Therapeutic Antibodies Targeting Glycosylation

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Contributor: Ron Amon , Ronit Rosenfeld , Shahar Perlmutter , Oliver C. Grant , Sharon Yehuda , Aliza Borenstein-Katz , Ron Alcalay , Tal Marshanski , Hai Yu , Ron Diskin , Robert J. Woods , Xi Chen , Vered Padler-Karavani

We generated a platform for designing optimized functional therapeutic antibodies against cancer glycans. The target tumor-associated carbohydrate antigen is commonly expressed in colon and pancreatic cancers. We developed a system for selection of potent antibodies by yeast surface display against this carbohydrate antigen, then showed that elite clones have potent affinity, specificity, cancer cell binding, and therapeutic efficacy. These tools have broad utility for manipulating and engineering antibodies against carbohydrate antigens, and provide major innovative avenues of research in the field of cancer therapy and diagnostics.

cancer, antibody, carbohydrate, immunotherapy

1. Introduction

Cancer is a leading cause of death worldwide and selective targeting by therapeutic monoclonal antibodies (mAbs) shows increasing success in modern oncology, mostly targeting proteins[1][2][3][4]. Cell surface glycosylation expression pattern is altered on cancer cells, leading to abnormal tumor-associated carbohydrate antigens (TACA) that are selectively and abundantly expressed on cancer cells[5][6]. These potentially valuable cancer cell surface targets are poorly immunogenic, hindering functional TACA-based cancer vaccines or immunotherapies, thus far [7][8][9]. Anti-carbohydrate antibodies typically have much lower affinities than antibodies recognizing proteins or peptide antigens (by 3–5 orders of magnitude), further complicated by carbohydrates large diversity in linkage types and modifications[10][11].

In 2018, The Nobel Prize in Chemistry was awarded to Frances H Arnold for inventing the directed evolution of enzymes, conjointly with George P. Smith and Sir Gregory P. Winter for their discoveries on phage display of peptides and antibodies. Yeast surface display (YSD) is one of the leading antibody engineering technologies to date, for both isolating novel antibodies and for directed evolution by *in vitro* affinity maturation of selected clones[12][13][14][15][16], allowing to identify mAb leads with good specificity and affinity. This system takes advantage of the agglutinin mating proteins (Aga1p and Aga2p) that are normally expressed on the yeast cell surface. These are expressed at 10^4–10^5 copies per cell, where Aga1p domain is anchored to the yeast cell wall and Aga2p is covalently attached to Aga1p through disulfide bonds[15][16]. In YSD, an antibody fragment is fused to the Aga2p allowing its cell surface presentation in accordance with the expression of the agglutinin proteins [18]. Most commonly, single chain fragment variable (scFv) or Fab antibody fragments are used in YSD, with surface

expression and antigen binding monitored by flow cytometer allowing a very efficient sorting of large libraries according to antigen binding[18]. This system had mostly been employed against protein antigens[19][20].

Sialic acids (Sias) are acidic sugars found in vertebrates, topping cell surfaces glycans and glycoconjugates. Their expression patterns are altered on cancer cells[21][22][23], correlating with advanced stage, progression, and/or metastasis[24][25][26]. Thus, sialylated-TACA are promising targets for cancer therapy[27]. These include sialyl Lewis a (SLe^a) found on pancreas, colorectal, stomach and liver cancers that suffer very short five-year survival rates [28][29]. The SLe^a tetrasaccharide Neu5Acα2−3Galβ1−3(Fucα1−4)GlcNAcβ1-R, namely CA19−9 antigen, is a cancer-associated marker widely used in clinical practice[30][31][32][33]. It is the only FDA-approved test for pancreatic cancer, but is also used in colorectal, gastric, or biliary cancers. It is utilized to monitor response to therapy; however, it is not useful for early detection, diagnosis, or therapy[33][34][35]. Different antibodies are used to measure CA19-9; however, there is great variability between measured outcomes[36][37]. Taken together, these findings suggest that antibodies of greater specificity and affinity against SLe^a carbohydrate antigen could potentially serve as better cancer theranostic tools. Importantly, such potent antibodies should recognize their glycan target in the right context as presented on cancer cells or shed from them. As a proof of concept for development of efficient cancer therapeutics targeting glycosylation, we cloned the most commonly used antibody against SLe^a (1116-NS-19-9)[38] into YSD platform that was used to obtain specific anti-SLe^a antibodies of high affinity and potency in cancer cells binding and cytotoxicity.

2. Cloned Antibodies Are Effective at Cancer Cells Binding and Cytotoxicity

Cancer cell binding is critical for antibody therapeutic and diagnostic applications. Hence, cancer cell binding was examined to further evaluate antibody clones potency in the natural context. SLe^a expression was determined by native antibody and found positive in some cell lines (WiDr, Capan2, BxPC-3), while negative in others (MCF-7, MDA-MB-231) (Figure 1). Thus, we followed up investigation on antigen-positive cells. We compared the binding of native and RA9-23 antibody clones to the SLe^a-positive cancer cell lines WiDr and Capan2. RA9-23 showed higher binding efficiency compared to the native antibody clone in both cell lines, at various concentrations (Figure 1), and with high specificity since binding was reduced dramatically after removal of sialic acids from the cell surface by a sialidase treatment (Figure 1). Thus, RA9-23 antibody clone has high affinity against nanoparticle multivalent-glycans, and this is also reflected in the whole cell context, despite their heterogenous glycan expression patterns. It would be interesting to examine these novel antibody variants on patient samples in comparison to the native antibody. We next examined whether this improved cancer cell binding also translates into cancer cell killing. Antibodies of IgG1 isotype can facilitate cell killing by complement recruitment. Hence, complement-dependent cytotoxicity (CDC) was evaluated showing that RA9-23 clone has higher cytotoxicity in both WiDr and Capan2 cancer cell lines compared to the native clone (Figure 1). These results exemplify the potential of this improved antibody clone for both detection of SLe^a-positive tumors, and as cancer therapeutics.

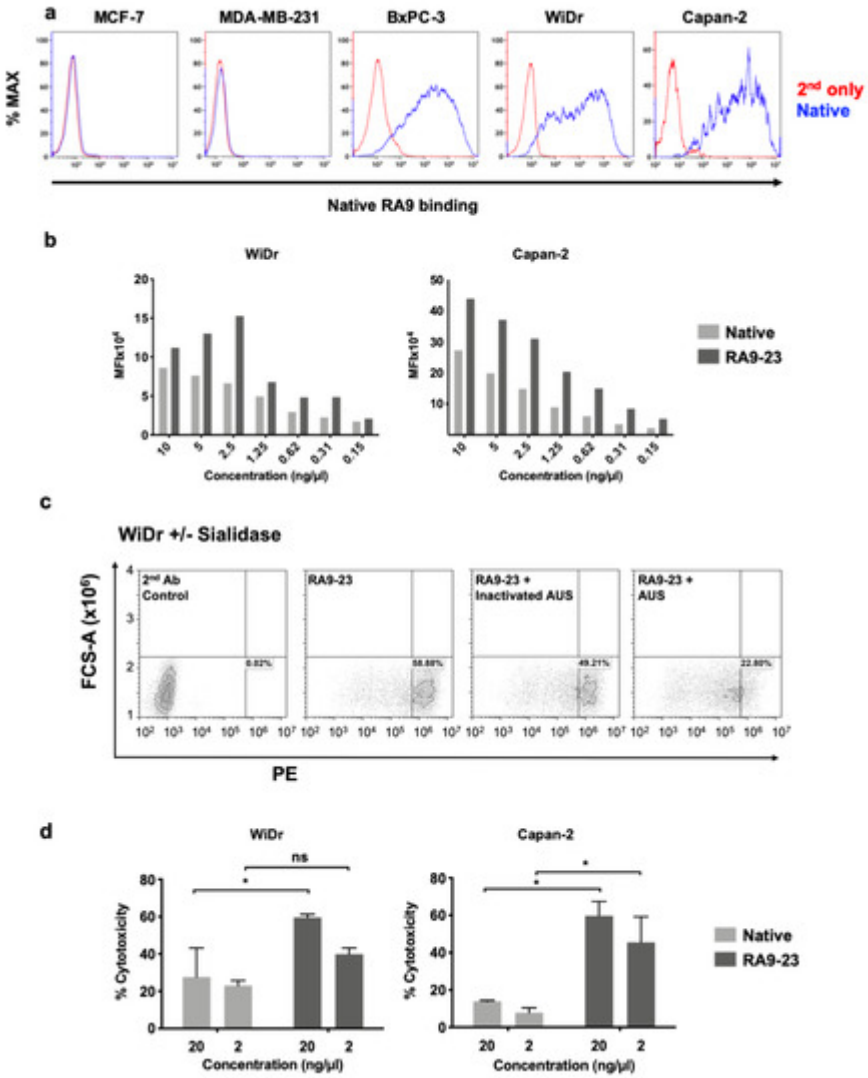

Figure 1. Antibody binding and cytotoxicity against cancer cells. (**a**) Binding of native antibody was examined by FACS against different cancer cell lines at 10 ng/µL (blue), compared to secondary antibody control (red). (**b**) Binding of native and RA9-23 IgGs to SLe^a-expressing cancer cells (WiDr and Capan2) was examined by FACS at various concentrations (10–0.15 ng/µL). (**c**) Specificity of binding to cells was demonstrated by treatment of cells with *Arthrobacter Ureafaciens Sialidase* (AUS) that abrogated binding of RA9-23 IgG to SLe^a-expressing WiDr cells, in comparison to direct binding of the antibody or its binding to cells treated with heat-inactivated AUS. (**d**) Complement-dependent cytotoxicity (CDC) of native and RA9-23 IgGs was examined. WiDr and Capan2 target cells were incubated with antibodies at concentrations 20 ng/µL and 2 ng/µL, then rabbit complement was added. Cytotoxicity was determined by LDH detection kit (representative of 2 independent experiments; 2-way ANOVA, * $p < 0.05$).

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