Glycosyltransferases regulating leukocyte-endothelial cell adhesion

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The glycosyltransferases (glycoTs) are a family of ~200 Golgi-resident enzymes that facilitate the biosynthesis of a variety of carbohydrate structures. This includes the sialofucosylated glycan epitopes on the surface of blood leukocytes that function as the natural ligands for E- and P-selectin expressed on the inflamed endothelium. By engaging members of the selectin family of adhesion molecules, the cell-surface glycoconjugates control the rate of leukocyte accumulation at sites of inflammation. This presentation will highlight recent progress in our laboratories that develop systems biology based methods, both theoretical and experimental, to construct, simulate and analyze glycosylation reaction networks. The focus is on the glycoTs that regulate leukocyte selectin-ligand biosynthesis. Two examples will be discussed:

In one aspect, I will present the development of a novel toolbox called GNAT (Glycosylation Network Analysis Toolbox) for the *in silico* visualization and simulation of glycosylation reaction networks. The program uses MATLAB and JAVA to integrate XML (eXtensible Markup Language) based glycan structures into glycosylation reaction networks that are described using SBML (Systems Biology Markup Language). These computer simulations rely on 'system perturbation' wet-lab experiments where one or more of the glycosylation reactions are disrupted. This is achieved in our studies using lentivirus based shRNA delivery strategies that aid the construction of human leukocytic cells that lack up to three different glycoTs. Following this methodology, results will be presented that implicate a novel role for the myeloid α1,3fucosyltransferase FUT9 during the biosynthesis of human E-selectin-ligands.

In a second aspect, I will discuss recent developments where we have advanced glycomic and glycoproteomic analysis methods to elucidate the precise glycans that form the pan-selectin-ligand at the N-terminus of the leukocyte cell surface receptor PSGL-1, P-selectin glycoprotein ligand-1. In particular, the discussion will highlight new computer programs for the analysis of glycoproteomic mass spectrometry data. Taken together with wet-lab experiments, our studies demonstrate that a competition between the core-2 GlcNAc transferase and ST6GalNAc transferase regulate the synthesis of the leukocyte selectin-ligand on P-selectin glycoprotein ligand-1.

Together, the development of theoretical and experimental analysis tools at the systems-level has aided the identification of new enzymes and pathways that regulate selectin mediated leukocyte adhesion processes. Methods to perturb these enzyme activities may be applied to control leukocyte adhesion during immunity and inflammation.