HHS Public Access

Author manuscript

ACS Chem Neurosci. Author manuscript; available in PMC 2019 January 17.

Published in final edited form as:

ACS Chem Neurosci. 2018 January 17; 9(1): 51–72. doi:10.1021/acschemneuro.7b00271.

Glycosylation Changes in Brain Cancer

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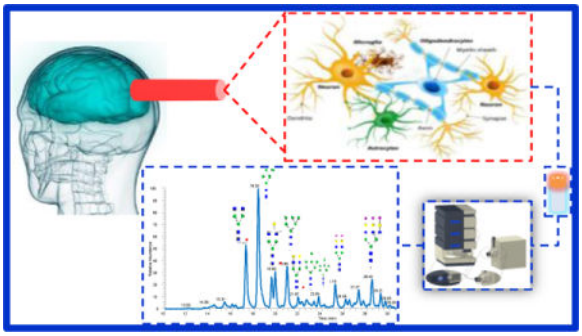
Abstract

Protein glycosylation is a posttranslational modification that affects more than half of all known proteins. Glycans covalently bound to biomolecules modulate their functions by both direct interactions, such as the recognition of glycan structures by binding partners, and indirect mechanisms that contribute to the control of protein conformation, stability, and turnover. The focus of this review is the discussion of aberrant glycosylation related to brain cancer. Altered sialylation and fucosylation of N- and O-glycans play a role in the development and progression of brain cancer. Additionally, aberrant O-glycan expression has been implicated in brain cancer. This review also addresses the clinical potential and applications of aberrant glycosylation for the detection and treatment of brain cancer. The viable roles glycans may play in the development of brain cancer therapeutics are addressed as well as cancer-glycoproteomics and personalized medicine. Glycoprotein alterations are considered as a hallmark of cancer while high expression in body fluids represents an opportunity for cancer assessment.

Graphical abstract



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Author Contributions

All Authors were involved in the writing and proofreading of the document. YM provided the article outline and materials to be covered. Each author was assigned specific sections of the manuscript while LV was responsible for the assembly and proofreading of the final document. LV was responsible for proof reading. YM was responsible for the final proofreading and submission of the manuscript.

Keywords

brain cancer; posttranslational modification of proteins; glycosylation; central nervous system; aberrant glycosylation; bone marrow-derived human mesenchymal stem cells; glioblastoma; cancer stem cells; glioma stem cells; small cell lung carcinomas; human mucin family; carcinoembryonic antigen

1 Introduction

Protein glycosylation is both a co- and post-translational modification that affects more than half of all known proteins.[1] Glycans covalently bound to biomolecules modulate their function by both direct interactions, such as the recognition of glycan structures by binding partners, and indirect mechanisms that contribute to the control of protein conformation, stability, and turnover. During, or shortly after, protein biosynthesis amino acids may be modified by the covalent attachment of a variety of functional groups. This type of protein modification is known as posttranslational modification (PTM). In the case of glycosylation, asparagine, serine, threonine, tryptophan,[2] hydroxyproline,[3] hydroxylysine[3] and rarely tyrosine residues are modified by the attachment of carbohydrate moieties. Although there are over 200 PTMs characterized to date; glycosylation is unique in that it is a template free process that results in the addition of heterogeneous structures that cause a large mass shift (typically greater than 800 Da), relative to other PTMs. Glycosylation modifications are involved in several pathological and normal conditions including host–pathogen interaction, tumor invasion, cell infiltration as well as cell migration.[4–6] Interactions and cell-cell adhesion are mediated by glycan moieties that are found on cellular membranes and secreted proteins.[7–10]

Protein glycosylation is generally classified as N- or O-linked. N-linked glycans are attached to asparagine residues that occur in the subsequence Asn-XXX-Ser/Thr, where XXX represents any amino acid except proline. While this is the norm, it should be noted that several studies have reported N-glycosylation sites at non-canonical protein consensus motifs such as Asn-XXX-Gly,[11] Gln-GlyThr,[12] and Asn-XXX-Cys.[13] All N-linked glycans share a common carbohydrate sequence, Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAcβ1-Asn-X-Ser/Thr, that is referred to as the “core” structure. The multi-step process of N-glycan biosynthesis starts on the cytosolic surface of the endoplasmic reticulum (ER) membrane and is finalized in the Golgi complex.[14] N-glycans found in humans are composed of GlcNAc, mannose, galactose, N-acetylgalactosamine (GalNAc), fucose, and N-acetylneuraminic acid (sialic acid) monosaccharide residues and are classified as either high mannose, complex or hybrid. High mannose structures have only mannose residues attached to the core structure, whereas complex structures have other monosaccharides. Hybrid structures possess characteristics of both high mannose and complex structures on different branches. Due to the diverse biological functions of sialic acid and fucose, glycans are also commonly classified as sialylated or fucosylated when these residues are present within the glycan structure.

With the exception of glycosaminoglycan biosynthesis, which represents an independent mechanism involving both the Golgi apparatus and the endoplasmic reticulum, O-glycan

biosynthesis is a less complicated process than N-glycan biosynthesis. The process begins with direct covalent attachment of glycans to the serine, threonine or, less commonly, tyrosine hydroxyproline[3] and hydroxylysine[3] residues of polypeptide chains in the Golgi apparatus.[15, 16] Despite the relative simplicity of their biosynthesis, O-glycan structures are still highly variable due to trimming and the addition of monosaccharides by various glycosyltransferases and glycosidases.[17] A unique feature of O-glycosylation is the opportunity for attachment of glycans on consecutive and adjacent amino acids,[18–23] a phenomenon not commonly observed in N-glycosylation perhaps due to steric hindrance.[24] There are also a variety of other O-glycosylation forms documented, such as β-linked O-GlcNAc and α-linked O-fucose, β-linked O-xylose, α-linked O-mannose, α- or β-linked O-galactose, and α- or β-linked O-glucose glycans which were recently reviewed by Moremen *et al.*[25] Additionally, while in-depth characterization of the mechanisms involved in the biosynthesis of all O-linked structures is beyond the scope of this review, those interested may refer to reviews of mucin-type glycan biosynthesis,[26] glycosaminoglycan/proteoglycan biosynthesis[27, 28] as well as a general review that describes all O-glycan biosynthesis by Wopereis *et al.*[29]

Cancer is characterized by aberrations in glycolipid and glycoprotein content; both at the structural and concentration levels.[5, 6] Aberrant glycosylation has been associated with the mechanisms underlying tumorigenesis and metastasis.[30] Several studies, which utilized experimental approaches that were correlative in nature have associated glycosylation and development of metastasis. For example, glycosylation staining of paraffin sections utilizing the helix pomatia (HPA) lectin, that binds to α-GalNAc residues of cellular structures, was employed to correlate the occurrence of metastasis to other parameters such as tumor size or histological stage.[31, 32] Similarly, other approaches used glycosylation enrichment techniques such as *Datura stramonium* agglutinin (DSA)-sepharose column binding to show that metastasized carcinomas harbor elevated levels of glycosylation compared to normal or primary carcinomas.[33] More recent studies, utilizing advanced techniques to dissect the stages of cancer metastasis, have been conducted providing more in depth information into pathological glycosylation mechanisms.[34–37]

Metastatic spreading is a multistep process that involves the ability of cancerous cells to escape normal tissue boundaries and detach from primary tumors. This process is coupled with degradation of the extracellular matrix (ECM) and invasion of the surrounding tissues or entry into the lymphatic/blood vessels to form metastatic lesions.[38] Metastasis and invasion are regulated by alterations in the ECM[39–42] impacting cell–cell interactions as well as structural changes in glycosylation that occur on cell surface components.[43] Mechanistically, aberrant secretions of cell surface and /or secreted proteins by the malignant cells or surrounding adjacent cellular tissues is crucial for the metastatic process. These molecules include the ECM glycoproteins cytokines, growth factors, and cell surface proteins, and their altered glycosylation promotes self and contact-dependent interactions enabling propagating tumor cells to extravagate to remote tissues.[44] A hallmark feature of several tumor cell-types is the upregulation of sialic acid sugars attached to glycolipids and glycoproteins.[45]

Gliomas represent one of the most prevalent primary brain tumors, which are difficult to treat due to their invasive characteristics.[46] Owing to this characteristic feature, gliomas are able to invade normal tissues in a diverse and infiltrative manner compared to peripheral tumors that metastasize to the brain but are not able to penetrate the host nervous tissue, although they can colonize next to it.[47, 48] Central nervous system (CNS) gliomas are able to interact with surface receptors which involve both the lectican family chondroitin sulfate proteoglycans and CD44 via binding to the hyaluronic acid-based ECM.[49, 50]

In the context of the invasiveness of gliomas, BEHAB/brevican protein, a CNS–specific lectican that is regulated in a spatiotemporal manner in the brain,[51, 52] has been shown to be upregulated in conditions of glial cell motility, injury, and gliomas.[51, 53, 54] It is of interest that being exposed to proteolytic cleavage, alternative splicing, differential glycosylation, and variable expression, in human gliomas, contributes to its progression and plays a key role in its capacity for invasiveness into naive, nervous tissues.[55, 56] In an elegant study, Viapiano *et al.* assessed the role of BEHAB/brevican in gliomas; two novel specific isoforms were identified which were found to carry altered glycan structures due to differential glycosylation regulation. B/b_{sia} and B/$b_{\Delta g}$, isoforms were detected; B/b_{sia} is an over-sialylated isoform that was identified in high- and low-grade gliomas, while B/ $b_{\Delta g}$ was shown to be an under-glycosylated isoform lacking the carbohydrates detected in the high-grade gliomas and absent in normal tissues.[57] As they are absent in benign gliomas, this finding highlighted the role of modulated forms of glycosylation as diagnostic markers for glioma progression and as a putative aim for immunotherapy by targeting cell surface antigenicity.[57]

As previously mentioned, aberrant glycosylation has been closely linked to the development and progression of several cancers, including brain cancer. The primary focus of this review is to discuss the association of aberrant N- and O-glycosylation with human brain cancer, although some studies are utilizing murine and rat models will also be presented. This review also highlights and discusses the potential utility of glycans as biomarkers, and the role glycosylation plays in therapeutics.

2 Aberrant N-Linked Glycosylation in Brain Cancer

N-glycan changes commonly associated with cancer include increased terminal glycan sialylation,[58, 59] and increased formation of extensively branched structures.[60–62] The three glycosyltransferases, which are known to take part in the biosynthesis of cancer associated branched N-glycan structures, are β1,4-n-acetylglucosaminyltransferase (GnT-III), β1,6-N-acetylglucosaminyl transferase (GnT-V) and α1,6-fucosyltransferase (FUT8) (Fig. 1).[63] The product of GnT-III is referred to as a bisecting GlcNAc linkage, where GnT-III catalyzes the addition of GlcNAc to a mannose residue linked through a β1,4-linkage. The introduction of the bisecting GlcNAc prevents further processing because this structure cannot act as a substrate for other glycosyltransferases.[64] GnT-III is considered an essential enzyme in the biosynthetic pathway of N-glycans that inhibits metastasis.[65]

The activity of GnT-V is in stark contrast to that of GnT-III, as the catalytic products of GnT-V are β1,6-GlcNAc branching structures known to play essential roles in tumor metastasis

(Fig. 1).[66, 67] GnT-V, which is encoded by the *MGAT5* gene, exhibits increased expression in cancers of the colon, brain, and breast and is documented as decreasing cellular adhesion while promoting tumor invasion and metastasis.[65, 68–70] Moreover, when studies were carried out with transplantable tumors in mice, the product of GnT-V was shown to contribute to both cancer growth and metastasis.[68, 71] Glycans with β1,6-GlcNAc structures have also been demonstrated to be biomarkers for tumor progression in colon and breast cancer.[61, 72] With regards to brain cancer specifically, when GnT-V was overexpressed in glioma cells changes in focal adhesions and increased tumor cell invasion was observed.[69]

Bone marrow-derived human mesenchymal stem cells (BM-hMSCs) have the intrinsic ability to seek out and engraft in tumors, including glioblastoma (GBM) of the brain. As a result, they have generated interest for use in cell-mediated delivery of therapeutic agents in GBM. When transcriptomic and glycomic profiling strategies were combined for the profiling of glioma stem cell xenografts, cells that did not attract BM-hMSCs were found to display increased truncated high mannose and sialylated N-glycan structures. Whereas cells that attracted BM-hMSCs exhibited N-glycan structures that terminated in either GlcNAc or galactose. The authors hypothesized that increased upregulation of sialic acid expression in cells that do not attract BM-hMSCs might facilitate a reduction in reactive oxygen species-mediated inflammation,[73] an assertation supported by studies that indicate sialic acids act as antioxidants by scavenging free radicals.[74–79]

FUT8 catalysis results in the transfer of fucose residues from GDP-fucose to the sixth position on the inner GlcNAc residue of complex and hybrid glycoprotein N-linked glycans, a process referred to as core fucosylation (Fig. 1). FUT8 activity has been shown to be higher in brain tissues than in other non-diseased tissues, and it is the only core fucosyltransferase expressed in mammals.[80] Core fucosylated glycoproteins have been shown to be altered during pathological states, such as hepatocellular carcinoma and cirrhosis of the liver,[81, 82] and increased FUT8 expression was demonstrated to have a direct relationship with tumor size and lymph node metastasis in papillary cancer.[83] Additionally, the removal of core fucose residues from Immunoglobulin G (IgG) 1 molecules was shown to increase antibody-dependent cell-mediated cytotoxicity (ADCC) by as much as 100-fold, indicating that core fucosylated structures are significantly involved in the ADCC process.[84]

In regard to the normal brain, Tsuchiya *et al.* characterized 46.4% of the total N-linked oligosaccharides in normal brain tissue. A2G2 was found expressed in normal brain. While, no tri- or tetra-antennary structures were expressed, unlike bi-antennary N-linked oligosaccharides that were observed most frequently. Oligomannose structures were also detected, with the largest proportion corresponding to M5A then M6B and M9A.[85] Another study discerned the existence of O-glycosylation at the synaptosomes and identified three neuron specific proteins, involved in intracellular signaling, that carry glycomic modifications including collapsin response mediator protein-2 (CRMP-2), ubiquitin carboxyl hydrolase-L1 (UCH-L1) and β-synuclein. The presence of such O-GlcNAc modifications is reasonable since O-GlcNAc transferase (OGT), and N-acetyl- β-D-glucosaminidase (O-GlcNAcase) activity has been detected in the cytosol of nerve terminal regions.[86]

In one study by Zamze *et al.*, it was shown that rodent rat brain proteins carry complex types of abundant N-linked oligosaccharides.[87] These include neutral N-linked glycans comprising the “brain type” of N-glycosylation.[88, 89] Interestingly, this analysis has been further investigated to assess other forms of N-linked oligosaccharides, including sialylated entities which were found to account for ~40% of the N-glycan pool.[87] The remainder of the acidic structures included sulfate groups; in addition to, sialic acid (NeuAc). These altered acidic structures carry high relevance to neural tissue where they are involved in important functions such as neural plasticity[90, 91]; an excellent example of the involvement of such moieties are α(2-8)-linked polysialic acid and sulfoglucuronyl moieties which are present on the neural-cell-adhesion molecule (N-CAM), and the L2/HNK-1 epitope; respectively[92, 93].

Recently, the concept of a hierarchy of cells within a tumor has been widely studied, and cancer stem cells (CSCs) are taking the lions share in this[94]. Glioma stem cells (GSCs) are found within GBMs and are considered among the main contributors for the initiation and perpetuation of these tumors.[95] GSCs adopt the most undifferentiated profile and retain the ability of self-renewal and aberrant proliferation.[96] A study by Boa *et al.* revealed that GSCs are able to defy ionizing radiation induced death through the activation of the DNA repair system.[97] Thus, GSCs are resistant to the most exhaustive treatments, such as chemotherapy and radiotherapy, contributing to cancer recurrences.[97, 98] During tumor re-initiation, GSCs undergo several molecular changes that are dependent on alterations in glycosylation. Of interest, the glycosylation status of cell surface glycoprotein epitopes has been associated with differentiation state, tumor transformation and progression.

2.1 Altered Sialylation Associated with Brain Cancer

Sialic acids are monosaccharides that are commonly observed as terminal residues of glycoprotein oligosaccharide chains. Sialic acids are involved in a number of biological processes, such as immune modulation and cell adhesion, and are known to bind proteins such as siglecs, selectins, and lectins.[99, 100] Negatively charged sialic acid residues, on the cell surface, are capable of impacting tumorigenesis by decreasing cell adhesion, which may result in increased cancer cell motility and greater metastatic potential.[101–104] Numerous studies have reported elevated glycoprotein sialic acid levels in malignant cells,[105–107] and it has been suggested that the changes in sialic acid abundance take place early on in the process of tumorigenesis.[108] Cell surface sialic acid epitopes have the ability to mask antigenic sites, preventing recognition of foreign or cancerous cells by the immune-defense system.[101] An example of such masking is provided by the binding of tumor cell sialic acids to Siglec7 on NK cells, which prevents cancer cell immune recognition by NK T cells. Hudak *et al.* demonstrated this by inserting sialylated glycopolymers into the membrane of cancer cells, to emulate cancer associated sialylation, and showing that the ensuing localization of Siglecs to the activation site increases SHP-1 and SHP-2 phosphatase recruitment. This recruitment resulted in inhibition of the phosphorylation cascade, thus preventing NK cell activation (Fig. 2).[109]

A large number of glycomics and glycoproteomics studies have established a strong correlation between glycosylation and brain cancer. These studies have investigated the role of glycomic changes in the transformation and progression of neural cells. Several of these

studies have shown that cancer transformation, progression, and metastasis are coupled with alterations in glycosylation phenotypes, as discussed later. Hu *et al.*[110] evaluated the mechanisms by which MYCN gene amplification relates to neuroblastoma transformation. The study revealed that N-linked glycomic changes relate to the progression of these cells. The study was performed on two neuroblastoma cell lines: SY5Y, where *MYCN* was not amplified, and NLF which is a cell line that has elevated levels of the *MYCN* gene. Their results revealed that the more aggressive neuroblastoma cell line, NLF, had increased sialylation and expression of larger glycans compared to SY5Y. These cell lines demonstrated significant alterations in sixteen different glycans as detected by LC-MS/MS. Among the sialylated glycans that showed the most significant upregulation were HexNAc5Hex6NeuAc4, HexNAc5Hex6NeuAc3 and HexNAc6Hex7dHex1NeuAc2. Furthermore, SY5Y contained small glycans like HexNAc2Hex4 whereas NLF expressed larger glycans such as HexNAc4Hex5dHex1NeuAc2.[110]

Most of the proteins found in the sera of humans are glycosylated, and many of these glycosylated proteins contain sialylated structures. Alterations to sialylated glycan structures on serum glycoproteins are downstream of cancer induced changes in the behavior of glyco-enzymes, such as the sialyltransferase family of enzymes.[63] As a result, a number of research groups have proposed the use of aberrant serum protein glycan sialylation as a cancer biomarker.[111–115] A study involving the quantification of total serum sialic acid in healthy individuals versus lung cancer patients indicated that total serum sialic acid was significantly elevated in lung cancer patients when compared to healthy individuals, supporting the utility of serum glycoprotein sialylation as a cancer biomarker.[116]

Terminal N-glycan sialic acids can be attached via α2,3- or α2,6-linkages. α2,3-Linked sialic acids, and their associated sialyltransferase have been reported to be the only detectable form of sialic acid in patients with GBM.[117, 118] Additionally, *in vivo* studies have indicated that α2,3- and α2,6-linked sialic acids promote opposing processes, with α2,3-linked sialic acid promoting tumor cell invasion and α2,6-linked sialic acid decreasing it. It was also reported that, along with reduced cancer cell invasion, α2,6-linked sialic acid was associated with changes in adhesion-mediated signaling and a reduction in tumor growth. Yamamoto *et al.* further postulated that because integrin-extracellular matrix interactions are critically involved in anchorage-dependent tumor growth, that *ST6Gal1* expression, through glycosyltransferase gene delivery, may alter α3β1 integrin function in a manner useful for ortho-adhesion and signaling therapy for malignant brain tumors.[70]

The increased ST3GAL1 expression has been characterized as defining an invasive fraction of GBM cells with the capacity for self-renewal and a loss of its function has been shown to prolong survival in mouse models.[119] It was also demonstrated, in the same study, that the transcriptomic program that is associated with ST3GAL1 is associated with poor prognosis in glioma patients and leads to higher tumor grades of the mesenchymal molecular classification.[119] Additionally, hyper sialylated β1 integrin has been demonstrated to be endogenously expressed in human astrocytoma cell lines, and it has been suggested that sialylation may represent a mechanism for the regulation of β1 integrin signal transduction in glioma cells.[120]

When normal human astrocytes were transformed to mimic human brain tumor grades I-IV, the changes observed included elevated expression of α2,6-sialylation with a concomitant decrease in core 2 (GlcNAcβ1-6(Galβ1-3)GalNAcαSer/Thr) O-glycan biosynthesis.[121] Furukawa *et al.* employed a multi-step human glioma model to unveil the glycosylation profiles and their contribution to tumor progression. The model consisted of consecutive introduction of hTERT, SV40ER, H-RasV12 and myrAKT, thereby transforming normal human astrocytes (NHA) in a way that mirrors the four stages (I – IV) of gliomas. Although it does not comprise the same complexity of brain cancer *in vivo*, this model allows for the modification assessment of N- and O-glycans along with glycosphingolipids (GSL) through the successive brain tumor stages[121]. The introduction of hTERT increases the life of NHAs; thus, establishing grade I glioma cells. At this stage, alterations in N-glycans were demonstrated by an elevation in paucimannose and monoantennary glycans as well as β1,6-branched glycans. In addition, an upregulation of the GSL GD3 was also identified. It was also shown that SV40ER immortalized the cells, that up to this stage were still benign, simulating grade II glioma cells. A decrease in total N-glycans and GSL occurs at this step. The N-glycans expressed are multi-fucosylated with an elevation in N-glycolylneuraminic acid (Neu5Gc) expression. The changes in GSL were displayed as a transition from ganglio-series GSLs to globo-series. Next, transformation and malignancy manifest in grades III and IV that are obtained by H-RasV12 and myrAKT induction, respectively. Of interest, Grade III phase cells exhibited an elevation in branched GlcNAc N-glycans and a downregulation of core 2 O-glycan biosynthesis. Moreover, transformed cells had higher levels of α2,6-sialylation. However, the fully transformed grade IV cells exhibited reduced levels of bisecting GlcNAc N-glycans and increased levels of fucosylated glycans. Furthermore, (neo) lacto-series GSLs were also upregulated. Thus, data from this study indicate that glycosylation modifications during glioma progression are transitory rather than stable.[121]

Polysialic acid (PSA) is a linear carbohydrate homopolymer composed of α2,8-linked sialic acids, that is primarily bound to N-CAM. N-CAM in most adult tissues lack PSA, while poly-sialylated N-CAM in embryonic tissues is abundant.[122–124] Further, during the process of embryonic development, poly-sialylated N-CAM is localized specifically to migrating cell types.[125, 126] The presence of large negatively charged PSA molecules on N-CAM regulate its adhesive properties, and a large body of research suggests that PSA regulates its function, as well.[127] Despite the reduction of PSA expression in most adult tissues, some tumors have been shown to “re-express” PSA. Therefore, PSA is considered an oncodevelopmental antigen.

Human lung cancer cells, small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC), as well as astrocytomas, are known to express PSA,[128–130] and it is believed that either decreased N-CAM or a reduction in its adhesive properties, by PSA, result in increased cell migration and metastasis.[127] PSA and N-CAM expressions correlate positively with both cell proliferation and metastatic potential, in SCLC.[128, 131] Additionally, in NSCLC, PSA expression is found in 77% of stage IV tumors, whereas only 21% of stage I tumors are positive for PSA expression.[129] This general trend is also observed in astrocytomas, where PSA expression is 10 times higher in world health organization grade III and IV tumors compared to grade II tumors.[130]

When the expression of PSA-NCAM, a neurodevelopmental protein, was examined in GBM it was found to be an adverse prognostic factor for overall survival and disease free survival. In the same study, PSA-NCAM expression was also found to correlate with olig2, which is a transcription factor required for gliomagenesis.[132] In a different study, PSA-NCAM was found to be present in 46.3% of typical human pituitary tumors and 85% of human pituitary tumors regarded as highly aggressive, while being absent in the healthy pituitary gland, and PSA-NCAM expression was found to be strongly related to tumor invasion.[133] Fibronectin and sialic levels have also been demonstrated to be significantly higher in human pituitary adenomas than in normal brain tissue, and infiltrative pituitary adenomas were found to contain higher levels than non-infiltrative adenomas.[134] In a study conducted by the same authors, fibronectin, and sialic levels were also demonstrated to be significantly higher in human meningiomas and gliomas than in nondiseased control brain tissue, and grade III-IV gliomas were found to contain higher levels than grade I-II gliomas. Based on these findings, one can conclude that both sialic acid and PSA modifications have a high probability of involvement in the lung, brain, and other cancers.

2.2 Altered Fucosylation Associated with Brain Cancer

Fucosylation is a common modification that takes place when the deoxyhexose fucose is transferred from GDP to glycolipids, O-glycans, N-glycans or polypeptide chains. Fucosylation is known to be involved in a number of biological functions, such as ABO blood group determination (Fig. 3), host-microbe interactions, selectin dependent leukocyte-endothelial adhesion,[135, 136] ontogenesis, including Notch receptor signaling, as well as several pathological conditions, such as atherosclerosis and cancer.[137] An example of aberrant glycosylation in cancer involving fucosylated glycans is provided by the loss of A (GalNAcα1-3(Fucα1-2)Gal-) and B (Galα1-3(Fucα1-2)Gal-) blood group antigens, coupled with an increase in H (Fucα1-2Gal-) antigen and Lewis-Y (Fucα1-2Galβ1-4(Fucα1-3)GlcNAc-) antigen expression, in many tumors. Changes of this nature are of particular importance because they have been found to correlate with poor clinical prognosis.[138–141] α-Fetoprotein provides an additional example, as its increased α1,6-fucosylation is used as a clinical diagnostic marker to distinguish between hepatocellular carcinoma and chronic liver disease.[81]

Sialyl Lewis-X (Siaα2-3Galβ1-4(Fucα1-3)GlcNAc-) and –A (Siaα2-3Galβ1-3(Fucα1-4)GlcNAc-) antigens (Fig. 3), glycan structures bound by the endothelial-leukocyte adhesion molecule E-selectin, are commonly expressed at higher levels in carcinoma cells.[142–144] These higher expression levels are correlated with both advanced tumor grade and poor prognosis. Additionally, because sialyl Lewis-X and -A antigens function as ligands for selectin molecules, these glycans may facilitate hematogenous metastasis through direct binding of cancerous cells to P- and E-selectins present on the endothelium.[138, 145] Additional potential scenarios where metastasis is facilitated by the fucose-containing structures sialyl Lewis-X and –A antigens include the formation of cellular thromboemboli by way of interaction with P-selectin[146] and the blockage of tumor leukocyte infiltration through the secretion of inhibitors, that contain sialyl Lewis-X and -A antigen structures, of endothelial-leukocyte adhesion.[138]

The Lewis-X (Galβ1-4(Fucα1-3)GlcNAc-) antigen (Fig. 3), also known as stage-specific embryonic antigen 1 (SSEA-1) and CD15, is a fucose containing glycan proposed to be a marker for tumor-initiating cells/tumor stem cells among GBM cell populations.[147] When SSEA-1+ cells were selected for from acutely isolated tumor cells from fresh GBM patient tumors they were found to be enriched in glioma tumor-initiating cells. Further, SSEA-1+ acutely isolated cells and cultured in vitro-established tumor-initiating cells were determined to exhibit, at least, a 100-fold tumorigenic enrichment in mouse xenograft models when compared to SSEA-1- cells and demonstrated capabilities for self-renewal and multi-lineage differentiation.[147]

Moreover, in a study that aimed to define the N-linked oligosaccharides present in GBM, Tsuchiya *et al.* identified 16 oligosaccharides from GBM tissue.[85] They determined that major oligosaccharide structures were similar between the normal brain and GBM tissue. Interestingly, they reported an elevation in the levels of biantennary bigalactosylated sugar chains with one core fucosylation (A2G2F) moiety normally expressed in the brain at embryonic stages only. Owing to the core fucose in A2G2F, GBM cells were able to bind to lens culinaris agglutinin (LCA) lectin which subjected them to apoptosis, indicating the potential importance of A2G2F in targeted therapy.[85]

3. Aberrant O-Linked Glycosylation in Brain Cancer

Glycans covalently linked to the polypeptide chain of a protein by the hydroxyl group of serine or threonine are referred to as O-linked glycans. Mucin O-glycans are α-linked via GalNAc. Mucins are heavily O-glycosylated glycoproteins produced by the epithelia of most animals that come in both secreted and transmembrane varieties. They are integral to the formation of mucus barriers and are documented as being intimately involved in both inflammation and cancer (Fig. 4).[148] Alternatively, when O-glycans are covalently bound through a β-linkage to a GlcNAc, the modification is known as O-GlcNAc. O-GlcNAcylation is comparable to protein phosphorylation in that it is a dynamic process where the PTM can be removed or added in response to changes in the local environment related to nutrients, hormones or stress.[149] O-GlcNAcylation is one of the most abundant PTMs to occur in the cytoplasm and nucleus and is known to regulate a variety of essential biological processes, including metabolism, insulin recognition, cell proliferation, protein degradation, gene transcription and cell signaling pathways.[63]

3.1 O-GlcNAc Glycan Changes Associated with Brain Cancer

The process of O-GlcNAcylation is regulated by a pair of enzymes that catalyze the addition of GlcNAc, which is transferred from uridine diphospho-N-acetylglucosamine (UDP-GlcNAc) and catalyzed by O-GlcNAc transferase (OGT), and the cleavage of GlcNAc, which is catalyzed by O-GlcNAcase (OGA). The O-GlcNAc modification is known to affect a variety of proteins involved in a number of diverse processes including insulin resistance (Akt, insulin receptor substrate 1),[150] oncogene transcription (c-Myc, NF-κB),[151, 152] tumor suppression (p53),[153] hepatic gluconeogenesis (transducer of regulated cyclic adenosine monophosphate response element-binding protein 2),[154] maintenance of neuronal health and prevention of Alzheimer's disease (α-synuclein, amyloid precursor protein, tau, protein

kinase A),[155–159] regulation of mitosis and neuronal development (Cdh1),[160] among other things.

The substrate for OGT, UDP-GlcNAc, is an end product of the hexosamine biosynthesis pathway that branches from glycolysis, directly linking cellular metabolism with O-GlcNAcylation (Fig. 5).[161, 162] Additionally, nine out of the 10 enzymes required for glycolysis are potential substrates for OGT.[163] Cancer cells produce energy by the non-oxidative breakdown of glucose, with tumors cells demonstrating glycolytic rates as high as 200 times that of normal tissues.[164] O-GlcNAcylation serves as a nutrient and stress sensor that links the metabolic state of cells with a myriad of signaling pathways;[165] therefore, it is plausible that glycosylation with O-GlcNAc may play a role in the regulation of glycolysis and the ensuing metabolic abnormalities observed in cancer cells. A study by Yi *et al.* supported this hypothesis by demonstrating that O-GlcNAcylation of phosphofructokinase 1 (PFK1) was induced by hypoxic conditions,[166] which commonly occur in cancer cells. Further, glycosylation was shown to inhibit PFK1 activity and redirect glucose flux through the pentose phosphate pathway, resulting in a selective growth advantage for human NSCLC cells.[166]

3.2 O-GalNAc Glycan Changes Associated with Brain Cancer

Members of the mucin family contain tandem repeat structures that have a high proportion of prolines, threonines, and serines, which are known as PTS domains. The PTS domains of mucins are heavily glycosylated with glycans attached through GalNAc residues O-linked to serine and threonine. Members of the human mucin family (MUC) are designated MUC1 through MUC21 and fall into secreted or transmembrane sub categories. Different glycosyltransferases synthesize the four most common mucin-type O-glycans which are the core1 or T-antigen (Galβ1-3GalNAcαSer/Thr), core2 (GlcNAcβ1-6(Galβ1-3)GalNAcαSer/Thr), core3 (GlcNAcβ1-3GalNAcαSer/Thr) and core 4 (GlcNAcβ1-6(GlcNAcβ1-3)GalNAcαSer/Thr) structures.

Abnormal patterns of mucin expression in the human respiratory mucosa have been found to be associated with lung carcinogenesis.[167–169] An example of a secreted mucin playing a role in cancer progression is provided by MUC2, which has been shown to functionally suppress inflammation in the intestinal tract and inhibit the development of colorectal cancer.[170, 171] When the expression of core Tn (GalNAcαSer/Thr), sialyl-Tn (Siaα2-6GalNAcαSer/Thr) and core 1) and terminal fucosylated and sialylated (Lewis antigens) glycans in different NSCLC lung tumors were compared, it was found that truncated structures were observed in both tumor types, but core and terminal structures were detected more frequently in adenocarcinoma than in squamous cell carcinoma, excepting Lewis-Y structures which are substantially expressed in both NSCLC types.[172] This finding is interesting in that it implies that different combinations of glycosyltransferases are expressed in adenocarcinoma and squamous cell carcinoma. Further, it has been demonstrated that while most Lewis structures are expressed in NSCLC tumors, Lewis type 1 antigens are detected less frequently than Lewis type 2 antigens and Lewis-Y structures represent the most strongly expressed antigen.[173, 174]

MUC1 is a transmembrane mucin that is localized to the apical membrane of secretory epithelial cells under normal conditions.[175] During the process of transformation when epithelial cells lose their polarity, MUC1 is known to exhibit high levels of expression on the entire surface of a diverse array of carcinoma cells. After being translated into a single polypeptide MUC1 splits, through auto proteolysis, into N-terminal (MUC1-N) and C-terminal (MUC1-C) subunits.[148] Glycosylation of the tandem repeats present in MUC1-N have been shown to be altered in carcinomas, a change at least partially attributed to alterations in glycosyltransferase expression observed in tumors.[176] Atypical glycosylation of MUC1 also has demonstrated involvement in cancer immunosurveillance.[177]

MUC1 is anchored to the cell surface when MUC1-N non-covalently associates with its corresponding transmembrane subunit, MUC1-C, forming a stable complex. MUC1-N, which is the mucin component of the complex, blocks both cell-extracellular and cell to cell interactions.[178] When MUC1-N departs from the cellular surface, it is believed that MUC1-C remains, putatively functioning as a receptor interacting with a variety of signaling pathways associated with transformation and tumor growth.[179] When the cellular distribution of MUC1 was examined, using immunohistochemistry, in patients with NSCLC, it was found that a depolarized cellular distribution of MUC1 was associated with modal metastasis, advanced pathological state, and poor prognosis.[180–182] Additionally, immunohistochemical analyses also indicated that a high ratio of MUC1 to surfactant apoprotein A expression was strongly associated with poor outcomes in patients with small-sized lung adenocarcinoma.[183] It should be noted that one study examining the expression of a novel carbohydrate-induced conformational tumor-associated MUC1 epitope, termed TA-MUC1, found it to be a favorable prognostic factor for NSCLC patients with lymph node metastases[184], in contrast to many other findings.

MUC1 has also been proposed as a marker enabling researchers to follow the type II pneumocyte lineage through the process of lung carcinogenesis. It was reported that MUC1 expression was preserved in Type II alveolar pneumocytes, which serve as progenitor cells for neoplastic and normal epithelium during the repair of injury and cancer carcinogenesis, but was decreased when NSCLC cells reverted to more normal phenotypes.[169] In addition to lung cancer, the MUC1 expression has also been shown to correlate with prognosis in ovarian cancer, where low expression of MUC1 was found to associate with early stage and good outcomes for patients with invasive tumors.[185] In an indirect role, MUC1-induced tumorigenesis has been demonstrated to contribute to the regulation of genes highly associated with poor clinical outcomes in both lung and breast cancer patients.[186, 187] Signal transducer and activator of transcription 3, better known as STAT3, is known to be abnormally activated in human lung cancer and was demonstrated to regulate expression of MUC1 mediating metastasis both in vitro and in vivo (Fig. 4).[188] Additional findings have also indicated that NSCLC cells depend on MUC1-C for activation of the PI3K/Akt pathway and survival.[189]

The smoking of cigarettes accounts for 87% of lung cancer deaths and increases the risk for lung cancer 20-fold. Zhang *et al.* reported that cultured primary human bronchial epithelial cells treated with smoke-concentrated medium generated a novel 400 kDa glycoform of MUC1-N that differed from the 230 and 150 kDa glycoforms observed in untreated cells.[190]

Further, the smoke-induced shedding of glycosylated MUC1-N resulted in the exposure of the membrane spanning MUC1-C subunit, enabling it to serve as a putative receptor for the epidermal growth factor receptor (EGFR), proto-oncogene tyrosine-protein kinase Src and p120-catenin. The glycosylation of MUC1-C, induced by smoke exposure, modulated phosphorylation of MUC1-C tyrosine which was essential for interaction of MUC1-C with p120-catenin through bridging of Src-MUC1-C-galectin-3-EGFR signalosomes,[190] a process upstream of the degradation of E-cadherin, reduction of cell-cell adhesion and the loss of cellular polarity that's associated with the epithelial–mesenchymal transition and lung carcinogenesis. This body of work strongly suggests that aberrant O-glycosylation of MUC1 contributes to the pathogenesis of lung cancer.

The version of MUC1 associated with cancer is thought to be distinct from the normal protein. The malignant-associated form is reported to contain a lower percentage of O-glycosylated serine and threonine residues. Additionally, the O-glycans that are attached tend to be shorter and truncated forms relative to that of the normal MUC1.[63, 191, 192] It has been proposed that MUC1 on cancerous cells may exhibit anti-adhesive effects, due to a combination of its net negative charge, stemming from sialic acid residues, and its ability to mask surrounding cell surface molecules inhibiting their interactions with adjacent binding partners.[178] Examples of MUC1 exhibiting anti-adhesive effects have been provided by studies examining both integrin-[193] and E-cadherin[194] mediated cell matrix and cell-cell adhesion, respectively. An additional mechanism by with MUC1 and other glycoproteins may contribute to tumor growth was proposed by Paszek *et al.* who demonstrated that a bulky glycocalyx, as a result of the expression of bulky glycoproteins such as MUC1, may initiate metastasis by mechanically enhancing integrin-dependent growth factor signaling promoting growth and survival of tumor cells.[195]

MUC1 is a transmembrane protein expressed on epithelial cells that are characterized by aberrant O-glycosylation when in a cancerous state. These altered glycosylation patterns allow the exposure of the protein core which in turn facilitate the binding of tumor cells to other tissues; which is a major feature of metastasis[196]. MUC1 protein under-glycosylation is also associated with more aggressive stages. Therefore it represents a diagnostic marker.[197] In addition, MUC1 associates with CIN85 protein forming a complex in tumor cells mediating cell migration and invasion.[198] Finally, MUC1, MUC1-core, and Thomsen–Friedenreich (TF) proteins are expressed in mucinous tumors.[199] On the other hand, galectins are galactoside-binding lectins involved in cell adhesion and growth regulation.[200] Differential expression and function of the galectin family (elevated galectin-1 binding and downregulated galectin-3) have been correlated with lymph node metastasis.[200] Binding to matrix metalloproteinase 9 (MMP-9) and laminin are among the roles of galectin-3. Upon decreased MMP-9 binding to ECM in tumor cells, the specific MMP cleaves the ECM-proteins leading to the activation of the metastatic process.[201] The cancer-associated MUC1 protein serves as a ligand for galectin-3 mediated by the Thomsen–Friedenreich antigen (TF-Ag), and it promotes cancer cell adhesion to the endothelium, which initiates metastasis.[202, 203]

While the findings of Gordower *et al.*, who demonstrated that galectin-3 expression significantly decreases in most tumor astrocytes from low to high grade,[204] corroborates the

aforementioned study it should be mentioned that conflicting evidence has been reported. In their study, Gordower *et al.* also observed some tumor cell clones, that were highly malignant, expressing galectin-3 at high levels.[204] Additionally, it has been demonstrated that galectin-3 expression levels were significantly associated with astrocytic tumor grade, [205] and a number of other studies have also reported that astrocytic tumors demonstrate high galectin-3 expression levels.[206–210]

In the interest of clarifying these conflicting findings Strik *et al.* implemented immunohistochemistry to pinpoint the cellular origin and degree of galectin-3 positivity in glioma samples.[208] They demonstrated that galectin-3 expression was observed in B- and T-lymphocytes, endothelial cells, macrophages/microglial cells and neoplastic astrocytes and showed that tumor-infiltrating macrophages exerted considerable influence on galectin-3 positivity.[208] Therefore, one can conclude that galectin-3 expression levels appear to be dependent on non-tumor cells such as endothelial cells or macrophages/microglial cells, which may explain, in part, the conflicting data that have been presented on the expression of galectin-3 in human gliomas.[208, 211]

The involvement of galectin-1 in cancer biology is more clear cut as it has been shown to have numerous functions that include involvement in cell migration, through interactions with integrins and the ECM,[212] the formation of metastases,[213] angiogenesis stimulation via ORP150 regulation,[211] radio- and chemo- resistance through interactions with Ras[214, 215] and modulation of p53 nuclear migration.[214] Galectin-1 has also been shown to inhibit T-cell effector function through the promotion of apoptosis which has the effect of enabling tumor invasion of the immune system.[216, 217] In light of the role galectin-1 plays in radio- and chemo-resistance, it is considered a therapeutic target and synthetic lactulose amines[218] as well as galactomannan (Davanat®)[219] have been used to bind and modulate its activity. The latter, Davanat®, has received approval for colorectal cancer treatment from the FDA and appears promising, but more research is required to determine its utility for GBM treatment. [220]

GBM is the most common primary brain tumor and results in the largest number of central nervous system tumor-related deaths. The expression of MUC4, a highly O-glycosylated protein, has been studied in a number of cancers.[221–229] When MUC4 expression was examined in GBM cell lines, it was found to be overexpressed. Additionally, the capacity for GBM cell proliferation and invasion was dramatically increased by ectopic expression of MUC4. The expression of EGFR was found to be modulated by MUC4, and small interfering ribonucleic acid experiments targeting MUC4 and EGRF reduced proliferation and invasion, in both instances. Therefore, one can draw the conclusion that MUC4 expression plays a part in GBM proliferation and invasion, potentially through upregulation of EGFR.[229]

4. Clinical Potential and Applications of Aberrant Glycosylation for the Detection and Treatment of Brain Cancer

4.1 Identification of Glycan Biomarkers for Brain Cancer

It is well established that aberrant glycosylation commonly coincides with cancer and many instances of specific structures correlating with advanced disease states have been presented. Numerous studies, many of which are reviewed herein, indicate that aberrant glycosylation may contribute to the progression of cancer. Therefore, identification and characterization of glycan biomarkers have great potential for early diagnosis and favorable prognoses. This is particularly true in light of studies that demonstrate early detection of the brain, lung, and other cancers is correlated with improved prognoses and patient survival.[230, 231] The importance of early detection, especially in cancers such as lung and brain where early disease is asymptomatic, is highlighted by Miller *et al.* who reviewed cancer treatment and survivorship statistics for 2016 and reported that the five year survival rate for lung cancer is 55% for cases detected early when the disease is localized to its place of origin, but only 4% when detected at an advanced stage.[232] Brain cancer is similar to lung cancer in that by the time a glioma becomes symptomatic it is nearly always far too late in its clinical course for successful treatment.[233] Therefore, if we hope to improve brain cancer prognoses, early detection is critically needed, and biomarker screening represents a minimally invasive option with much potential.

As was previously stated, over half of all proteins are glycosylated, and it is estimated that the majority of human serum are glycosylated.[1] Changes in glycosylation reported to accompany pathological conditions range from minimal to significant and can be extremely specific to certain conditions, therefore it's believed that the investigation of serum protein glycosylation provides a viable avenue for diagnosis and prognosis of many diseases.[234, 235] Pathological states both acute and chronic in nature have been associated with changes in glycosylation[236] and in the case of acute pathology changes may occur very early on.[237, 238] IgGs are glycoproteins secreted by the adaptive immune system to impart long-term defense against antigens of previous exposure. IgGs exhibit significant diversity in the position and number of conserved N-linked glycosylation sites that are found on both the crystallizable fragments (Fc) and antigen-binding fragments (Fab) of the proteins. IgG glycans play multiple roles and are critical for IgG structure, binding events, solubility, conformation and function.[239] Accordingly, IgG glycosylation status are viewed as potential biomarkers for specific diseases such as cancer. An example of this is provided by Chen *et al.* who reported a marked increase in IgG1 Fc-agalactosylation (a decrease in galactose) in lung cancer patients when 259 lung cancer patients were compared to 410 control individuals.[240]

Specific ablation of fucose and/or sialic acid of particular glycan chains can trigger ADCC, resulting in the death of cancer cells.[241–243] The majority of the serological assays utilized for prognosis, diagnosis and surveying cancer development assess glycoconjugates including glycoproteins, proteoglycans, and glycosphingolipids, etc.[4] One example is the mucin glycoprotein CA 125 assay, of ovarian cancer (detected in 80% of ovarian patients), where levels are correlated with regression and prognosis.[244–246] Elevated levels of CA125 are detected in 50% of patients with stage I ovarian cancer.[247] Currently, there are several

glycolipid biomarkers found in tissues and biofluids, that are FDA approved, that are used in cancer diagnosis and prognosis, including CA27-29-Breast cancer, CEA Carcinoembryonic antigen-Colon cancer; CA19-9-Gastrointestinal cancer, etc. (please refer to Kim *et al.* for a detailed discussion[248]).

Cheray *et al.*[95] examined two well-defined GBM cell lines, U87-MG and U251, at distinct phases: floating undifferentiated and adherent differentiated cells. The main focus of the study was to scrutinize the implication of changes in the glyco-genome expression during the differentiation of GSCs. They analyzed 559 glycosylation-related genes using TLDA and were able to identify eight chiefly upregulated genes in the GSC-enriched undifferentiated state in both cell lines. These genes comprised members of the glycosyltransferase family that are potentially involved in tumor aggression and invasion. In addition to glycosyl-hydrolases that are involved in tumor angiogenesis.[95] As a result, the authors concluded that the identified genes might be considered as potential biomarkers for the characterization of the most aggressive and undifferentiated cells and provide new targets for GBM therapeutics.

Increased levels of expression of sialylated and fucosylated glycans have been associated with negative prognosis in a number of human cancers including breast, colorectal, gastric, urinary bladder carcinoma and pulmonary adenocarcinoma.[249] The potential utility of fucosylation as a marker for cancer is reported by Kossowska *et al.*[250] Elevated sialyl Lewis-X or –A, both of which are fucose-containing structures, expression has been observed in metastatic cancer cells and leads to selectin-mediated extravasation. The authors hypothesized that extensive fucosylation of the serum microenvironment might contribute to the loss of adhesion observed during the process of tumor formation. When the fucosylation of serum glycoproteins in SCLC and NSCLC patients was examined, fucosylated glycans were determined to be present in higher amounts in cancer patient sera when compared to healthy patient sera and differences in the degree of fucosylation were observed between SCLC and NSCLC patients. Therefore, the degree of fucosylation has been proposed to be a marker to estimate the glycosylation status of serum proteins in patients with cancer. Cluster analysis also pointed to the potential use of fucosylation status as a predictive factor for patient survival.[250]

As previously discussed, aberrant mucin O-glycosylation has been shown to accompany lung cancer, and the monitoring of mucins has been proposed to have prognostic value for individuals with lung cancer.[180–183] MUC1 expression, in particular, has been demonstrated to correlate with poor prognoses in several different types of cancer.[148] A study published in 2015, by Song *et al.*, showed that the under-glycosylated form of MUC1 overexpressed in human malignant cancer cell lines (BT20, HT29, and LS174T) could be differentiated, using chemical exchange saturation transfer (CEST) magnetic resonance imaging (MRI), from the normally glycosylated form expressed in a benign human epithelial cell line (MCF10A) and a tumor cell line (U87) that did not express the under-glycosylated form of MUC1. Moreover, when tumor cells from the LS174T and U87 cell lines were implanted in the brains of mice, CEST MRI was able to differentiate between the two *in vivo*.[251] These findings suggest that CEST MRI may represent a non-invasive method for the investigation of mucin glycosylation and the malignant transformation of brain tumors. In addition to

MUC1, MUC4 has also been identified as a potential biomarker for diagnosis and prognosis of cancer. Examples of MUC4 overexpression, and often the loss of localization to the apical membrane, correlating with cancer include malignancies of the gall bladder,[223] breast,[226] pancreas,[222, 252] ovaries,[224, 225] lung,[227, 228] and brain.[229]

The monitoring of glycopeptides is a potential strategy for the identification and utilization of cancer biomarkers, although enrichment procedures prior to analysis are necessary. A 2016 study by Zacharias *et al.* described a strategy where hydrophilic interaction liquid chromatography and electrostatic repulsion liquid chromatography enrichment techniques were used in a complementary fashion, with mass spectrometry, for the identification of glycopeptide biomarkers of cancer. The method was capable of distinguishing glycopeptides that were associated with brain cancer from those that were not when breast and brain cancer cell lines were examined.[253]

An additional candidate cancer biomarker is represented by sialylation which has reported involvement in a number of cancers,[101–108] one such example is the increased expression of α2,3-linked glycoprotein sialylation on malignant gliomas.[254] Sialic acids have been proposed as biomarkers for endocrinal cancers, including cancers of the thyroid, ovary, pancreas, thyroid, adrenal and pituitary gland, a topic reviewed by Ghosh in 2015.[255] The use of the altered expression of sialic acids and or their linkages as a prognostic tool for human cancer is supported by *in vivo* findings that indicate, in mice, α2,6-linked sialic acids mediate tumor progression via β1 integrin.[58] A study utilizing quantitative proteomic analyses revealed 35 cell surface sialoproteins that were overexpressed in human GBM tumors compared to normal human astrocytes.[256] Investigations into the expression levels of lipid-associated sialoprotein (LSP) in the cerebrospinal fluid (CSF) of individuals with brain tumors revealed patients with newly diagnosed primary or metastatic brain tumors displayed on average 3.7 fold higher CSF LSP levels compared with CSF from tumor-free patients. Further, the CSF from progressive disease patients with brain tumors not responsive to treatment contained LSP at levels similar to the newly diagnosed patients while treatment-responsive patients exhibited CSF LSP levels comparable to non-diseased patients.[257] Therefore, LSP levels in CSF appear to be a biomarker useful for the detection of brain malignancies and the assessment of treatment responsiveness.

A separate study examining total serum or plasma sialic acid and lipid bound sialic acid found that total sialic acid was significantly elevated in a variety of brain tumors, particularly in the microsomal fraction, and lipid bound sialic acid was found to significantly increased in the serum and tissue of patients with brain and thyroid tumors.[258] The measurement of serum sialic acid concentration has also been demonstrated as a valid method for the differentiation between benign and malignant intracranial tumors.[259] In a study examining the efficiency of biochemical tumor markers, patients with non-tumorous disease of the central nervous system, that coincide with brain tissue lesions, were described as having extremely high serum sialic acid levels, additionally while serum sialic acid levels in brain cancer patients were found to increase with increasing brain tumor malignancy the differences were not deemed statistically significant.[260] However, when the sialic acid levels in CSF were examined in patients with brain tumors, CSF sialic acid concentration was found to be significantly increased in patients with malignant and semi-malignant brain

tumors compared to CSF from healthy controls.[261] The authors concluded that measurement of CSF sialic acid concentration represents a valid method for the diagnosis and assessment of brain tumor malignancy.[261]

4.2 Glycans and Development of Brain Cancer Therapeutics

4.2.1 Neurotherapeutics and Brain Neoplasms—A handful number of therapeutic interventions have been developed and tested in animal models of brain tumors though less on human subjects. Nevertheless, the variety of approaches utilized are promising especially when combination therapy is utilized as described below. Some researchers have used pharmacological agents while others employed microRNAs, viral vector delivery systems and nanotechnology (Table 1).

Given Temozolomide's limited efficacy, which is used in chemotherapy for malignant gliomas, its combination with other agents might be beneficial.[262] When combined with doxorubicin, temozolomide notably affected glioma-bearing rat survival via modulating P-glycoprotein (P-gp) transport activity when compared to treatment with a single agent.[263] The importance of targeting P-gp lies in the fact that recently, de Gooijer *et al.* revealed that Palbociclib, an inhibitor of cyclin dependent kinase (CDK) 4/6, is a P-gp glycoprotein substrate restricting Palbociclib's access to the CNS.[264] Also, the brain tumor penetrance of two PI3K/mTOR kinase inhibitors, GDC-0980 and GNE-317, has been shown to be affected by the activity of P-gp.[265] Given the recent evidence that low-intensity ultrasound (LIUS) may promote glioma cell apoptosis, Zhang *et al.* identified the underlying mechanism for this outcome characterized by the downregulation of P-gp expression levels along with multidrug resistance protein 1 (MDR1) orchestrated by the PI3K/Akt/NF-kappaB pathway.[266] This finding suggests that LIUS can be utilized to enhance the sensitivity of glioma cells to therapy via downregulation of the P-gp glycoprotein.

Recently, patients with GBM were shown to exhibit good prognoses if elevated expression levels of CD40/CD40L were observed.[267] The study revealed that the administration of FGK45 alone, an agonistic antibody for CD40, or in combination with OX86, an agonistic antibody for OX40, enhanced survival in GL261 and NSCL61 mouse glioma models. From a different perspective, in GBM patients, γ9δ2 T cells were found to be decreased when compared to healthy donors.[268] Enriching these cells *in vitro* mediated cytotoxicity to GBM-derived cell lines along with decreased interferon (IFN) γ secretion, an effect that was abolished when γ9δ2 cells were removed.[268] Overall, this suggests that CD40/CD40L may be utilized as a favorable prognostic marker with the promise of using the therapeutic potential of FGK45 in clinical applications.[269] Friedmann-Morvinski *et al.* identified the *Spp1* gene (secreted phosphoprotein 1) which encodes for the osteopontin (OPN) protein, a RGD-containing glycophosphoprotein with properties of both a cytokine and a chemokine. In GBM-bearing mice, inhibition of OPN, using miROPN, ceased the formation of neurospheres (stem cell markers showed decreased expression), altered neuron proliferation and prolonged mouse survival.[270]

In the interest of targeting glycoproteins in neuro-oncology as a novel approach, it was reported that podoplanin (PDPN), a type I transmembrane mucin-like glycoprotein, is overexpressed in mesenchymal GBM.[271, 272] Using a lentiviral vector expressing a 3rd-

generation chimeric antigen receptor (CAR), as a targeted PDPN-specific antibody, it was shown that systemic injection of CAR-transduced T cells into immunodeficient mice limited the survival of intracranial glioma xenografts.[273] Also, LRIG1 (leucine-rich repeat and immunoglobulin domain-containing protein-1), a tumor suppressor gene, showed efficacy by acting as a negative regulator of the ErbB and Met receptors which are oncogenic tyrosine kinases receptors.[274] Similarly, comparison of athymic nude mice injected with *LRIG1*-transfected RC-4B/C cells to those injected with normal pituitary tumor RC-4B/C cells showed that *LRIG1*-transfected tumors displayed significantly diminished tumor growth. This was mediated via inhibiting Ras/Raf/ERK as well as PI3K/Akt signaling pathways.[275] Gruslova *et al.* developed a novel adenoviral gene therapy characterized by VB-111, a non-replicating Adenovirus 5 with a pro-apoptotic Fas-chimera A, targeting the tumor vasculature.[276] Bioluminescence and MRI of U87MG-luc2-bearing rats showed that 4-week treatment with VB-111 significantly inhibited tumor growth, indicating its potential in the treatment of highly vascularized solid GBM tumors.

Along the same line, decorin targeting was assessed as a potential target. Decorin, a proteoglycan that mediates its anti-cancer activity using EGFR, transforming growth factor-beta (TGF-beta), and p21 was also investigated in brain tumors.[277] Using decorin delivered into a mouse xenograft U87MG glioma tumor via an adeno-associated viral gene delivery system, cell growth was found to be inhibited with decorin by enhancing cellular differentiation and prolonging mouse survival.[278] Additionally, employing a mass spectrometry-based approach was shown to help in identifying proteins involved in apoptosis, proliferation, resistance to chemotherapy, and fatty acid metabolism mediated by decorin overexpression.[278, 279]

On the level of epigenetics, the inhibition of histone deacetylase 6 (HDAC6i), either pharmacologically by valproic acid or using oncolytic engineered herpes simplex virus (oHSV) increased nucleus-targeted trafficking of post-entry oHSV.[280] Nakashima *et al.* also observed improved survival in GSC-bearing mice treated with both oHSV and HDAC6i in comparison to oHSV treatment alone. Friedman *et al.* showed that pediatric medulloblastoma is an excellent target for oHSV virotherapy via viral adhesion to nectin-1 (CD111).[281] The combinatorial treatment of HDACi such as the use of MS275, Valproic acid and Delta24-RGD, a genetically modified serotype-5 adenovirus, enhanced the anticancer activity in GBM stem-like cells.[282]

4.2.2 Glycosylation, Nanotechnology and Drug Delivery—On a different front, overcoming the tight constraints of the blood brain barrier (BBB) and the rapid removal of metabolites, drugs, and proteins from circulation constitute two major obstacles for CNS drug delivery. For this reason, nanotechnology has become instrumental for such treatment. For instance, Fang *et al.* developed a novel lactoferrin-based delivery system of dual-targeting magnetic polydiacetylene nanocarriers (PDNCs), which is found on the cell surface of GBMs.[283, 284] Fang *et al.* showed an enhanced trans-BBB activity of the PDNCs along with improved retention time of traced lactoferrin. Furthermore, administrations of etoposide, which targets VEGF secretion, using cross-linked Lf and folic acid (FA) on poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs), across the BBB revealed a two-fold increase in permeability.[285] Further, Lf/FA/PLGA NPs were superior to FA/PLGA NPs in

antiproliferative efficacy against U87MG cells, a human primary GBM cell line. In terms of diffusion capacity, solid lipid nanoparticles (SLN) may diffuse through the BBB due to their lipophilic nature. In addition, lactoferrin conjugated on the surface of SLN (C-SLN) increased the targeting potential for brain neoplasms and cytotoxicity.[286]

Wang *et al.* developed a PEGylated, RGD peptide modified, and disulfide cross-linked short polyethyleneimines (DSPEIs) functionalized gold nanorod (RDG) for shRNA delivery.[287] Stable nanoparticles of shRNAs were formed by the RDG; thus, it is marked to defined human brain cancers (U-87 MG-GFP) via integrin-mediated endocytosis in U-87 MG-GFP tumor bearing BALB/c mice. Also, the high stability and long blood bioavailability, imparted by PEGylation, suggests a promising non-viral vector approach for effective tumor targeting and intra-tumor gene silencing.[287] Interestingly, it has been shown that the administration of an ultra-small hyaluronic acid (HA) paclitaxel nanoconjugate leads to better survival levels, assessed in preclinical brain-breast metastases of cancer model, compared to controls.[288] The passive trans-BBB diffusion of ultra-small molecules provides a sound option to optimize chemotherapeutic drug efficacy.

Given the anti-mitogenic impacts of vitamin D3 (VD3) on C6 glioma tumor cells,[289] in an attempt to utilize the resistance-reducing capacity of combinatorial therapy, Maleklou *et al.* used VD3-loaded nanoparticles (VD3NPs) paradoxically revealing a significant increase in drug resistance once exogenous VD3NP-doxorubicin and VD3NP-epirobicin combinations were administered to C6 glioma cells.[290] Taken together, in light of the roles glycoproteins play in brain cancers, it is of high importance to initiate the development of brain tumor therapies with glycobiology in mind. In particular, in the case of GBM, one of the most clinically devastating cancers, it is of the utmost necessity to treat CNS tumors utilizing drug delivery systems and neurotherapeutics, such as those that have been described herein. Moving forward, indubitably, further experimental studies and clinical interventions are needed to validate and implement the abovementioned interventions on human subjects.

4.2.3 Cancer Glycoproteomics and Personalized Medicine—Within the scope of recent advances in therapy against CNS cancers, personalized medicine has emerged as a concept and practice aimed at selecting and optimizing therapies based on cellular or genetic analyses. Glycoproteomics paved the way for employing a particular therapy on a subset of patients carrying a unique glycomic signature.[291, 292] For instance, neo antigens can be identified via the addition of several of these glycosylation modifications; for example tumor-associated carbohydrate antigens (TACA), such as the dietary sialic acid Neu5Gc, which inserts on cell surfaces producing an immunogenic neo-TACA.[293, 294] Additionally, it should be noted that several FDA-approved assays are used for the detection of cancer antigens; an excellent example is provided by the Truquant BR radioimmunoassay, which uses the monoclonal antibody B27.29 to quantify the MUC1 gene product CA 27.29.[295]

Recently, Thanabalasingham *et al.* identified a markedly lower glycan index in hepatocyte nuclear factor 1-α (HNF1A)-maturity-onset diabetes of the young than in controls or other diabetes subtypes.[296] Mucins, carriers of cancer-associated O-glycans, showed a significant increase in expression in a variety of malignancies such as esophageal, colon, breast, gastric, bladder, pancreatic and ovarian cancers.[297] Furthermore, in prostate cancer, α2,3 NeuAc

levels were significantly increased whereas seminal fluid free PSA had more sialylation and core fucosylation.[298] These findings make N- and O-glycans potential vaccination targets against the particular overexpressed glycans identified in certain individuals; thereby, highlighting the importance of glycosylation in personalized medicine. Furthermore, alterations in PTMs also occur at the level of the glycosyltransferases/glycosidases enzymes mediating the generated TACAs, such as UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase (ppGalNAc-T), alpha6-sialyl-transferase-I (ST6GalNAc-I), N-acetylgalactosaminyltransferase 6 (GALNT6), UDP-N-acetyl-D-glucosamine: N-acetylglucosamine transferase V (GlcNAcT-V) and α2-3 sialyltransferase I (ST3Gal-I).[299–303] Specifically for alpha-fetoprotein (AFP-L3), increased fucosylation of AFP has been found to be due to the increased expression of α1-6 fucosyltransferase in hepatoma tissues[304]

Finally, given this brief overview of the pathological and regulatory roles of glycoprotein PTMs and the fact that unique cancer-associated-glycan profiles reflect the underlying genetic disruptions that are translated into distinctive glycosylation patterns, the novel development of methodologies that help in differentiating physiologic versus aberrant glycosylation patterns observed in cancer can be considered a promising strategy for shaping treatment and therapy. Personalized theranostics have the potential to be instrumental in the areas of vaccination, therapy, and diagnosis.

5. Concluding Remarks

In conclusion, alterations in glycoproteins represent a hallmark of cancer development and offer biomarker targets due to the presence of differential carbohydrate modifications. This, coupled with their heterogeneity and complexity of expression as well as their involvement in regulatory pathways render them as key markers worthy of investigation. The high expression of glycoproteins both in bodily fluids, such as serum and CSF, as well as on cell surfaces is a source of encouragement for researchers aiming to assess their roles in cancer-related and other pathological conditions.[305]

Acknowledgments

This work was supported by NIH grant (1R01GM112490-03) and CPRIT (RP130624).

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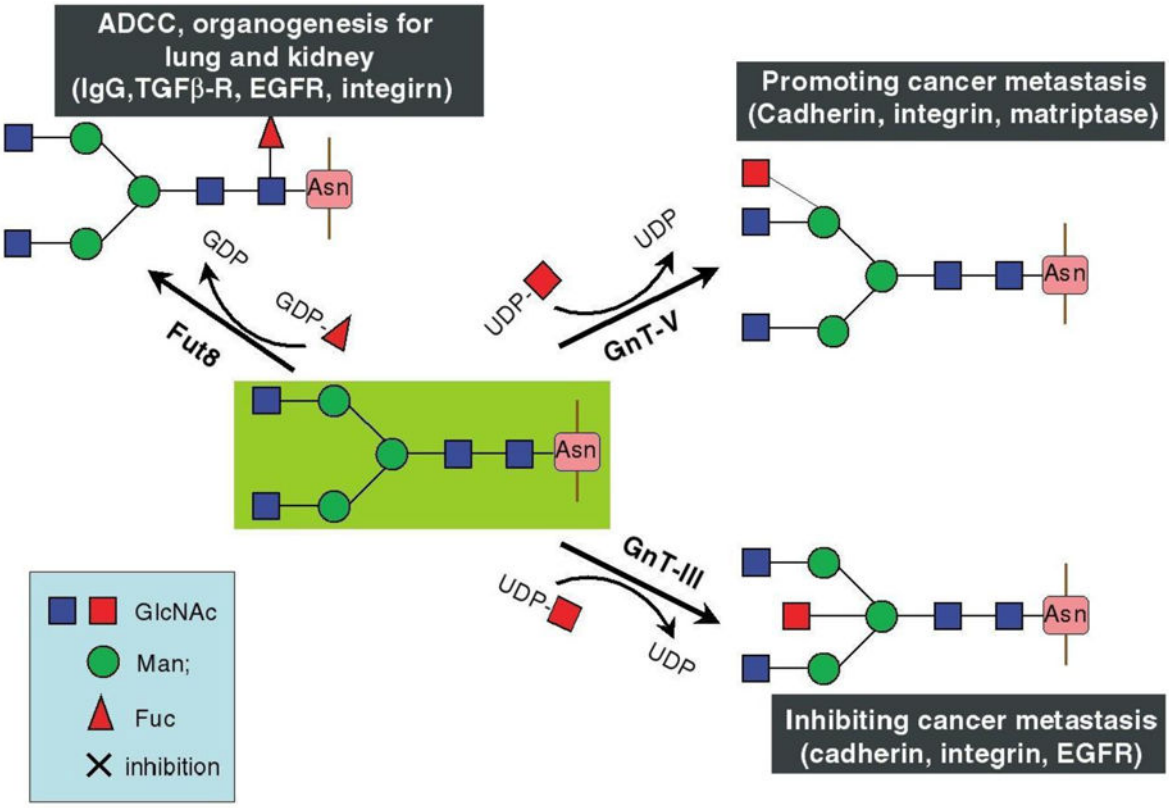

Figure 1.

Glycosylation reactions catalyzed by the glycosyltransferases GnT-III and GnT-V, as well as by Fut8, and their biological functions. Reprinted from[65], with permission.

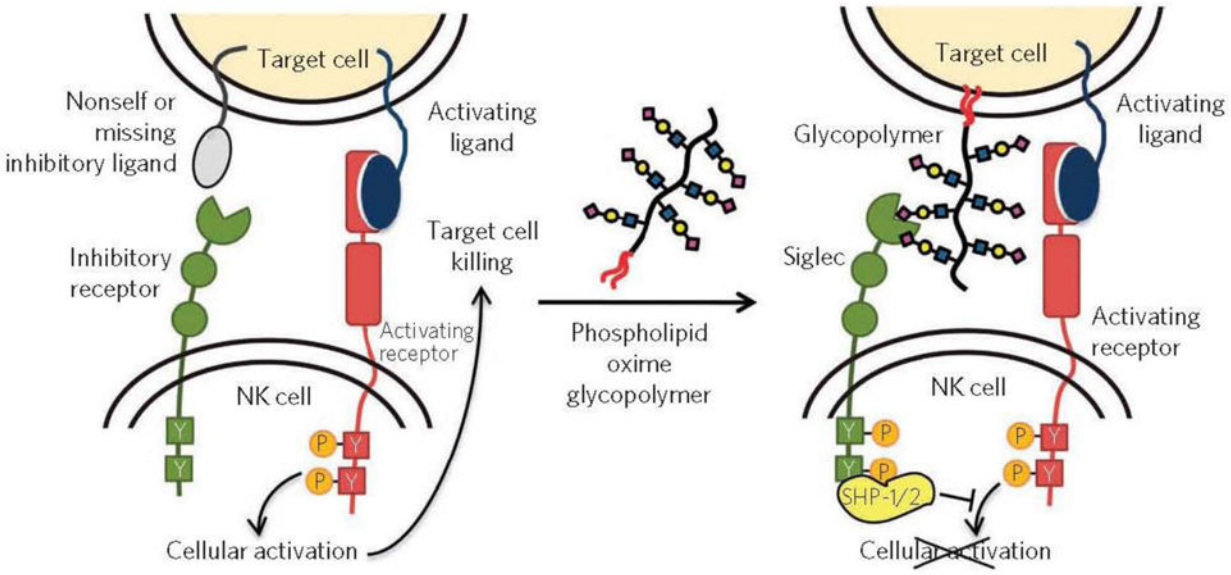

Figure 2.

In the presence of activating ligands and absence of inhibitory ligands on the target cell, NK cells are activated to release cytotoxic effectors and cytokines. Coating cancer cells with sialylated glycopolymers by membrane insertion can emulate cancer associated glycosylation changes that engage the Siglec family of inhibitory receptors. Localization of Siglecs to the site of activation enhances SHP-1/2 phosphatase recruitment to halt the phosphorylation cascade before cellular activation. Reprinted from[109], with permission.

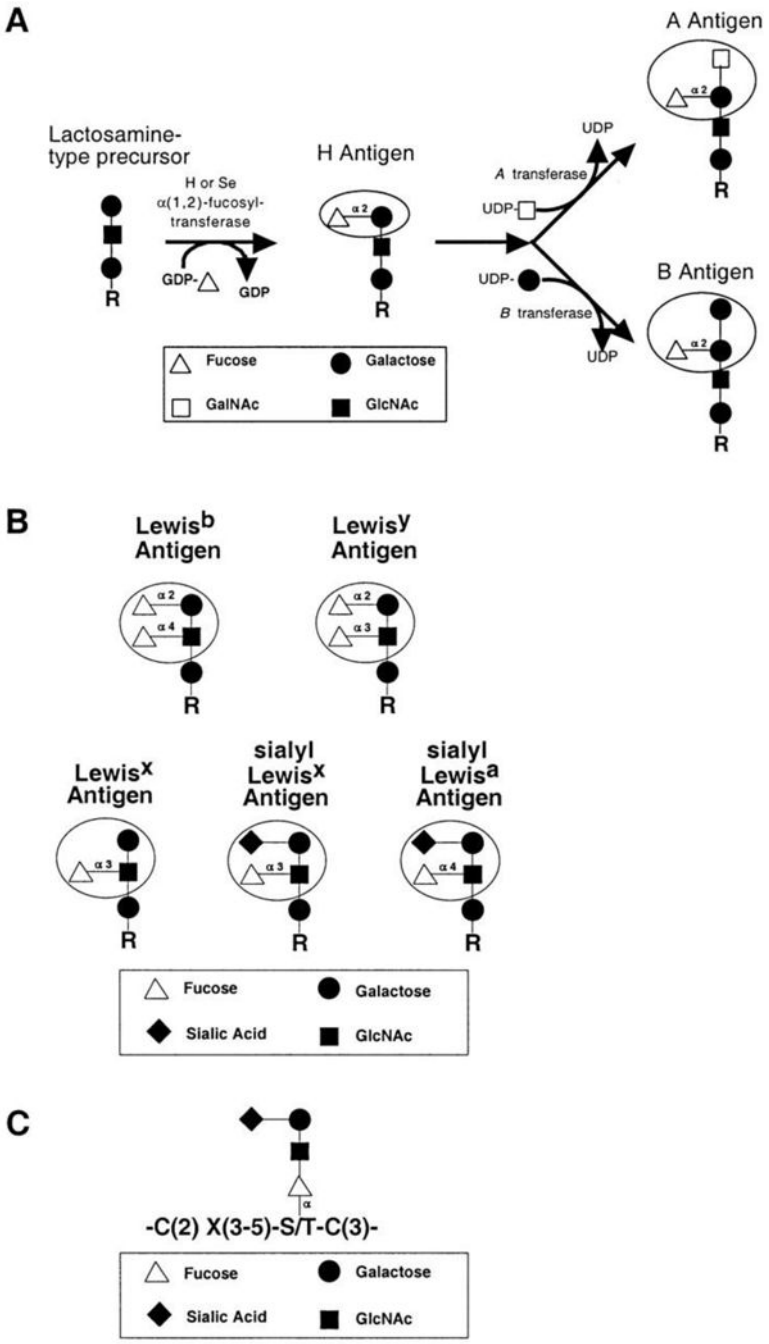

Figure 3.

Structures of common fucosylated glycans. (**A**) Synthesis of ABO blood group antigens. The H and Se transferases are a pair of α(1,2)-fucosyltransferases that synthesize the H antigen in a variety of tissues. The *ABO* locus encodes a glycosyltransferase that further modifies the H antigen. The *A* allele at the *ABO* locus encodes an N-acteylgalactosaminyltransferase. The *B* allele encodes a galactosyltransferase that differs from the A transferase at four amino acid positions. The *O* allele at the *ABO* locus encodes a truncated, enzymatically inactive protein. (**B**) Lewis-related antigens. Circles indicate the

immunodominant portion of each antigen. (**C**) A representative O-linked fucose glycan. Fucose modifies serines or threonine within the broad consensus site shown here, and in Table I. R indicates glycolipid and N- and O-linked glycoprotein precursors. Reprinted from[137], with permission.

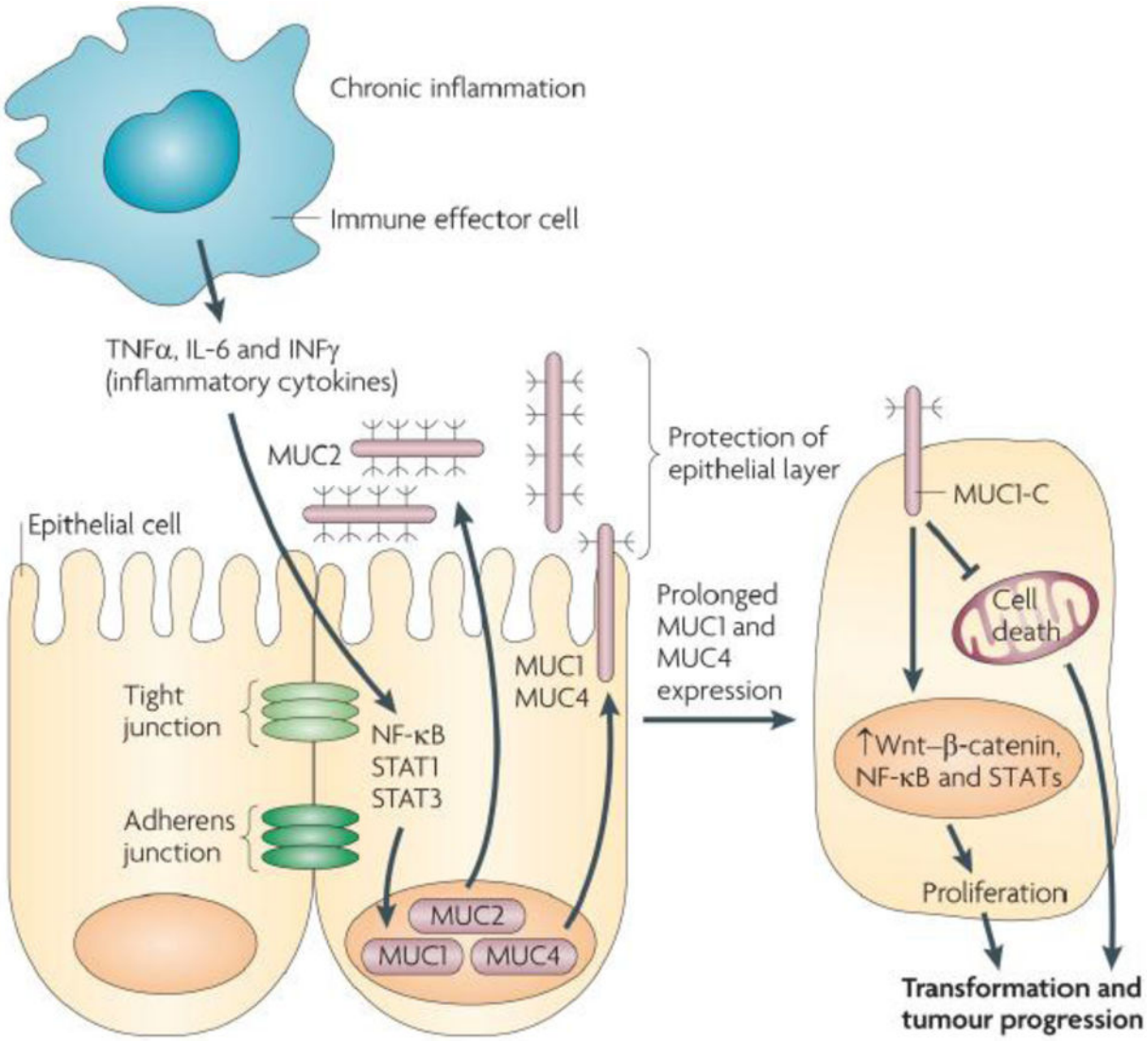

Figure 4.

Mucins, chronic inflammation and cancer. In this proposed model of the association of mucins with chronic inflammation and cancer, the production of inflammatory cytokines by immune effector cells activates transcription factors, for example nuclear factor-κB (NF-κB), signal transducer and activator of transcription 1 (STAT1) and STAT3, in epithelial cells. In turn, these transcription factors upregulate mucin expression to enhance the mucous barrier and protect the epithelial layer. Mucin 2 (MUC2) limits the inflammatory response at the apical membrane and inhibits transformation. Upregulation of the MUC1 and MUC4 transmembrane mucins similarly contributes to the protective barrier and loss of polarity in the epithelial stress response. Activation of MUC1 is associated with targeting of the MUC1 C-terminal transmembrane subunit (MUC1-C) to the nucleus, where it promotes a gene programme for proliferation and survival. Targeting of MUC1-C to the mitochondria also blocks cell death to prevent loss of the epithelial barrier. However, with chronic inflammation and prolonged stimulation of this protective response, epithelial cells may

become susceptible to the accumulation of genetic mutations that induce transformation in a setting with downregulation of pathways that would otherwise protect against oncogenic events. IL-6, interleukin-6; IFNγ, interferon-γ; TNFα, tumour necrosis factor-α. Reprinted from[148], with permission.

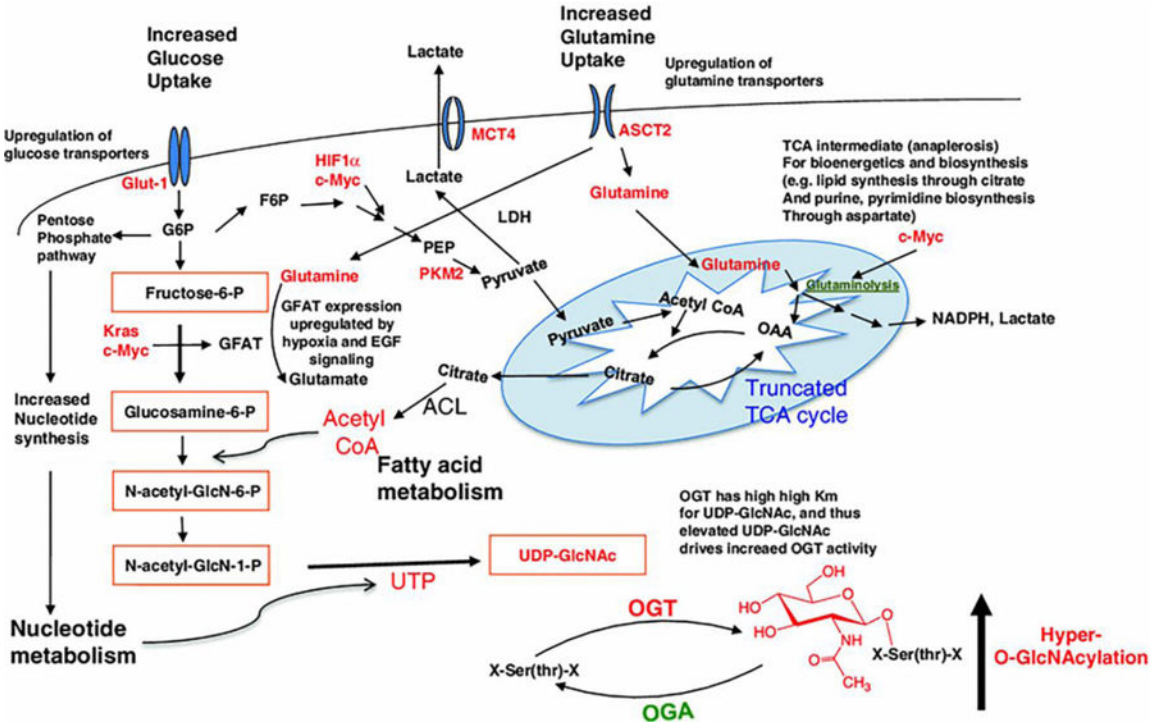

Figure 5.

Cancer cell metabolic changes linked to hyper-*O*-GlcNAcylation. The hexosamine biosynthetic pathway (HBP) outlined in *orange boxes* integrates metabolic intermediates to generate the end-product UDP-GlcNAc. Glucose is transported into cells by glucose transporters such as Glut1 and then first phosphorylated by hexokinase to generate glucose-6-phosphate. Glucose-6-phosphate can be shunted into the PPP which produces nucleotides and NAPDH, or converted into fructose-6-phosphate. While most fructose-6-phosphate continues through glycolysis to produce pyruvate, some is directed into the HBP. GFAT, the HBP rate-limiting enzyme, irreversibly transfers the amino group from glutamine to fructose-6-phosphate, generating glucosamine-6-phosphate and glutamate. Glucosamine-6-phosphate is ultimately converted to UDP-GlcNAc, which is used by OGT to attach *O*-GlcNAc to hydroxyl groups of serine and/or threonine residues of cytosolic and nuclear proteins. *O*-GlcNAc is removed by OGA. Cancer cell metabolic changes including increased glucose uptake (due to “Warburg effect”) and increased glutamine uptake (along with elevated UTP and acetyl-CoA production) cooperate to maximize flux through the HBP. Oncogenes such as *HIF1α*, *Kras*, and *c-Myc* regulate cancer cell shifts to aerobic glycolysis and glutaminolysis, including upregulation of glucose and glutamine transporters and increased expression of GFAT. Additionally, the level of OGT is increased and the level of OGA is decreased. In sum, cancer cell metabolic reprogramming leads to increased HBP flux, elevated UDP-GlcNAc, and ultimately hyper-*O*-GlcNAcylation. Proteins and metabolic intermediates in *red* are increased in cancer cells. G6P: Glucose-6-phosphate; F6P: fructose-6-phosphate; *FBP* fructose 1,6-bisphosphate, *PEP* phosphoenolpyruvate, *GFAT* glutamine: fructose-6-phosphate amidotransferase, *MCT4* monocarboxylate transporter,

OAA oxaloacetate, *PFK1* phosphofructokinase 1, *PKM2* pyruvate kinase M2. Reprinted from[161], with permission.

Table 1

Therapeutic interventions in brain neoplasms and the observed outcomes.

Therapy	Target	Outcome	+/−	Model	Ref.
Temozolomide + doxorubicin	P-gp	Increased survival	+	Rats	263
Palbociclib	CDK4/6	Activity limited by P-gp	−	Mice	264
LIUS	P-gp and MDR1	Apoptosis and CNS delivery	+	Rats and C6 cells	266
FGK45	CD40	Increased survival Decreased proliferation	+	Mice	267
γ9δ2 T cells	IFN γ	Increased cytotoxicity	+	GBM cells	268
miROPN	OPN	Less stem cell markers Less proliferation Increased survival	+	Mice	270
CAR-transduced T cells	PDPN	Inhibition of intracranial glioma xenografts growth	+	Mice	273
LRIG1	Oncogenic receptor tyrosine kinases	Slowing of tumor growth. Apoptosis	+	Mice	275
VB-111	Endothelial cells	Decreased tumor vasculature and growth	+	Rats	276
Decorin	EGFR, TGF- beta and p21	Cellular differentiation Survival	+	Mice	278
HDAC6i	HDAC6	Increased survival	+	Mice	280
oHSV	nectin-1 (CD111)	Increased cytotoxicity	+	Pediatric patients	281
HDACi + Delta24-RGD	HDAC and p16INK4/Rb pathway	Apoptosis and necrosis	+	Patient-derived GSCs	282
PDNCs	CNS delivery	Increased trans-BBB delivery and CNS retention time	+	Rats	284
Lf/FA/PLGA NPs	VEGF secretion	Less proliferation	+	U87MG cells	285
C-SLN	CNS delivery	Improved delivery and cytotoxicity	+	Mice	286
RDG	CNS delivery	Increased delivery and CNS retention	+	Mice	287
HA-paclitaxel nanoconjugate	Microtubules	Survival	+	Mice	288
VD3NPs combined with doxorubicin or epirobicin	Sphingomyelin pathway	Increase in drug resistance	−	C6 glioma cells	290

+ indicates a favorable outcome.

−indicates an unfavorable outcome.