the original antigen. In a phase III randomised trial in non-small-cell lung cancer, Racotumomab conferred a significant survival advantage [139] and induced anti-NeuGc antibodies capable of killing tumour cells by a mechanism similar to oncosis, validating the approach and highlighting the beneficial value of antibodies mediating oncosis for therapeutic purposes [140].

5. Conclusion

Glycoconjugates are major components of cells. They are involved in defining and modulating multiple key physiological processes in normal tissues. Aberrant glycosylation in cancer lead to the modification of glycosylations, resulted in the generation of TACAs, which drive several biological processes in cancer. Investigation of the molecular basis underlying these glycan modifications will aid in the understanding of cancer immunology as well as the development of anti-glycan therapeutic mAbs. Furthermore, rapid advances in glycomics and glycoproteomics will have a major impact on the unravelling of novel targets for cancer treatment.

Author details

Jia Xin Chua and Lindy Durrant*

*Address all correspondence to: lindy.durrant@nottingham.ac.uk

University of Nottingham, Nottingham, United Kingdom

References

[1] Tuccillo FM, de Laurentiis A, Palmieri C, Fiume G, Bonelli P, Borrelli A, et al. Aberrant glycosylation as biomarker for cancer: focus on CD43. BioMed Research International 2014;2014:742831.

[2] Fuster MM, Esko JD. The sweet and sour of cancer: glycans as novel therapeutic targets. Nature Reviews Cancer 2005;5:526–42.

[3] Padler-Karavani V. Aiming at the sweet side of cancer: aberrant glycosylation as possible target for personalized-medicine. Cancer Letters 2014;352:102–12.

[4] Spiro RG. Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds. Glycobiology 2002;12:43R–56R.

[5] Nagae M, Yamaguchi Y. Function and 3D structure of the N-glycans on glycoproteins. International Journal of Molecular Sciences 2012;13:8398–429.

[6] de Beer T, Vliegenthart JF, Loffler A, Hofsteenge J. The hexopyranosyl residue that is C-glycosidically linked to the side chain of tryptophan-7 in human RNase Us is alpha-mannopyranose. Biochemistry 1995;34:11785–9.

[7] Hofsteenge J, Muller DR, de Beer T, Loffler A, Richter WJ, Vliegenthart JF. New type of linkage between a carbohydrate and a protein: C-glycosylation of a specific tryptophan residue in human RNase Us. Biochemistry 1994;33:13524–30.

[8] Haynes PA. Phosphoglycosylation: a new structural class of glycosylation? Glycobiology 1998;8:1–5.

[9] Ferguson MA. The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research. Journal of Cell Science 1999;112(Pt 17):2799–809.

[10] Durrant LG, Noble P, Spendlove I. Immunology in the clinic review series; focus on cancer: glycolipids as targets for tumour immunotherapy. Clinical and Experimental Immunology 2012;167:206–15.

[11] Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, et al. A toll-like receptor that prevents infection by uropathogenic bacteria. Science 2004;303:1522–6.

[12] Lingwood CA. Glycosphingolipid functions. Cold Spring Harbor Perspectives in Biology 2011;2–3.

[13] Ichikawa S, Sakiyama H, Suzuki G, Hidari KI, Hirabayashi Y. Expression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first glycosylation step of glycosphingolipid synthesis. Proceedings of the National Academy of Sciences of the United States of America 1996;93:4638–43.

[14] Futerman AH, Pagano RE. Determination of the intracellular sites and topology of glucosylceramide synthesis in rat liver. The Biochemical Journal 1991;280(Pt 2):295–302.

[15] Jeckel D, Karrenbauer A, Burger KN, van Meer G, Wieland F. Glucosylceramide is synthesized at the cytosolic surface of various Golgi subfractions. The Journal of Cell Biology 1992;117:259–67.

[16] Maccioni HJ, Giraudo CG, Daniotti JL. Understanding the stepwise synthesis of glycolipids. Neurochemical Research 2002;27:629–36.

[17] Hakomori S. Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. Advances in Experimental Medicine and Biology 2001;491:369–402.

[18] Dube DH, Bertozzi CR. Glycans in cancer and inflammation – potential for therapeutics and diagnostics. Nature Reviews Drug Discovery 2005;4:477–88.

[19] Pochechueva T, Jacob F, Fedier A, Heinzelmann-Schwarz V. Tumor-associated glycans and their role in gynecological cancers: accelerating translational research by novel high-throughput approaches. Metabolites 2012;2:913–39.

[20] Dennis JW, Laferte S, Waghorne C, Breitman ML, Kerbel RS. Beta 1-6 branching of Asn-linked oligosaccharides is directly associated with metastasis. Science 1987;236:582–5.

[21] Kumamoto K, Goto Y, Sekikawa K, Takenoshita S, Ishida N, Kawakita M, et al. Increased expression of UDP-galactose transporter messenger RNA in human colon cancer tissues and its implication in synthesis of Thomsen-Friedenreich antigen and sialyl Lewis A/X determinants. Cancer Research 2001;61:4620–7.

[22] Kellokumpu S, Sormunen R, Kellokumpu I. Abnormal glycosylation and altered Golgi structure in colorectal cancer: dependence on intra-Golgi pH. FEBS Letters 2002;516:217–24.

[23] Chen L, Zhang W, Fregien N, Pierce M. The her-2/neu oncogene stimulates the transcription of N-acetylglucosaminyltransferase V and expression of its cell surface oligosaccharide products. Oncogene 1998;17:2087–93.

[24] Taniguchi N, Miyoshi E, Ko JH, Ikeda Y, Ihara Y. Implication of N-acetylglucosaminyltransferases III and V in cancer: gene regulation and signaling mechanism. Biochimica et Biophysica Acta 1999;1455:287–300.

[25] Miyoshi E, Terao M, Kamada Y. Physiological roles of N-acetylglucosaminyltransferase V(GnT-V) in mice. BMB Reports 2012;45:554–9.

[26] Ishida H, Togayachi A, Sakai T, Iwai T, Hiruma T, Sato T, et al. A novel beta1,3-N-acetylglucosaminyltransferase (beta3Gn-T8), which synthesizes poly-N-acetyllactosamine, is dramatically upregulated in colon cancer. FEBS Letters 2005;579:71–8.

[27] Mori S, Aoyagi Y, Yanagi M, Suzuki Y, Asakura H. Serum N-acetylglucosaminyltransferase III activities in hepatocellular carcinoma. Journal of Gastroenterology and Hepatology 1998;13:610–9.

[28] Song EY, Kang SK, Lee YC, Park YG, Chung TH, Kwon DH, et al. Expression of bisecting N-acetylglucosaminyltransferase-III in human hepatocarcinoma tissues, fetal liver tissues, and hepatoma cell lines of Hep3B and HepG2. Cancer Investigation 2001;19:799–807.

[29] Sewell R, Backstrom M, Dalziel M, Gschmeissner S, Karlsson H, Noll T, et al. The ST6GalNAc-I sialyltransferase localizes throughout the Golgi and is responsible for the synthesis of the tumor-associated sialyl-Tn O-glycan in human breast cancer. The Journal of Biological Chemistry 2006;281:3586–94.

[30] Julien S, Ivetic A, Grigoriadis A, QiZe D, Burford B, Sproviero D, et al. Selectin ligand sialyl-Lewis x antigen drives metastasis of hormone-dependent breast cancers. Cancer Research 2011;71:7683–93.

[31] Ogawa J, Inoue H, Koide S. Expression of alpha-1,3-fucosyltransferase type IV and VII genes is related to poor prognosis in lung cancer. Cancer Research 1996;56:325–9.

[32] Togayachi A, Kudo T, Ikehara Y, Iwasaki H, Nishihara S, Andoh T, et al. Up-regulation of Lewis enzyme (Fuc-TIII) and plasma-type alpha1,3fucosyltransferase (Fuc-TVI) expression determines the augmented expression of sialyl Lewis x antigen in non-small cell lung cancer. International Journal of Cancer 1999;83:70–9.

[33] Ju T, Wang Y, Aryal RP, Lehoux SD, Ding X, Kudelka MR, et al. Tn and sialyl-Tn antigens, aberrant O-glycomics as human disease markers. Proteomics Clinical Applications 2013;7:618–31.

[34] Terasawa K, Furumoto H, Kamada M, Aono T. Expression of Tn and sialyl-Tn antigens in the neoplastic transformation of uterine cervical epithelial cells. Cancer Research 1996;56:2229–32.

[35] Cao Y, Stosiek P, Springer GF, Karsten U. Thomsen-Friedenreich-related carbohydrate antigens in normal adult human tissues: a systematic and comparative study. Histochemistry and Cell Biology 1996;106:197–207.

[36] Springer GF. Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. Journal of Molecular Medicine (Berlin, Germany) 1997;75:594–602.

[37] Brockhausen I, Yang J, Dickinson N, Ogata S, Itzkowitz SH. Enzymatic basis for sialyl-Tn expression in human colon cancer cells. Glycoconjugate Journal 1998;15:595–603.

[38] Dalziel M, Whitehouse C, McFarlane I, Brockhausen I, Gschmeissner S, Schwientek T, et al. The relative activities of the C2GnT1 and ST3Gal-I glycosyltransferases determine O-glycan structure and expression of a tumor-associated epitope on MUC1. The Journal of Biological Chemistry 2001;276:11007–15.

[39] Marcos NT, Cruz A, Silva F, Almeida R, David L, Mandel U, et al. Polypeptide GalNAc-transferases, ST6GalNAc-transferase I, and ST3Gal-transferase I expression in gastric carcinoma cell lines. The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society 2003;51:761–71.

[40] Wagner KW, Punnoose EA, Januario T, Lawrence DA, Pitti RM, Lancaster K, et al. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. Nature Medicine 2007;13:1070–7.

[41] Forrester JA, Ambrose EJ, Macpherson JA. Electrophoretic investigations of a clone of hamster fibroblasts and polyoma-transformed cells from the same population. Nature 1962;196:1068–70.

[42] Forrester JA, Ambrose EJ, Stoker MG. Microelectrophoresis of normal and transformed clones of hamster kidney fibroblasts. Nature 1964;201:945–6.

[43] Gasic G, Gasic T. Removal and regeneration of the cell coating in tumour cells. Nature 1962;196:170.

[44] Bull C, Stoel MA, den Brok MH, Adema GJ. Sialic acids sweeten a tumor's life. Cancer Research 2014;74:3199–204.

[45] Altevogt P, Fogel M, Cheingsong-Popov R, Dennis J, Robinson P, Schirrmacher V. Different patterns of lectin binding and cell surface sialylation detected on related high- and low-metastatic tumor lines. Cancer Research 1983;43:5138–44.

[46] Yogeeswaran G, Salk PL. Metastatic potential is positively correlated with cell surface sialylation of cultured murine tumor cell lines. Science 1981;212:1514–6.

[47] Schneider F, Kemmner W, Haensch W, Franke G, Gretschel S, Karsten U, et al. Overexpression of sialyltransferase CMP-sialic acid:Galbeta1,3GalNAc-R alpha6-Sialyltransferase is related to poor patient survival in human colorectal carcinomas. Cancer Research 2001;61:4605–11.

[48] Angata T, Varki A. Chemical diversity in the sialic acids and related alpha-keto acids: an evolutionary perspective. Chemical Reviews 2002;102:439–69.

[49] Pillai S, Netravali IA, Cariappa A, Mattoo H. Siglecs and immune regulation. Annual Review of Immunology 2012;30:357–92.

[50] Kim YJ, Varki A. Perspectives on the significance of altered glycosylation of glycoproteins in cancer. Glycoconjugate Journal 1997;14:569–76.

[51] Brockhausen I. Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. EMBO Reports 2006;7:599–604.

[52] Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. Nature Reviews Cancer 2004;4:45–60.

[53] Dall'Olio F, Chiricolo M. Sialyltransferases in cancer. Glycoconjugate Journal 2001;18:841–50.

[54] Gessner P, Riedl S, Quentmaier A, Kemmner W. Enhanced activity of CMP-neuAc:Gal beta 1-4GlcNAc:alpha 2,6-sialyltransferase in metastasizing human colorectal tumor tissue and serum of tumor patients. Cancer Letters 1993;75:143–9.

[55] Hedlund M, Ng E, Varki A, Varki NM. Alpha 2-6-Linked sialic acids on N-glycans modulate carcinoma differentiation in vivo. Cancer Research 2008;68:388–94.

[56] Wang PH, Lee WL, Lee YR, Juang CM, Chen YJ, Chao HT, et al. Enhanced expression of alpha 2,6-sialyltransferase ST6Gal I in cervical squamous cell carcinoma. Gynecologic Oncology 2003;89:395–401.

[57] Julien S, Adriaenssens E, Ottenberg K, Furlan A, Courtand G, Vercoutter-Edouart AS, et al. ST6GalNAc I expression in MDA-MB-231 breast cancer cells greatly modifies their O-glycosylation pattern and enhances their tumourigenicity. Glycobiology 2006;16:54–64.

[58] Alley WR, Jr., Novotny MV. Glycomic analysis of sialic acid linkages in glycans derived from blood serum glycoproteins. Journal of Proteome Research 2010;9:3062–72.

[59] Vasseur JA, Goetz JA, Alley WR, Jr., Novotny MV. Smoking and lung cancer-induced changes in N-glycosylation of blood serum proteins. Glycobiology 2012;22:1684–708.

[60] Saldova R, Fan Y, Fitzpatrick JM, Watson RW, Rudd PM. Core fucosylation and alpha2-3 sialylation in serum N-glycome is significantly increased in prostate cancer comparing to benign prostate hyperplasia. Glycobiology 2011;21:195–205.

[61] Yamamoto H, Saito T, Kaneko Y, Kersey D, Yong VW, Bremer EG, et al. Alpha2,3-sialyltransferase mRNA and alpha2,3-linked glycoprotein sialylation are increased in malignant gliomas. Brain Research 1997;755:175–9.

[62] Wang PH, Lee WL, Juang CM, Yang YH, Lo WH, Lai CR, et al. Altered mRNA expressions of sialyltransferases in ovarian cancers. Gynecologic Oncology 2005;99:631–9.

[63] Ravn V, Dabelsteen E. Tissue distribution of histo-blood group antigens. Acta Pathologica, Microbiologica, et Immunologica Scandinavica 2000;108:1–28.

[64] Yuriev E, Farrugia W, Scott AM, Ramsland PA. Three-dimensional structures of carbohydrate determinants of Lewis system antigens: implications for effective antibody targeting of cancer. Immunology and Cell Biology 2005;83:709–17.

[65] Krug LM, Milton DT, Jungbluth AA, Chen LC, Quaia E, Pandit-Taskar N, et al. Targeting Lewis Y (Le(y)) in small cell lung cancer with a humanized monoclonal antibody, hu3S193: a pilot trial testing two dose levels. Journal of Thoracic Oncology 2007;2:947–52.

[66] Soejima M, Koda Y. Molecular mechanisms of Lewis antigen expression. Legal Medicine (Tokyo, Japan) 2005;7:266–9.

[67] Ballare C, Barrio M, Portela P, Mordoh J. Functional properties of FC-2.15, a monoclonal antibody that mediates human complement cytotoxicity against breast cancer cells. Cancer Immunology, Immunotherapy 1995;41:15–22.

[68] Cazet A, Julien S, Bobowski M, Burchell J, Delannoy P. Tumour-associated carbohydrate antigens in breast cancer. Breast Cancer Research 2010;12:204.

[69] Kannagi R, Izawa M, Koike T, Miyazaki K, Kimura N. Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. Cancer Science 2004;95:377–84.

[70] Ragupathi G, Damani P, Srivastava G, Srivastava O, Sucheck SJ, Ichikawa Y, et al. Synthesis of sialyl Lewis(a) (sLe (a), CA19-9) and construction of an immunogenic sLe(a) vaccine. Cancer Immunology, Immunotherapy 2009;58:1397–405.

[71] Croce MV, Isla-Larrain M, Rabassa ME, Demichelis S, Colussi AG, Crespo M, et al. Lewis x is highly expressed in normal tissues: a comparative immunohistochemical study and literature revision. Pathology and Oncology Research 2007;13:130–8.

[72] Heimburg-Molinaro J, Lum M, Vijay G, Jain M, Almogren A, Rittenhouse-Olson K. Cancer vaccines and carbohydrate epitopes. Vaccine 2011;29:8802–26.

[73] Shimodaira K, Nakayama J, Nakamura N, Hasebe O, Katsuyama T, Fukuda M. Carcinoma-associated expression of core 2 beta-1,6-N-acetylglucosaminyltransferase gene in human colorectal cancer: role of O-glycans in tumor progression. Cancer Research 1997;57:5201–6.

[74] Ogawa J, Sano A, Inoue H, Koide S. Expression of Lewis-related antigen and prognosis in stage I non-small cell lung cancer. The Annals of Thoracic Surgery 1995;59:412–5.

[75] Birkle S, Zeng G, Gao L, Yu RK, Aubry J. Role of tumor-associated gangliosides in cancer progression. Biochimie 2003;85:455–63.

[76] Handa K, Hakomori SI. Carbohydrate to carbohydrate interaction in development process and cancer progression. Glycoconjugate Journal 2012;29:627–37.

[77] Lopez PH, Schnaar RL. Gangliosides in cell recognition and membrane protein regulation. Current Opinion in Structural Biology 2009;19:549–57.

[78] Fredman P, Hedberg K, Brezicka T. Gangliosides as therapeutic targets for cancer. BioDrugs 2003;17:155–67.

[79] Mujoo K, Cheresh DA, Yang HM, Reisfeld RA. Disialoganglioside GD2 on human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytolysis and suppression of tumor growth. Cancer Research 1987;47:1098–104.

[80] Grant SC, Kostakoglu L, Kris MG, Yeh SD, Larson SM, Finn RD, et al. Targeting of small-cell lung cancer using the anti-GD2 ganglioside monoclonal antibody 3 F8: a pilot trial. European Journal of Nuclear Medicine 1996;23:145–9.

[81] Zhang S, Cordon-Cardo C, Zhang HS, Reuter VE, Adluri S, Hamilton WB, et al. Selection of tumor antigens as targets for immune attack using immunohistochemistry: I. Focus on gangliosides. International Journal of Cancer 1997;73:42–9.

[82] Livingston PO, Hood C, Krug LM, Warren N, Kris MG, Brezicka T, et al. Selection of GM2, fucosyl GM1, globo H and polysialic acid as targets on small cell lung cancers for antibody mediated immunotherapy. Cancer Immunology, Immunotherapy 2005;54:1018–25.

[83] Cheresh DA, Pytela R, Pierschbacher MD, Klier FG, Ruoslahti E, Reisfeld RA. An Arg-Gly-Asp-directed receptor on the surface of human melanoma cells exists in an divalent cation-dependent functional complex with the disialoganglioside GD2. The Journal of Cell Biology 1987;105:1163–73.

[84] Wang X, Sun P, Al-Qamari A, Tai T, Kawashima I, Paller AS. Carbohydrate-carbohydrate binding of ganglioside to integrin alpha(5) modulates alpha(5)beta(1) function. The Journal of Biological Chemistry 2001;276:8436–44.

[85] Crocker PR, Clark EA, Filbin M, Gordon S, Jones Y, Kehrl JH, et al. Siglecs: a family of sialic-acid binding lectins. Glycobiology 1998;8:v.

[86] Ito A, Handa K, Withers DA, Satoh M, Hakomori S. Binding specificity of siglec7 to disialogangliosides of renal cell carcinoma: possible role of disialogangliosides in tumor progression. FEBS Letters 2001;504:82–6.

[87] Khatib AM, Kontogiannea M, Fallavollita L, Jamison B, Meterissian S, Brodt P. Rapid induction of cytokine and E-selectin expression in the liver in response to metastatic tumor cells. Cancer Research 1999;59:1356–61.

[88] Kojima N, Shiota M, Sadahira Y, Handa K, Hakomori S. Cell adhesion in a dynamic flow system as compared to static system. Glycosphingolipid-glycosphingolipid interaction in the dynamic system predominates over lectin- or integrin-based mechanisms in adhesion of B16 melanoma cells to non-activated endothelial cells. The Journal of Biological Chemistry 1992;267:17264–70.

[89] Hakomori S. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco) lipid metabolism. Cancer Research 1996;56:5309–18.

[90] Biswas K, Richmond A, Rayman P, Biswas S, Thornton M, Sa G, et al. GM2 expression in renal cell carcinoma: potential role in tumor-induced T-cell dysfunction. Cancer Research 2006;66:6816–25.

[91] Biswas S, Biswas K, Richmond A, Ko J, Ghosh S, Simmons M, et al. Elevated levels of select gangliosides in T cells from renal cell carcinoma patients is associated with T cell dysfunction. Journal of Immunology 2009;183:5050–8.

[92] Chu JW, Sharom FJ. Gangliosides inhibit T-lymphocyte proliferation by preventing the interaction of interleukin-2 with its cell surface receptors. Immunology 1993;79:10–7.

[93] Irani DN, Lin KI, Griffin DE. Brain-derived gangliosides regulate the cytokine production and proliferation of activated T cells. The Journal of Immunology 1996;157:4333–40.

[94] Morioka N, Furue M, Tsuchida T, Ishibashi Y. Gangliosides inhibit the proliferation of human T cells stimulated with interleukin-4 or interleukin-2. The Journal of Dermatology 1991;18:447–53.

[95] Heitger A, Ladisch S. Gangliosides block antigen presentation by human monocytes. Biochimica et Biophysica Acta 1996;1303:161–8.

[96] Uzzo RG, Rayman P, Kolenko V, Clark PE, Cathcart MK, Bloom T, et al. Renal cell carcinoma-derived gangliosides suppress nuclear factor-kappaB activation in T cells. The Journal of Clinical Investigation 1999;104:769–76.

[97] Thornton MV, Kudo D, Rayman P, Horton C, Molto L, Cathcart MK, et al. Degradation of NF-kappa B in T cells by gangliosides expressed on renal cell carcinomas. The Journal of Immunology 2004;172:3480–90.

[98] Gonzalez SA. Novel biomarkers for hepatocellular carcinoma surveillance: has the future arrived? Hepatobiliary Surgery and Nutrition 2014;3:410–4.

[99] Kirwan A, Utratna M, O'Dwyer ME, Joshi L, Kilcoyne M. Glycosylation-based serum biomarkers for cancer diagnostics and prognostics. BioMed Research International 2015;2015:490531.

[100] Bergstrand CG, Czar B. Demonstration of a new protein fraction in serum from the human fetus. Scandinavian Journal of Clinical and Laboratory Investigation 1956;8:174.

[101] Kobayashi M, Kuroiwa T, Suda T, Tamura Y, Kawai H, Igarashi M, et al. Fucosylated fraction of alpha-fetoprotein, L3, as a useful prognostic factor in patients with hepatocellular carcinoma with special reference to low concentrations of serum alpha-fetoprotein. Hepatology Research: The Official Journal of the Japan Society of Hepatology 2007;37:914–22.

[102] Johnson PJ, Poon TC, Hjelm NM, Ho CS, Blake C, Ho SK. Structures of disease-specific serum alpha-fetoprotein isoforms. British Journal of Cancer 2000;83:1330–7.

[103] Ito Y, Miyauchi A, Yoshida H, Uruno T, Nakano K, Takamura Y, et al. Expression of alpha1,6-fucosyltransferase (FUT8) in papillary carcinoma of the thyroid: its linkage to biological aggressiveness and anaplastic transformation. Cancer Letters 2003;200:167–72.

[104] Osumi D, Takahashi M, Miyoshi E, Yokoe S, Lee SH, Noda K, et al. Core fucosylation of E-cadherin enhances cell-cell adhesion in human colon carcinoma WiDr cells. Cancer Science 2009;100:888–95.

[105] Kyselova Z, Mechref Y, Al Bataineh MM, Dobrolecki LE, Hickey RJ, Vinson J, et al. Alterations in the serum glycome due to metastatic prostate cancer. Journal of Proteome Research 2007;6:1822–32.

[106] Peracaula R, Barrabes S, Sarrats A, Rudd PM, de Llorens R. Altered glycosylation in tumours focused to cancer diagnosis. Disease Markers 2008;25:207–18.

[107] Ohyama C, Hosono M, Nitta K, Oh-eda M, Yoshikawa K, Habuchi T, et al. Carbohydrate structure and differential binding of prostate specific antigen to Maackia amurensis lectin between prostate cancer and benign prostate hypertrophy. Glycobiology 2004;14:671–9.

[108] Peracaula R, Tabares G, Royle L, Harvey DJ, Dwek RA, Rudd PM, et al. Altered glycosylation pattern allows the distinction between prostate-specific antigen (PSA) from normal and tumor origins. Glycobiology 2003;13:457–70.

[109] Tajiri M, Ohyama C, Wada Y. Oligosaccharide profiles of the prostate specific antigen in free and complexed forms from the prostate cancer patient serum and in seminal plasma: a glycopeptide approach. Glycobiology 2008;18:2–8.

[110] Sarrats A, Comet J, Tabares G, Ramirez M, Aleixandre RN, de Llorens R, et al. Differential percentage of serum prostate-specific antigen subforms suggests a new way to improve prostate cancer diagnosis. The Prostate 2010;70:1–9.

[111] Isono T, Tanaka T, Kageyama S, Yoshiki T. Structural diversity of cancer-related and non-cancer-related prostate-specific antigen. Clinical Chemistry 2002;48:2187–94.

[112] Kannagi R. Carbohydrate antigen sialyl Lewis a – its pathophysiological significance and induction mechanism in cancer progression. Chang Gung Medical Journal 2007;30:189–209.

[113] Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P. Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genetics 1979;5:957–71.

[114] Ballehaninna UK, Chamberlain RS. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: an evidence based appraisal. Journal of Gastrointestinal Oncology 2012;3:105–19.

[115] Duraker N, Hot S, Polat Y, Hobek A, Gencler N, Urhan N. CEA, CA 19-9, and CA 125 in the differential diagnosis of benign and malignant pancreatic diseases with or without jaundice. Journal of Surgical Oncology 2007;95:142–7.

[116] Liao Q, Zhao YP, Yang YC, Li LJ, Long X, Han SM. Combined detection of serum tumor markers for differential diagnosis of solid lesions located at the pancreatic head. Hepatobiliary & Pancreatic Diseases International 2007;6:641–5.

[117] Safi F, Roscher R, Bittner R, Schenkluhn B, Dopfer HP, Beger HG. High sensitivity and specificity of CA 19-9 for pancreatic carcinoma in comparison to chronic pancreatitis. Serological and immunohistochemical findings. Pancreas 1987;2:398–403.

[118] Vestergaard EM, Hein HO, Meyer H, Grunnet N, Jorgensen J, Wolf H, et al. Reference values and biological variation for tumor marker CA 19-9 in serum for different Lewis and secretor genotypes and evaluation of secretor and Lewis genotyping in a Caucasian population. Clinical Chemistry 1999;45:54–61.

[119] Weiner LM, Murray JC, Shuptrine CW. Antibody-based immunotherapy of cancer. Cell 2012;148:1081–4.

[120] Cheung NK, Saarinen UM, Neely JE, Landmeier B, Donovan D, Coccia PF. Monoclonal antibodies to a glycolipid antigen on human neuroblastoma cells. Cancer Research 1985;45:2642–9.

[121] Svennerholm L, Bostrom K, Fredman P, Jungbjer B, Lekman A, Mansson JE, et al. Gangliosides and allied glycosphingolipids in human peripheral nerve and spinal cord. Biochimica et Biophysica Acta 1994;1214:115–23.

[122] Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. The New England Journal of Medicine 2010;363:1324–34.

[123] Hellstrom I, Garrigues HJ, Garrigues U, Hellstrom KE. Highly tumor-reactive, internalizing, mouse monoclonal antibodies to Le(y)-related cell surface antigens. Cancer Research 1990;50:2183–90.

[124] Tolcher AW, Sugarman S, Gelmon KA, Cohen R, Saleh M, Isaacs C, et al. Randomized phase II study of BR96-doxorubicin conjugate in patients with metastatic breast cancer. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 1999;17:478–84.

[125] Ross HJ, Hart LL, Swanson PM, Rarick MU, Figlin RA, Jacobs AD, et al. A randomized, multicenter study to determine the safety and efficacy of the immunoconjugate

SGN-15 plus docetaxel for the treatment of non-small cell lung carcinoma. Lung Cancer 2006;54:69–77.

[126] Scott AM, Geleick D, Rubira M, Clarke K, Nice EC, Smyth FE, et al. Construction, production, and characterization of humanized anti-Lewis Y monoclonal antibody 3S193 for targeted immunotherapy of solid tumors. Cancer Research 2000;60:3254–61.

[127] Farrugia W, Scott AM, Ramsland PA. A possible role for metallic ions in the carbohydrate cluster recognition displayed by a Lewis Y specific antibody. PLoS One 2009;4:e7777.

[128] Shitara K, Hanai N, Kusano A, Furuya A, Yoshida H, Wada K, et al. Application of anti-sialyl Lea monoclonal antibody, KM231, for immunotherapy of cancer. Anticancer Research 1991;11:2003–13.

[129] Sawada R, Sun SM, Wu X, Hong F, Ragupathi G, Livingston PO, et al. Human monoclonal antibodies to sialyl-Lewis (CA19.9) with potent CDC, ADCC, and antitumor activity. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 2011;17:1024–32.

[130] Weerasinghe P, Buja LM. Oncosis: an important non-apoptotic mode of cell death. Experimental and Molecular Pathology 2012;93:302–8.

[131] Weerasinghe P, Hallock S, Brown RE, Loose DS, Buja LM. A model for cardiomyocyte cell death: insights into mechanisms of oncosis. Experimental and Molecular Pathology 2013;94:289–300.

[132] Ostman A, Augsten M. Cancer-associated fibroblasts and tumor growth – bystanders turning into key players. Current Opinion in Genetics & Development 2009;19:67–73.

[133] Krysko O, Love Aaes T, Bachert C, Vandenabeele P, Krysko DV. Many faces of DAMPs in cancer therapy. Cell Death and Disease 2013;4:e631.

[134] Chua JX, Vankemmelbeke M, McIntosh RS, Clarke PA, Moss R, Parsons T, et al. Monoclonal antibodies targeting leclex-related glycans with potent antitumor activity. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 2015;21:2963–74.

[135] Tan HL, Fong WJ, Lee EH, Yap M, Choo A. mAb 84, a cytotoxic antibody that kills undifferentiated human embryonic stem cells via oncosis. Stem Cells 2009;27:1792–801.

[136] Malykh YN, Schauer R, Shaw L. N-Glycolylneuraminic acid in human tumours. Biochimie 2001;83:623–34.

[137] Nguyen DH, Tangvoranuntakul P, Varki A. Effects of natural human antibodies against a nonhuman sialic acid that metabolically incorporates into activated and malignant immune cells. The Journal of Immunology 2005;175:228–36.

[138] Roque-Navarro L, Chakrabandhu K, de Leon J, Rodriguez S, Toledo C, Carr A, et al. Anti-ganglioside antibody-induced tumor cell death by loss of membrane integrity. Molecular Cancer Therapeutics 2008;7:2033–41.

[139] Alfonso S, Valdes-Zayas A, Santiesteban ER, Flores YI, Areces F, Hernandez M, et al. A randomized, multicenter, placebo-controlled clinical trial of racotumomab-alum vaccine as switch maintenance therapy in advanced non-small cell lung cancer patients. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 2014;20:3660–71.

[140] Hernandez AM, Rodriguez N, Gonzalez JE, Reyes E, Rondon T, Grinan T, et al. Anti-NeuGcGM3 antibodies, actively elicited by idiotypic vaccination in nonsmall cell lung cancer patients, induce tumor cell death by an oncosis-like mechanism. The Journal of Immunology 2011;186:3735–44.