Proc. Indian Natl. Sci. Acad. **B71** No. 1 pp 1-8

Protein Profiling by SELDI–TOF ProteinChip System for Disease Marker Discovery

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(Received on 2nd September 2004;after revision Accepted on 24 September 2005)

Protein profiling is a powerful method for biomarker discovery. Protein profiling can be accomplished by different technologies including two-dimensional electrophoresis (2-DE) and protein microarray screening. 2-DE is an established technique that is still considered as one of the best options for protein profiling. Recent advancement in protein profiling for biomarker identification by utilizing protein microarrays, especially the ProteinChip/SELDI-ToF system, enables researchers to overcome problems in identifying low abundance proteins from complex biological samples. In this review, we summarize recent progress in biomarker identification through proteomic profiling, with emphasis on how ProteinChip/SELDI-ToF technology fits into protein profiling and biomarker discovery. The aim is to provide a reference for the researchers seeking new analytical tools that are now available in contemporary biological profiling and medical study.

Key Words: Protein profiling, Proteomics, Proteinchip array system, Biomarker identification

Introduction

Proteins, not genes, are functional molecules in cells and represent important targets for the clinical therapeutic intervention. Protein expression is subjected to post-translational modification that exists in hundreds different types and the protein-expression level changes under different biological conditions. Disease development is a multi-step process involving different biological pathways. Many proteins are altered in expression levels and/or expression types such as modification during this process. These altered proteins can be detected in tissue, blood, urine, or other body fluids and thus may be used as biomarkers to provide indicators for the disease. Biomarkers are therefore usually disease-associated proteins that can be quantitatively measured for disease diagnosis, staging, prognosis, and treatment monitoring. An ideal biomarker should have high specificity for a certain disease condition; this kind of biomarkers is rare, however. Most of biomarkers are those proteins expressed by many different types of diseases but with variant expression levels from type to type. Combining several unspecific biomarkers together may lead to a specific index for a particular disease. In this regard, proteomics offers a suitable and powerful technological platform for the biomarker identification, characterization, and evaluation by globally examining the protein expression profiles under given conditions (He & Chiu 2003). Protein profiling is a major approach in proteomics for the large-scale identification, characterization, and quantitation of proteins expressed in a cell line, tissue or serum. Two-dimensional electrophoresis (2-DE) is an established technique for protein profiling and 2-DE has been extensively used for profiling protein expression since introduced. Other technologies such as

Abbreviations used: *2-DE*, 2-dimensional gel electrophoresis; *MS*, mass spectrometry; *LC-ESI MS/MS*, liquid chromatography-electrospray ionization tandem MS; *MALDI-TOF*, matrix-assisted laser desorption ionization time of flight; *SELDI*, surface enhanced laser desorption ionization; *MW*, molecular weight; pI, isoelectric point; *HBV*, hepatitis B virus; *HCC*, hepatocellular Carcinoma

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2-DIGE and ICAT have also been widely used for proteome profiling in proteomics. Recently, a new technology of protein profiling, surface enhanced laser desorption/ionization mass time of flight (SELDI-ToF) ProteinChip system is getting popular for biomarker identification. This new technique provides an easy, effective and sensitive approach of protein profiling for identifying biomarkers especially from crude samples such as serum and urine. This report attempts to summarize the recent progress in the application of SELDI-ToF ProteinChip system in biomarker identification with different kinds of diseases.

SELDI-ToF ProteinChip System

SELDI-ToF mass spectrometry (MS) is a patented technology introduced by Hutchens and Yip (Hutchens & Yip 1993). SELDI-ToF ProteinChip Array System is built based on the SELDI-ToF MS technology, combining with protein separation on chromatographic or biochemical surfaces. Surface modification on the ProteinChip array is another key technology in the system. Array surfaces are individually modified either chemically by varying chromatographic properties (e.g. anion exchange, cation exchange, metal affinity and reverse phase) or biochemically by attaching a target protein such as antibody for binding an antigen (figure 1)(Haleem et al. 2002). Depending on the types of modified chip surfaces, different groups of proteins can be captured on the surfaces and thus protein separation can be achieved. The captured proteins can be detected by the SELDI-ToF MS and the corresponding MS spectra are generated. By comparing the MS spectra of control samples, proteins with altered expressions in diseased specimens can be identified. Using this SELDI-ToF ProteinChip Array System, subsets of proteins can be selectively profiled, separated and characterized directly from crude biological materials such as serum, blood, intestinal fluid, urine, cell lysates and cellular secretion products.

Compared to 2-DE that is commonly used for protein profiling, SELDI-ToF ProteinChip System has a number of advantages. For example, proteins which have molecular weights (MW) less than 10 kDa and isoelectric points (p*I*) less than 3 and higher than 11 cannot be well separated or displayed in 2-DE gels. SELDI-ToF ProteinChip technology is much useful in analyzing proteins with low MWs covering the entire p*I* range. Other advantages of ProteinChip SELDI-ToF include its high sensitivity for samples with limited amounts and straightforwardness especially in examining crude body fluids such as serum samples. Table 1 gives out the comparison of different protein profiling technologies (Willard & Scott 2004). On the other hand, as many other new technologies, SELDI-ToF ProteinChip System has its limitation. It mainly aims at providing the differences between specimens in low MWs proteins and it is difficult to identify proteins directly. Protein or peptide identification is not straightforward and the *m/z* peaks may contain more than one species. The variation in sample treatment leads lab-to-lab comparison to be inconsistent. In addition, the cost of the SELDI-ToF ProteinChip System is relatively high.

The procedure to perform an experiment with SELDI-ToF ProteinChip System is simple. A few microliters of samples are loaded onto the ProteinChip surfaces under specific binding conditions. By applying corresponding buffers to wash out unbound proteins and interfering substances such as salts and

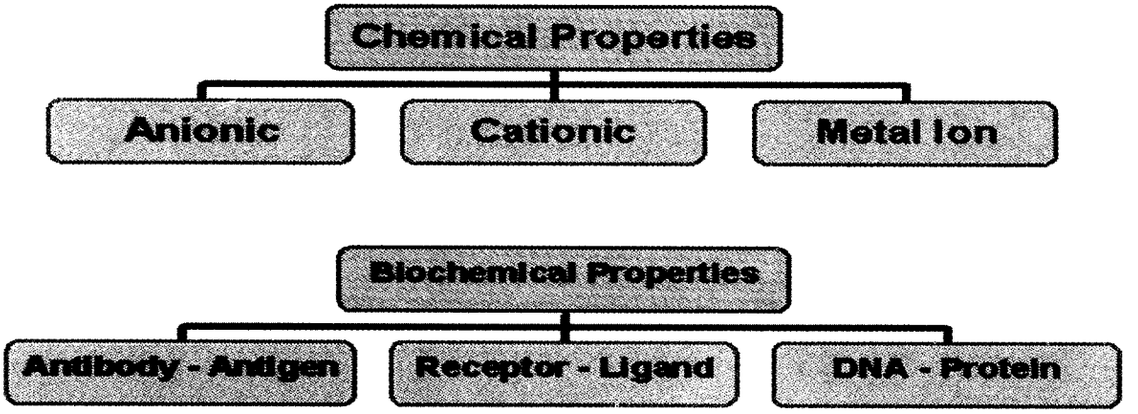

Figure 1. The arrays constitute 8 or 16 sports comprised of a specific chromatographic surface, including chemical and biochemical modified properties. Chemical modified surfaces can retain proteins based on a specific property such as hydrophobicity, charge, etc, while biochemical modified surfaces can be used to isolate a specific protein or a functional class of proteins.

Table 1: Comparison of Different Protein Profiling Technologies

	2-DE	2-DIGE	ICAT	SELD1
Separation	Electroporesis: IEF PAGE	Electroporesis: IEF PAGE	LC/LC of peptides	Binding different chips, MS
Quantitation	Densitometry of stains	Imaging of Cy3 and Cy5 normalized to Cy2	Heavy and light tags	Comparison of MS peaks
Identification	PMF	PMF	MS/MS	Difficult, need second MS
Advantages	Low cost, easy	High quality	Wide range of proteome	Eeasiest MS instrumentation, High sensitivity

detergents, proteins that have affinities with the modified surfaces can be retained. After being allowed to dry, a solution containing energy absorbing molecule (EAM) is added to help ionization of the proteins and the array is analyzed through a ProteinChip Reader (SELDI-ToF MS) to produce a mass spectrum with MWs for the bound proteins. By proceeding and comparing normal control and diseased samples in parallel, proteins exhibiting altered MS intensities (protein expressions) can be identified as potential biomarkers.

Application of SELDI-ToF ProteinChip System to Biomarker Identification

The major applications of the ProteinChip Array System are: (a) protein expression profiling; (b) protein purification development and monitoring; (c) protein characterization and identification; and (d) protein interaction assays (Yip & Lomas 2002). SELDI-ToF ProteinChip technology provides an effective platform to profile proteins and polypeptides for biomarker identification. Currently, cancer is the primary disease that received extensive studies by the ProteinChip array system. Other diseases that have also been addressed in the protein profiling include cardiovascular diseases and neurological disorders.

Cancer

Prostate cancer

Prostate-specific antigen (PSA) is probably the best example for using as a single biomarker in aid of the diagnosis of prostate cancer (PCA). However, its low specificity in distinguishing PCA from benign prostatic hyperplasia (BPH) limits its utility as an early detection biomarker. By combing SELDI-ToF ProteinChip technology with bioinformatics, multiple proteins as signature proteomic patterns for distinguishing cancer from non-cancer have been analyzed and evaluated as an index of biomarkers for accurate detection of prostate cancer (Adams et al. 2002, Petricoin et al. 2002, Qu et al. 2002, Banez et al. 2004, Lij et al. 2004, Gretzer et al. 2004). Using SELDI-ToF ProteinChip and tissue microdissection techniques, a specific 4.3 kDa MS peak was identified with an increased intensity (expression) in the prostate tumor stroma and glands compared to normal prostate proper and transitional zone stroma and glands (Wellmann et al. 2002). Besides being a potential biomarker, the identification of the tumor specific protein provided opportunities to understand molecular events underlying prostate carcinoma development. In addition, the Early Detection Research Network (EDRN) validation study for SELDI for prostate cancer has presented a rigorous evaluation for the new detection method for prostate cancer (Grizzle et al. 2004).

Lung cancer

By using a specifically designed anti-Fus1-antibody-capture ProteinChip array, Uno and other researchers have identified that myristoylation is required for Fus1-mediated tumor-suppressing activity and suggested a novel mechanism for the inactivation of tumor suppressors in lung cancer and a role for deficient posttranslational modification in tumor suppressor-gene-mediated carcinogenesis (Uno et al. 2004). Also, a number of potential tumor markers have been found in tissue and sera by ProteinChip Array System coupled with an artificial intelligence classification algorithm and laser capture microdissection technologies (Xiao et al. 2004, Zhukov et al. 2003). To study the arsenic-induced cell transformation of lung cancer, we have performed a proteomic analysis by using SELDI-ToF ProteinChip technology. Differential protein profiling between control and arsenic-induced transformed lung cells distinguished several prominent protein peaks, indicating the potential of SELDI proteomics for identifying biomarkers for lung cancer (He et al. 2003).

Breast cancer

SELDI-ProteinChip has also been applied as a sensitive tool to detect new biomarkers in serum for breast cancer (Lij et al. 2002, Vlahou et al. 2003). As a unique case, the biomarker discovery in nipple aspirate fluid (Pawaletz et al. 2001, Coombes et al. 2003) by the ProteinChip system featured an even special significance as it may lead to a potential non-invasive method in the diagnosis of the disease. In a study headed by Laronga et al. 2004, three potential

applications, which are breast cancers prior to surgery and post-surgery, BRCA-1 mutation carriers and sentinel lymph node positive and sentinel lymph node negative patients were evaluated and the results indicated the potential of SELDI-ToF ProteinChip Array System profiling approach for developing new diagnostic and prognostic assays for breast cancers.

Liver cancer

Since chronic infection with hepatitis B or C virus (HBV, HCV) is a major risk factor for the development of hepatocellular carcinoma (HCC), most proteomic studies concerning biomarker identification focused on the HBV- and HCV- related HCC (Seow et al. 2001, Steel et al. 2001, Le Naour et al. 2002). SELDI-ToF ProteinChip System has also been recently employed to identify biomarkers for liver cancer and cirrohosis(Paradis et al. 2004, Poon et al. 2003, Zhu et al. 2004). In particular, Poon et al have successfully used two types of ProteinChip arrays to comprehensively profile serological proteins to identify proteomic signatures for detection of HCC and its subtypes (Poon et al. 2003). By applying bioinformatics and cluster analyses, the tumor-specific proteomic features obtained from the protein profiling were evaluated and proved useful for detection and classification of HCC liver cancers. Most recently, two independent laboratories reported the utilization of SELDI-ProteinChip system to identify serum biomarkers for detection of HCV-related liver diseases progression to HCC and demonstrated the encouragement and high potential of applying SELDI peaks to classify chronic liver diseases (Paradis et al. 2005, Schwegler et al. 2005).

In order to search for biomarkers that can be used to predict HCC incidents in HBV-infected patients, we have employed SELDI-ProteinChip system to perform protein profiling for serum samples from healthy subjects and patients in HBV immune tolerance phase (early infection), HBV immune clearance phase (chronic infection) and with HBV-induced HCC liver cancer (unpublished results). Several proteomic signals with consistent trends of changes in MS intensity in regard with disease aggravation were identified. More clinical specimens are recruited for screening to test the sensitivity and specificity and the possibility of using these proteomic features as a biomarker index for predicting HCC incidence is being evaluated.

Digestive cancers

Researchers have utilized ProteinChip array system in protein profiling and biomarker identification for various types of digestive carcinomas. Helicobacter pylori is one of the most prevalent human pathogens and is the aetiological agent of gastritis, peptic ulcer disease and gastric malignancies. Investigators at Lund University have used ProteinChip technology to analyze Helicobacter pylori-related proteome and found that two protein peaks increased their expressions under bile stress (Hynes et al. 2003). In the study of colorectal carcinomas, Krieg et al. identified some specific protein bands in the 3.4 to 3.6 kDa range showing much differences between colon tumor epithelium and associated stroma by coupling microdissection with SELDI (Krieg et al. 2004). Another group of scientists evaluated colon cancer cells and normal colon cells to demonstrate that the ProteinChip platform could perform the whole process of biomarker discovery from screening to evaluation of the identified markers (Shiwa et al. 2003).

So far, pancreatic adenocarcinoma is the fifth leading cause of cancer death. The most widely used serum marker for pancreatic cancer, CA 19-9, is not sufficiently accurate for screening test, especially for identifying patients with small surgically resectable cancers. Researchers from Johns Hopkins Medical Institutions used SELDI technology to perform a case-control study, analyzing serum samples from three groups of subjects including pancreatic adenocarcinoma, nonmalignant pancreatic diseases and healthy controls (Koopmann et al. 2004). They determined a minimum set of protein peaks able to discriminate between patient groups and used the unified maximum separability algorithm to compare the performance of the individual marker in the panels alone or in conjunction with CA19–9. A sensitivity of 78% and specificity of 97% were obtained with pancreatic cancer from healthy controls, which is much superior than CA19–9 alone.

Other cancers

Other cancers that received ProteinChip System analysis for protein profiling and biomarker discovery include endometrial carcinoma, (Yang et al. 2004) renal cell carcinoma, (Wu et al. 2004, Tolson et al. 2004, Won et al. 2003) head and neck cancers, (Soltys et al. 2004, Wadsworth et al. 2004) cervical cancer, (Wong et al. 2004), ovarian cancer, (Jacobs & Menon 2004, Vlahou et al. 2003, Ardekani et al. 2002, Petricoin et al. 2002), melanoma, (Wilson et al. 2004), neuroblastoma (He et al. 2005) and follicular lymphoma (Lin et al. 2004). Similar strategy was implemented in which proteins from tumor and control samples were profiled and compared in parallel and protein alterations in expression were further evaluated with masked specimens to test the potentials as biomarkers. Among these new progresses of cancer profiling by

applying SELDI-TOF ProteinChip Array System, the most worth-emphasized project utilized a serological proteomic pattern generated through SELDI technology as a screening tool to classify 116 masked serum samples, which yielded a sensitivity of 100% and specificity of 95% in discriminating ovarian cancer from non-cancer (Petricoin et al. 2002). Recently, scientists cooperating from different universities and research centers developed a five-center case-control study to analyze 153 patients with invasive epithelial ovarian cancer, 42 with other ovarian cancers, 166 with benign pelvic masses, and 142 healthy women (Zhang et al. 2004). Data from patients with early stages of ovarian cancer and from healthy women in two centers were analyzed independently and the results were cross-validated to discover potential biomarkers. The results were further verified using the samples from other two remaining centers. After protein identification, potential biomarkers were tested by immunoassay on samples in the fifth center, which include 41 healthy women, 41 patients with ovarian cancer, and 20 each with breast, colon, and prostate cancers. Three biomarkers were eventually identified as follows: (*a*) apolipoprotein A1 (down-regulated in cancer); (*b*) a truncated form of transthyretin (down-regulated); and (*c*) a cleavage fragment of inter-α-trypsin inhibitor heavy chain H4 (up-regulated). In an independent validation to detect early stage invasive epithelial ovarian cancer from healthy controls, the sensitivity of a multivariate model combining the three biomarkers with CA125 [74% (95% CI, 52–90%)] was higher than that of CA125 alone [65% (95% CI, 43–84%)] at a matched specificity of 97% (95% CI, 89–100%). When compared at a fixed sensitivity of 83% (95% CI, 61–95%), the specificity of the model [94% (95% CI, 85–98%)] was significantly better than that of CA125 alone [52% (95% CI, 39–65%)]. These biomarkers demonstrated the potential to improve the detection of early stage ovarian cancer. This is a successful cross-validated demonstration of developing potential biomarkers by combining SELDI-ToF ProteinChip Array System with other proteomic technologies.

Other Diseases

Many other diseases have been investigated using ProteinChip Array System. Alzheimer's disease (AD) is an increasingly prevalent type of neurodegenerative dementia, accounting for approximately 50-60% of overall cases of dementia among people over 65 years old. Pathological AD hallmarks include cerebral beta-amyloid (Abeta) deposition, amyloid accumulation, and neuritic plaque formation. However, the characters of these proteins related to AD remain uncertain. Researchers have dedicated to the AD study concerning these biomarkers by means of ProteinChip Array System together with other technologies. Protein signals with molecular masses of 4525.1, 4846.8 and 7755.8 Da were observed corresponding to three novel Abeta peptides, which have not previously been described and thus may be novel biomarkers of AD (Lewczuk et al. 2004). A novel carboxyterminally elongated Abeta peptide was found by studying and comparing the patterns of Abeta peptides in human cerebrospinal fluid (CSF) and brain homogenates through SELDI-ToF MS with those obtained by Abeta-SDS-PAGE/immunoblot (Lewczuk et al. 2003). Sheng et al. validated the hypothesis that ERC may be the primary source of amyloidogenic Abeta in the dentate gyrus, and suggested an important role of corticocortical and corticolimbic forward connections in determining patterns of amyloid deposition in AD (Sheng et al. 2002). Trends toward an increase in soluble Abeta peptide in the brain and a decrease in assayable Abeta peptide in the serum of immunized animals were detected (Vehmas et al. 2001). Other studies involving SELDI technology include monitoring the production of Alzheimer's betaamyloid in transfected cells (Austen et al. 2000) and the role of cholesterol in the biosynthesis of beta-amyloid (Frears et al. 1999).

SELDI-ToF protein profiling has also been extensively employed to study other diseases and various physiological conditions. For example, proteomic analyses were carried out in tears after ocular surface surgery (Zhou et al. 2004), in placenta (Batorfi et al. 2003), pluripotent stem cells (Hayman & Przborski 2004)and tissue-engineering blood vessels (Opitz et al. 2004). Biomarker identifications were also attempted for alcoholism (Nomura et al. 2004, Patel et al. 2003), inflammatory hyperalgesia, (Ginestec et al. 2003), diabetes and cardiovascular diseases (Dayal & Ertel 2002), arthritis (Uchida et al. 2002), ischemic and hemorrhagic stroke (Allard et al. 2004), sweat (Flad et al. 2002), Creutzfeldt-Jakob disease (Snchez et al. 2004), ectopic pregnancy (Gerton et al. 2004), urolithiasis (Cadieux et al. 2004), hemodialysis(Langlois et al. 2004) and African trypanosomiasis (Papadopoulos et al. 2004). Among these, Papadopoulos et al. and his group used SELDI-ToF ProteinChip System combing with three other powerful data-mining tools to discover a biomarker pattern, determining African trypanosomiasis patients with a sensitivity of 100% and a specificity of 98.6% (Papadopoulos et al. 2004). This pilot research revealed that the novel approach using SELDI protein chip is much more accurate than any other diagnostic test in differentiating African trypanosomiasis patients from normal people.

Concluding remarks

SELDI-ToF ProteinChip Array System has demonstrated its powerful capacity in protein profiling for biomarker identification though its application is still at its early stage. Many studies in disease profiling showed that this new technology is straightforward, easy to handle and with high sensitivity and accuracy although its limitations need to be improved in protein binding affinity and restricted detection ranges. Other analytic techniques should be combined with SELDI system to enhance its ability in protein identification and result validation. High effectiveness would be achieved if bioinformatics algorithm methods can be incorporated in identifying patterns of protein alterations as specific biomarkers. Nevertheless, as a key player in protein profiling, SELDI-ToF ProteinChip technology has been proved to be able to facilitate the discovery of new and better biomarkers and it has potential of being developed into a novel diagnostic assay in clinical setting.

Acknowledgements

This work was partially supported by Hong Kong Research Grants Council Grants HKU 7227/02M (to Q.Y.H.), HKU 7218/02M and HKU 7395/03M (to J.F.C.), the Department of Chemistry, and the Areas of Excellence scheme of Hong Kong University Grants Committee.

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