Glycomics & Lipidomics

Extended Abstract

Glycomics based biomarkers: A sweet alternative for biomarker development in liver transplantation

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Abstract

The study of ???clinical glycomics??? has led to the discovery of a multitude of glycomics-based biomarkers in a variety of disease conditions. Using DNA-sequencer assisted fluorophore assisted carbohydrate electrophoresis (DSA-FACE), our research group was able to define specific alterations of the N-glycome on the whole serum protein content. These specific glycomic signatures can be used to diagnose liver fibrosis, liver cirrhosis and non-alcoholic steatohepatitis with a high sensitivity and specificity. Recently, we also showed that the glycomic profile diagnostic for cirrhosis, is also predictive of the development of hepatocellular carcinoma in cirrhotic patients. Based on our large experience in glycomics-based biomarkers for liver disease, we explored the potential of glycomics-based biomarkers in liver transplantation. Two projects are potentially relevant for clinical practice. First, we analysed serum samples of patients after liver transplantation. In a single center cohort of 127 liver transplant patients, a specific glycomic signature in serum, seven days after liver transplantation was independently related to graft loss during the first year after liver transplantation with a hazard ratio of 7:22 ($p<0.001$; 95% CI 2.35???22.12) for graft loss at three months after liver transplantation. In a second project, the N-glycomic profile of perfusate, the fluid in which the donor liver is transported to the acceptor, was analysed and a profile predictive of primary non-function (PNF) of the liver after transplantation was discovered. PNF is an acute liver failure in the first hours after liver transplantation which requires prompt retransplantation in order to avoid patient death. This novel biomarker answers a huge medical need. Both biomarkers illustrate the validity of glycomics based biomarkers in liver disease and liver transplantation. Furthermore, the DSA-FACE technology allows easy implementation on routine laboratory equipment.

Protein glycosylation is a post-translational modification that provides proteins with structural diversity required for their interactions with other proteins and cells. The diversity of the glycome (repertoire of glycans structures in an organism) reflects their multiple functions in cells, and glycans act as an interface between the cell surface and the environment.1 Glycans regulate folding and functions of proteins and lipids.2 The glycome is affected by genetic and environmental factors, and changes have been associated with development of inflammation. Disruption of genes that encode specific glycosyltransferases causes alterations in the immune system that range from immune suppression to autoreactivity,2, 3, 4 indicating the importance of glycans in regulation of the immune response.

Glycans regulate the cellular and humoral immune responses,5 including assembly of peptide-loaded major histocompatibility complexes antigens,6,7 reorganization of T-cell receptor complexes,8 modulation of immune receptor clustering, endocytosis, receptor signaling,9 and immunoglobulin functions.10,11 Some glycan motifs serve as danger-associated molecular patterns or pathogen-associated molecular patterns.12 The immune system contains different classes of glycan-binding proteins, including C-type lectins, galectins, and siglecs, which are expressed by immune cells and can be secreted. Glycan-binding proteins regulate leukocyte trafficking, pathogen recognition, immune cell activation, and immunosuppression.13, 14, 15, 16, 17 Changes in protein glycosylation are associated with the pathogenesis of diseases, including cancer, infections, and autoimmune diseases.2,4,18 These include gastrointestinal and liver disease, such as inflammatory bowel diseases (IBDs),19 liver fibrosis20 and cirrhosis,21 nonalcoholic fatty liver disease (NAFLD),22 gastric cancer,23 colorectal cancer,24,25 and hepatocellular carcinoma.

There are many challenges to diagnosis of IBDs and liver diseases, and to predicting responses to treatment or outcomes of patients. Despite recent advances in IBD therapy, a high proportion of patients are refractory to treatment, and half of patients with ulcerative colitis (UC) do not achieve sustained remission.27 For example, despite the fact that corticosteroids are effective in controlling acute flares of IBD, 20%–30% of patients have only a partial response and 16% do not respond to corticosteroids.28 It is a challenge to select the best treatment for patients due to the lack of reliable biomarkers of response or adverse events. Biomarkers are also urgently needed to differentiate patients with liver steatosis from those with nonalcoholic steatohepatitis (NASH).

Changes in protein glycosylation might be used as diagnostic and prognostic markers, as well as targets of therapy for chronic inflammatory gastrointestinal diseases. We review how alterations in protein glycosylation affect development of chronic inflammatory gastrointestinal and liver diseases and the application of glycomic analysis to their diagnosis and prediction of patients' response to therapy and outcome.

Fibrosis is associated with increased levels of under-galactosylated IgGs.20,70,117 Profiles of protein N-glycans were generated from patient serum samples using 8-aminopyrene-1,3,6-trisulfonic acid-labeled (N-glycan) profiling on a high-throughput DNA sequencer (DNA sequencer-assisted fluorophore-assisted capillary electrophoresis).118,119 Serum samples from patients with chronic hepatitis C virus (HCV)20 or hepatitis B virus infection120,121 had gradual increases in proportions of under-galactosylated core fucosylated glycans and a decrease in the proportion of triantennary N-glycans was observed.

Other groups reported an increased core fucosylation and the presence of bisecting GlcNAc residues using matrix-assisted laser desorption/ionization, quadrupole ion trap time of flight analysis.122 Glyco-alterations, including fucosylation and desialylation, on specific serum proteins are also present during fibrosis development like α1-acid glycoprotein.123 The Fast-Lec Hepa, an automated immune assay that measures fibrosis-related alterations in glycosylation of serum hyperglycosylated galectin 3 binding protein (LGALS3BP, also called MAC-2-BP),124, 125, 126 shows an excellent correlation with fibrosis stage.

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