COMPREHENSIVE INVESTIGATIONS OF MUC1 O-GLYCOSYLATION PROCESS REVEAL INITIAL SITE PREFERENCE BY THE POLYPEPTIDE GALNAC TRANSFERASES

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Mucin1 (MUC1) is an attractive target for anticancer vaccines, due to its overexpression and highly aberrant O-GalNAc glycosylation in many prevalent cancers. The distribution and pattern of O-GalNAc glycosylation on MUC1 are essential for its biological activity, which is regulated by 20 members of the polypeptide N-acetyl-α-galactosaminyltransferase (GalNAc-T) family in human cells. However, the site-specific O-glycosylation process of MUC1 by each GalNAc-T isoform is still incompletely understood.

In this study, we successfully obtained 14 members of the human GalNAc-T family with high catalytic activity based on a simple bacterial expression system. Using MUC1-derived peptides as substrates, we comprehensively investigated the substrate specificity and site selectivity of GalNAc-Ts through chromatographic and mass spectrometric analyses. The results reveal that based on their initial acceptor sites, GalNAc-Ts can be grouped into two clusters: cluster1 enzymes preferentially initiate O-glycosylation at the GVTS motif on MUC1, and cluster2 enzymes initiate at the GSTA motif, leading to the high O-glycosylation occupancy of both motifs. Furthermore, molecular dynamics simulations and site-directed mutagenesis revealed that the initial O-glycosite preferences of GalNAc-Ts are governed by two key residues located within the catalytic flexible loop, which plays a critical role in regulating peptide substrate binding. Swapping these residues between representative members of the two clusters not only influences the initial glycosylation of MUC1, but also affects the O-glycosite selectivity of interferon alpha-2b (IFNα2b) and granulocyte colony-stimulating factor (G-CSF). In addition, we identified that the lectin domain of GalNAc-Ts is involved in the cooperative regulation of substrate selectivity. Taken together, these results establish a classification of the GalNAc-T family based on initial site selectivity and uncover the enzymatic mechanism underlying MUC1 O-glycosylation, providing insights into its aberrant O-glycosylation and roles in cancer immunomodulation.

References:

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