Combination of glycoproteomics technologies utilized in successful development of diagnostic systems for liver fibrosis, M2BPGi

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Introduction

It is widely accepted that glycan structures have tissue/cell specificities and reflect cell status such as differentiation and canceration. Recent advancement of glycoproteomics technologies are remarkable; large-scale analysis of site-specific glycome analysis, as well as ultra-sensitive detection system of glycan structures have been developed and receiving attention for their application in biological and clinical studies. We have been focusing on the development of novel glycoproteomics technologies and their application for practical use, such as development of diagnostic markers.

A strategy was established for development of clinically useful glycobiomarkers. Our unique technologies enabled comprehensive identification of qualitative changes and sensitive detection of quantitative changes of glycan structures specific to the disease cells. The principle of our strategy is as follows: The lectin microarray is used for selection of the probing lectins that can discriminate the diseased from control regions/cells. The isotope-coded glycosylation site-specific tagging (IGOT)-LC/MS analyzes diseased and control samples for large-scale identification of candidate glycoproteins that bind to the selected lectins. The selected candidate glycoproteins are prioritized by bioinformatics, and assessed for feasibility. For clinical use, the lectin-antibody sandwich ELISA system consisted of the most appropriate set of lectin and glycoprotein is established in consideration of practical applicability.

Results and Discussion

We have developed a liver fibrosis marker based on the glyco-isomer of serum M2BP (M2BPGi), which received the marketing authorization recently in Japan. We also identified feasible marker candidates for liver cirrhosis, cholangiocarcinoma, and ovarian cancer.

Recently, we reported development of two unique glyco-biomarkers for liver fibrosis and cholangiocarcinoma (Kuno A. et al. Sic Rep 2013; Matsuda A. et al. Hepatology 2010). Basically, these biomarkers are measured by the sandwich assay, but the conventional assays had two major disadvantages for clinical application: long (18 hours) reaction time and manual assay.

Through the intimate collaboration with Sysmex Co., the company developed a liver fibrosis marker kit, M2BPGi (Mac-2 binding protein glycosylation isomer), that enabled the onsite assay of liver fibrosis at the clinical sites. Here we report three successful features of our kit: 1. Establishment of

the high-throughput clinical diagnostic system (17 min measurement) using fully automated immunoassay system (HISCL-5000). 2. Construction of the standard calibrator using a recombinant glycoprotein. 3. Providing the standardized value (cut off index) capable of distinguishing between the healthy group and chronic hepatitis group (Kuno A. et al. Proteomics Clin Appl. 2013).

Very recently, we reported an interesting clinical usefulness of M2BPGi (Yamasaki K et al. Hepatology, 2014), i.e., the serum levels of M2BPGi predict the development of hepatocellular carcinoma in HCV patients.

The kit for the cholangiocarcinoma marker (e.g., MUC1 glycosylation isomer) is currently under development. We will also introduce some of the preliminary results in cholangiocarcinoma diagnosis as well as the useful and powerful instrument, HISCL for measuring the glyco-biomakers using lectins and antibodies.

Conclusions

Our approach demonstrated that the unique strategy and technologies based on glycoproteomics are effective for development of clinically useful biomarkers. Recent remarkable progress of glycoproteomics would contribute to the medical progress as well as the research activity in other scientific fields.