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Antibody recognition of aberrant glycosylation on the surface of cancer cells

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Carbohydrate-binding antibodies and carbohydrate-based vaccines are being actively pursued as targeted immunotherapies for a broad range of cancers. Recognition of tumor-associated carbohydrates (glycans) by antibodies is predominantly towards terminal epitopes on glycoproteins and glycolipids on the surface of cancer cells. Crystallography along with complementary experimental and computational methods have been extensively used to dissect antibody recognition of glycan epitopes commonly found in cancer. We provide an overview of the structural biology of antibody recognition of tumor-associated glycans and propose potential rearrangements of these targets in the membrane that could dictate the complex biological activities of these antibodies against cancer cells.

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Introduction

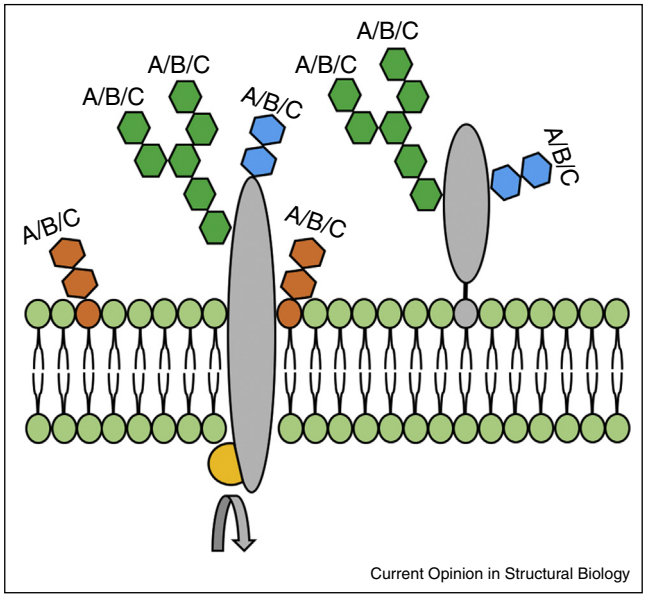
Aberrant carbohydrate determinant (epitope) presentation on the surface of cancer cells has resulted in opportunities for tumor targeting by antibodies for diagnostic and therapeutic purposes. Several crystallographic studies of antibody fragments (e.g. Fab, fragment antigen binding) as complexes with tumor-associated carbohydrate antigens (TACAs) have revealed how the terminal glycan epitopes are easily and specifically recognized by cavity or groove shaped binding sites. In addition, structural recognition of TACAs by antibodies has been extensively studied by computational approaches (e.g. docking and molecular dynamics) and complementary structural and binding methods. In this review, we provide an overview of the structural biology of TACA recognition by antibodies and propose how they could reorganize and cluster glycan targets, and how such membrane reorganization may explain their multimodal activities against cancer cells.

Tumor targeting by carbohydrate-binding antibodies

Of all the cellular changes in carcinogenesis, modification of glycosylation machinery and consequent aberrant glycosylation is considered to be a universal change [1]. Glycosylation changes include truncations, decreases, and increases in the density of glycan epitopes on the surface of cancer cells [2]. These altered antigenic profiles allow antibodies and lectins (carbohydrate-binding proteins) to readily distinguish malignant cells from normal cells and provide the basis for tumor-targeted immunotherapy [2,3•]. Major categories of TACAs being considered include: (i) truncated O-linked glycans that represent cryptic epitopes, (ii) fucosylated blood-group related carbohydrates such as the Lewis system carbohydrates, and (iii) glycosphingolipids, particularly the sialylated versions termed gangliosides [4,5•].

A distinct difference between a TACA and a conventional protein antigen is the occurrence of the same glycan epitope on a range of cell surface glycoproteins and glycolipids (Figure 1). Consequently, the biological activity of TACA-binding antibodies can be complex. Similar to other antibodies, binding of cancer cells can initiate effector functions including antibody-dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and phagocytosis [6]. Many cell surface glycoprotein receptors are associated with intracellular signaling pathways that among other functions are involved in cancer cell proliferation and survival [7]. Thus, carbohydrate-binding antibodies can activate or inhibit a wide range of receptor-mediated signaling pathways, some of which can result in novel antitumor activity [5•]. Additionally, antibodies against TACAs can kill cancer cells by direct action, including apoptosis and oncosis, which is characterized by rapid membrane changes (membrane protrusions and pore formation) resulting in cell swelling and lysis. Both apoptosis and oncosis have been observed with carbohydrate-binding antibodies towards glycoprotein and more commonly

Figure 1



Simplified schematic of the presentation of altered carbohydrate epitopes on tumor cell membranes. For clarity the membrane bilayer has been simplified to a generic lipid component (e.g. phospholipids). Carbohydrate epitopes (indicated as A/B/C) are often displayed at the end of oligosaccharide chains on glycolipids (orange) and glycoproteins as N-linked (green) and O-linked (blue) glycans. Truncated carbohydrate epitopes can also occur revealing hidden or cryptic neoantigens not present on normal cells. Glycoprotein antigens can be extracellular (e.g. glycophosphatidylinositol-anchored) or associated within intracellular signaling systems (yellow circle) that can be triggered or inhibited (indicated by the arrow) by binding of the glycans on the extracellular protein domains.

glycolipid targets [8,9•], but the structural mechanism for the membrane reorganization that occurs with antibody-induced cell death is largely unknown.

Structural recognition of tumor-related carbohydrates by antibodies

Crystal structures have been determined for several tumor targeting antibodies in complex with carbohydrates and glycopeptides and others without bound ligand (Table 1). Several of the antibody candidates with known structures have been through preclinical and some through early stage clinical testing against a range of cancer types [3•,10]. A ganglioside-binding (GD2 specific) antibody, dinutuximab (marketed as Unituxin) has been approved as a combination therapy in high-risk pediatric neuroblastoma [11]. Dinutuximab is a chimeric version of the murine monoclonal antibody (14G2a), for which recognition of GD2 has recently been structurally characterized by X-ray crystallography [12••]. Although dinutuximab is the first-in-class of TACA-specific antibodies to be approved, there are many more in various stages of development [5•,13], similar to the numerous monoclonal antibodies in the burgeoning oncology area [7,14].

A prominent feature of carbohydrate binding by antibodies is the end-on insertion and anchored recognition, predominantly of one or two saccharide units, which are bound in a cavity formed by up to six complementarity determining regions (CDR) of the variable domains of the light (VL) and heavy (VH) chains (Table 2) [10,15]. Direct carbohydrate–protein and water-mediated hydrogen bonding networks contribute to the exquisite specificity for the target carbohydrate epitopes on cancer cells, especially as these are often very similar to tissue and blood group antigens on healthy tissues [3•]. Hydrophobic interactions also contribute to carbohydrate recognition, mainly mediated by aromatic side-chains in the antibody binding site. Unlike lectins, antibodies generally do not use metal ions to interact directly with their target carbohydrate ligands [16••]. The molecular details and conformational aspects of carbohydrate recognition by antibodies have been reviewed in detail elsewhere [10,16••,17,18]. Here we provide an overview of select examples of crystal structures and modeling studies that examine antibody recognition of the three main categories of TACA.

Antibody recognition of truncated O-linked glycan epitopes

Due to the inactivation of certain glycosyltransferases in many tumors, truncated O-glycans are displayed on cancer cells and two are actively being investigated: Thomsen Friedenreich disaccharide (TF) and Thomsen nouvelle monosaccharide (Tn) antigens. Both glycans are linked to threonine or serine residues, are expressed at high densities on cancer-expressed glycoproteins (particularly mucins), and are not found on healthy cells as they are cryptic antigens [19].

Crystal structures of a Tn-specific antibody (237mAb) have been determined with a bound GalNAc monosaccharide and a Tn-glycopeptide from the mucin-like podoplanin glycoprotein, which is associated with tumor invasion and metastasis [20]. Regardless of its presentation as a free monosaccharide (Figure 2a) or as a glycopeptide (Figure 2b), the GalNAc is anchored in a cavity and participates in several hydrogen bonds with binding site residues from LCDR1, LCDR3, HCDR2 and HCDR3 (Table 2). Specificity for the podoplanin glycopeptide is ensured by interactions between amino acids of the Tn-glycopeptide and CDR residues in the large groove-shaped binding site of 237mAb. Thus, the glycan and peptide components are important for recognition by 237mAb, and different Tn-binding antibodies are specific for other glycoproteins containing the Tn determinant.

The basis for specificity of the JAA-F11 antibody raised against the TF disaccharide has been explored by glycan