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Advance in Proteomics Research and Application

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Abstract: Completion of the human genome project marked the life sciences had entered the post-genomics and as an important element in the post-genome, proteomics had already gone deep into the field of life sciences and medicine. In recent years, proteomics technology developed sharply. In this study, the concept of proteomics, the background was introduced and summarized the technical route and progress of proteomics.

Key words: Proteomics, 2-DE, bio-mass spectrometry, bionformatcs, element, China

INTRODUCTION

April 14, 2003, the International Human Genome Sequencing Organization announced the successful conclusion of the human genome project (Human Genome Project HGP). It marked that life sciences had been substantially entered the post genome era (Collins *et al*., 2003). In post genome era, the researching focus had transferred from revealing all the life genetic information to studying the biological function on overall level and the functional genomics was born. As the most important disciplines in large-scale study of gene function, proteomics had also been a rapid development (He, 1999; Aebersold and Mann, 2003) and became an important pillar in the functional genomics.

PROTEOMICS

In 1994, proteome was first proposed by Macquarie University, Wilkins and Williams (Reynolds, 2002) as a new word proteome was protein and genome by splicing together and it referred to all the expressed proteins by a genome a cell or a tissue.

Proteomics studied the dynamic changes of cells growth and differentiation on overall level and compared cells or organization of the protein differences under the different circumstances through observation the genes expression products on quantity and structure's different in normal and disease states it contributed to disease's early detection to improve the pathogenesis understanding and then we could establish the rational therapeutic measures (Haynes *et al*., 1998; Wang and Tsou, 1998; Anderson and Anderson, 1998; Humphery-Smith and Blackstock, 1997; Wilkins *et al*., 1996). It mainly included two aspects 1st was expression proteomics, it primarily created the quantitative map for cell or tissue extracts and this was the foundation of the proteome research 2nd was cell map proteomics. It was mainly determined the protein cellular localization interactions and function and this was an important goal of proteomics research.

RESEARCH ON PROTEOMICS TECHNOLOGY

The method on proteome research mainly was separation protein through high throughput two-Dimensional gel Electrophoresis (2-DE) and processed image analysis by professional computer software (e.g., Melanie3) then using Mass Spectrometry (MS) and protein data information analysed and identified protein spots in gel (Yang and Li, 1999).

2-DE principle: At present 2-DE was considered to be indispensable center technology in protein separation. This technology was invented by O'Farrell (1975), it was privileged among the forms of any other techniques for separation protein (Mary, 1999; Gorg *et al*., 2004). O'Farrell made use of this technique to analyze mouse tissues and *E. coli* protein extracts, respectively and got 300 protein spots (dyeing) and 1100 protein spots (radiation imaging) by separation, far better than dimensional gel electrophoresis (Sperling, 2001; Klose and Kobalz, 1995; Molloy, 2000). 2-DE principle was based on different isoelectric point and molecular weight for different protein, first, depending on Protein Isoelectric point (PI) in pH gradient gel processed Isoelectric Focusing (IEF) for first separation and then turn 90° according to the molecular weight size in Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) for second separation. By 2-DE separation it was needed to dye (gel dyeing methods mass high blue stain, colloidal silver dye, zinc dye, negative dye, fluorescence staining techniques, etc.) and then tested on protein spots. When it was tested, we needed to process the scan-stained

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electrophoresis map on computer and acquired the 2-DE electrophoresis map then we could get the changes of the protein in the corresponding physiological or pathological conditions.

Advantages and disadvantages on 2-DE and improved methods: 2-DE had advantages of high resolution, good repeatability and trace preparation to separate protein but it also had its limitations such as many of hydrophobic protein (membrane protein) could not be dissolved in sample buffer, relative molecular mass was too large (>200×10^3), extremely acidic or basic protein was easy to lost in the course of electrophoresis and 2-DE could not distinguish them, low content components were susceptible to be shielded by high content components, so 2-DE resolution was reduced (Ye *et al*., 2006; Zhou *et al*., 2002). To further enhance the work efficiency of the 2-DE, Fluorescence Difference in Gel Electrophoresis (DIGE) technology came into being (Zhao *et al*., 2006). This method was that all of the two compared protein samples were marked by two different fluorescent labeling reagents (cy2, cy3 or of cy5), respectively and balanced mix then separated them by 2-DE. Researchers used two different wavelength excitation to image in 2-DE gel imager and software for quantitative analysis then identified with MALDI-TOF-MS or ESI-MS for the differential protein spots. Fluorescence difference gel electrophoresis technology separated protein in the same gel, so it could avoid the human error and the difference between two piece of gels when researchers carried through electrophoresis and it could accurately reflect the differential protein expression between two samples (Jungblut *et al*., 1999). DIGE compared with the conventional 2-DE had the advantages of simpler, lower labor intensity and more efficient.

2-DE technology in cancer research: Today, proteomics technologies for cancer had become a hot topic research. Cancer was a disease that was one of the most harmful to human health. There were >7.5 million cancer patients in China, about 1.3 million people died of cancer every year and its morbidity and mortality was still increasing year by year. It was well known, cancer was a long-term, multi-factor and phased process. It was a genetic disease including activation of oncogenes and tumor suppressor gene inactivation. And genetic changes would cause the changes of its translated protein, proteomics was based on all of the protein within cells as the object of study. So, it could provide a powerful tool for study cancer mechanism. In inducing mouse liver cancer model with N-methyl-N-nitrosourea, Peter used 2-DE and the amino acid micro-sequencing technology to distinguish aldose reductase protein in hepatoma cells (35×10^3/PI 7.4), these protein had highly expression in liver and fetal liver. Confirmed by immunization experiments, the aldose reductase protein could not be expressed in human liver. Meanwhile researchers found that these protein were expressed strong in pre-cancerous and liver cancer while in the surrounding normal tissues of the liver were not expressed. It indicated that these protein might be related to the causes of liver cancer. The occurrence of colorectal cancer was due to polygenes mutation what caused the tumor suppressor gene inactivation and oncogene activation. Sanchez *et al*. (1995) separated protein with 2-DE for 15 of rectal cancer cases and 13 of normal rectal epithelial and the results showed that the protein in relative molecular mass of 13000 and isoelectric point of 6 only appeared in the tissue of patients with rectal cancer. The protein with 13000/PI 5.6 for 13/15(87%) of patients with cancer was up regulated. In addition, these protein were not only over expression in the moderate low differentiation and a 24 years history of ulcerative proctitis patients but the different degree of differentiation of adenoma precancerous lesions in seven cases were found however the control group rarely was found. This suggested that the emergence of these proteins for the early detection of colorectal cancer had important clinical significance. Colon cancer was a common gastrointestinal malignancy in the colon and the incidence occupied third in gastrointestinal tract tumors. The usual site is the rectum and the unction in the rectum and sigmoid colon, 65% people of onset age were after 40 years old and the ratio of the men and women were 2~3-1, its pathogenesis was not clear but researchers conjectured that it was caused by a variety of factors. Friedman *et al*. (2004) analysed the different stages of colon cancer cases with 2-DE, 1500 spots were found in gel and 52 kinds of protein included cytokeratin, Annexin IV, creatine kinase, fatty acid binding protein were identified by MS. Among them, there were 12 kinds of protein for colon cancer-specific protein. The discovery of all these specific protein would help people to do in depth study of cancer pathogenesis.

Biological mass spectrometry: In 1906, Thomson invented mass spectrometry and for the next several decades mass spectrometry technology gradually developed into cutting edge methods for research analysis and identification biological macromolecules (Domon and Aebersold, 2006). In the 1980s, there had been a landmark soft-ionization mass spectrometry Matrix-assisted Laser Desorption Ionization Time Offlight Mass Spectrometry(MALDI-TOF-MS) and Electrospray Ionization Mass Spectrometry (ESI-MS) (Li *et al*., 2000;

Mark *et al*., 2000) and thus it opened up the new mass spectrometry areas. Biological mass spectrometry and made mass spectrometry to be extensive application and development in the field of life science (Yates, 1998; Jasminka and Brown Larry, 2001; Mann *et al*., 2001).

The principle of biological mass spectrometry: When biological mass spectrometry analysed samples, first, sample molecules (or atoms) were ionized into single-charged molecules, ions or fragment ions which had different qualities and these were all cation. Second, these singly-charged ions were accelerated when they came into the accelerating field and formed ion beam with the same kinetic energy. Third these ion beam came into separator for the electric and magnetic fields the ion trajectory happened deflection in electric field ions at low velocities had a larger deflection angle and faster had small deflection angle ions happened opposite deflection with angular velocity vector when they transited magnetic field, ions at low velocities still had a larger deflection angle and faster had small deflection angle when the deflection of the two fields were cancelled each other out, these ions track would intersect at one point. At the same time, the ions with different quality occurred separation in magnetic field. On this occasion, the same charge-mass ratio (m/z) ions were focused at the same point, the different charge-mass ratio ions were focused on the different points and its focal plane was close to a plane. Fourth, detection system at the focal plane could detect the different charge-mass ratio spectrum, it was called mass spectrometry (Cong, 1987; Hua, 1986). By mass spectrometry, we could acquire sample molecular weight molecular formula, the molecular isotopic composition and molecular structure and many other informations. Mass spectrometry for protein and other bioactive molecules had the following advantages high sensitivity for sub-microgram sample, the most effectively with the hyphenated chromatographic, identification or structure determination of trace substances in the complex system, accurate, easy, fast and good universality (Wang and Liu, 2007).

Biological mass spectrometry identification of protein peptide mass: The molecular weight of protein and peptide was an important parameter to study protein biological activity and the determination of the protein or peptide structure was often represented by molecular weight, sometimes it meant the discovery of a new protein. Biological mass spectrometry mainly used full spectrum analysis peptide mass fingerp rinting and Tandem Mass Spectrometry (MS^n) for peptide and protein quality to identify the protein spots which were separated by 2-DE.

Full spectrum analysis for proteins and peptides quality: Full spectrum analysis for protein and peptide quality was also called component analysis analysis object was the full tissues, body fluids or extracts. The goal of analysis was to separate the components in the mixture of peptide and protein as much as possible. Generally, full spectrum analysis for protein and peptide quality did not need to separate protein or peptide samples from the mixture and researchers need only extraction or simply component fractionation. In this way, researchers could save a lot of time and effort. It was far more than the applicable scope of the radioimmunoassay test and chemical test (Serafii, 1993; Takamatsu and Tamemoto, 1992). The application of proteomics by mass spectrometry had been many successful examples such as Michael used LC/LC-MS/MS to identify >70 proteins in yeast ribosome, Shevchenko used MALDI-TOF-MS to identify yeast genome and found that there were still unknown protein in yeast organism (Michael and Kelvin, 2000; Shevchenko *et al*., 2001).

Identification proteins by peptide mass fingerp rinting: PMF technology was that mass spectrometry identified the quality of peptide mixture after protein with specific enzyme digestion, got a characteristic peptide quality map and compared with theoretical protein enzymatic peptide mass mapping in database thus protein could be identified. Identification protein by peptide fingerp rinting was mass spectrum analysis for peptide mixture which came from degradation protein spots by 2-DE with enzymatic hydrolysis or chemical methods. There were two most commonly methods First was treated the separation of protein spots by 2-DE for enzymolysis in gel and extracted, collected protein hydrolysates (if necessary the appropriate desalination or the enrichment) for processing by mass spectrometry. Another was transferred the separation of protein spots by 2-DE to Polyvinylidene Fluoride (PVDF) with coupled protease for processing by mass spectrometry (Uardroni and James, 1999). Compared peptide for mass spectrometry with theoretical protein enzymatic peptide mass mapping in database, researchers could determine that the protein was known or unknown. Different protein had different amino acid sequence, so peptide for different protein had fingerprint characteristics (Wise *et al*., 1997). In short, the success of peptide mass fingerp rinting depended on quality determination, accuracy of the database retrieval and purity of the protein. Therefore, sometimes, researchers needed to be conclusive evidence for the second method such as N-terminal sequencing, amino acid composition analysis and tandem mass spectrometry sequencing (Liu *et al*., 2003).

Identification peptide sequence by tandem Mass Spectrometry (MS^n): The peptide for protein enzymolysis was <4, it could not use the PMF for protein identification and identification for some protein were not reliable by PMF, so if researchers continued to need peptide sequence information, researchers could use tandem mass spectrometry directly on the peptide amino acid to sequence. Tandem mass spectrometry determinated peptide sequence by different mass spectrometry techniques, it chose the ions with specific charge-mass ratio to process collision induced dissociation and extrapolate to the fracture peptide then researchers could deduce this peptide sequence (Fisato *et al*., 2004).

The application of mass spectrometry in food safety: With the development of economic globalization, human's food culture was increasingly diverse, food hygiene and safety had become a hot concern topic. The detection of pesticide and veterinary drug residues, microorganisms, biological toxins and additives in food could enhance food quality and safety control, safeguard human's health and prevent food-borne diseases. Mass spectrometry with strong anti-interference ability, high detection sensitivity and accuracy detection information was widely recognized and as a common testing method, it was used for food safety analysis. Li *et al*. (2009) used ESI-MS Method to direct determine melamine in the milk-site without sample pretreatment. The functional ingredients in drinks such as taurine, caffeine, lysine, inositol, niacinamide, Vitamin B6 and the main ingredients for soft drinks (e.g., Coca Cola, Pepsi) such as caffeine, phosphoric acid, fructose and dehydration of fructose could be text in site by high throughput within a few seconds for a sample (Hu *et al*., 2010) used ESI-MS Method to screen cocaine which was trace banned substances in drinks and detection limit was 7~15 pg L^{-1}. (Chen *et al*., 2007) used ND-ESI for fruit emission volatiles to fast fingerprint analysis, it could distinguish between bananas, grapes and strawberries in different maturity. With the same as fruit maturity detection, Chen distinguished between the freshness of the fish by microbial metabolism of biogenic amines in fish and detected contaminated vegetables by the *E. coli*. Therefore, mass spectrometry techniques were widely used in the identification of food safety analysis.

BIOINFORMATICS

Through acquisition, processing, storage, retrieval and analysis biological experimental data, bioinformatics could interpret the data which contained the biological significance (Spengler, 2000). Bioinformatics was a emerging disciplines for intersect among biology, computer science and applied mathematics. Bioinformatics was widely used in proteomics, for example, 2-DE and biological mass spectrometry had to rely on bioinformatics method to identify protein types, quantitied, structures and functions to obtain data. Besides, bioinformatics had played an important role in the prediction about protein structure and function.

Table 1: Proteomics common searchable database website

Proteomics	Website
Mascot	http://www.matrixscience.com
Espasy	http://www.espasy.ch/tools
Peptide Search	http://www.narrador.embl-heidelberg.de/services/ peptide search
Protein Prospector	http://www.prospector.ucsf.edu
Profound	http://prowl.rockefeller.edu/PROWL/prowl.html
Sequest	http://www.thomposn.mbt.wa shing.edu/sequest
MassSearch	http://cbrg.inf.ethz.ch/Serve/MassSearch.html
Mowse	http://srs.hgmp.mrc.ac.uk/cgi-bin/mowse
Sherpa	http://www.hairfatguy.com/sherpa

Table 1 was the commonly used retrieve database in proteomics. In fact, there was no uniform standard for evaluation of databases searching results so far, scoring methods for various search software were not the same. So, when we did in experiment, researchers needed according to the actual situation and considering the various possibilities then make judgments carefully and cautiously.

So far, China had made tremendous contributions for the development of bioinformatics in improving the existing database system, developping sequence annotation systems and bioinformatics algorithms. In 2002, Chinese scientists were in cooperation with EM Bnet which was the European Molecular Biology Network Organization and built the Bioinformatics Resource Center which had types of biological databases, data and timely updates characteristics, it served biological database searching and download of data resources for various fields of biotechnology (Wang *et al*., 2006).

CONCLUSION

Proteomics had many ways but every method had its inadequacies. With the development of science, researchers believed that proteomics technology would constantly improve and progress. It was not only provide the material basis to clarify the law of life activity but also could provide important theoretical basis and practical solution for discussion the mechanism of major animal diseases, disease diagnosis (Nilofer, 2006), meat quality control and development of new drugs thus it could make a significant contribution to research in human production practices and sciences.

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