Development of cancer immune therapy by antibody-recruitment using metabolic labeling

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α-Gal epitope (Gal-α(1,3)-Gal-β(1,4)-GlcNAc) is a trisaccharide antigen which is widely expressed in many animals, but not in humans. Instead, humans produce large amount of antibody which specifically interact with α-gal, termed anti-Gal antibodies. Anti-Gal antibody is the most abundant natural antibodies found in human, consisting of 1-2% of total IgG and 3-8% of total IgM in serum.[1] To develop cancer immune therapy, our group has previously demonstrated the induction of immune responses through antibody recruitment using antibody-α-gal conjugates.[2]

In this study, we investigated α-Gal presentation on the cancer cell surface by metabolic glycan labeling (MGL) followed by copper-free click chemistry (Fig. 1). MGL has provided facile and powerful method to label cell surface with chemical tags. Raji cells were first treated with *N*-azidoacetyl-D-mannosamine (Ac_4ManNAz) to introduce azide group on the cell surface. After confirming the expression of azide group on the cell surface by bioorthogonal copper-free click chemistry using carboxyfluoroscein (FITC)-conjugated DBCO, α-gal was introduced using α-gal-conjugated DBCO (α-gal-DBCO). Furthermore, we investigated complement-dependent cytotoxicity (CDC) assay for the α-gal-presented Raji cells. The α-Gal-presented Raji cells were effectively killed by anti-Gal antibody treatment and the potency was depended on the Ac_4ManNAz and α-Gal-DBCO concentration (Fig. 2).

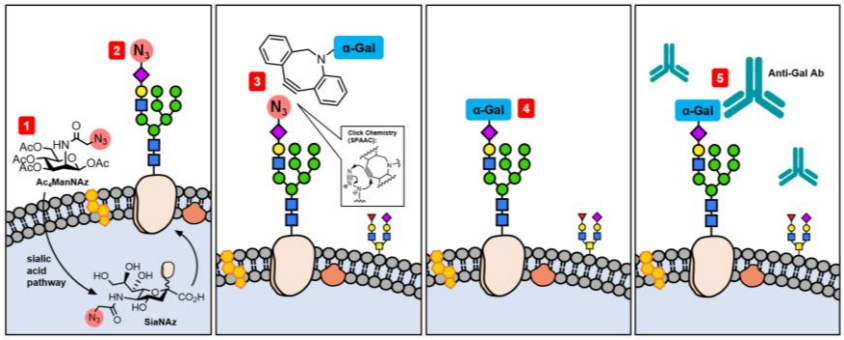

Fig. 1. Combination of MGL and antibody-recruiting strategy

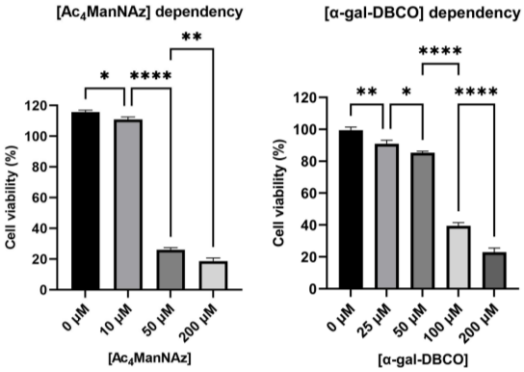

Fig. 2. CDC to α-Gal-presented Raji cell.
**p*<0.05, ***p*<0.005, *****p*<0.0001

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