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Glycokinomics: Emerging Therapeutic Approaches for Malignant Brain Tumors

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Abstract

The oligosaccharide chains, or glycans, that decorate cell surface glycoproteins and glycolipids are among the most complex and diverse structures in vertebrate cells. It is estimated the well over half of all human proteins are glycosylated. Their expression is exquisitely regulated and is the result of the coordinated activity of distinct glycosyltransferases and glycosyl hydrolases that add or remove individual sugars to complete each glycan chain. Aberrantly expressed cell surface glycoconjugates are associated with malignant transformation, tumor progression, and metastasis and are predominantly the result of alterations in their biosynthetic machinery. They mediate key pathophysiological events during tumorigenesis including altered cellular adhesion and invasivity, molecular trafficking, receptor activation, and intracellular signal transduction in tumors.

Keywords: Glycoconjugate; Glycosyltransferases; Glyco-transcriptome; GalNAc; Galectins

Introduction

This review focuses on the linkage between glycoconjugate structure/function and intracellular protein kinase activity modulation. Tumor-associated, aberrant glycosylation of cell surface glycoconjugates has significant impact on intracellular phosphorylation cascades. Some recent studies have shown that altering specific glycosyltransferase gene expression in malignant human glioblastoma models not only suppresses tumor growth in vivo, but alters the activities of specific kinases as well [1,2]. These data suggest that glycoconjugate-mediated protein kinase/phosphatase activity modulation may help explain how altered glycogene expression can lead to enhanced metastasis and invasivity and provide a new approach for the creation of effective anti-cancer therapeutics.

N- And O-Glycan-Mediated Signaling

N-linked glycans (asparagine-linked glycans) are the major post-translational constituents of glycoproteins in eukaryotes. Their biosynthesis takes place largely in the endoplasmic reticulum and Golgi apparatus where the potential to synthesize a large diversity of structures from high-mannose N-glycans into a large repertoire of hybrid and complex N-glycan subtypes that are secreted or positioned at the vertebrate cell surface. Since the cloning and sequencing of the entire glyco-transcriptome has been completed, studies on the manipulation of genes that regulate the N-glycan diversification pathways aimed at characterizing their physiological function have been very productive over the past several decades. A number of glycoprotein N-glycan structures, particularly growth factor receptors and adhesion molecules such as integrins, galectins, selectins, and cadherins, appear to contribute to folding, stability, and biological function of the proteins.

The modification of serine or threonine residues on proteins by addition of a GalNAc residue results in an O-linked oligosaccharide or O-glycan. O-glycan biosynthesis is simpler than asparagine (N)-linked oligosaccharide generation in that a lipid-linked oligosaccharide precursor for transfer to protein is not required. The initiating event is the addition of the monosaccharide GalNAc (from UDP-GalNAc) to serine and threonine residues catalyzed by a polypeptide GalNActransferase (GalNAcT). O-glycans are commonly biantennary structures, less branched than most N-glycans(Figure 1). The frequency of O-glycosylation varies and, on certain tumor cells, O-glycans may be as abundant as N-glycans. O-glycosylation plays a key role in the control of cell differentiation and growth through both proliferative and apoptotic pathways.

Growth factor receptor signaling

The remodeling of cell surface growth factor receptors through modification of their oligosaccharide structures is associated with the functions and biological behavior of most tumor cells, including highly malignant glioblastomas. For example, theepidermal growth factor receptor (EGFR) is highly glycosylated and EGFR amplification is among the most prevalent molecular event during gliomagenesis and development. As such, modulation of EGFR function by altered glycan structures has been extensively characterized over the past several decades by a number of laboratories.

Core fucosylation of EGFRs, mediated by α1, 6 fucosyltransferase 8 (FUT8), has been shown to contribute to tumor malignancy and to their invasive and metastatic potential. FUT8 catalyzes the transfer of fucose from GDP-fucose to N-linked type complex glycopeptides and is distinct from other, more common fucosyltransferases which catalyze α1, 2, α1, 3, and α1, 4 fucose additions. Loss of core fucosylation results in downregulation of EGFR-mediated signaling pathways, primarily involving decreased phospho-ERK and phospho-JNK [3]. In addition, core fucosylation by FUT8 also regulates the high affinity binding of EGFR, which is both required and sufficient for EGF-induced responses [3]. EGFR is also decorated with N-glycans synthesized by β1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase 3 (GnT-III) in many tumor types, and these alterations dramatically influence tumor progression [4]. In addition, forced overexpression of GnT-III, a glycosyltransferase that

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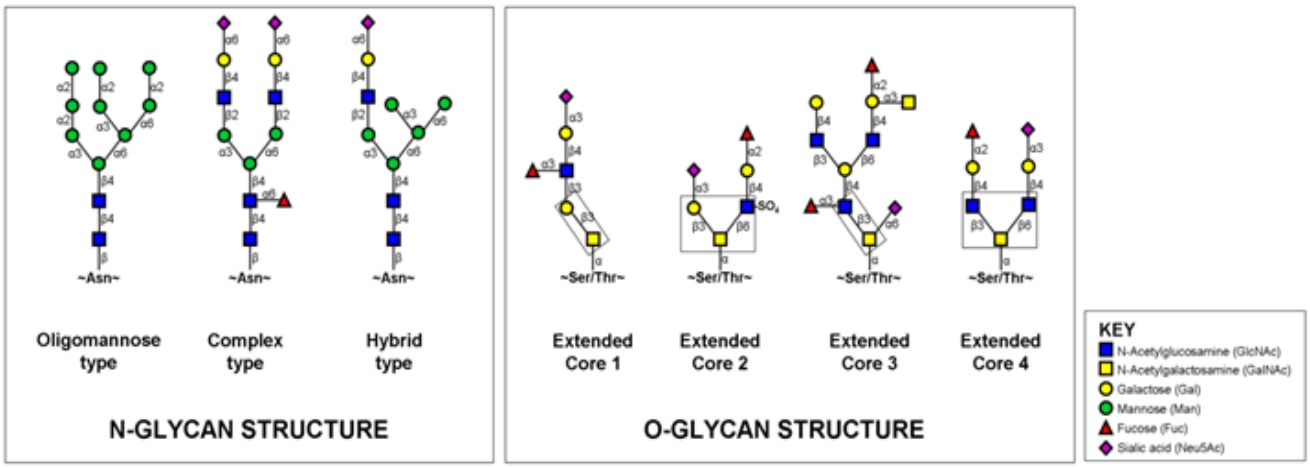

Figure 1: N-Glycan structure and O-Glycan structure.

plays a major role in the biosynthesis of hybrid and complex types of N-linked oligosaccharides [5], significantly reduces the ability of EGF to bind to its receptor, reduces EGFR autophosphorylation, and subsequently blocks EGFR-mediated Erk phosphorylation in U373MG glioma cells [6] and in PC12 cells [7].

Glycan structures on other growth factor receptors also influence intracellular signaling cascades. In addition to targeting the EGFR, GnT-III-mediated glycan changes have also been described on the PDGF receptor, similarly influencing tumor progression[8]. Nerve growth factor binds to its receptor, TrkA, on the surface of PC12 cells, resulting in TrkA receptor dimerization and phosphorylation [9]. TrkA-mediated neurite outgrowth and its tyrosine phosphorylation are blocked as the result of the transfection of GnT-III into PC12 cells, suggesting that bisecting structures also participate in the regulation of TrkA functions [10].

Fibroblast growth factor receptor (FGFR) forms a ternary complex at the cell surface with its ligand, FGF, and heparan sulfate proteoglycan which in turn leads to activation and phosphorylation of the receptor tyrosine kinase that triggers various intracellular signaling cascades, including the MAPK pathway [11-14].Lastly, the N-glycan β1,6GlcNAc branching associated with GnT-V activity can promote the cell motility through specifically triggering Rho family signaling. Upregulation of Rac1 but downregulation of RhoA, together with dephosphorylation of cofilin play the indispensable roles for GnT-V- and β1,6GlcNAc-driven cell motility and phenotypic changes [15].

Integrin-mediated Signaling

The effects of cellular adhesion to the extracellular matrix (ECM) are primarily mediated by integrins, a family of heterodimeric cell surface receptors that bind to distinct, although partially overlapping, subsets of ECM proteins. The resultant mechanical and chemical signals regulate the activities of cytoplasmic kinases, growth factor receptors, and ion channels and control the organization of the intracellular actin cytoskeleton. These signals also control the action of receptor tyrosine kinases (RTKs), determining whether cells proliferate and migrate in response to soluble growth factors and cytokines. Most integrins activate focal adhesion kinase (FAK) and Src Family Kinases (SFKs), causing phosphorylation and signaling from p130-CAS and paxillin. A subset of integrins, α1β1, α5β1, and αvβ3, also activate the adaptor protein Shc.

Alterations in integrin glycosylation profoundly affect their capacity to transduce intracellular signals. Integrin engagement during normal cell adhesion leads to intracellular phosphorylation, primarily phosphorylation of focal adhesion kinase (FAK), and normal regulation of gene expression, cell growth, cell differentiation and survival from apoptosis [16]. Forced overexpression of GnT-III in human gliomas inhibited α5β1 integrin-mediated cell spreading and migration, and phosphorylation of FAK [17]. In addition, increased expression of the glycosyltransferase β1,3-N-acetylglucosaminyltransferase 6 (core3-synthase) increased core3 structure on α2β1 integrin, leading to decreased tumorigenesis by attenuating the maturation, heterodimerization, and phosphorylation of focal adhesion kinase (FAK) [18]. Expression of the normally quiescent, α2,6sialyltransferase ST6Gal1, in gliomas was shown to lead to the replacement of terminal α2,3-linked sialic acids on the β1 subunit of the a3b1 integrin with α2,6-linked sialic acids. This in turn led to inhibition of adhesion-mediated tyrosine phosphorylation of FAK, modification of actin cytoskeletal dynamics, and marked decreases in invasivity and tumorigenesis both in vitro and in vivo models [1,2,19,20]. Thus, alterations in signaling thru FAK and its downstream effectors appears to be an overarching theme and implies that indirect modulation of downstream kinase(s) by altering glycosyltransferase gene expression, rather than direct kinase activity modulation, may be a key to how differential glycogene expression is related to alterations in tumor cell metastasis, invasivity and growth control.

Cell adhesion molecule (cam)-mediated signaling

The galectins, selectins, and cadherins are families of lectins that have been strongly implicated in many cancers [21,22]. Although they are typically not themselves glycosylated, they play pivotal roles in the recognition of cell surface glycoconjugates and activation of intracellular signals.

The galectins are defined by shared sequence elements and by affinity for β-galactosides. Galectins are intimately involved in the modulation of the cell cycle, apoptosis, tissue invasion, metastasis, angiogenesis and tumor immune surveillance. Galectin-3 has been proposed to enhance tumor growth by being antiapoptotic, proangiogenic, and to promote metastasis by mediating effects on cellular adhesion. Binding of galectin-3to branched N-glycan

ligands modulate focal adhesion remodeling through FAK and phosphoinositide 3-kinase (PI3K) activation, local F-actin instability, and α5β1 integrin translocation to fibrillar adhesions [23]. The overexpression of galectins has been correlated with the aggressiveness of a large number of tumors and therapeutic approaches targeting their functional inactivation have shown promise [24].

Selectins are also a family of cell adhesion molecules (CAMs). All selectins are single-chain transmembrane glycoproteins that themselves bind other sialic acid rich glycoproteins. The vast majority of highly invasive or metastatic tumors express significantly increased cell surface sialoglycoproteins although to date there have been few selectin-directed therapeutic approaches reported [25].

Cadherins mediate cell adhesion and migration and play a fundamental role in normal development in that they participate in the maintenance of proper cell-cell contacts. Cadherins are extensively modified post-translationally via both glycosylation and phosphorylation. Oligosaccharide remodeling via many diverse approaches regulates E-cadherin function [26,27].On the cell surface, cadherins tend to be concentrated at cell-cell junctions and proximally associated with actin bundles. The cytoplasmic domains of the cadherins are associated with cytoplasmic proteins termed catenins. Deletion of the cytoplasmic domain destroys these interactions and also eliminates cadherin function. Selective changes in N-glycosylation also directly affect the tyrosine phosphorylation of β-catenin. For example, forced overexpression of GnT-III leads to suppression of tyrosine phosphorylation of β-catenin after EGF stimulation in stable GnT-III transfectants[28]. Recently src, yes, and lyn kinases have also been found co-expressed with cadherin at cell-cell junctions and it has been proposed that these kinases may be responsible for cadherin phosphorylation used for inter- and intracellular signaling [29,30].

In neuroblastomas and rhabdomyosarcomas, the addition of polysialic acid (PSA) to CAMs, predominantly neural cell adhesion molecule (NCAM), is mediated by the differential expression of the polysialyltransferaseST8SiaII, ST8SiaIV and ST8SiaV [31] and has been shown to be a positive modulator of tumor malignancy [32-34]. NCAM is highly concentrated at cell-cell contact sites, and the number of NCAM-positive cell-cell contacts has been shown to increase following PSA removal [35]. Moreover, previous studies have demonstrated that PSA affects NCAM-dependent signaling, is involved with regulation of tumor cell proliferation, survival, and differentiation, and that these effects are mediated via the direct involvement of the p44/p42 MAPK ERK1/2 pathways [36-38].

Modulation of Additional Signaling Pathways

The role of the AKT and MAPK pathways in gliomas have been a primary focus for decades and large therapeutic development programs focusing on small molecule inhibitors of key members of these pathways have been established. Modulation of the kinase activities in these pathways for therapeutic gain may also be achievable via glycobiology-based approaches. For example, β1,4GalTV functions as a positive growth regulator in gliomas via activation of AKT and MAPK pathways [39], both of which are important for facilitating tumor cell proliferation, inhibiting apoptosis, and maintenance of the tumor phenotype [40-42]. Secondly, FUT4 overexpression promotes cell proliferation through crosstalk of the MAPK and PI3K/Akt signaling pathways increased S-phase via augmenting cyclins and CDKs, specifically by decreasing cyclin-dependent kinase inhibitor 1 (p21) and $p27^{kip1}$, and increasing pRb. Interestingly FUT4 also directly activates ERK1/2, p38 MAPK, and AKT. Lastly, the reduction in the expression of GalTV leads to a reduction of the levels of phospho-AKT (ser473/thr308) and phospho-JNK1/2 (thr183/tyr185). Clearly, although complex, there are multiple ways in which crosstalk between major glyco- and phospho-mediated signaling pathways can take place.

Ganglioside-Mediated Signaling

Gangliosides are sialic acid-bearing glycosphingolipids that are an important component of the cell surface glycoconjugates expressed on all vertebrate cells Figure 2, [43]. They influence tumor growth and progression through modulation of adhesion, migration, and angiogenesis, and their expression is markedly altered in a variety tumors. Gangliosides are important transducers of cell signal transduction events due to both direct and indirect interactions with growth factor receptor tyrosine kinases (GFRTKs), membrane-associated or cytosolic protein kinases, and membrane microdomain-associated protein kinases.

Aberrantly expressed cell surface gangliosides directly impact intracellular signaling by affecting intracellular localization of integrins, src, and caveolin into or out of glycolipid-enriched microdomains[44]. The association of GD2 with the integrin/FAK macromolecular complex has also been demonstrated. Ganglioside alterations in epithelial cells leading to changes in (i) adhesion to specific extracellular matrix components, (ii) relative rates of cellular proliferation and apoptosis, (iii) protease activation and function, and (iv) disruption of cell surface integrin:growth factor receptor associations have also been described [45]. The direct binding of GT1b to α5β1 integrin directly leads to increased apoptosis via decreased activity of the integrin-linked kinase/protein kinase B/AKT pathway [46]. Although it remains to be determined how glycosphingolipid modulation affects gliomainvasivity and tumor metastasis to the brain, it is clear that aberrant signal transduction plays a pivotal role.

Ganglioside-dependent modulation of several well-known cytosolic protein kinase activities have also been described, identified primarily in cell-free systems [47]. Gangliosides suppress phospholipid and Ca+2-dependent activity of protein kinase C (PKC) [48]. Contrary to this, PKC is activated by GM3 along with phorbol ester as a substitute for the phospholipids [49]. Cyclic-AMP dependant protein kinase A (PKA) activity is stimulated by gangliosides[50], while cAMP-independent activity of catalytic subunit of PKA is suppressed by gangliosides[51]. CaMKII activity itself is modulated by gangliosides[52]; in the absence of Ca+2/calmodulin, gangliosides activate CaMKII, while higher concentrations of gangliosides prevent its activation. The mechanisms of this complex regulation of CaMKII activity by gangliosides are due to direct interactions between the gangliosides and regulatory domains of the kinase and between the gangliosides and Ca+2/calmodulin[53,54].

TrkA is the high-affinity tyrosine kinase-type receptor for nerve growth factor (NGF). TrkA activity is enhanced by GM1 ganglioside, again by direct interaction of the ganglioside and the receptor [55]. Conversely, the activity of other growth factor receptors including the epidermal growth factor receptor (EGFR) is unaffected by modulation of GM1. EGFR activity is, however enhanced by GM3 and GD1a, also by the direct interaction of the extracellular domain of the receptor and the gangliosides.

The ganglioside GM3, the first ganglioside in the step-wise biosynthesis of the ganglioside series of glycosphingolipids, has been among the most studied. The PTEN gene is a tumor suppressor gene frequently mutated in glioblastoma[56]. GM3 induces a marked

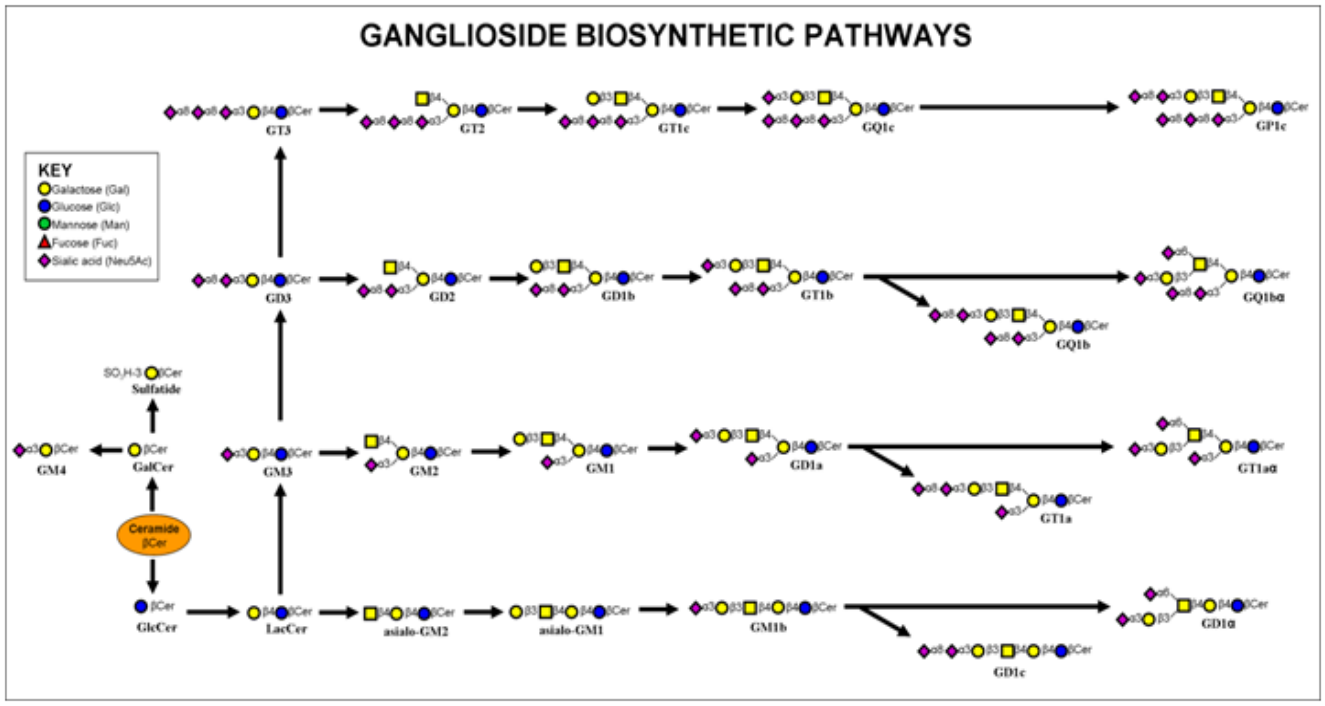

Figure 2: Ganglioside biosynthetic pathways.

expression of PTEN which, in turn, blocks PI-3K/AKT survival signaling. PTEN expression stimulated by ganglioside GM3 sustains the function of p53 as a transcriptional factor by inhibiting of MDM2 activity through the inactivation of PI-3K/AKT signal pathway [57]. Localized in the membrane, GM3 is known to interact with other transmembrane proteins such as the motility-regulatory protein (CD9) and EGF receptor (EGFR) to form a complex, which facilitates cell adhesion, cell motility, and cell signaling [58-60]. Endogenous depletion of the GM3 content by sialidase gene transfection in A431 cells results in increased EGFR autophosphorylation and activity [61]. Conversely, direct exogenous administration of GM3 results in inhibition of EGFR function [62], likely due to inhibitory effects on receptor autophosphorylation. It has been demonstrated in a number of tumors that GM3 exerts its effect on EGFR though the activation of a tyrosine phosphatase [63].

Disialogangliosides, including GD1a, has been found to increase EGFR dimerization and enhance receptor signaling in either the presence or absence of the ligand [64]. GD1a has also been shown to suppress FBJ cell metastasis[65] and bind c-Met to suppress signal transduction following HGF binding in FBJ-LL cells; in GD1a-rich cells, the phosphorylation of c-MET by HGF is suppressed compared to FBJ-LL cells [66]. Interference of NF-kB activation by increased GD3, has been observed in the past in various cell types [67-70].

Increases in GM2αganglioside by overexpression of ST6GalNAC5 in U373MG glioma cells leads to decreased invasivity, decreased adhesivity to fibronectin, increased adhesion-mediated tyrosine phosphorylation of HSPA8, and the inhibition of glioma growth in vivo [1].

Changes in glycogene expression and expression of gangliosides by GSC11 glioma stem cells in response to STAT3 phosphorylation inhibition by WP1193 have been studied [71]. WP1193 treatment resulted in decreased expression of gangliosides GM3, GM1b, GD1, and 3-sulfoglucuronylparagloboside and correlated with decreased transcripts for UDP glucose ceramideglucosyltransferase-like 2 (UGCGL2) and ST6GALNAC2. In a parallel phosphoproteomic study of the same cell line (refer to [72]), α-glucosidase (GANAB), ribophorin 1 (RPN1) and dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B were found to be increased at the protein level, consistent with the lipidomic findings. Changes in the cell surface glycolipids would be expected given that WP1193 treatment results in release of glioma stem cells (GSCs) from the characteristic clusters (neurospheres) formed by GSCs in cell culture (Table:1).

Direct Glycosylation of Signaling Molecules

In addition to the indirect effects that altered N- and O-glycans and gangliosides have on intracellular signaling, there is an emerging literature that describes the effects of differential glycosylation of the signaling molecules themselves.

Notch

Notch is a transmembrane receptor which plays an important role in numerous developmental processes and cell fate decisions [73]. Notch is modified by O-fucose[74] added to serine or threonine on extracellular EGF-like repeats by protein-O-fucosyltransferase 1 (POFUT1). This modification is essential for proper Notch function [75-77]. Notch signaling acts primarily as a result of the formation of an active nuclear transcription factor and interactions between the Notch and Ras pathways have both antagonistic and synergistic effects in different contexts [78]. As is becoming increasingly clear in the case of other (cross-talking) pathways, these interactions can be cooperative or antagonistic and multiple levels of feedback are possible depending on the context. Previous studies have demonstrated a correlation between the expression of Ras and Notch1 in breast cancers [79] suggesting a possible interaction between these two pathways. A

Glycogen ID	Involved kinase pathway
B3GALT5	AKT/JNK1/2/MAPK
B3GNT1	CCT6A (RAK/JRM, unpublished observation)
B3GNT6 (CORE 3 SYNTHETASE)	FAK/SRC/SHC
FUT4	MAPK/PI3K-AKT
FUT8	ERK
GnT-III	ERK
TRKA	FAK/SRC/SHC
GnT-V	MAPK/RHO/RAC/COFILINRPTPK
GnT-VB	RPTPB
OGT	CDK1, MYT1, CDC25C, CK2, GSK-3B, IRS1/2, PI3K, P65, I2PP2A
POFUT1	NOTCH/RAS
ST6GAL1	FAK
ST3GAL3	FAK
ST6GALNAC5	HSPA8
ST8SIAII, ST8SIAIV, ST8SIAV	p44/p42 MAPK ERK1/2
SIALYLTRANSFERASE/ SIALIDASE (NEU1)	SHP2, SRC, TYROSINE PHOSPHATASE

Table 1: Linkage between individual glycogenes and signal transduction pathways.

functional cooperation between constitutively active Notch1 and Ras in the transformation of immortalized breast epithelial cells as well as in breast stem cell self-renewal has been described [80]. Dysregulation of both Notch1 and Ras signaling is also characteristic of high grade gliomas[81,82]. That POFUT1 is also differentially expressed in these tumors [83] provides an alternative, glyco-based strategy targeting the Notch pathway for therapeutic gain.

Pecam

PECAM (platelet endothelial cell adhesion molecule) is considered to be an inhibitory receptor, and its cytoplasmic region possesses an immunoreceptor tyrosine-based inhibitory motif that becomes tyrosine-phosphorylated and subsequently recruits and activates Src homology 2 domain-containing protein-tyrosine phosphatase 2 (SHP2) for the transduction of inhibitory signals to the cell [84]. SHP-2 in other cell types has been implicated as a multifunctional signaling molecule, acting both as a phosphatase to activate nearby Src family kinases and/or as an upstream mediator of p21 ras activation via its ability to bind the Grb2/Sos complex. Homophilic interactions of PECAM in endothelial cells are dependent on cell surface, ST6Gal1-dependent, α2,6-linked sialic acids. In the absence of α2,6-sialic acid, PECAM is unable to remain on the cell surface and results in incomplete transduction of inhibitory signals, including those required for its antiapoptotic role. In PECAM-deficient endothelial cells, the absence of α2,6-sialic acid down-regulates the tyrosine phosphorylation of PECAM and subsequent recruitment of SHP-2 and ultimately renders these cells more sensitive to apoptotic signals [85].

Receptor protein-tyrosine phosphatase kappa (rptpκ)

GnT-V activates EGF-mediated signaling and, in part, promotes cell migration through the modification of N-glycans on receptor protein tyrosine phosphatase kappa (RPTPκ). Overexpression of GnT-V in the human hepatoma SMMC-7721 cell line has been demonstrated to induce the addition of β1,6 GlcNAc branch to N-glycans of RPTPκ and decrease the level of RPTPκ protein expression, ultimately contributing to the decreased phosphatase activity of RPTPκ and thereby activating subsequent EGFR signaling [86].

Receptor protein-tyrosine phosphatase beta (rptpβ)

Results using SH-SY5Y neuroblastoma cells indicate that GnT-Vb activity promotes the addition of the O-mannosyl-linked HNK-1 modification found on the developmentally regulated and neuron specific receptor protein-tyrosine phosphatase β (RPTPβ). The HNK-1 epitope is a terminal sulfoglucuronyl carbohydrate structure that plays important roles in neural cell adhesion and migration [87,88] and has been shown to be expressed on O-mannosyl-linked glycans[89]. These changes in glycosylation accompany decreased cell-cell adhesion and increased rates of migration on laminin. In addition, expression of GnT-Vb promotes RPTPβ dimerization and inhibits its intrinsic phosphatase activity, resulting in higher levels of phosphorylated β-catenin, suggesting a mechanism by which GnT-Vb glycosylation couples to changes in cell adhesion. GnT-Vb-mediated glycosylation of RPTPβ also promotes galectin-1 binding and RPTPβ retention on the cell surface [90].

CD45

CD45 is a receptor-like protein tyrosine phosphatase expressed on the cell surface of all hematopoietic cells and glioma stem cells. Its phosphatase activity is important for removing a negatively regulating COOH-terminal phosphate on Src family kinases, making CD45 critical for the induction of signaling events in these cells. A direct role of glycosylation in regulating CD45 function has been described. CD45 catalytic activity is curtailed by its dimerization; enzymatic blockade is mediated by an "inhibitory wedge", by which one CD45 molecule masks the phosphatase-active site of its partner [91,92]. Mutation of a key residue in this "wedge" leads to chronic activation of the phosphatase [92]. Other data suggest that CD45 dimer formation is determined by inclusion of the alternative exons and sialylation[93]. CD45RA dimers can form if glycosylation is perturbed through the removal of sialic acids or prevention of normal O-linked glycan synthesis, and this leads to a reduction in the capacity of CD45RA to support TCR-triggered activation [93]. An interesting prediction from these data is that CD45RA expression per se will not enhance signaling; rather, this effect will depend on sialylation and be conditioned, therefore, by the expression of sialyltransferases and neuraminidases within and, in the case of neuraminidases, outside, the cell.

Direct competition with o-linked β-n-acetylglucosamine (o-glcnac)

Like phosphorylation, the addition of a single O-GlcNAc (O-GlcNAcylation) by O-GlcNActransferase (OGT) is a ubiquitous, reversible process that modifies serine and threonine residues on both nuclear and cytoplasmic proteins. Unlike most glycans, however, it is not elongated to more complex structures. In many cases, O-GlcNAcylation is in direct competition with phosphorylation at the same sites [94,95]. For example, forced overexpression of OGT increased the inhibitory phosphorylation of cyclin-dependent kinase 1 (CDK1) and reduced the phosphorylation of CDK1 target proteins. The increased phosphorylation of CDK1 is explained by increased activation of its upstream kinase, MYT1, and by a concomitant reduction in the transcript for the CDK1 phosphatase, CDC25C [95].

O-GlcNAc has been detectedon a myriad of other proteins, including RNA polymerase II and manyof its associated transcription factors, on kinases, phosphatases,cytoskeletal proteins, nuclear hormone receptors, nuclear poreproteins, signal transduction molecules, and actin regulatoryproteins (reviewed in [96]). Among the kinases and adaptor proteins modified by O-GlcNAcylation described thus far are casein kinase II (CKII) [97], glycogen synthase kinase-3ß (GSK-3ß) [97], insulin receptor substrate 1 and 2 (IRS-1 and IRS-2) [98-100], and PI3 kinase (p85) [99]. O-GlcNAcylated phosphatases characterized thus far include nuclear tyrosine phosphatase p65 [101], and phosphatase 2a inhibitor (i2pp2a) [102].

There is no doubt that glycoconjugates and kinase activities are intimately linked. Repeatedly we see that alterations in the oligosaccharides of cell surface glycoproteins such as adhesion molecules, growth factor receptors and even kinases themselves, leads to alterations in various kinase activities that themselves play key roles in regulating normal cell function and cellular processes gone awry in cancer cells.

While there are a significant number of reports linking glycoconjugates and kinases, all in all there has been no truly systematic effort with any particular tumor cell type to establish true functional linkages between the observed changes in oligosaccharide expression found on tumor cells and the mechanism of kinase activity alteration. Nevertheless the correlational data are compelling.

We have examined glycogene expression patterns in malignant brain tumors. From these studies we have shown that increasing the expression of selective glycogenes in gliomas has led to the complete suppression of their growth in vitro and in vivo. We have also shown that in each case a unique kinase[s] was markedly expressed, again suggesting the possibility that expression of tumor suppressing glycogenes in brain tumors may lead to alterations in kinase activities that underlie their ability to inhibit tumor formation. Put another way, these studies suggest that specific glycogenes are linked to specific kinases.

There are numerous intracellular signaling pathways affected by aberrant glycosylation in tumors. It will be fruitful to contrast the difference in kinomic involvement between those transducers that are directly phosphorylated/dephosphorylated by differential glycosylation (e.g., Notch, PECAM, or O-GlcNAcylated molecules) with those that are more indirectly involved (e.g., select gangliosides and sialoglycoconjugates).

Glycogene expression is developmentally regulated and cell type specific. Thus when thinking about glycogene-based therapeutic strategies, cellular context must be considered. For example, compare the effect of α2,6sialylation in gliomas vs. colon tumors. In malignant, highly invasive gliomas, only α2,3-linked sialoglycoconjugates are expressed on the cell surface. Switching the N-glycan profile to predominantly α2,6-linked N-glycans (by forced overexpression of ST6Gal1) inhibits invasivity and tumorigenicity in vivo. In highly metastatic colon cells, α2,6sialoglycans predominate, and overexpression of ST6Gal1 would likely be an ineffective therapeutic approach. Molecular context (i.e., the endogenous glycotranscriptomic fingerprint) will, of course, also influence the relative roles of the actual signal transducer versus the downstream effector molecules.

While there has been a significant amount of research linking glycobiology and protein phosphorylation, combining comprehensive glycotranscriptomic and glycolipidomic analyses [71,83,103] with more detailed, global level phosphoproteomic analyses such as those reported by Nilsson and coworkers [72] will be required to set the stage for the kind of structure-function studies necessary to establish the mechanistic links that will then provide the foundation for developing glycogene-based cancer therapeutics.

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