Chemical tools for functional studies of tumor-associated glycans of MUC1

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One of the main barriers to explaining the functional significance of glycan-based changes in cancer is the natural epitope heterogeneity found on the surface of cancer cells. To help address this knowledge gap, we synthesized for the first time a MUC1-derived positional scanning synthetic glycopeptide combinatorial library (PS-SGCL) that vary in number and location of cancer-associated α-GalNAc (Tn antigen). This focused combinatorial library with defined structural complexity allowed us to evaluate the effect of neighboring residue glycosylation, glycan density, and/or the presence of unique patterns of *O*-glycan clusters on binding to lectins, thus helping us understand the multivalent carbohydrate-lectin recognition processes at the molecular level. Glycopeptide library was prepared by "tea-bag" approach using standard Fmoc-SPPS. Enzyme-linked lectin assay (ELLA) was used to screen PS-SGCL against two plant lectins, *Glycine max soybean agglutinin* (SBA) and *Vicia villosa* (VVA). Results revealed a carbohydrate density-dependent affinity trend and site-specific glycosylation requirements for high affinity binding to these lectins. The Tn antigen on Thr^{9} in the PDTR epitope of MUC1 showed the highest affinity for SBA, followed by $Thr^{16}$and Ser^{15}, and lastly, Ser^{5} and Thr^{4}, therefore, suggesting that interaction depends not only on the carbohydrate moiety but also on the peptide region surrounding the glycan site of attachment. In conclusion, PS-SGCLs provide a platform to systematically elucidate MUC1-lectin binding specificities, which in long term may provide a rational design for novel inhibitors of MUC1-lectin interactions involved in tumor spread and glycopeptide-based cancer vaccines.

Bibliographic references:

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