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Applications of proteomics and mass spectrometry to laboratory medicine

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Proteomics can be applied to laboratory medicine basically in two ways; 1) an attempt to discover and identify new diagnostic markers by comprehensive proteome analysis, and 2) taking advantage of proteomic profiles for diagnostic purposes.

Early diagnosis of hepatocellular carcinoma (HCC) greatly improves its prognosis. However, the pathological distinction between benign and malignant tumors is not necessarily an easy task and novel immunohistochemical markers are necessary. Using agarose two-dimensional fluorescence difference gel electrophoresis, we analyzed surgically resected HCC tissues. The fluorescence volumes of 48 spots increased and 79 spots decreased in tumor tissues compared with adjacent nontumor tissues, and 83 proteins were identified by mass spectrometry. Immunoblot confirmed that the expression of clathrin heavy chain (CHC) and Ku86 significantly increased, whereas formiminotransferase cyclodeaminase (FTCD), rhodanase, and vinculin decreased in tumor. The protein expression in tumor and nontumor tissues was further evaluated by immunostaining. Interestingly, CHC and FTCD expression was strikingly different between tumor and nontumor tissues, which could make substantial contributions to the early pathological diagnosis of HCC[1].

Gastric cancer is divided into two major histological types: diffuse (undifferentiated) and intestinal (differentiated). While the incidence of the diffuse-type gastric cancer (DGC) has been increasing, its early diagnosis is difficult and no practical biomarkers are known. We conducted integrated analysis of cancer secretomics together with publicly available bioinformatics resources; by this combination of proteomics and transcriptomics, we identified growth/differentiation factor 15 (GDF15) as a potential molecule involved in DGC progression[2].

The most successful application of proteomic profiles for laboratory medicine is MALDI-TOF MS based bacterial identification.[3] Until recently, clinical microbiology laboratories relied heavily on conventional methods that often initially involve culturing, followed by morphologic phenotyping and cumbersome biochemical testing. These procedures are time-consuming, laborious, and require well-trained technicians for correct interpretation of the results. By contrast, proteome analysis of bacterial proteins by MALDI-TOF MS is a quick, simple and reliable method for identification of microorganisms. Indeed, there has been a revolutionary shift in clinical diagnostic microbiology.

A small portion of a cultured bacterial colony is directly subjected to MALDI-TOF MS. The final mass spectral signature is composed of peaks originating from bacterial proteins including mainly ribosomal proteins. The spectral profiles are compared with a library of known spectra and a result is generated within 10 min instead of almost one day when conducted by the traditional methods.
The rate of successful identification at the species levels, however, is still not necessarily satisfactory. One of the reasons for the problematic identifications may be incomplete databases. We have found that in-house refinement of commercial database can be achieved relatively easily and effectively by incorporating MS spectra of clinical isolates obtained in each clinical laboratory , which in turn could reduce problematic identifications[4]. Also, for some pathogens such as Nocardia,

extensive pretreatment of the samples is mandatory to obtaine appropriiate proteomic profiles[5]. Progress has been made for direct analysis of bacteria in three types of specimens; urine, cerebrospinal fluid (CSF), and blood. We reported on a rapid identification of a pathogen in a case with bacterial meningitis by MALDI-TOF MS based method[6]. In addition to identification of bacteria, other uses of MS in clinical microbiology are being actively investigated particularly for detection of antibiotic resistance. For this purpose, LC/MS/MS could be useful as well.

Mass spectrometry (MS) is a powerful analytical tool used in an increasing number of clinical laboratories around the world. LC/MS/MS has been used for newborn screening, toxicology, therapeutic drug monitoring, endocrinology, and more recently for measurement of targeted proteins and peptides. Although immunoassay-based methods have dominated clinical chemistry analyses for many years, LC/MS/MS offers significant advantages over immunoassays in terms of analytical sensitivity and specificity. Moreover, LC/MS/MS enables the simultaneous analysis of multiple target analytes.

The quantification of serum 25-hydroxyvitamin D [25(OH)D] as an indicator of vitamin D status has been traditionally conducted by immunoassays, yet LC/MS/MS would allow more accurate determination. Furthermore, LC/MS/MS would allow simultaneous measurement of multiple analytes. The aim of this study was to develop and validate an LC/MS/MS method to simultaneously measure four vitamin D metabolites (25(OH)D3, 3-epi-25(OH)D3, 25(OH)D2, and 24,25(OH)2D3) in serum for clinical laboratory applications. Serum samples were first prepared in a 96-well supported liquid extraction plate and the eluate was derivatized using the Cookson-type reagent 4-(4'-dimethylaminophenyl)-1,2,4-triazoline-3,5-dione (DAPTAD), which rapidly and quantitatively reacts with the s-cis-diene structure of vitamin D metabolites. The derivatized samples were subjected to LC-MS/MS, ionized by electrospray ionization (positive-ion mode), and detected by selected reaction monitoring. The lower limits of quantification for 25(OH)D3, 3-epi-25(OH)D3, 25(OH)D2, and 24,25(OH)2D3 were 0.091, 0.020, 0.013, and 0.024 ng/mL, respectively. The accuracy values and the extraction recoveries for these four metabolites were satisfactory. Serum 25(OH)D levels determined by our LC/MS/MS were compared with those obtained by conventional radioimmunoassay (RIA) that cannot distinguish 25(OH)D3 from other structurally similar metabolites including 25(OH)D2. Indeed, the values obtained by the RIA method exhibited a mean bias of about 8.35 ng/mL, most likely as a result of cross reaction of the antibody with low-abundance metabolites, including 24,25(OH)2D3. Various preanalytical factors, such as long sample sitting prior to serum separation, repeated freeze-thaw cycles, and the presence of anticoagulants, had no significant effects on these determinations. This high-throughput LC/MS/MS simultaneous assay of the four vitamin D metabolites 25(OH)D3, 3-epi-25(OH)D3, 25(OH)D2, and 24,25(OH)2D3 required as little as 20 μL serum. This method will aid further understanding of low-abundance vitamin D metabolites, as well as the accurate determination of 25(OH)D3 and 25(OH)D2[7].

It is obvious that LC/MS/MS has advantages over immunoassays in terms of specificity. As a matter of fact, the editorial board of the Journal of Clinical Endocrinology and Metabolism stated in 2015 that “manuscripts reporting sex steroid assays as important endpoint must use MS-based assays”. Although still controversial, it is very likely that this statement will extend to other analytes as well in the near future.

Although LC/MS/MS has a number of advantages over traditional immunoassay, MS applications in clinical chemistry laboratories, however, has remained very limited because of substantial

challenges. These challenges include the high capital cost of equipment, requirements for a skilled labor force, lack of standardization and automation, and regulatory uncertainty and reimbursement issues.

To meet with rapid progress in applications of MS to laboratory medicine, it is mandatory to foster MS oriented personnels. The Japanese Society for Biomedical Mass Spectrometry has started to certify medical mass spectrometrist since 2013. As of June 2017, a total of 234 have been certified and the majority of them are medical technologists. It is obvious that MS is now one of the key technologies in laboratory medicine.

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