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Review

Structural biology of antibody recognition of carbohydrate epitopes and potential uses for targeted cancer immunotherapies

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ABSTRACT

Monoclonal antibodies represent the most successful class of biopharmaceuticals for the treatment of cancer. Mechanisms of action of therapeutic antibodies are very diverse and reflect their ability to engage in antibody-dependent effector mechanisms, internalize to deliver cytotoxic payloads, and display direct effects on cells by lysis or by modulating the biological pathways of their target antigens. Importantly, one of the universal changes in cancer is glycosylation and carbohydrate-binding antibodies can be produced to selectively recognize tumor cells over normal tissues. A promising group of cell surface antibody targets consists of carbohydrates presented as glycolipids or glycoproteins. In this review, we outline the basic principles of antibody-based targeting of carbohydrate antigens in cancer. We also present a detailed structural view of antibody recognition and the conformational properties of a series of related tissue-blood group (Lewis) carbohydrates that are being pursued as potential targets of cancer immunotherapy.

1. Alterations in glycosylation in cancer form the basis for antibody-based targeting

Carcinogenesis and cancer progression are characterized by multiple genetic and epigenetic changes that result in different gene expression profiles in tumor cells compared to healthy cells (Agrawal et al., 2014; Kobata and Amano, 2005). Importantly, many changes occur in the expression of enzymes that are involved in glycosylation of lipids and proteins, which include increased, decreased, and aberrant expression of various glycosyltransferases in cancer cells (Brockhausen and Gao, 2012; Durrant et al., 2012; Milde-Langosch et al., 2014; Singhal and Hakomori, 1990).

Increased sialylation and fucosylation are two of the most common glycosylation changes in carcinogenesis, cancer progression, and prognosis (Cazet et al., 2010a; Dube, 1987; Miyoshi et al., 2008). Decreases in glycosylation also commonly occur in cancer and are most apparent with glycoproteins where *O*-linked glycosylation can be reduced or absent revealing tumor neoantigens or cryptic epitopes. For example, mucins that represent some of the most studied tumor glycoprotein antigens, often have truncated and missing *O*-linked carbohydrates (or glycans) in their serine- and threonine-rich tandem repeat regions (Brockhausen, 2006; Devine and McKenzie, 1992). Aberrant expression may involve the occurrence of glycan epitopes (determinants) that are not normally

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytolysis; CDR, complementarity determining region; Fab, fragment antigen binding; H, antibody heavy chain; L, antibody light chain; Le, Lewis glycan epitope; PDB, protein data bank; TACA, tumor-associated carbohydrate antigen; TF or T, Thomsen–Friedenreich antigen; Tn, Thomsen nouvelle antigen (*s*Tn, sialyl-Tn); V, variable domain.

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present on healthy cells such as the presence of A, B, or Lewis b epitopes on tumors from individuals with different tissue-blood groups (Tempero et al., 1987; Yuan et al., 1985). Other cases of aberrant expression involve the expression on tumors of developmental or fetal glycan antigens (oncofetal antigens) or glycans that are not normally found or are in low abundance on the cell or tissue from which the tumor originated (Heimburg-Molinaro et al., 2011).

Alterations in glycosylation machinery result in dramatic changes in the antigenic profile at the tumor cell surface so that carbohydrate-binding proteins can be used to discriminate between malignant and normal adult cells (Bara et al., 1986; Feizi, 1985). Carbohydrate-binding proteins commonly used to profile the glycosylation of tumors are antibodies and lectins (*i.e.*, non-enzymatic carbohydrate-binding proteins excluding antibodies). In particular, carbohydrate-binding antibodies are considered promising candidates for immunotherapy of primary and metastatic tumors through their dual ability to target (bind) cancer cells and to mediate immune effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytolysis (CDC) (Brodzik et al., 2006; Kircheis et al., 2012; Rabu et al., 2012; Roque-Navarro et al., 2008; Sawada et al., 2011; Scott et al., 2000). Some glycan binding antibodies have also been shown to directly kill tumor cells by a complement-independent mechanism, termed oncosis, that resembles necrosis (Loo et al., 2007; Noble et al., 2013; Roque-Navarro et al., 2008) or apoptosis (Durrant et al., 2006). The capacity of many glycan binding antibodies to rapidly internalize is considered applicable for the delivery of cytotoxic payloads to the tumor cells (Feridani et al., 2007; Ma et al., 2011). Thus, with respect to developing antibody-based therapeutics, specificity for both primary and metastatic lesions is considered important as well as optimization of antibody properties for particular functions including ADCC/CDC/oncosis, payload delivery and immunotherapy (Clarke et al., 2000; Durrant et al., 2006; Kanazawa et al., 2000; Kelly et al., 2009; Kircheis et al., 2012; Ma et al., 2011; Orbom et al., 2013). The diverse mechanisms of action and approaches for developing antibodies for cancer immunotherapy have been expertly reviewed elsewhere (Scott et al., 2012; Sliwkowski and Mellman, 2013).

2. Antibody recognition of carbohydrate epitopes of glycolipids and glycoproteins in tumor cells

While antibodies have the capacity to discriminate between cancerous and healthy adult tissues, this discrimination or specificity is by no means guaranteed or absolute. In practice, antibody selectivity is determined by stronger binding to a particular glycan epitope, compared to binding to other epitopes, and the relative degree that the epitope is uniquely expressed on the target tumor cell population (Manimala et al., 2007; Mollicone et al., 1996). Therefore, glycan epitopes recognized by antibodies are often referred to as tumor-associated carbohydrate antigens (TACAs) rather than tumor-specific antigens (Heimburg-Molinaro et al., 2011). Effective antibody targeting of a single TACA may be possible with some tumors, but in many cases combinations of TACAs may better define a tumor or the tumor stage (*e.g.*, primary and metastatic lesions have different TACA profiles) (Cazet et al., 2010b; Durrant et al., 2012; Izawa et al., 2000; Kannagi, 2007; Tempero et al., 1987).

Distribution of TACAs on the surface of tumor cells is more complex when compared to conventional protein antigen targets. Glycan epitopes are presented as glycoconjugates of glycolipids or glycoproteins (Durrant et al., 2012; Heimburg-Molinaro et al., 2011; Kobata and Amano, 2005). Glycoproteins display carbohydrates as either *N*-linked or *O*-linked sugars with epitopes usually displayed at the exposed termini of the *N*- and *O*-glycans. Collectively, what distinguishes TACAs from defined protein antigens is the broad distribution of glycan epitopes on a variety of lipids and glycoproteins. Binding of antibodies to glycan epitopes on particular glycoproteins can result in a series of downstream biological consequences such as inhibition or activation of cellular receptors and associated intracellular signaling pathways (Aziz and Qiu, 2014; Farhan et al., 2006; Liu et al., 2009).

There are several categories of TACAs that are defined by their presentation as glycolipids and glycoproteins, or both. Gangliosides, a group of sialylated glycosphingolipids (oligosaccharide–ceramide conjugates), have been recognized as TACAs and include mono-sialylated GM1 and GM2 or di-sialylated GD2 and GD3 glycans (Hakomori, 2001). Gangliosides have restricted distribution in normal tissues, but are over-expressed in various tumors, particularly melanoma and tumors of neuroectodermal origin such as neuroblastoma and glioma (Hanai et al., 2000; Zhang et al., 1997). Gangliosides have also been found to be over-expressed on many small lung cell cancers and renal carcinomas (*e.g.*, GM1) as well as other tumors, suggesting their targeting by antibodies may be applicable to a broad range of cancers.

A second category of TACAs is formed by defects in *O*-glycosylation, which is one of the universal changes in cancer. The three main truncated *O*-glycans expressed on tumors are the Thomsen nouvelle (Tn), sialyl-Tn (sTn), and the Thomsen–Friedenreich (TF or T) antigens, which are GalNAcα1-O-Ser/Thr, Neu5Acα2-3GalNAcα1-O-Ser/Thr, Galβ1-3GalNAcα1-O-Ser/Thr, respectively (Brockhausen, 2006; Ju et al., 2011). Due to the proximity of the glycan epitope to the attached polypeptide, antibodies raised against Tn, sTn or T may preferentially recognize the epitope in the context of the protein sequence (Brooks et al., 2010). Antibody reactivity is also influenced by antigen density and for Tn, sTn, and T antigens this is determined by the number of *O*-linked glycan attachment sites in the protein (Heimburg-Molinaro et al., 2011).

A major class of TACAs expressed as glycolipids and glycoproteins are tissue (histo) and blood group related carbohydrate determinants (Feizi, 1985). These glycan epitopes include the A, B, and H (O) blood group epitopes and Lewis system carbohydrates (for details see Section 2.1). Glycosylation changes can involve the loss of expression of histo-blood groups, which has been reported in carcinomas, and the aberrant or incompatible expression of blood group antigens on tumors (Tempero et al., 1987; Yuan et al., 1985). Several histo-blood group related carbohydrates that are not normally expressed on adult tissues, but are expressed during fetal development, are also often over-expressed on tumor cells (Kobata and Amano, 2005; Yuan et al., 1985). This group of antigen falls into the broader category of oncofetal antigens or stage specific embryonic antigens (SSEA). Several SSEAs are well known such as SSEA-1 (CD15 or Lewis x) and SSEA-3 (Globo H), which have been pursued as potential targets for both antibody-based immunotherapies and vaccines (Buskas et al., 2009; Wang et al., 2008; Wilson and Danishefsky, 2013).

The abundance of certain gangliosides, truncated *O*-glycan epitopes, and histo-blood group related carbohydrates on the surface of tumor cells has resulted in several of these TACAs showing promise as targets for antibody-based therapies (Durrant et al., 2012; Rabu et al., 2012; Scott and Renner, 2001). However, the varied expression on normal tissues of certain antigens and the potential for cross-reactivity of a therapeutic antibody with a related histo-blood group epitope is of constant concern during development and optimization of antibodies in preclinical and clinical studies. Understanding the three-dimensional (3D) structural basis of selective binding of TACAs and cross-reactivity with related histo-blood group glycans may be of use in the structure-based optimization of antibodies for tumor targeting. Of the different TACAs that have been studied by structural techniques (Agostino et al., 2012a), the Lewis histo-blood group and related

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