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Affinity of monoclonal antibodies for Globo-series glycans

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ABSTRACT

Globo-series glycans are human cell-surface carbohydrates that include stem-cell marker SSEA-4 and cancer-cell antigen Globo H. These two hexasaccharides differ only in their terminal saccharide: *N*-acetylneuraminic acid in SSEA-4 and L-fucose in Globo H. Herein, we evaluated the affinity of the monoclonal antibodies α-SSEA-4 and α-GH for the glycans SSEA-4 and Globo H. Using fluorescence polarization, we find that the two monoclonal antibodies have affinity for their cognate glycan in the low nanomolar range, and have negligible affinity for the non-cognate glycan. Using surface plasmon resonance, we find that each cognate affinity is ~20-fold greater if the glycan is immobilized on a surface rather than free in solution. We conclude that the terminal saccharide plays a dominant role in the ability of monoclonal antibodies to recognize these Globo-series glycans and that the extraordinary specificity of these antibodies supports their use for identifying and sorting stem-cells (α-SSEA-4) and as an agent in cancer immunotherapy (α-GH).

1. Introduction

1.1. Globo-series glycans

Globo-series glycans comprise a group of neutral glycosphingolipids in which a ceramide is linked to a glycan with a root structure of GalNAcβ3Galα4Galβ4Glc.[1,2] Typically, these glycans are retained on the plasma membrane and cluster into lipid rafts.[3] The endogenous function of this glycan family is largely unknown. Their expression does, however, occur during early stages of development and is thought to mediate cell contact and adhesion.[4] Importantly, changes in these glycans are observed throughout differentiation and during tumorigenesis.[5,6] Two notable hexasaccharide members of this family are stage-specific embryonic antigen-4 (SSEA-4) and Globo H (Fig. 1). These glycans share a common precursor, SSEA-3 (Galβ3GalNAcβ3Galα4Galβ4Glc), but vary in the terminal monosaccharide: β3-linked *N*-acetylneuraminic acid for SSEA-4 and α2-linked L-fucose for Globo H.

1.2. SSEA-4, a stem-cell marker

SSEA-4 was discovered using the monoclonal antibody, MC-813-70 (α-SSEA-4), produced by immunization against human embryonic stem cells.[7] Subsequent analyses found expression of this epitope on many stem cell types as well as induced pluripotent stem cells and embryonic carcinoma cells.[8] Although SSEA-4 expression is not required for stem-cell pluripotency, a decrease in expression is observed upon differentiation.[9] In addition the pentasaccharide precursor, SSEA-3, is also used to identify stem cells and is depleted rapidly from the cell surface upon differentiation. Hence, commercial antibodies for both glycans are often used to identify undifferentiated cells.[10] The use of α-SSEA-3 (MC-613) and α-SSEA-4 enables the identification of spontaneous differentiation and the collection of live stem cells.[11,12] Such live-cell sorting has distinct advantages in stem cell and regenerative therapies,[13] and is not enabled by other known stem-cell markers, such as nuclear transcription factors.[14] More recently, SSEA-4 has been detected on malignant glioma cells,[15] which form the most aggressive and common brain tumors in adults, as well as on breast cancer cells.[16,17] As a result, antibodies against SSEA-4 can illicit complement-dependent cytotoxicity and support the targeting of SSEA-4 in cancer vaccines.

1.3. Globo H, a cancer-cell antigen

Globo H was isolated originally from human breast cancer cell line MCF-7.[18] High-level expression of Globo H has been observed on a variety of other cancer cells, including colon, ovarian, prostate, and lung.[16,19] Identification of this cancer-cell antigen was made possible using the antibody MBr1 (α-GH), which was raised

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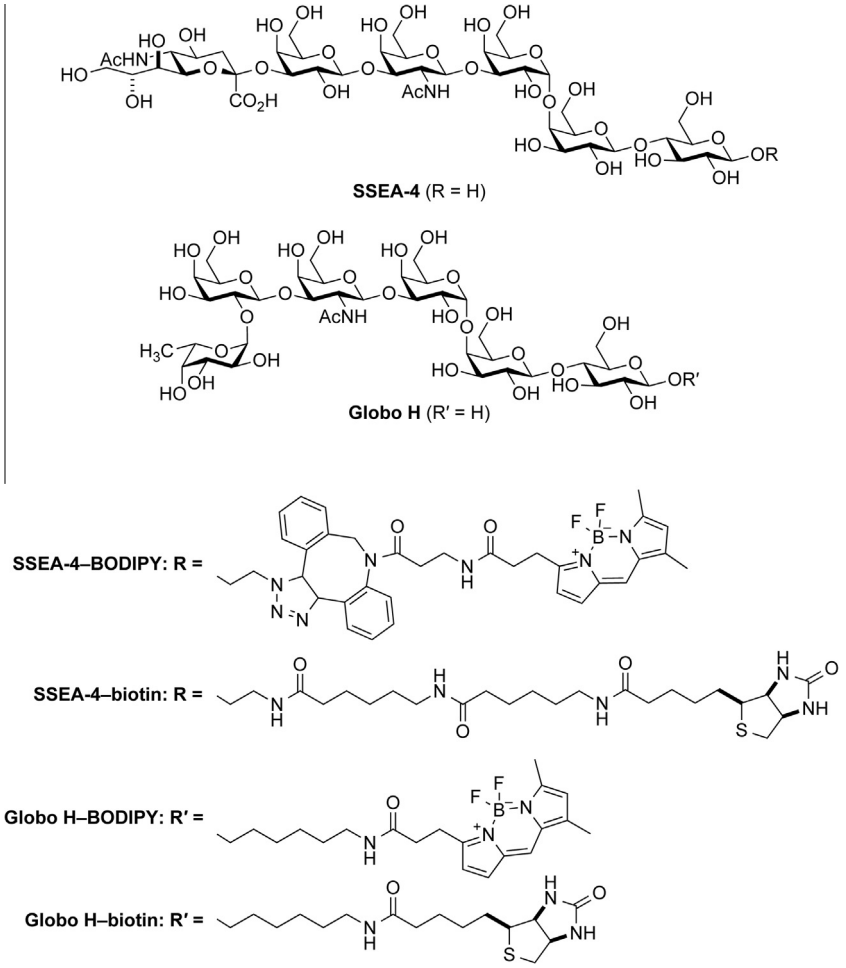

Figure 1. Chemical structures of SSEA-4, Globo H, and their conjugates with BODIPY and biotin.

specifically against MCF-7 cells.[20] Binding assays using printed microarrays demonstrated that α-GH recognizes the terminal tetrasaccharide moiety with 10-fold less affinity than the intact hexasaccharide, and does not bind to the SSEA-3 precursor of Globo H that lacks the terminal L-fucose.[21] Endogenous Globo H expression remains in the apical surface of epithelial tissue, an area somewhat inaccessible to the immune system.[21] As such, Globo H is an attractive target for cancer immunotherapy.[22]

Toward this end, chemical synthesis has been used to access the soluble moiety of Globo H on a large scale.[23] Conjugation of Globo H to other cancer-cell antigens, such as GM2, STn, TF, and KLH, can lead to potential vaccines that induce the production of IgM antibodies that direct the immune system to tumor cells.[17,24,25] Such experimental vaccines are undergoing clinical trials for the treatment of metastatic breast, prostate, lung, and ovarian cancers.[26]

The value of SSEA-4 and its antibody in stem-cell identification and therapies, and of Globo H as an epitope for cancer vaccines is unequivocal. Given the similar structures of SSEA-4 and Globo H (Fig. 1), we sought to determine the specificity of common monoclonal antibodies for each antigen. Investigations of the binding of proteins to cell-surface glycans typically involve printed microarrays, which can provide false-positives and are often less quantitative than other methods.[27] By using synthetic glycan conjugates, fluorescence polarization, and surface plasmon resonance, we provide a quantitative assessment of the affinity of α-SSEA-4 and α-GH for SSEA-4 and Globo H. Our findings provide guidance for a wide range of investigations in biomedicine.

2. Experimental

2.1. Materials

BODIPY-Fl succinimidyl ester was from Invitrogen (Carlsbad, CA). Dibenzocyclooctyne-amine was from Jena Biosciences (Jena, Germany). β-(azidoethyl)SSEA-4 (Compound No B295, Lot S270-1) and SSEA-4–biotin (Compound No B295, Lot S284-1) were provided by the Consortium for Functional Glycomics (San Diego, CA). β-(4-Pentene-1-yl)Globo H was synthesized as described previously.[23,28] α-SSEA-3 IgM monoclonal antibody (MC-613) and α-SSEA-4 IgG3 monoclonal antibody (MC-813-70) were from Thermo Fisher Scientific (Rockford, IL). α-Globo H IgM monoclonal antibody (MBr1) was from Enzo Life Sciences (Farmingdale, NY). Phosphate-buffered saline (PBS; Ca^{2+}- and Mg^{2+}-free) was from Life Technologies (Grand Island, NY). Bovine serum albumin (BSA), biotin, Tween-20, solvents, and other reagents were from Sigma–Aldrich (St. Louis, MO).

2.2. Instrumentation

The identity of synthetic compounds was confirmed by both NMR spectroscopy using a 500 MHz instrument and mass spectrometry using a ULTRAFLEX® III instrument, both from Bruker (Billerica, MA). LC/MS was performed with an LCMS-2020 instrument from Shimadzu (Kyoto, Japan). Fluorescence polarization was recorded on M1000 fluorimeter from Tecan Group