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Development of Proteomics and Applications of Proteomics in Toxicology

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Abstract

Proteomics has recently received intense scientific interest after the completion of the Human Genome Project, because this genome-based high technology allows to search new drug targets or diagnostic markers. Many proteome projects including Human plasma proteome projects (HPPP), Human liver proteome projects (HLPP), Human brain proteome projects (HBPP), and Mouse and Rat Proteome Project (MRPP) have been carried out and proteomic analytical techniques have been developed in second dimensional electrophoresis (2-DE) and LC/MS system. This powerful method has been applied in toxicology producing a new term "Toxicoproteomics". In this review, recent proteome projects, proteomic technologies, and toxicoproteomics will be discussed.

Keywords: LC/MS, plasma, proteomics, second dimensional electrophoresis, toxicology

After the completion of the Human Genome Project, many genome-based high technologies have been developed for searching new drug targets or diagnostic markers.

Now, we have to face the fact that the genome sequence and protein function cannot be directly correlated. It also has become increasingly clear that genetic alterations will ultimately influence proteins and their function. In this view, proteomics has been focused to study protein changes and functions in cells or organisms. Therefore, proteomics has been widely applied in drug discovery and development of pre-diagnostic biomarkers. Internationally, various proteome projects including Human plasma proteome projects (HPPP), Human liver proteome projects (HLPP), Human brain proteome projects (HBPP), and Mouse and Rat Proteome Project (MRPP) have been carried out by many research teams, which organized Human Proteome Organization (HUPO) at 2001[1].

In addition, proteomic analytical techniques have been developed very quickly in second dimensional electrophoresis (2-DE) and LC/MS system, which allow users to determine proteome in cells or organism. In specialty, plasma proteomic study has been focused to find diagnostic biomarkers in clinical and toxicological fields. Furthermore, the application of proteomics in toxicology has recently received intense scientific interest. The term "Toxicoproteomics" is used to describe two broad over looping areas (1) investigative or mechanistic studies and (2) screening or predictive toxicology[2].

In this review, we will discuss about recent international proteome projects, proteome techniques including plasma proteomic technology, and application of proteomics in toxicology.

Proteomic Researches

1) Human proteome organization (HUPO)

The first world congress of Human proteome organization was held in Versailles, France. In this meeting, Human plasma proteome projects (HPPP) was established and initial role of participating laboratories in the world also was discussed. The second HUPO congress was held in Monteal, Canada. Human liver proteome projects (HLPP), Human brain proteome projects (PBPP), and Mouse and Rat Proteome Project (MRPP) were established and each goal of project was discussed. The third congress was held last year in Beijing, China. Monoclonal antibody initiative was formed and new technologies and studies were reported.

2) Human plasma proteome projects (HPPP)

HPPP was started by proteome research team in University of Michigan, An harbor. The initial roles

of HPPP were 1) Provide HUPO PPP reference specimen 2) Comparison of proteome between EDTA v Heparin v citrate-anticoagulated plasma v serum from same individual, 3) Database development: ① Develop methods for identification, enumeration, and comparison of proteins, ② Propose system for identifying structure and function of related proteins, ③ Build inventory of plasma/serum proteins from HUPO PPP studies, ④ Link with other databases (Swissprot, EBI; DOE/PNNL; etc). Several committees were elected and challenges and issues were raised for HPPP; ① Sensitivity of Various Techniques, ② Technical aspects of specimen; collection, handling, storage, anti-protease, ③ Depletion of high abundant plasma proteins, ④ Comparison of serum and plasma, ⑤ Liquid phase multi-dimensional methods vs gel-based, ⑥ Parameters for high-through put link to MS, ⑦ MALDI and SELDI comparison, ⑧ Various labeling methods. However, these challenges and issues have been solved by international participating proteomics laboratories. Three plasma samples were discussed for HPPP ① National Industry Biological Standard & Control : Freeze and Dried Plasma of 25 individuals (HIV, HBV and HCV tests; 5000 each/1 ml), ② American Red Cross: Several thousands donors (K-EDTA, Sodium Citrate, Lithium heparin, anti-protease) ③ BD (Bruce Haywood & David Warunek) Biosciences Co.: Healthy, fasting, female and male donors (K-EDTA, Sodium Citrate, Lithium heparin, anti-protease) and finally American Red Cross sample was selected and provided to international HPPP participating laboratories[1].

3) Human liver proteome projects (HLPP)

First meeting for HLPP was held in Bethesda, MD on July, 2003 and a global team was built to map the human liver proteome as major initiate of HUPO. Collection of liver specimen (Banking), Protein expression profiles, and antibody bank of human liver proteins were discussed as major works and China research team led by Dr. Fuchu He had a key role to establish HLPP[1].

4) Human brain proteome projects (HBPP)

HBPP started under the patronage of the Human Proteome Organization (HUPO) and their aims: are 1) to analyze the brain proteome of human as well as mouse models in healthy, neurodiseased and aged status with focus on Alzheimer's and Parkinson's Disease, 2) to perform quantitative proteomics as well as complementary gene expression profiling on disease-related brain areas and bodily fluids, 3) to advance knowledge of neurodiseases and aging in order to push new diagnostic approaches and medications, 4) to exchange knowledge and data with other HUPO projects and national / international initiatives in the neuroproteomic field, 5) to make neuroproteomic research and its results available in the scientific community and society[1].

5) Mouse and Rat Proteome Project (MRPP)

MRPP started by Canadian research team and has following scientific deliverables: 1) collection and banking of rat and mouse tissue specimens using rigorous global standards. 2) determination of proteome expression profile of mouse and rat liver, 3) elucidation of protein modification profiles of liver, 4) intracellular localization of mouse and rat liver, 5) antibody bank for mouse and rat liver proteins, 6) Bank of genetically modified rat and mouse with tagged liver cells and liver proteins[1].

Development of Proteomics Technology

1) Two dimensional electrophoresis (2-DE) proteomics

Nowadays, the most common proteomic analytic technique is two dimensional polyarcylamide gel electrophoresis (2DE-PAGE). In the original procedure the first dimension, isoelectric focusing, is run in thin polyacrylamide gel rods in glass or plastic tubes. The gel rods contain urea, detergent, reductant and carrier ampholytes to form the pH gradient in electric field. In present time, the immobilized pH gradient strips with various size (7-24 cm) and range (3-11 pH) were developed for achieving higher resolution by several companies. Two types of immobilized pH gradients are available as linear and nonlinear gradients. New instrument, IPGphor was also developed to rehydrate, reswell and separate protein sample in strips with muti-IPG Drystrip tray. Gel with about 20×20 cm or 24×20 cm have been standard for an adequate spatial resolution. Very few groups manage to handle giant gel sizes like 35×45 cm^3.

2) LC/MS proteomics

Liquid chromatographic mass spectrometers (LC/MS) has been applied to analyzed the peptide fragmentation with combination electro spray ionization (ESI), which allows to separate polar biological compounds. In general, ESI ionizes liquid samples and is most often used for small molecules or peptides. For analysis, time of flight (TOF) is most frequently used with matrix assisted laser dissociation (MALDI), whereas ESI is usually coupled to quadrupole or ion-trap analyzer. There are two types of mass spectrometers, MS and MS/MS. MS is the faster, easier-to-operate option. In other hands, MS/MS can take some of the ion that have been separated and measured fragment them further, and then generate spectra of

those parts. In addition, MS/MS analysis enables to determine which amino acids the peptides contain and to identify the sequence of these amino acids within the peptides. Recently, nano-LC/MS system has been developed to enlarge the resolving power of separation of tryptic fragmented peptides and coupled ESI-ion-trap-quadrupole for precise proteome study[4,5].

3) Plasma proteomics

Plasma is the soluble component of human blood and contains 60-80 mg of proteins/ml. The 22 major proteins including albumin (35-45 mg of proteins/ml), Immunoglobulins (IgG, A, M: 12-18 mg of proteins/ml), Fibrinogen (2-6 mg of proteins/ml), Lipoprotein (LDL, HDL: 4.6-8.5 mg of proteins/ml), Transferrin, Macroglobulin, Haptoglobin, Antitrypsin, etc., constitute 99% of the protein content of plasma. Only 1% of the entire protein content of plasma is made up of proteins that are considered to be in low abundance and of great interest in proteomic studies in search of potential biomarkers[6]. However, plasma or serum proteins are useful target molecules to search

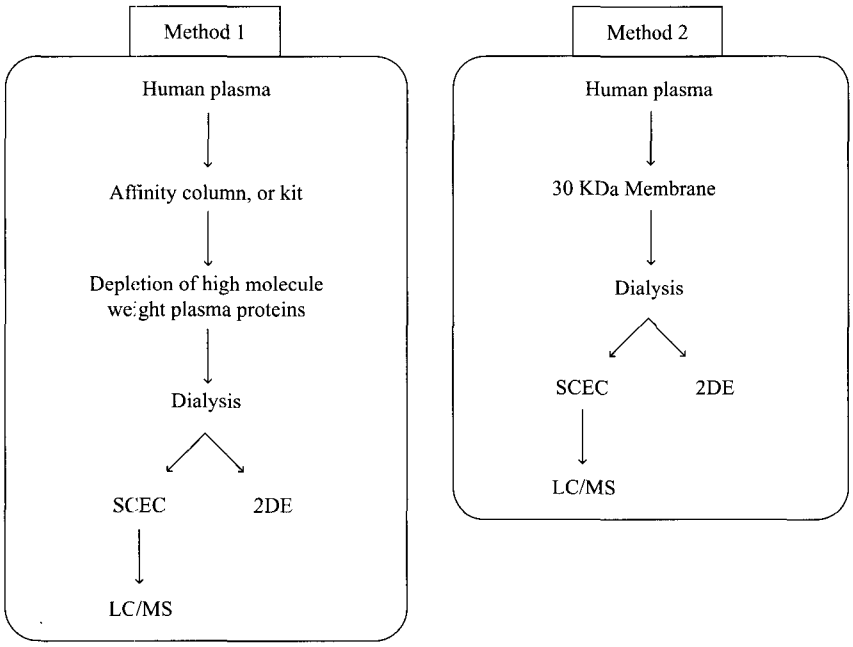

Fig. 1. Two Depletion Methods of High Abundant Plasma Proteins Using Multiple Affinity Columns and Cut Off Membranes

Table 1. Comparison of Plasma Proteome Profile

No.	Journal	Separation	Analysis	Number of identified proteins
1	Adkins J.N. *et al.* Mol Cell Proteomics, 2002	AC(IgG) / SCX / RPLC	LC/MS/MS	58(ID)/490
2	Pieper R. *et al.* Proteomics, 2003	AC(Alb,IgG) / AEC / SCX / 2-DE	MALDI-TOF/MS & LC/MS/MS	325(ID)/3700
3	Tirumalari R.S. *et al.* Mol Cell Proteomics, 2003	Cut off M/RPLC	LC/MS/MS	340(ID)
4	Fujii K. *et al.* J proteome Res, 2004	Affinity(Alb,IgG)/ SCX / 2-DE	LC/MS/MS	174(ID)
5	Marshall J. *et al.* J proteome Res, 2004	1-DE	MALDI-qTOF/MS & LC/MS/MS	600(ID)
6	Shen Y. *et al.* Anal Chem, 2004	SCX / n-RPLC	LC/MS/MS	>800(ID)
7	N. Leigh Anderson *et al.* Mol Cell Proteomics, 2004	MARC / SCX / Cut off M	MALDI-TOF LC/MS/MS	1175(ID)

AC:Affinity Chromatography, AEC:Anion Exchange Chromatography, COM:Cut Off Membrane, DE:Dimension Electrophoresis, SCX:Strong Cation Exchange Chromatography, RPLC:Reverse Phase Liquid Chromatography, MARC:Multiple Affinity Removal Chromatography

clinical and toxicological biomarkers. In general, the major high molecule plasma proteins are deleted before proteomic analysis of 2-DE or LC/MS. Two different depletion methods of major high molecule plasma proteins have been developed. Multi affinity column (depletion of 6 major plasma proteins) and cut off membrane (30 KDa or 50 KDa) are used to prepare plasma sample for proteomic analysis. In case of multi affinity column method, six major plasma proteins including albumin, transferrin, antitrypsin, haptoglobin, immunoglobulin G, and A were depleted from the plasma, but 16 high abundant plasma proteins still interrupts proteomic analysis (Fig. 1). The cut off membrane is designed originally to delete salts or low molecule weight proteins from the sample for obtaining target molecules. However, in proteomic analysis 30 KDa cut off membrane is applied to delete higher molecule weight of plasma proteins than 30 KDa to use low abundant plasma proteins (Fig. 1)[6].

Many plasma proteome studies have been reported (Table 1). Recently 1175 plasma proteins are identified by MALDI-TOF and LC/MS/MS. However, there are still a lot of problems to be solved in further plasma proteome project. Proteomic technologies using 2-DE or LC/MS should be developed and provide better proteomic analytical results[6-12].

Toxicoproteomics

Toxicological effects between normal and exposed subjects show differences in absorption, distribution, metabolism and excretion of the toxicant, or DNA damage and repair, which causes changes in genes regulating protein expression or controlling cell growth and differentiation. Although a great number of environmental and genetic risk factors have been identified, high-throughput technologies for the assessment of toxicological properties are needed[13].

A number of studies about toxicological proteomics have been reported in animals and human exposed to various kinds of toxicants. In these studies, toxicological biomarkers have been discovered and toxicological mechanisms were postulated[14-18]. In vitro, toxicological proteomic experiments were carried out in the low level of toxicants exposure, which could produce practical biological markers that are applied to human monitoring to environmental toxicants. Several assays including MTT assay, Comet assay, and LDH assay, were used to evaluate proper concentration of toxicants to cells for the development of toxicological biomarkers[14](Fig. 2). In general, chemical or environmental toxicants cause gene mutation, DNA damage, DNA adducts, and other types of genotoxic effects that induce the changes in genes controlling protein expression.

Oh *et al.* reported that low level benzo(a)pyrene exposure causes DNA single strand breakage, which

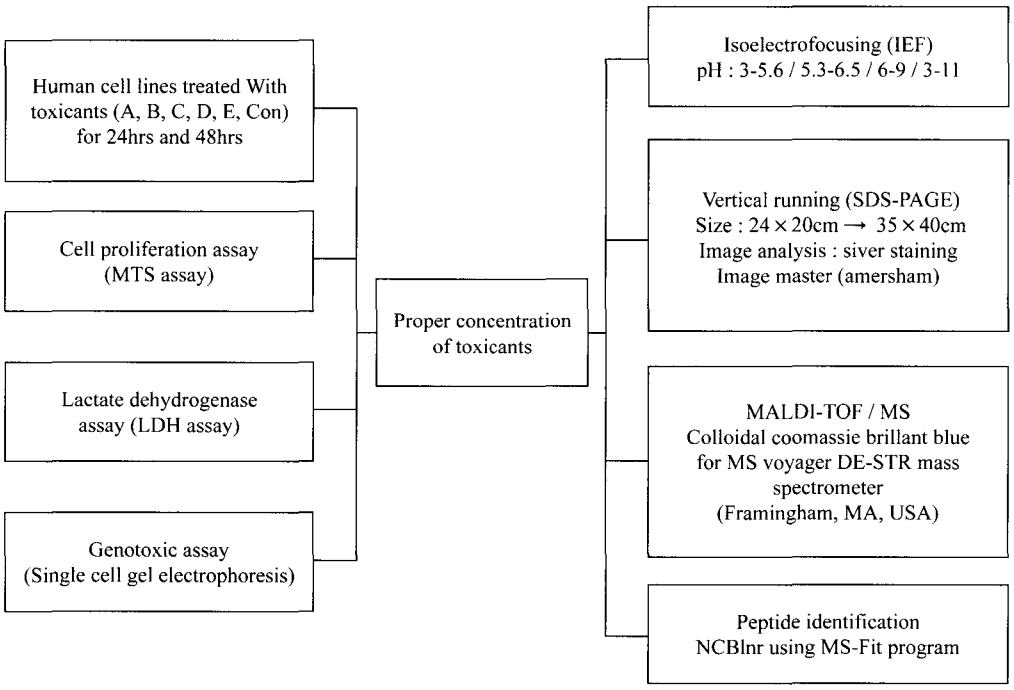

Fig. 2. Schematic Procedure for Evaluation of Toxicological Biomarkers in Cells; Comet Assay for Determination of Genotoxicity, MTT Assay for Determination of Viability, and LDH Assay for Cytotoxicity

can change gene expression and the proteome in Jurkat cells. In this study, they chose the human T-lymphocytes and B(a)P as target cells, and B(a)P as the toxic compound at two different concentrations (2.5 μM and 10 μM) for the cytotoxic and genotoxic assays, because 2.5 μM of B(a)P did not cause the cell death bu: did cause DNA damage because 10 μM of B(a)P, which caused significant cytotoxicity and genotoxicity also induced greater changes in protein expression in human T-lymphocyte cells. DNA damage was evaluated by the alkaline version of the comet assay in Jurkat cells exposed to 0, 2.5, 5, 10, 20, or 40 μM of B(a)P for 24 or 48 hr. MTS and LDH assays were carried out to evaluate the cytotoxicity in human T-lymphocyte cells treated with B(a)P. In Jurkat cells treated with 2.5 μM B(a)P, the LDH and MTS assays showed no significant cytotoxic damage. In this study, they postulated that toxicities capable of causing DNA damage without cell death could change of protein expression in human exposed to low concentrations of PAHs containing B(a)P as a major toxic components.

Finally, for proteomic analysis, cells were treated with 2.5 μM or 10 μM of B(a)P for 48 hrs and their protein profiles were then analyzed and identified by 2-DE and MALDI-TOF[14].

Recently, proteome analysis offered a mean of conveniently analyzing differential gene expressions at the protein level by comparing the 2-DE patterns of proteomes under different conditions after expo-sure to compounds of toxicological relevance.

Moreover, large range of immobilized pH gradients (IPG) strips and more advanced two-electrophoresis analysis make possible to identify a number of proteins whose level significantly increased or decreased after treatment with toxic compounds in cells and animals, moreover, responding to environmental challenges[13]. With increasing of interest in toxicological effects on human health, toxicological proteomics will provide a powerful tool to search the way that evaluates the risk assessment of chemical environmental toxicants in human. In addition, toxicological proteomic researches will open the new pre-diagnostic or preventive clinical areas and support the basic scientific development.

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