Cutting back on the carbs

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Glycans are essential components in maintaining many important biological processes within the body, and can be found in forms ranging from simple monomers to complex polymeric assemblies. On the cell surface, oligosaccharides are presented and recognized by an array of carbohydrate-binding proteins (lectins), such as sialic acid-binding Ig-like lectins (Siglecs), selectins, and galectins. The interactions play critical roles in cellular adhesion, migration, inflammation, and immunological responses through cellular recognition and signaling activities (1). Dysregulation of glycán–lectin interactions has been implicated in both autoimmune diseases and cancer (2, 3). Consequently, the glycome and its interacting partners have attracted a great deal of attention as targets for therapeutics, ranging from small molecules (4) to antibodies (5, 6). In PNAS, Xiao et al. (7) describe a new method to cut back on the carbohydrate structures of cancer cells in a manner that leads to the activation of the immune system. The work represents a new glycoengineering strategy that may have therapeutic applications.

Drugs leading to the inhibition of glycán–lectin interactions have had considerable success in the treatment of pathogenic infections and several other glycán-based diseases (4, 8). These agents function by disrupting interactions between binding partners or by inhibiting enzymes within the glycome machinery, such as glycosidases or glycosyltransferases (9). Heparin is the most widely used glycán-derived drug, acting as an anticoagulant by binding to antithrombin, leading to the inactivation of enzymes within the coagulation system (10). Oseltamivir (Tamiflu) and zanamivir (Relenza) (11) are two successful carbohydrate-inspired antiviral agents that inhibit the influenza viral neuraminidase, which is responsible for virus release from the host cell and propagation (12). Antidiabetic agents, miglitol (Glyset), voglibose (Glustat), and acarbose (Precose), target glucosidases and amylases in the gut to inhibit the digestion of carbohydrates (13). These examples are but a few instances where modification of glycán structures can have pronounced biological and medical impacts.

Glycoengineering represents an alternative approach toward altering glycán–lectin interactions. With antibodies, alteration of these interactions has been effectively achieved through modification of glycán structures residing on the heavy chains, not only having an impact on inherent glycán heterogeneity and immunogenicity but also leading to more optimized antibody pharmacokinetics and efficacy (5). These glycán alterations have been accomplished through new expression host systems or through enzymatic or chemical methods postexpression. In one powerful example of engineered cell lines, Chinese hamster ovary cells were designed to express β-1,4-N-acetylglucosaminyl transferase III, which adds a bisecting N-acetylglucosamine (GlcNAc) onto the antibody N-glycan core. The addition of GlcNAc results in glycoengineered antibodies with increased antibody-dependent cellular cytotoxicity (ADCC) activities (14, 15). Further studies have shown that incorporation of GlcNAc blocked the fucosylation of the antibody Fc, which increased ADCC through improved binding to FcγRIII (16). There are many advanced programs to produce afucosylated (16–20) antibodies, and one such agent, obinutuzumab, has been approved for the treatment of various CD20-positive lymphomas (6).

In PNAS, Xiao et al. (7) present an interesting glycoengineering twist. Instead of changing the glycán profile of the therapeutic as has been done with antibody-based drugs, the new approach involves the development of an antibody–enzyme conjugate designed to alter the cell-surface glycans of antigen-positive cancer cells catalytically, allowing for increased ADCC downstream effects. The investigators note that cancer cells are often able to circumvent the immune system through the overexpression of “self-ligands” that inhibit the onset of immune cell signaling. Clinically approved drugs, such as nivolumab and ipilimumab, enhance the immune system by targeting PD-1 and CTLA-4, respectively (21, 22). In PNAS, Xiao et al. (7) highlight a method to combat cancers that thwart the immune system by capping their cell-surface glycans with sialic acid to generate a “self” signature (23).

Sialylated cell-surface glycans on cancer cells evade the immune system in several ways. They can serve as ligands for Siglecs found on natural killer (NK) cells...
(Fig. 1A), which mediate immune surveillance and suppress immune activation upon binding (24). Sialylation of glycans may also potentially reduce NK cell activity by disrupting the interaction of the NK-activating receptor natural killer group 2D (NKG2D) with its ligand (25). Xiao et al. (7) hypothesize that the protective role of sialic acid is reversed via desialylation to increase NK activity on cancer cells (Fig. 1B). The authors aim to remove the cell-surface sialic acids on breast cancer cells that overexpress the human epidermal growth factor receptor 2 (HER2) antigen through the use of a trastuzumab-sialidase conjugate. Click chemistry was used to attach Vibrio cholerae sialidase to the C terminus of the antibody, creating an antibody enzyme conjugate dubbed “T-Sia.” Despite conjugation to an antibody, the sialidase did not suffer from significant loss of function and was shown to remove cell-surface sialic acid, resulting in enhanced ADCC activities. This cytotoxicity roughly correlated with Siglec-7-binding levels, but not with NKG2D binding on NK cells, which was originally thought to be a modulator of ADCC activity. Interestingly, Xiao et al. (7) found that the cell lines expressing lower levels of HER2 benefitted the most from treatment with T-Sia, a finding that may have implications on how this approach may eventually be applied.

Xiao et al. (7) demonstrate a new and potentially powerful method to alter glycosylation at the cell surface by targeting an enzyme to the cancer via an antibody–enzyme conjugate. The in vitro effects are promising, and suggest several avenues for further research. For example, it may be of interest to apply human sialidase enzymes or abzymes (26, 27) to the strategy because the V. cholerae enzyme source is likely to be immunogenic. In addition, alternative constructs using carriers other than the 150-kDa anti-HER2 antibody may expand applications of the approach, and potentially address issues surrounding penetration and retention of the conjugate within tumor masses. Given the general expression of Siglecs on immune cells other than the NK T cells studied, it will be interesting to elucidate what other immune pathways are affected by desialylation of the cancer cell-surface glycan. Cutting back on the carbohydrate content of tumor cells through targeted enzymatic hydrolysis is a new idea with high potential.

Fig. 1. Interactions of sialylated glycans with proteins. (A) Crystal structure of Siglec-7 associated with a sialylated ligand (Protein Data Bank ID code 2DF3) (28). (B) Cancer cells containing sialylated glycans can bind to Siglec and disrupt binding of NKG2D with its ligand potentially to inactivate NK cell killing. Desialylation can reverse the protective role of sialic acid.