**Glycoengineering of LacNAc Motifs and ABO Blood Group Antigens to Elucidate their Physiological Roles in Cancer**

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Cell-surface glycans appended to protein or lipid carriers (i.e. glycoconjugates) are involved in many important physiological processes that often occur through highly specific recognition events mediated by glycan-binding proteins (GBPs). Type 2 LacNAc (Gal-β4-GlcNAc-β3) and histo-blood group antigens are important glyco-motifs recognized by many GBPs, such as galectins, which are often involved in immunomodulatory processes. Additionally, the aberrant expression of poly-LacNAc motifs has been implicated in cancer immune evasion, whereas the expression of the histo blood-group H-antigen has been hypothesized to be involved in cancer metastasis. As such, glycan editing strategies are needed to label or modify glycans on living cells as tools to study and elucidate their physiological roles. One such approach is selective exo-enzymatic labeling (SEEL), which is designed to install specific glyco-motifs on cell-surface glycoconjugates using reporter-modified nucleotide sugar probes and recombinant glycosyltransferase enzymes. Using this approach, our group has designed two SEEL strategies to introduce reporter-functionalized type 2 LacNAc or histo-blood group glyco-motifs on the surface of cells to better understand their involvement in cancer. First, a library of UDP-GlcNAc and UDP-GalNAc derivatives bearing azide, alkyne, or diazirine functionalities was chemo-enzymatically synthesized for SEEL applications. To install LacNAc motifs onto cells, we developed a two-step SEEL approach, which first employs a concurrent sialidase treatment with the human β1,3GlcNAc-transferase B3GNT2 and reporter-functionalized UDP-GlcNAc derivatives followed by treatment with the human β1,4Gal-transferase B4GalT1 and UDP-Gal to form the disaccharide epitope. Here, we observed that B3GNT2 had limited tolerability toward donor derivatives with bulky modifications while B4GalT1 was promiscuous toward modified GlcNAc acceptor substrates, ultimately allowing us to install native and azide-modified type 2 LacNAc moieties on cells. To label H-antigen-presenting cells by SEEL, we employed reporter-functionalized UDP-GalNAc donor derivatives with the human α1,3GalNAc-transferase ABO-GTA to install unnatural histo-blood group A glyco-motifs. SEEL with ABO-GTA was amenable with all UDP-GalNAc derivatives, demonstrating broad donor substrate promiscuity by ABO-GTA. As such, we successfully labelled H-antigen presenting glycoconjugates with clickable handles (azide; alkyne) used for detection and enrichment studies. Additionally, the utility of the photo-activatable diazirine-modified probe was also demonstrated with successful photo-crosslinking to the GSL-I lectin, providing a proof-of-concept work for the covalent capture of GBP interactions with the histo blood group A-antigen. Overall, our work highlights SEEL as a broadly applicable chemical biology tool for the elucidation and study of cell-surface glycoconjugates to interrogate their involvement in health and disease.