Cell surface polysialic acid is part of a developmental program required for germ layer formation from human pluripotent stem cells

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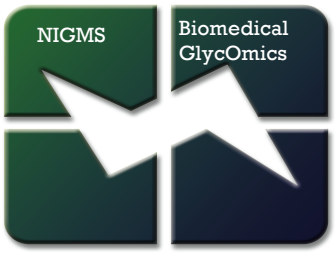

The National Center for Biomedical Glycomics has pioneered a comprehensive platform to quantify changes in glycogene expression that has been utilized in many collaborations, as well as Driving Biomedical Projects. One of the first applications of this platform was to analyze changes in glycogene expression during embryonic stem cell differentiation into the three germ layers as a first step to understand how glycan expression is regulated. Our first experiments discovered that the two enzymes responsible for polysialic acid (PSA) expression are drastically upregulated in the differentiation of both mouse and human pluripotent stem cells (hPSCs) down the three primary germ layer pathways. Polysialic acid (PSA) is a carbohydrate polymer of repeating α-2,8 sialic acid residues that decorates multiple targets, including neural cell adhesion molecule (NCAM). PST and STX encode the two enzymes responsible for PSA modification of target proteins in mammalian cells. Changes in polysialylation can be attributed to lineage-specific expression of polysialyltransferase genes; PST is elevated in endoderm and mesoderm, while STX is elevated in ectoderm. In hPSCs, PST and STX genes are epigenetically marked by overlapping domains of H3K27 and H3K4 trimethylation, indicating that they are held in a 'developmentally-primed' state. Activation of PST transcription during early mesendoderm differentiation is under control of the T-Goosecoid transcription factor network, a key regulatory axis required for early cell fate decisions in the vertebrate embryo. This establishes polysialyltransferase genes as part of a developmental program associated with germ layer establishment. We have recently collaborated with the P41 Resource for Integrated Glycotechnology, who has produced STX and PST in large amounts. These enzymes have been used by our Resource to develop further a new technology for labeling of cell surface glycoproteins by Selective Exo-Enzymatic Labeling (SEEL) to be utilized in subsequent proteomic analysis. This technology allows precise identification of cell surface glycoproteins without contamination from the rest of a cell's proteome. Moreover, use of these enzymes to produce PSA *in vitro* will facilitate collaboration with the P41 National Center for Functional Glycomics in which PSA can be coupled to one of the many glycan microarrays produced by this Center. These arrays are probed by carbohydrate binding proteins, prokaryotes and viruses sent by investigators from around the world to determine their potential binding specificities; therefore, agents that bind PSA are likely be identified.