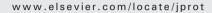
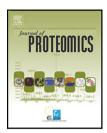


#### available at www.sciencedirect.com







## Development of quantitative plasma N-glycoproteomics using label-free 2-D LC-MALDI MS and its applicability for biomarker discovery in hepatocellular carcinoma

Takeshi Ishihara <sup>a, b</sup>, Isao Fukuda <sup>a</sup>, Atsushi Morita <sup>a,\*</sup>, Yoshihiko Takinami <sup>a</sup>, Hiroyuki Okamoto <sup>a</sup>, Shin-Ichiro Nishimura <sup>c</sup>, Yoshito Numata <sup>a</sup>

#### ARTICLEINFO

### Article history: Received 3 March 2010 Accepted 12 June 2011 Available online 17 June 2011

Keywords:
Biomarker
Glycoproteomics
2-D LC-MALDI MS
Hepatocellular carcinoma

#### ABSTRACT

There has been rapid progress in the development of clinical proteomic methodologies with improvements in mass spectrometric technologies and bioinformatics, leading to many new methodologies for biomarker discovery from human plasma. However, it is not easy to find new biomarkers because of the wide dynamic range of plasma proteins and the need for their quantification. Here, we report a new methodology for relative quantitative proteomic analysis combining large-scale glycoproteomics with label-free 2-D LC-MALDI MS. In this method, enrichment of glycopeptides using hydrazide resin enables focusing on plasma proteins with lower abundance corresponding to the tissue leakage region. On quantitative analysis, signal intensities by 2-D LC-MALDI MS were normalized using a peptide internal control, and the values linked to LC data were treated with DeView™ software. Our proteomic method revealed that the quantitative dynamic ranged from 10<sup>2</sup> to 10<sup>6</sup> pg/mL of plasma proteins with good reproducibility, and the limit of detection was of the order of a few ng/mL of proteins in biological samples. To evaluate the applicability of our method for biomarker discovery, we performed a feasibility study using plasma samples from patients with hepatocellular carcinoma, and identified biomarker candidates, including ceruloplasmin, alpha-1 antichymotrypsin, and multimerin-1.

© 2011 Elsevier B.V. All rights reserved.

#### 1. Introduction

Proteomic approaches using human plasma samples are now being used to discover biomarkers against a number of clinical diseases [1–4]. However, it is very difficult to find new biomarkers from plasma samples by these proteomic approaches because the "classical proteins" account for 99% of total plasma proteins with a high dynamic range of 10 orders of magnitude

[5]. In addition, the plasma proteins are known to have many variations of posttranslational modifications, such as phosphorylation, glycosylation, glycation, etc. Several effective procedures have been reported for improving these issues by removing high-abundance proteins from the plasma by affinity chromatography, e.g., multiple affinity removal system (MARS) [6], and concentrating the proteins focusing on a specific modification of interest coupled with proteomic analysis [7–9].

E-mail address: atsushi.morita@shionogi.co.jp (A. Morita).

<sup>&</sup>lt;sup>a</sup> Discovery Research Laboratories, Shionogi & Co. Ltd., Fukushima-ku, Osaka 553–0002, Japan

<sup>&</sup>lt;sup>b</sup> Shionogi Innovation Center for Drug Discovery, Shionogi & Co. Ltd., Kita-ku, Sapporo 001–0021, Japan

<sup>&</sup>lt;sup>c</sup> Graduate School of Advanced Life Science, Hokkaido University, Kita-ku, Sapporo 001–0021, Japan

<sup>\*</sup> Corresponding author at: Discovery Research Laboratories, Shionogi & Co. Ltd., 12–4, Sagisu 5-chome, Fukushima-ku, Osaka 553–0002, Japan. Tel.: +81 6 6458 5861; fax: +81 6 6458 0987.

Protein glycosylation is one of the major posttranslational modifications in plasma, which is considered to have a number of functions, including roles in protein stability, cellto-cell communication, and adhesion [10,11]. Some protein biomarkers in cancer are known to have glycosylation modifications, such as AFP in hepatocellular carcinoma, CA125 in ovarian cancer, Her2/neu in breast cancer, and prostate-specific antigen in prostate cancer [12-14]. The carbohydrate moieties of plasma proteins are changed in the case of cancer and rheumatoid diseases, and these changes are considered to be useful markers [15,16]. In addition, the differences in carbohydrate structures on total serum proteins demonstrated the possibility of the efficient detection of patients with hepatocellular carcinoma [17]. These findings suggest that changes in the glycosylation modification profiles (expression levels and carbohydrate modification patterns) of plasma glycoproteins are closely linked to disease

Proteomic analysis is usually carried out by separating proteins or peptides prior to their quantification and identification by MS. Separation is mainly performed by gel-based techniques, such as 2-DE [18] and LC-based techniques, such as ionic exchange and reverse phase column chromatography [19]. LC-based separation is easier and exhibits high-throughput performance because samples globally digested with trypsin can be used and automated on-line LC systems can be constructed. In the case of plasma protein separation, gelbased technology has some disadvantages with respect to spot resolution because of the heterogeneity of posttranslational modifications of plasma proteins.

In some types of MS quantification, LC-separated peptides are quantified by labeling using stable isotopes, such as iTRAQ reagents [20]. Although this technology is very useful and effective, the labeling reagents are expensive and the labeling steps generate additional complexity, which reduces quantitative accuracy. Theoretically, a label-free method would be suitable for comprehensive analysis because sample preparation without labeling steps reflects the non-biased diversity of the original protein levels. There have been many previous reports regarding discovery of biomarkers by label-free methods using LC-ESI MS systems with continuous data acquisition and analysis [21]. On the other hand, there have been only a few reports describing label-free quantification by LC-MALDI MS [22-24]. LC-MALDI MS methods are not used widely due to the discontinuous separation on LC (or LCs) needed to drop onto the MALDI target plate followed by integration and rearrangement of all of the intermittent MS data by analytical informatics. Thus, LC-MALDI MS methods have some issues for systematic and automatic high-throughput data analysis in contrast to LC-ESI MS methods. However, the peptides/ proteins identified using MALDI MS are different from those determined by ESI MS [25,26] because of the differences in ionization type. Therefore, it may be possible to find unknown novel biomarkers when using LC-MALDI MS for biomarker

In this study, we developed a new methodology for largescale N-glycoproteomic analysis combined with a quantitative label-free 2-D LC-MALDI MS system. Using this method, we analyzed plasma samples from patients with hepatocellular carcinoma as a feasibility study to evaluate its applicability for the discovery of new biomarkers in human plasma.

#### 2. Materials and methods

#### 2.1. Chemicals

Dithiothreitol, iodoacetamide, and formic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). RapiGest SF was from Waters (Milford, MA) and sequencing-grade trypsin was from Promega (Madison, WI). PNGase F was purchased from New England Biolabs Inc. (Ipswich, MA). Ammonium carbonate and [Sar¹, Thr³]-angiotensin II were purchased from Sigma-Aldrich (St. Louis, MO). Alpha-1-antichymotrypsin monoclonal antibody was from LifeSpan Biosciences (Seattle, WA). Blocking reagent and horseradish peroxidase-conjugated anti-mouse IgG antibody were from GE Healthcare (Uppsala, Sweden). HPLC-grade acetonitrile was purchased from Merck Chemicals (Darmstadt, Germany). Ammonium acetate was from Kanto Chemical (Tokyo, Japan). Trifluoroacetic acid was obtained from Nacalai Tesque (Kyoto, Japan). Other chemicals were purchased from Sigma-Aldrich.

#### 2.2. Human plasma samples

Human EDTA-plasma samples from healthy volunteers were used for development and evaluation of the enrichment of N-glycopeptides and 2-D LC-MALDI MS. To perform a feasibility study, human EDTA-plasma samples from six patients with hepatocellular carcinoma (HCC) and three healthy volunteers were purchased from Asterand Plc. (Royston, UK). All plasma samples (100  $\mu L$ ) were passed through MARS-human 7 LC columns (4.6×100 mm; Agilent Technologies, Santa Clara, CA) using ÄKTAexplorer 10S system (GE Health-care) to deplete the seven high-abundance proteins in plasma: albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, and fibronectin.

#### 2.3. Enrichment of N-glycopeptides

An Affi-Gel Hz Immunoaffinity Kit (Bio-Rad Laboratories, Hercules, CA) was used for enrichment of N-glycopeptides. The MARS-depleted plasma samples were diluted to  $500 \, \mu L$ with the coupling buffer and oxidized with 10 mM sodium periodate at room temperature for 1 h in the dark. Excess oxidizer was removed using an equilibrated NAP-5 column (GE Healthcare) with the coupling buffer. The oxidized proteins were collected and allowed to react with 100 µL of hydrazide resin slurry at room temperature overnight. The resin was washed three times each with methanol and 8 M urea in 100 mM NH<sub>4</sub>HCO<sub>3</sub>. The reduction reaction was performed in 50 mM dithiothreitol at 37 °C for 90 min and the alkylation reaction in 25 mM iodoacetamide at 37 °C for 30 min, followed by exposure to 100 mM NH<sub>4</sub>HCO<sub>3</sub> containing 8 M urea in the dark. After washing the resin three times with  $1\,\mathrm{M}$  urea in 100 mM NH<sub>4</sub>HCO<sub>3</sub>, the reduced and alkylated N-glycoproteins on the resin were digested with 10 µg of trypsin in 100 mM NH<sub>4</sub>HCO<sub>3</sub> containing 1 M urea and 0.1% RapiGest SF at 37 °C overnight. The resin was washed three times with 2 M sodium

## Download English Version:

# https://daneshyari.com/en/article/10556125

Download Persian Version:

https://daneshyari.com/article/10556125

<u>Daneshyari.com</u>