GC-MS Based Glycan Analysis in Clinical Samples

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Introduction

- Analysis in Whole Biofluids and Tissues
- Targeted Multiplexed Bottom-up Approach
- Promising Results in Lung Cancer Pilot Study

Glycans are complex, heterogeneous biological sugar polymers found on certain lipids and on protein surfaces. The construction of abnormal glycans is an established hallmark of nearly every known type of tumor cell and appears to facilitate their ability to metastasize. Additionally, there are numerous types of organ cancers as well as other inflammation-related diseases that are able to induce aberrant glycosylation of abundant blood plasma proteins.

Ablation glycantransferase (GT) expression and/or activity is the immediate upstream cause of irregular glycan production. Unfortunately, the ability to directly track the activity of GTs in human biospecimens is technically difficult and/or generally precluded in common clinical samples where GTs tend to lose activity ex vivo or are simply absent.

GTs build at glycan polymer branch-points and chain link sites in a non-template-driven, first-come-first-build manner—yet individual GTs generally exhibit strict donor, acceptor, and linkage specificity. As a result, the altered expression of a single GT within a cell often results in the production of a complex, heterogeneous mixture of unique, abnormal whole-glycan structures (Fig. 1) that are difficult to fully characterize routinely. Thus, cancer markers based on intact glycan structures are generally based on one or a few particular aberrant glycan structures (out of many)—including those based on a unique antibody or lectin epitope.

Here we have reduced to practice the idea that monosaccharide-and-linkage-specific glycan polymer chain links and branch points (a.k.a. “glycan nodes”), if broken down and quantitated from the pool of all glycan structures in a biological sample may serve as direct, 1:1 molecular surrogates of aberrant GT activity. An initial assessment of the utility of this approach for routine measurement of novel glycan-based lung cancer markers is provided.

Methods

- Does Not Require Pre-Isolation of Proteins or Glycans
- Directly Applicable to Whole Biofluids and Homogenized Tissues
- Covers N-, O-, and Link-Lyphed Glycans

Results

Blood Plasma from Central and Eastern Europe (CEE) Lung Cancer Study

- 30 Newly Diagnosed Lung Cancer Patients
- 29 Age/Gender/Smoking Status Matched Non-Healthy Controls

Conclusions/Summary

Conceptual Advantages
- Analytes are 1:1 (or near 1:1) Molecular Surrogates of Glycan Transferase Activity
- Multiplexed for Ready Creation of Biosignatures

Practical Advantages
- Small Sample Size (10 µL)
- Applied Directly to Whole Biofluids or Tissues
- No Biological Reagents

Analytical Modality is GC-MS of Small Molecules - Already Clinically Accepted & Clinically Practiced & Part of Reference Lab Infrastructures

Routinely Used for Inter-Laboratory Standardization (Shared QC sample or Standard Curves)

Analytical Methods are Targeted and Validated

- Blind to Polysialic & Hexuronic Acids and Sulfated & Phosphorylated Residues
- Current Throughput (without Automation) for 1 Analyst = 60-75 Sample/Wk

References


Abbreviations: GN = Glycan Node, GNR = Glycan Node Ratio

DC = Dihydronicotinamide Tolerance, GT = Glycosyltransferase, T2D = Type 2 Diabetes, CVD = Cardiovascular Disease

A manuscript describing this work was recently accepted for publication in Analytical Chemistry. See http://pubs.acs.org/doi/abs/10.1021/ac3035579

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