Differential breast cancer glycosylation detected by Gas Chromatography nodal glycan analysis

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Breast cancer is a heterogeneous disease and understanding the molecular differences among these subtypes is necessary for effective clinical treatment. Traditional classification based on expression of glycoprotein receptors Her2, ER and PR is associated with clinical outcome and treatment response. Aberrant glycosylation is a hallmark of many cancers, including breast cancer, affecting cell communication, immune response and tumor invasion.

Here we investigate membrane associated glycan profile differences between the breast cancer cell lines, MCF-7 (ER+/PR+), SKBR3 (Her2+) and MDA-231 (triple negative). The membrane fraction was purified from 50 million cells in three biological replicates for each cell line. Enrichment of the plasma membrane was demonstrated by western blot.

To measure glycan content, we used glycan permethylation to pool all N-, O- glycans from the enriched plasma membrane, followed by quantitative gas chromatography as previously described [Anal. Chem. 2013, 85:2927-36]. Glycan monosaccharide identities and linkages preserved by permethylation were measured by GC-MS. Thirty three monosaccharide-and-linkage-specific glycan "nodes" which are associated with specific glycotransferases were detectable; three have not previously been described. The result support significant differences (P<0.005 in all the reported cases) in core fucosylation, 6-sialylation, bisecting GlcNAc, and beta 1-6 branching among the three cell lines. The triple negative breast cancer cell line, MDA-231, had evidence of higher sialyation compared with the other cell lines,in agreement with previous findings [Oncology reports. 2011, 25.5: 1365-1371]. MCF-7 had elevated 3, 6-Mannose which is a potential indicator of high-mannose structures [Mol. Cell. Proteomics. 2011, 10.1:M110-002717]. The Her2 positive breast cancer cell line SKBR3 has almost undetectable t-GlcNAc and 3, 6-GalNac. Bottom-up GC-MS based glycan profiling (aka glycan "node" analysis) provides a limited number of targeted analytes which individually represent a unique

glycan feature (often produced by a particular glycosyltransferase) condensed into a single analytical signal. These approaches may identify physiologically relevant differences in glycoproteins between breast cancer subtypes.