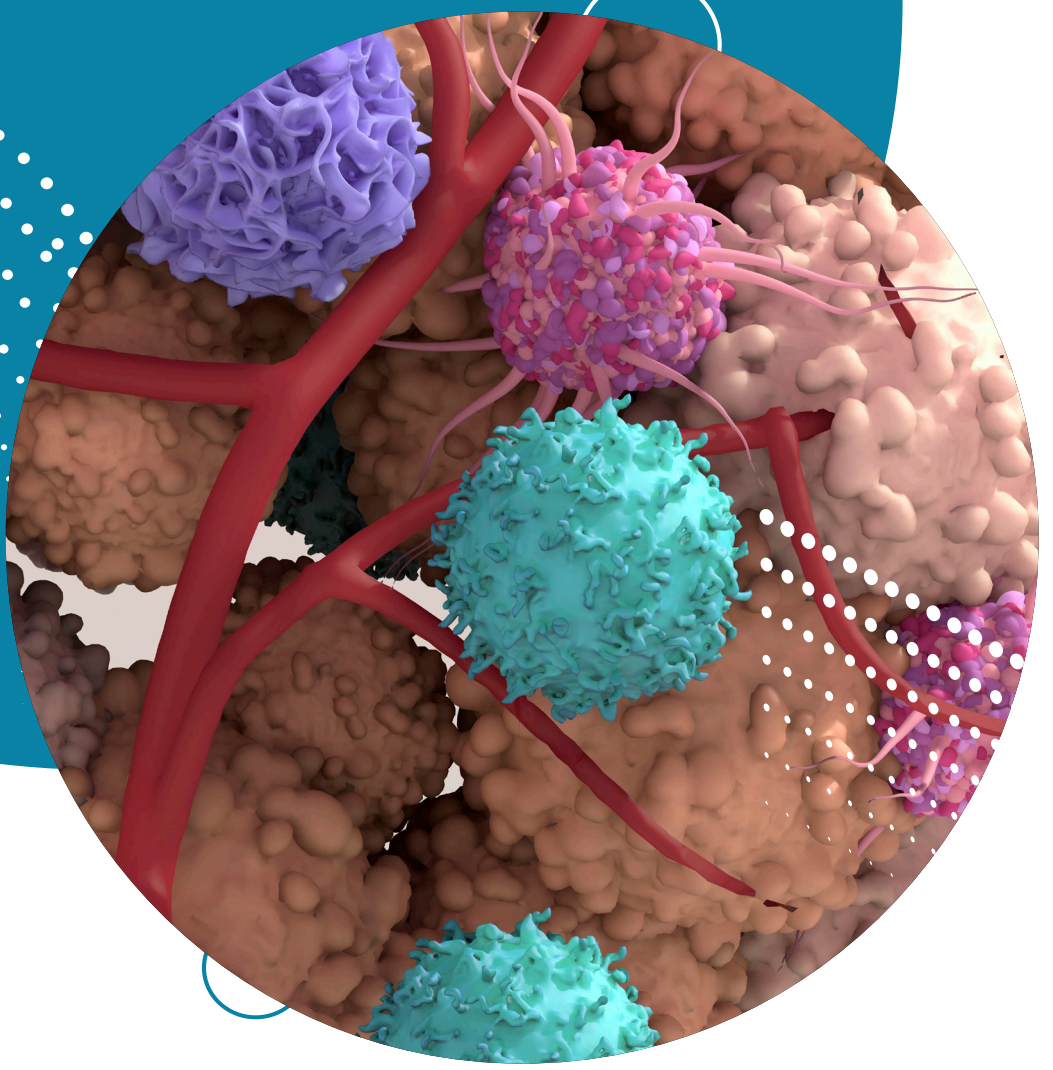
Examining Altered Glycobiology in Cancer



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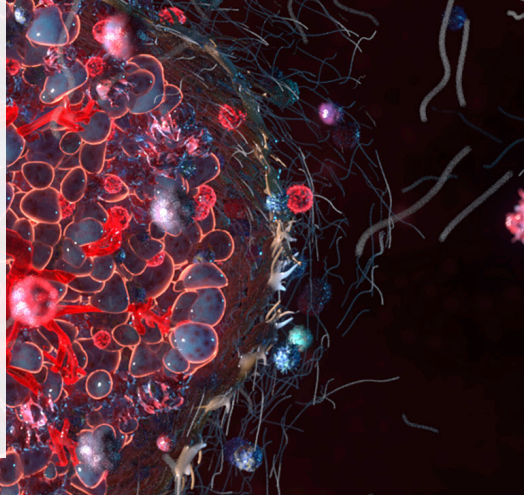
Glycosylation and the Tumor Microenvironment



Glycosylation refers to the covalent attaching of glycans to proteins during or after translation. It is integral to many processes, including protein folding and stability, and researchers have linked abnormal glycosylation to numerous diseases (1). Altered glycosylation is common in cancer that correlate with oncogenesis and disease progression (1). The full extent of glycoproteome alterations in cancer is presently unknown, but researchers believe that glycans have the potential to serve as diagnostic biomarkers and therapeutic targets for cancer (1).

How Glycobiology Affects Cancer

Glycans are known to participate in numerous fundamental biological processes that are affected by cancer, including inflammation, immune surveillance, cell adhesion, and cellular metabolism. As such, aberrant glycosylation contributes to tumor progression by enhancing tumor proliferation, invasion, metastasis, and angiogenesis (1,2). Glycosylation is a highly diverse process, drawing upon a large pool of glycoconjugate macromolecules, including *O*-glycans, *N*-glycans, glycosphingolipids, and glycoproteins (1), and potentially occurring at multiple sites on a given protein. Unsurprisingly, cancer-induced glycoproteomic profiles are heterogeneous and often case-specific.

Glycobiology and Proliferation

The glycome regulates cancer cell proliferation in multiple ways. First, heavily glycosylated proteoglycans produced by cancer-associated fibroblasts promote growth in nearby tumor cells. Glycosylation also impacts growth factor receptor activation and stabilizes transcription factors involved in cell cycle progression (2). Finally, glycosylation plays a critical role in programmed cell death by determining ligand-receptor sensitivity. For example, decreased fucosylation is linked to increased resistance to apoptosis in colon cancer cells (3), while *N*-glycan sialylation blocks Fas-mediated apoptosis (4). Furthermore, glycosyltransferases activate survival signals such as focal adhesion kinase to prevent cell death (5). Not all glycoproteins are pro-proliferative; researchers have identified proteins such as decorin or MGAT3 which can reduce tumor growth (2,6). Nonetheless, crosstalk between cancer cells and the tumor microenvironment (TME) generally creates a pro-proliferative glycosylation profile (2).

Glycobiology and the Tumor Microenvironment

The TME, largely through the creation of a hypoxic environment, plays a major role in driving cancer cell metabolism. Hypoxic induction of HIF-1α elevates *O*-GlcNAcylation by funneling glucose into the hexosamine biosynthetic pathway (HBP), which is linked with enhanced tumor progression (1). Hypoxia also induces the dynamic glycosylation of glucose-6-phosphate dehydrogenase and phosphofructokinase 1. Both of these mechanisms contribute to increased cancer cell proliferation (2). Finally, elevated *O*-GlcNAcylation in cancer cells indirectly stabilizes HIF-1α, resulting in further promotion of additional *O*-GlcNAcylation (2).

Glycobiology and the Immune System

The immune response is essential for limiting tumor progression, and the TME is well-known to aid immune evasion by recruiting immunosuppressive cell types and driving the adoption of tolerogenic phenotypes (2). Glycosylation dysregulation results in the presentation of abnormal protein structures at the cell surface. These structures are only mildly antigenic and rarely immunogenic, thus contributing to immune evasion (7). Glycans also interact with lectin receptors expressed by immune cells, such as sialic acid-binding immunoglobulin-like lectins (Siglecs) (8), to drive anti-inflammatory mechanisms (2). Finally, sialoglycans interact to induce an antigen-specific tolerogenic program, which enhances regulatory T cells at the expense of inflammatory counterparts (2).

Seeing Glycobiology

Mass spectrometry (MS) is commonly used to characterize glycoconjugates, as the technique can provide structural information with high sensitivity (9). However, MS has limited applicability for in vivo contexts, so researchers are turning towards immunohistochemistry and flow cytometry to analyze glycobiology within spatial and cellular contexts (10). These techniques employ a number of different probes, including lectins, antibodies, and aptamers, with lectins—carbohydrate-binding proteins ubiquitous in nature—being the most popular (11). Developments in probe technology have led to the creation and expansion of glycan arrays, capable of probing hundreds of glycans simultaneously (12). Advances like these will continue to aid researchers in their attempt to better understand the complexities of the glycome in cancer.

Please see references on page 7.

Glycobiology Biomarkers

The myriad glycomic and glycoproteomic changes that develop during oncogenesis and tumor progression provide researchers with opportunities to identify novel biomarkers. These can be diagnostic, such as the identification of glycosylation moieties, sites, or patterns that are specific to certain cancer types or indicative of certain degrees of progression. They can also potentially serve as therapeutic targets, with researchers seeking to correct or counterbalance aberrant oncogenic or cancer-promoting signaling or pathway modulations.

Where to Look?

Cancer can affect the glycome in a number of ways, generating many different types of biomarkers. Individual modulations at the epitope, glycan moiety, or glycosylation-site level may indicate the presence of cancer (1,2). These potential biomarkers are likely differentiated not just by their presence or absence, but by relative changes in either abundance or modification profile between cancer and healthy cells (2). As such, multi-glycoprotein panels offer better specificity and sensitivity for disease identification, with researchers moving towards global *N*- and *O*-glycan analysis for more comprehensive disease (and biomarker) screening and discovery (1).

Biomarkers for Disease Diagnosis and Prognosis

Researchers have made promising strides in identifying glycomic and glycoproteomic biomarkers for cancer diagnosis. For example, the FDA approved the glycoprotein antigens CA-125 (a.k.a. mucin 16), CA 19-9 (a.k.a. sialyl-Lewis^{A}), and CA 15-3 (derived from mucin 1) as serum markers for ovarian, pancreatic, and breast cancer, respectively. Similarly, kallikrein-3 (better known as prostate-specific antigen) is a glycoprotein-modulating enzyme that is widely used to screen for prostate cancer (1,2). These, along with many others, are readily detectable in patients' serum and have become routine elements of medical practice (3).

The work done exploring how cancer alters glycosylation mechanisms has the added benefit of highlighting potential markers that can be targeted to correct cancer-induced modulations.

Beyond this, researchers are also looking at the proteins that modulate key glycosylation mechanisms—namely glycosyltransferases and glycosidases (4). *O*-linked-*N*-acetylglucosamine transferase (OGT) and *O*-GlcNAcase (OGA) add and remove *O*-linked-*N*-acetylglucosamine (*O*-GlcNAc) moieties, respectively. Elevated *O*-GlcNAcylation stemming from increased OGT expression is found in lung, colon, and breast cancers. Indeed, a combination of increased OGT and decreased OGA expression is a possible prognostic marker for breast and prostate cancer (4). Similarly, sialyltransferases such as ST6GAL1 are abnormally upregulated in cancers. This phenomenon may have prognostic application for colorectal cancer and is linked to tumorigenesis in ovarian and pancreatic cancer cells (5).

Biomarkers as Therapeutic Targets

Exploring how cancer alters glycosylation mechanisms has the added benefit of highlighting potential markers that can be targeted to correct cancer-induced modulations. Tumor-associated carbohydrate antigens (TACAs) are cell surface structures resulting from cancer-induced alterations to glycosylation, making them a prime target for potential cancer vaccines (6,7). Researchers are also looking at cancer cell-specific glycans as binding targets for vectors carrying chemotherapeutics to improve specificity and efficacy, with promising in vitro and in vivo results (8,9).

In the same vein, targeted attenuation of glycosylation using small molecule inhibitors is a major avenue of investigation, with several candidates currently in clinical trials. The fact that various cell types can easily take up these small molecules, combined with the ubiquity of aberrant glycosylation mechanisms across multiple cancers, means that these agents can potentially treat multiple diseases. As a prime example of this, galectin inhibitors are currently part of clinical trials for melanoma, head, neck, colorectal, lung, breast, and prostate cancers (10).

Please see references on page 7.

Unraveling Glycobiology for Cancer Therapeutics

In response to the drawbacks of traditional cancer therapeutic techniques, including radiation, surgery, and chemotherapy, scientists are developing more targeted approaches such as small molecule inhibitors and immunotherapy for better precision and efficacy. Naturally, scientists are adapting these approaches for glycobiology targets as well. However, this process has been hampered by the inherent complexity associated with glycosylation, as well as difficulties in replicating glycobiology ex vivo. New advances, particularly in high-throughput screening (HTS), have made it easier to characterize the glycoproteome and identify modulatory agents (1).

Adapting Drug Discovery for Glycobiology

Today, cancer drug development typically starts by screening a library of candidate compounds for "hits" that potentially act on the target of interest, a process that has been greatly streamlined by automated HTS. To that end, researchers have adapted HTS for glycobiology, developing assays for individually targeting key glycosyltransferases (1). Because a given glycosylation enzyme can modulate multiple proteins, implementing HTS for cellular systems is vital. Researchers are making progress on that front, with a 2017 report documenting the first use of a cellular model to find an inhibitor for the transferase ppGalNAc-T3 (2).

Targeting Glycosylation

In addition to targeting key glycosylation enzymes, researchers are testing the feasibility of targeting specific glycan-protein interactions that drive cancer progression. Selectins and their glycan ligands, for example, are heavily implicated in metastasis (3), and selectin inhibition can limit or prevent bone marrow metastasis in acute myeloid leukemia (4). Similarly, sialic acid-binding immunoglobulin-like lectins (Siglecs), a receptor family that binds sialylated ligands, are important immune homeostasis regulators that facilitate immune evasion in cancer scenarios (3). Siglec or Siglec ligand antagonism—via desialylation or physical impediment—represents an interesting anticancer strategy that clinicians have employed against melanoma and breast cancer (3,5).

Glycobiology and Combination Therapeutics

The glycome not only directly affects cancer progression by modulating important mechanisms associated with survival, proliferation, immune evasion, and metastasis, but can also impact the efficacy of existing treatment strategies. Indeed, aberrant glycosylation has been implicated in chemotherapeutic resistance and poor tumor cell recognition by immunotherapeutic antibodies. Researchers are therefore investigating combination therapies that use glycobiology modulators alongside targeted therapeutic agents (1). For example, conjugating a sialidase to the anti-HER2 monoclonal antibody trastuzumab resulted in increased natural killer cell activation and elevated cytotoxicity against tumor cells (6).

These combination strategies are also important for better targeting, especially when using nanoparticle vectors (7). Researchers have used TKH2 monoclonal antibody binding to the tumor cell-exclusive sialyl-Tn antigen to selectively deliver the chemotherapeutic agent cisplatin, resulting in attenuated in vitro and in vivo tumor growth (8). Researchers have seen similar results when they treated sialyl-Lewis^A-expressing gastric cancer cells with targeted nanoparticles containing 5-fluorouracil and paclitaxel (9).

Glycans as Vaccine Targets?

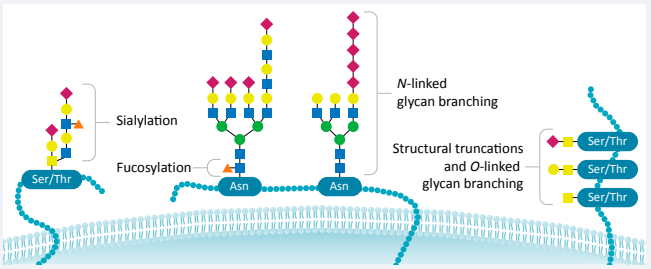
Finally, tumor-associated carbohydrate antigens (TACAs) are interesting candidates for anticancer vaccine development, owing to their potential commonality across different cancer types (3). Antigenicity has posed a problem, especially for monomeric vaccines, as carbohydrates alone are not highly immunogenic (3,10). To combat this, researchers are coupling TACAs to other elements such as T-cell epitopes or chemically modifying them in vitro (10). Multivalent vaccines using agents that simultaneously target multiple glycans may also be a solution, as recent studies investigating heptavalent and pentavalent vaccines induced better immune responses than prior monomeric attempts (3).

Please see references on page 7.

Glycobiology Under the Looking Glass

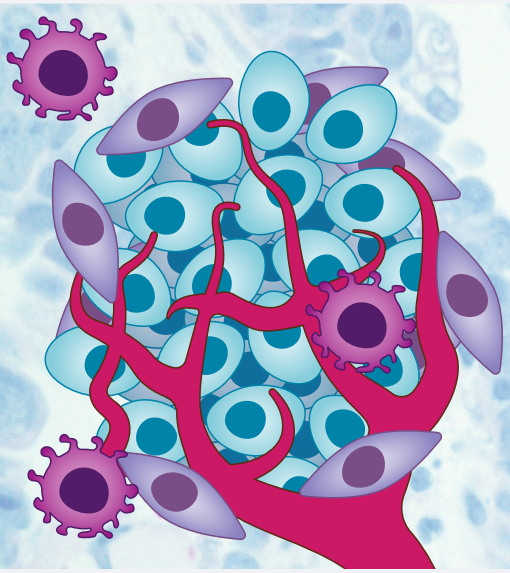
How Cancer Changes Glycosylation

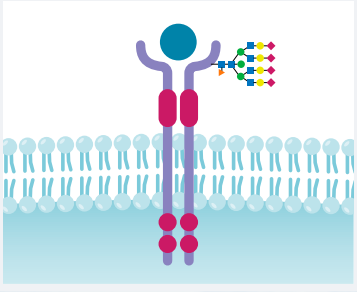
In addition to expression level shifts, tumor cells often display glycans not found in normal cells. The most common changes include increased sialylation, fucosylation, structural truncations, and *N*- and *O*-linked glycan branching (1).



Glycans Stimulate Cell Proliferation

Cancer-associated fibroblasts produce growth-promoting proteoglycans such as syndecan-1 and -2 that stimulate proliferation in nearby cancer cells. Other secreted glycans enhance growth factor binding and mitogenic activity either by acting on receptors (largely receptor tyrosine kinases) or downstream transcription factors (2).



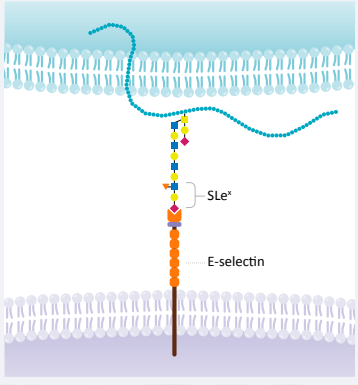


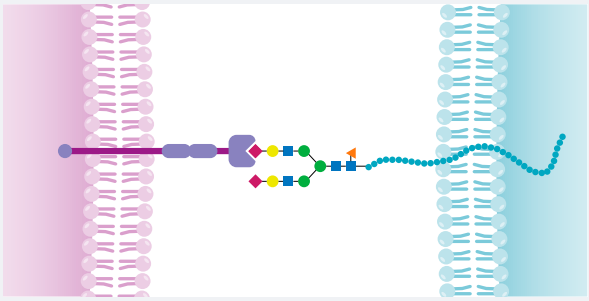
How Glycosylation Affects Metabolism

Hypoxic stress and subsequent HIF-1α activation significantly impact glycosylation, resulting in increased *O*-GlcNAcylation and sialylation. This contributes to the stabilization of oncogenic transcription factors, promoting oncogenesis and tumor aggression (2).

Glycosylation Promotes Migration and Metastasis

Selectins, as mediators of cell-cell adhesion, figure prominently in mechanisms of cancer cell migration, extravasation, and metastasis. Selectin binding by glycan ligands is a major promotor of metastasis in both solid tumor and blood-based cancers (3).





Glycobiology and Immune Evasion

Glycan-binding to cell surface lectins such as sialic acid-binding immunoglobulin-type lectins (Siglecs) interferes with cancer-cell antigen recognition by immune cells—particularly NK cells. Siglec binding also downregulates immune cell activation proteins and upregulates immunosuppressive cytokines (4).

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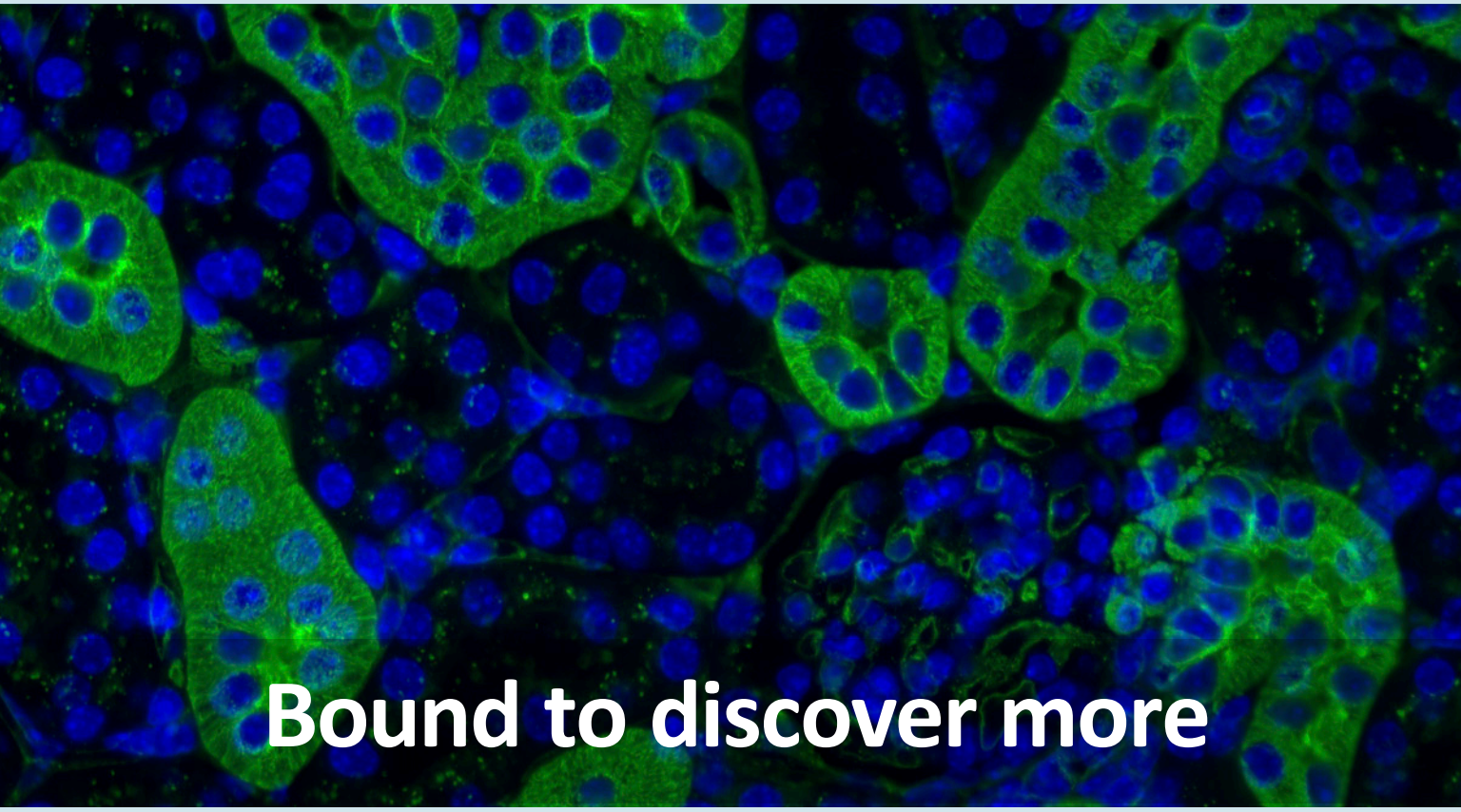
- Discriminate between oligosaccharide structures
- Isolate a specific glycoconjugate, cell, or virus from a mixture



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