Systems Biology of Glycosylation

Sriram Neelamegham

Chemical and Biological Engineering and the NY State Center for Excellence in Bioinformatics
State University of New York at Buffalo

Leukocyte adhesion during inflammation is initiated by the binding of sialofucosylated carbohydrates expressed on leukocytes to E-/P-selectin borne on the vascular endothelium. We apply *in silico* mathematical modeling of reaction networks along with *wet lab* glycan engineering of white blood cells to better understand critical rate limiting steps regulating this cell adhesion process.

For the computational modeling, object oriented programming concepts are developed to define glycans, enzymes, reactions, pathways and compartments for modeling cellular glycosylation reaction networks. These class definitions are combined with current biochemical knowledge to define potential reaction networks that participate in the formation of the sialyl Lewis-X (sLe^X) epitope on O-glycans linked to a leukocyte cell-surface glycoprotein, P-selectin Glycoprotein Ligand-1 (PSGL-1). Subset modeling, hierarchical clustering, principal component analysis and adjoint sensitivity analysis are applied to refine the reaction network and to quantify individual glycosyltransferase rate constants. Wet-lab enzymology experiments validate estimates from computer modeling. Such analysis predicts that sLe^X on leukocytes is regulated by the concerted action of α(2,3)sialyltransferases and α(1,3)fucosyltransferases.

With the goal of refining the mathematical model, we launched a program where specific enzymes that construct human selectin-ligands are engineered using RNA interference (RNAi) methods. In this regard, while much of our knowledge regarding glycosyltransferases (glycoTs) that construct selectin-ligands is derived from transgenic mice, accumulating evidence suggests that important differences may exist between human and mouse systems. To address these discrepancies, we developed a systematic lentiviral based shRNA delivery workflow to create a panel of human leukocyte cell lines that lack up to three glycoTs. Using this, the contributions of all three myeloid α1,3 fucosyltransferases (FUT4, FUT7 and FUT9) to selectin-ligand biosynthesis was evaluated. Results show that, like mice, FUT7 and to a lesser extent FUT4 synthesize the selectin-ligands that mediates leukocyte tethering and rolling on substrates bearing P- and L-selectin. Unlike mice, however, FUT9 plays a major role during the synthesis of human E-selectin ligands with FUT7 and FUT4 having smaller contributions.

Strategies to refine the mathematical model using experimental data will be discussed.

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