Heparan sulfate ligand identification in interaction with cell surface signaling receptor Robo1

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The Resource for Integrated Glycotechnology is focused on developing novel, integrated multidisciplinary technologies to examine protein-carbohydrate interactions in critical biological systems. One of our DBPs is focused on interactions between heparin sulfate (HS), a heterogeneously sulfated proteoglycan (PG), and a cell surface signaling receptor, Robo1. The integrated resource efforts produced wild type and mutant forms of Robo1 for affinity enrichment of HS fragments that were sequenced by MS-MS approaches developed in the resource (aided with informatics approaches developed in the P41 National Center for Biomedical Glycomics). Chemical synthesis of HS polymers and microarray generation of HS variants conforming to the affinity enriched HS sequences allowed further dissection of the structural determinants for ligand recognition. These studies were complemented by NMR structural studies on HS-Robo1 interactions and molecular dynamics simulations of docked complexes. Additional data on the interaction interface was developed through high-resolution hydroxyl radical footprinting. Confirmation of HS determinants required for interaction with Robo1 were examined by cell-based binding studies using mutant endothelial cell lines harboring defects in HS biosynthesis. The resulting models from the integrated studies revealed structural features of HS-protein interactions that will provide a framework for similar protein-carbohydrate interactions studies in other biological systems.