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REVIEW

Research Progress in SELDI-TOF MS and Its Clinical Applications

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Abstract: Proteinchip profiling is a powerful and innovative proteomic technology for the discovery of biomarkers and the development of diagnostic/prognostic assays. On the basis of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS), Ciphergen's proteinchip system offers a single, unified, and high throughput platform for a multitude of proteomic research applications. Proteins are the major functional components of the cell. The study of proteomics helps to better understand the mechanism of a disease. Remarkable findings in disease biomarkers have shed light on the early diagnosis, monitoring, and prognosis of various diseases, especially for cancer. In this paper, the development and technology of SELDI-TOF MS are introduced. The research progress and encouraging research results in malignancies, infectious diseases, neurological diseases, and diabetes mellitus using SELDI-TOF MS are reviewed. This paper concludes by evaluating the pros and cons, and the future perspectives are also expounded.

Key Words: proteinchip profiling; proteomics; tumor biomarker; surface–enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS)

Proteins are the chief actors in the cell, that is to say, carrying out the major duties specified by the information encoded in genes. The total complement of proteins present in a cell or cell type is known as its proteome. As the executive molecules in life, proteins play a crucial role in the physiological and pathophysiological states of a disease. The complicated and changing pattern of protein expression provides important information about the pathologic process taking place in the cells of a particular tissue. Proteomics is the study of various types of proteins and their interactions in a cell. Utilization of this information for the diagnosis and selection of suitable drugs or targets will be very useful. Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS), a technology invented in the early 1990s by Yip and Hutchens, represents a breakthrough in protein separation and analysis based on the property of analytes[1].

1 Principle of the technology

SELDI-TOF MS is a novel approach to biomarker discovery that combines two powerful technologies, retentate chromatography and mass spectrometry. The core of the SELDI-TOF MS platform is the proteinchip arrays, which have varying chromatographic properties, such as anion exchange, cation exchange, metal affinity, and reverse phase. Superior to the traditional two-dimensional gel electrophoresis, SELDI-TOF MS is capable of detecting proteins that are <10 kD, as well as proteins that are basically charged. The group of proteins in this lower molecular weight range has tremendous biologic significance because they contain cleaved or aberrantly shed proteins/peptides that may reflect the essential features of a disease. Until recently, these molecules were below the limit of detection. Various complicated biological materials can be uniformly captured,

concentrated, and purified on the small chemical surface of the proteinchip. A complex mixture of proteins from cells or body fluids can be reduced to sets of proteins with common properties by binding the sample to chips with differing surface chemistries in parallel and in series. After washing to remove unbound proteins, salts, and contaminants, the bound proteins/peptides are ionized by laser and read in a time-of-flight mass spectrometer. The resulting spectra provide a multidimensional binding profile on the basis of different types of interactions (Fig. 1).
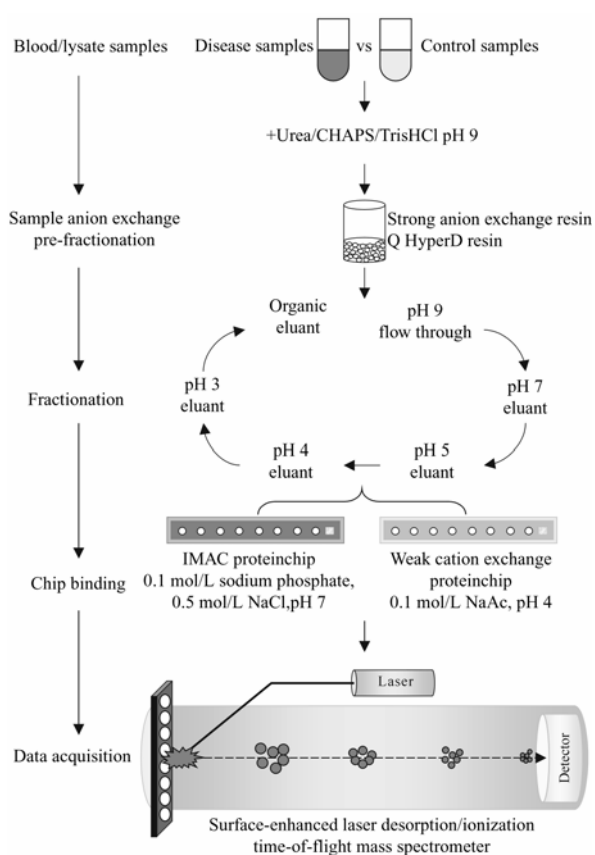

Fig. 1   Sample fractionation, chip binding, and data acquisition in proteinchip profiling

2   Bioinformatics and statistical analysis

For SELDI-TOF mass spectroscopy, the data come in the form of an array of intensity values and their corresponding mass-to-charge ratios. Each data array represents the spectrum of the ionized composition of a given sample separated by mass-to-charge ratio. The processing and analysis of these data arrays can be broadly divided into three stages using the ProteinChip Software or the CiphergenExpress Data Manager (Ciphergen Biosystems Inc, Fremont, CA, USA). Stage one processes the data arrays individually and it consists of noise filtering, baseline subtraction, and peak detection. Stage two analyzes the data arrays collectively and it consists of peak clustering and normalization. Stage three operates on peak clusters and it consists of statistical univariate and multivariate analyses of the clusters.

Univariate analyses uncover single potential biomarkers that can classify the sample groups by themselves. In some cases, univariate analyses alone are sufficient, but biological systems are usually complex and a disease can be caused by multiple factors. In that case, if univariate analyses show individual biomarkers that can be positively identified and linked to a disease pathway, multivariate analyses are needed for proper classification.

2.1   Univariate analysis tools

2.1.1   Mann-Whitney U test: The Mann-Whitney U test is a nonparametric statistical significance test for assessing whether the difference in the medians between two samples of observations is statistically significant. The null hypothesis is that the two samples are drawn from a single population and the two samples must be independently measured.

2.1.2   Receiver operating characteristic: Receiver operating characteristic (ROC) is a graphical plot representing the sensitivity versus (1-specificity) for a binary classifier system as its discrimination threshold is varied. The ROC can also be represented equivalently by plotting the fraction of true positives versus the fraction of true negatives. The ROC curve is used to evaluate the results of a prediction. The area under ROC curve is equivalent to the Mann-Whitney U test, which tests for the difference in the medians between the scores obtained by the two groups considered in the study. However, any attempt to summarize the ROC curve into a single number results in the loss of information about the pattern of tradeoffs of the particular discriminator algorithm.

2.1.3   Scatterplot: Scatterplot is a graph used in statistics to visually display and compare two or more sets of related quantitative or numerical data by displaying finite points, each having a coordinate on a horizontal and a vertical axis. It shows the position of all the cases in an x-y coordinate system. The relationship between interval variables can be identified from scatter graph. A dot in the chart represents the intersection of the data on the x and y axes. One advantage of a scatterplot is that it does not require a user to specify dependent or independent variables, either type of variable can be plotted on either axis. Actually, a scatterplot represents the association (not causation) between two variables.

2.1.4   Box and Whisker plot: Box and Whisker plot is a convenient way to graphically depict the five-number summary, which consists of the smallest nonoutlier observation, lower quartile, median, upper quartile, and the largest nonoutlier observation. Using a boxplot, it is possible to view different types of populations, without any assumption of the statistical distribution. The spaces between the different parts of the box help to indicate variance, skew, and outliers.

2.2   Multivariate analysis tools

2.2.1   Principal components analysis: Principal components

analysis (PCA) is used to simplify a dataset by reducing multidimensional datasets to lower dimensions for analysis. The PCA is a linear transformation that transforms the data to a new coordinate system, in which the greatest variance by any projection of the data comes to lie on the first coordinate (called the first principal component), the next greatest variance on the second coordinate, and so on. The PCA can be used for dimensionality reduction in a dataset while retaining the characteristics of the dataset that contribute most to its variance. It keeps the lower-order principal components and ignores the higher-order ones as the lower-order components often contain the most important aspects of the data.

2.2.2 Heat map: Heat map is a graphical dendrogram of data where the values of a variable in a two-dimensional map are represented in colors. A heat map is typically used in molecular biology to represent the levels and similarities in the expression patterns of genes or proteins across a number of comparable samples, e.g. samples from different patients.

2.2.3 Classification and regression tree analysis: Biomarker Patterns Software (Ciphergen Biosystems Inc, Fremont, CA, USA) shows the hierarchy of specific proteins using the classification and regression trees algorithm to predict multiple potential biomarkers and carry out cross-validation by applying the trees computed from learning samples to testing samples. The software builds a large number of small trees, and each is designed to correct the errors of its predecessors. The preselected variables allow a more focused analysis and improve the predictive accuracy.

2.2.4 Support vector machines: Support vector machine is a set of supervised learning methods used for classification and regression. It uses the kernel trick to apply linear classification techniques to nonlinear classification problems.

2.2.5 Artificial neural network: Artificial neural network (ANN) is an interconnected group of artificial neurons that uses a mathematical or computational model for information processing based on a connectionist approach to computation. In most cases, the ANN is an adaptive system that changes its structure based on external or internal information flowing through the network. In more practical terms, the ANN is a nonlinear statistical data modeling tool. It can be used to model complex relationships between inputs and outputs or to find patterns in data.

3   Research progress and applications

Differential proteomic analysis has been extensively applied to study cancer using SELDI-TOF MS profiling of blood and tissue derived from human or animal. There is an intense interest in applying proteomics to foster a better understanding of oncogenesis and discover new biomarkers for diagnosis because proteins are ultimately responsible for the malignant phenotype. The early detection of cancer has the potential to dramatically reduce mortality. Oncoproteomics is also addressing the discovery of new therapeutic targets and the study of drug effects[2].

Proteomic spectra were generated by SELDI-TOF MS, a cluster pattern was found to completely segregate ovarian cancer from noncancer serum. The results yielded a sensitivity of 100 %, specificity of 95 %, and positive predictive value of 94 %[3]. In another SELDI-TOF MS study, three biomarkers (apolipoprotein A1, a truncated form of transthyretin, and a cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4) were identified to detect early stage invasive epithelial ovarian cancer from healthy controls. The sensitivity and specificity of a multivariate model combining the three biomarkers were 83 % and 94 %, respectively[4]. Pathologic states within the prostate might be reflected by changes in serum proteomic patterns. To test this hypothesis, a predicted diagnosis of benign disease or cancer was rendered based on similarity to the discriminating pattern discovered by SELDI-TOF MS[5]. Besides, the SELDI technology discovered a panel of three biomarkers to separate stage 0-I breast cancer patients from noncancer controls. Bootstrap cross-validation demonstrated that a sensitivity of 93 % for cancer patients and a specificity of 91 % for controls were achieved[6]. The SELDI-TOF MS was also adopted to discover differential biomarkers for lung cancer serum, and the results have been filed in the Patent Corporation Treaty by our group. In another study that attempted to discover the specific biomarkers for the detection of lung cancer using SELDI-TOF MS, three protein peaks yielded a sensitivity of 93 % and a specificity of 97 %[7]. Besides, using proteinchip profiling analysis, two isoforms of serum amyloid A (SAA) protein that are useful in the diagnosis of relapse in nasopharyngeal cancer were identified. Monitoring the patients longitudinally for SAA level both by proteinchip and immunoassay showed a dramatic increase in SAA correlated with relapse, and a drastic fall in SAA correlated with response to salvage chemotherapy[8]. Subsequent tandem MS sequencing and immunoaffinity capture assay identified platelet factor-4 as a treatment-associated serum biomarker which might serve to triage patients with nasopharyngeal carcinoma for appropriate chemotherapy treatment[9]. When SELDI-TOF MS was carried out to identify differentially expressed proteins in hepato-cellular carcinoma serum, complement C3a was found to be elevated in patients with chronic hepatitis C and hepatitis C virus-related hepatocellular carcinoma[10]. On the other hand, the combination of SELDI with bioinformatics tools could help to find new biomarkers and establish patterns with a high sensitivity of 92 % and a specificity of 89 % for the detection of colorectal cancer[11]. Another study identified calgizzarin as a biomarker that could be used to distinguish colorectal carcinoma from colon adenoma. Their results might provide a better understanding of the molecular mechanisms in the process of tumorgenesis[12]. Benzene is an industrial chemical and environmental contaminant that causes leukemia.

SELDI-TOF analysis on the serum proteome of shoe factory workers showed that lowered expression of platelet factor-4 and connective tissue activating peptide-III proteins were potential biomarkers of benzene's early biologic effects, which might play a role in the immunosuppressive effects of benzene[13].

Apart from cancer, proteomic analyses are already extensively used to study a wide range of diseases, including infectious diseases, neuropsychosis, chronic nephropathy, organ transplant, rheumatoid arthritis, diabetes mellitus, and cardiovascular disease. Although SELDI analysis of these diseases was not as popular as oncoproteomics, there has already been quite a number of encouraging research results.

With regard to infectious diseases, SELDI-TOF MS has been applied to the analyses of acquired immunodeficiency syndrome (AIDS), severe acute respiratory syndrome (SARS), *Yersinia pestis*, *Mycobacteria*, *Caulobacteria*, *Streptococcus*, *Botulism*, and bubonic plague. Using SELDI technology, it was discovered that a protein named α-defensins found in patients with AIDS exhibited suppressive effect on human immunodeficiency virus. It might contribute to the therapy of this deadly viral disease. Moreover, proteinchip profiling could be useful to simultaneously examine the molecular weight of specific antigen in various diseases and to conduct tests during the window period. For example, diagnosis of pathogen could be carried out in the early stage of SARS and plague. Proteinchip array profiling analysis was applied to find SAA as a specific biomarker that was potentially useful in monitoring the severity of disease in patients with SARS[14].

In the aspect of neurological diseases, there have been researches on senile dementia, hypochondria, and schizophrenia using SELDI-TOF MS. A sensitive assay using SELDI proteinchip arrays was developed to monitor production of beta amyloid in transfected cells, which was the principal component of senile plaques found in the brains of patients with Alzheimer's disease. The assay was of great value in screening inhibitors of the proteases involved in its release from the amyloid precursor protein[15]. Another study discovered five novel possible cerebrospinal fluid biomarkers (a fragment of neurosecretory protein VGF, transthyretin, S-cysteinylated transthyretin, truncated cystatin C, and a fragment of chromogranin B) for frontotemporal dementia using SELDI-TOF MS. With the use of these potential biomarkers, frontotemporal dementia could be distinguished from control subjects with high accuracy[16].

Urine test by SELDI-TOF MS could be adopted as a noninvasive continuous monitoring tool to determine the acute response of renal transplant rejection. Using proteinchip profiling, a specific diagnostic biomarker (lipocalin-type prostaglandin D synthase) was detected in chronic nephropathy patients. This result provided important values for the dose adjustment of immune suppressor, the early detection of complications, and the determination of treatment response.

Moreover, SELDI technology and the use of decision tree boosting analysis also allowed the discovery of a pattern of protein peaks specific for rheumatoid arthritis[17].

On the other hand, there were also significant breakthroughs in the researches on diabetes mellitus and cardiovascular disease using SELDI-TOF MS. Applying proteinchips to monitor the complications of diabetes and cardiovascular disease, atherosclerosis and myocardial infarction could be detected early. The technology could even differentiate ischemic stroke from hemorrhage stroke. The SELDI-TOF MS was used to study the serum and tissue lysate of diabetic rats during different stages of diabetes. Several biomarkers were found to be associated with the disease and its complications, including islet amyloid polypeptide and resistin. These potential biomarkers might provide valuable insight into the pathogenesis of diabetes and its complications[18,19]. The high throughput proteomic approach was adopted to decipher the antidiabetic action of ginsenoside Re (a major component of ginseng) in diabetic rats. C-reactive protein was discovered to have significant decrease corresponding to ginsenoside Re treatment, indicating that the drug could improve diabetes and its complications by alleviating inflammation[20]. For cardiovascular disease, a novel SELDI-TOF MS assay was devised to offer a rapid, cost–effective, and functionally relevant test for timely diagnosis and management of thrombotic thrombocytopenic purpura[21].

Early diagnosis of cancer at a curable stage is crucial for the successful treatment of malignancy. Most of the current available tumor markers appear too late with low sensitivities and/or low specificities. As an additional burden for patients, the traditional tumor markers often require biopsy material instead of less invasively collected samples such as serum. The SELDI-TOF platform is a revolutionary approach in proteomic patterns analysis that can be applied at the bedside for discovering protein profiles to distinguish disease and disease-free states with high sensitivity and specificity. Scientists from leading research institutions have taken advantage of SELDI technology to reveal a large number of previously uncharacterized biomarkers for the diagnosis of a wide variety of cancers (Table 1).

4 Evaluation of pros and cons

One of the key features of SELDI-TOF MS is its ability to provide a rapid protein expression profile from a variety of biological and clinical samples, such as blood, urine, cerebrospinal fluid, synovial fluid, saliva, gastric juice, bronchial eluate, cell lysis solution, and various secretions. Recently, a new application of direct tissue proteomic analysis has been developed to expand the use of clinical proteomics as a complement to the anatomopathological diagnosis[22]. The SELDI technology offers many advantages, such as low

sample volume requirement, high speed of discovery, quantitative, easy to use, automated sample handling, high resolution, and high throughput. It is one of the most potential molecular diagnostic tools for clinical care[23–26].

However, the proteinchip and its associated reagents are quite expensive. It costs around $22 USD for the examination of one sample, excluding the cost of the instrument. A comparatively large amount of experimental fund is thereby required to accomplish modeling and the evaluation of the model. This imposes limitations on the popularity of large-scale studies. On the other hand, even with all its potential, studies must be carefully designed in order to differentiate true differences in protein expression from differences originating from variation in sample collection, variation in experimental conditions, and normal biological variability. The reproducibility, standardization, and feasibility of SELDI-TOF MS need to be addressed before it can become a tool for routine clinical use. To solve these problems, some projects adopt multicenter and/or multistage studies to ensure the consistency of research results, where the cost is enormous. Consequently, many research institutes flinch from SELDI technology for its high implementation costs[27].

One of the major bottlenecks facing SELDI-TOF MS is the protein identification. In recent years, SELDI has interfaced with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, new equipment such as Q-STAR can optimize protein/polypeptide identification. This is of great beneficial to the future development of SELDI proteinchip technology. On the other hand, the study of low abundant proteins in highly complex biological samples is also a major obstacle in proteomic research. Blood possibly constitutes the most complex of all biological samples with a concentration dynamic range from low pg/mL to high mg/mL. A significant portion of the total protein content in serum and plasma comprises just a few high abundant proteins, such as albumin and immunoglobulin. Low abundant proteins make up less than 1 % of protein content. In view of this, the newly developed deep proteome technology and laser capture microdissection are introduced to improve the detection of low abundant proteins on SELDI. The increased capacity allows the enrichment of low abundant proteins from larger sample volumes, proteins that would previously have been swamped by high abundant proteins become visible in the spectra[28–30].

Table 1   Comparison of proteinchip biomarkers and current tumor markers

Cancer	Proteinchip biomarker		Current tumor marker		
	Sensitivity/%	Specificity/%	Marker	Sensitivity/%	Specificity/%
Bladder[33]	80	90–97	NMP22[34]	31	95
Breast[35]	93	91	CA15-3[36]	58	96
Colorectum[37]	91	93	CEA[38]	43	* * * *
Stomach[39]	83	95	CEA[40]	31	* * * *
Liver[41]	94	86	AFP[42]	50	70
Lung[43]	87	80	Cyfra21-1[44]	63	94
Ovary[45]	83	94	CA-125[46]	58	91
Pancreas[47]	78	97	CA19-9[48]	75	80
Prostate[49]	83	97	PSA[50]	46	91

5   Future perspectives

The capability of SELDI-TOF MS to simultaneously and comprehensively examine changes in large numbers of proteins in the context of disease or other changes in physiological conditions holds great promise to unlock the diagnostic and therapeutic solutions for difficult clinical problems. The SELDI technology is a rapidly growing field that combines high throughput analytical methodologies with complex bioinformatics to study the role of proteins in the biology of disease, such as monitoring disease progression and evaluating the therapeutic and adverse effects of drugs.

The combinations of SELDI MS, retentate affinity chromatography, and statistical algorithms for pattern recognition have engendered enormous interest in proteomic profiling as a comprehensive set of tools to manage protein interaction and posttranslational modification studies involving in early detection of disease, development of drugs, molecular biology, forensic medicine, food hygiene, environmental monitoring, breeding, and improvement of agricultural products. The field of proteomics is expanding rapidly to provide greater volume and quality of protein information for a better understanding of the multifaceted nature of biological systems. To catalog all human proteins, ascertain their functions and interactions poses a challenge to scientists. Another major challenge will be the integration of proteomics with genomics and metabolomics data, as well as their functional interpretation in conjunction with clinical results and epidemiology.

Many oncologists believe that targeted personalized therapies are the oncotherapy in the future. As solid tumor continues to be viewed as a chronic condition and systemic

disease, methods for long-term treatment, with less adverse effects, continue to be investigated. Protein expression profiling is increasingly being used to discover, validate, and characterize biomarkers that can potentially facilitate the combination of therapeutics with diagnostics and will thus play an important role in the development of personalized medicine[31]. However, it is becoming increasingly recognized that the reproducibility and validation, as well as the origin and identity of these biomarkers should be addressed carefully. Discovering valid biological information from SELDI-TOF MS depends on clear experimental design, meticulous sample handling, and sophisticated data processing. If these efforts are made, protein profiling may contribute to the better diagnosis and management of patients, as well as the optimization of treatment[32].

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