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Trends In Proteomics : A Tool In Disease Diagnosis And Drug Discovery

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ABSTRACT

Proteomics is the large-scale and extensive study of proteins, which are the vital components in the physiological and metabolic pathways of living cells. The aim of the present work is to study the various trends in proteomics especially 2- Dimensional, Differential Gel Electrophoresis and Mass spectroscopy. 2-Dimensional Gel Electrophoresis and Mass Spectroscopy are the main tools which are used in the Proteomics field. 2-Dimensional gel electrophoresis is used for the separation of proteins from a group of proteins on the basis of their molecular weight and isoelectric pH. Mass spectrometry determines the molecular masses of proteins and peptides, as well as small pharmaceutical molecules, and defines their structural features based on analyses of MS data by bioinformatics database methods. Various techniques used in the proteomics field were studied. The role of proteomics in biomarker discovery, target identification and drug discovery were also studied. The use of proteomics in finding out the mechanism of drug action and presence of chemical warfare agents in the body were also taken into consideration. The use of latest sophisticated instruments, software and databases has revolutionized the field of proteomics. The knowledge about the various challenges and trends in this field has enlightened the future proteomics. From the present study we were able to conclude that the importance of proteomics in our biological life has tremendously changed in the recent years, thus making it an important tool in disease diagnosis and drug discovery.

WHAT IS PROTEOMICS ?

The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system [1]. This will vary with time and distinct requirements, or the stress, that a cell or organism undergoes. Drug actions largely involve interactions of the drug molecule with a specific protein that regulates physiological systems in health and disease. It is, therefore, essential to investigate the system of proteins that interact in pathways underlying disease mechanisms as a means for identifying new drug targets. Such study of proteins in pathways of biological systems is known as proteomics. 'Proteomics' is defined as the study of the structure, quantities, and coordinated functions of interacting proteins in a biological system. Proteomic research will facilitate development and discovery of new therapeutic agents for the treatment of human diseases. The attention of the scientific community has now switched to the proteome, the protein content of specific cell types at any one time.

Proteomic projects involving biochemical, genetic and computational methods are being used to elucidate the functions of thousands of proteins in healthy and diseased states. The results provide valuable information both for understanding basic physiological processes and for the development of novel therapeutics. However, the ability of a protein to moonlight, or to have more than one function, can complicate the interpretation and application of the results from these proteomics studies [2].

Proteomics or the study of protein expression may also play a role in drug discovery. Two-dimensional polyacrylamide gel electrophoresis (2-DE) coupled with mass spectroscopy has been the most widely accepted format to study protein expression. However, protein microarrays are now being developed and modified to a high-throughput screening format.

Compound discovery and development is an intense and lengthy process. For the pharmaceutical industry, it will take number of years to bring a drug from discovery to market & it will cost millions of dollars for a single drug. The percentage of

compounds, which fail or drop out of the process, is extremely high, over 99%. To address these issues, new genomic and proteomic technologies have been developed over the last several years. These methods are aimed at discovering new proteins, quantifying and analyzing their expression and assigning functionality[3].

TECHNOLOGIES USED IN PROTEOMICS

The prevailing operational definition for proteomics is the combined use of mass spectrometry (MS) with two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) analysis to study gene expression at the protein level. 2D-PAGE is still the most comprehensive, quantitative, high-resolution method for displaying proteins, while MS is the most sensitive method available to identify proteins.

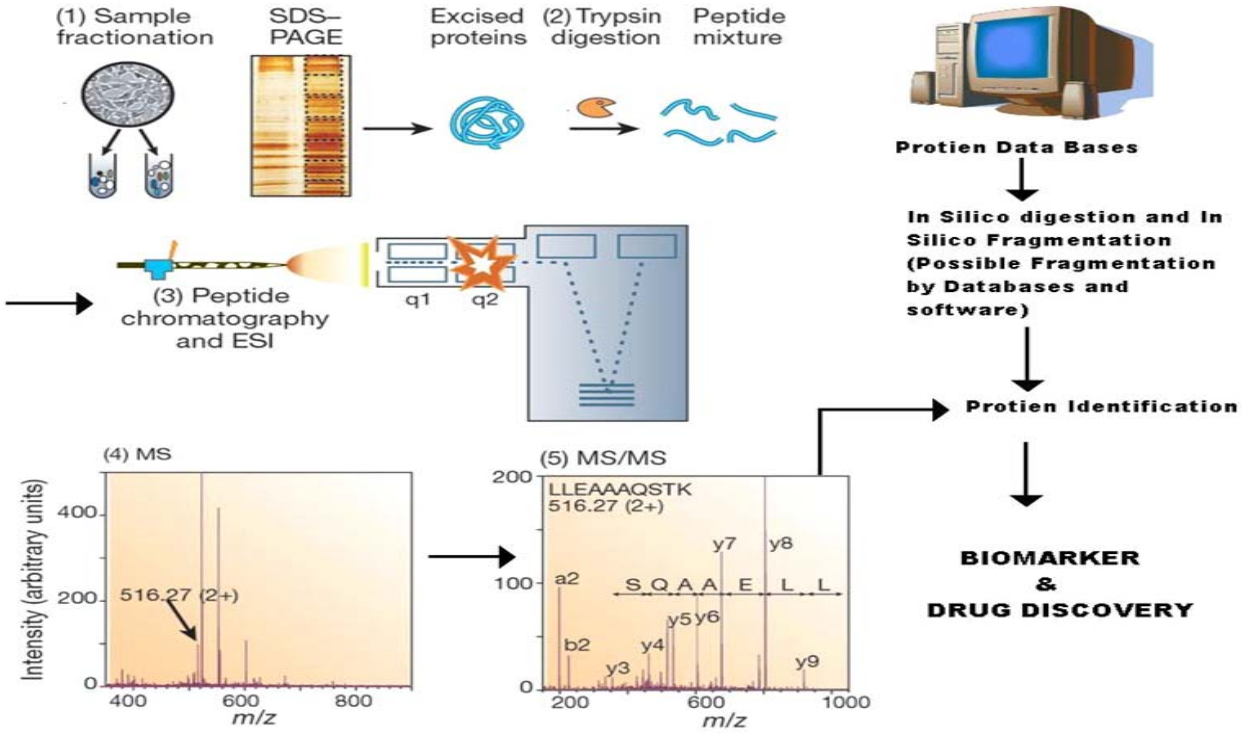

Fig 1 Schematic overview of two protein identification strategies commonly followed in proteomics and drug discovery

Proteomics uses various techniques [e.g. two-dimensional gel electrophoresis (2D-PAGE), mass spectrometry (MS), isotope coded affinity tags (ICAT), protein chip arrays and surface enhanced laser desorption/ionization (SELDI)] aiming to comprehensively profile the expressed proteins (proteome) in a particular system [4].

Protein samples are separated by either two-dimensional (2-D) or one-dimensional (1-D) polyacrylamide gel electrophoresis (PAGE)(5). 2D-PAGE technology has improved dramatically over the past five years. Since the introduction of Immobiline focusing strips, in which the pH gradient is covalently linked to the gel, 2D gels have been shown to be extremely reproducible. 2D gels are capable of resolving thousands of proteins (including isoforms and post-translationally modified proteins) and, when stained with silver or fluorescent dyes, provide a sensitive method for quantitating protein expression. Sample loading advances and larger gel formats have increased protein capacity to milligram levels, and improved sample extraction techniques are increasing the representation of total cellular protein loaded onto the gel [6-9]. Recent improvements in sample handling and instrumentation allows for more simplified sample loading and focusing. 2D-PAGE technology is able to separate complex protein mixtures into their individual polypeptide components, and can compare the protein expression of sample pairs (i.e. normal versus transformed cell) and also protein response of the cell under specific conditions(i.e. following the addition of a drug to a given cell type) [10].

MS has also been advancing at a rapid pace to position itself as the fundamental tool for high-throughput, high-sensitivity protein sequence analysis. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry has been adapted for high-throughput peptide mass fingerprinting for protein identification [11-15]. This technique is ideal as an initial screening of 2D gel spots to identify known proteins from the ever-expanding databases. More definitive identifications based on peptide sequence or identification of protein modifications can be achieved with tandem mass spectrometry (MS/MS) [16]. Triple quadrupole or ion trap mass spectrometers have been used for generating fragmentation data of peptides for database searches or sequence analysis [17-19]. The modification of the electro spray ion source to a micro- or nano spray configuration has increased the sensitivity by at least an order of magnitude [20, 21]. Consistent, successful identification of faint silver-stained 2D gel spots at low femtomole levels is now attainable [22]. Capillary Electrophoresis- Mass Spectroscopy (CE–MS) was extensively applied to protein and peptide analysis and biomarker discovery during the last 5 years [23]. Latest mass instrument parts like MALDI, Electron Spray Ionization (ESI) & TOF are combined with CE and used extensively for identifying protein and peptide disease biomarkers in urine, Cerebrospinal fluid & Other body fluid. It is also used for finding out the post translational protein modification [24].

However, ionization techniques that allow production of gas-phase molecular ions (electro-spray ionization, ESI) of proteins or liquid phase peptides (matrix-assisted laser desorption ionization, MALDI) have been adopted in mass spectrometry technology (ESI-MS, MALDI-MS). Both technologies are highly dependent on gene and protein bioinformatics, as the precise mass of any given protein is a unique feature that can be matched with a theoretically calculated mass obtained from information available in protein or genomic databases [10]. Laser capture micro-dissection (LCM) is another technology that allows separation of a cellular subpopulation from a complex tissue under direct microscopic visualization [25].

APPLICATIONS OF PROTEOMICS

In recent years, proteomic technologies have led to enormous advances in basic research and medicine. On the clinical side, proteomics is viewed as a promising new approach that will speed the discovery and validation of protein biomarkers that correlate with disease and allow for assessment of therapeutic regimens. Various Applications of proteomics are explained in details below:

BIOMARKER DISCOVERY AND DIAGNOSIS OF DISEASE

A biomarker can be any biomolecule which is an indicator of a change in any biological structure and function that can objectively measure the state of a living organism. The major objective is to evaluate an individual's state of health for diagnosis of any type of disease, preventive treatment, and/or treatment efficacy if a disease has been diagnosed. Disease biomarkers can be informative about underlying molecular mechanism of disease as well as treatment. Proteomics is the systematic evaluation of proteins produced by the cell under normal or pathological circumstances. Investigating protein production will allow us to identify and modify disease natural history and treatment through biomarker discovery [10, 26].

Serum/plasma, cerebrospinal fluid, urine, solid tissue biopsies, blood cells, tears etc. can be used as a sample material for proteomics analysis. Proteomic profiling of serum and plasma revealed that there is no technology platform that can analyze proteins quantitatively with a dynamic range of concentration as high as 10^{12} and that pre-fractionation of the samples is necessary. Removal of most abundant proteins from serum/ plasma and CSF samples became a standard first step in clinical proteomics analyses aiming at biomarker discovery. This widely used approach is now commonly accepted as the first step in sample preparation and it is quite obvious that immune depletion of the 12 most abundant proteins is necessary (i.e. serum albumin, IgG, brinogen, transferrin, IgA, IgM, haptoglobin, apoA-I, apoA-II, a1-antitrypsin, a1-acid glycoprotein, a2-macroglobulin). However, immune depletion of multiple proteins can increase the risk of losing proteins of interest or low-abundant candidate biomarkers that are removed along with those specically depleted. Albumin is the most abundant protein in plasma and is a carrier of many proteins and other compounds (e.g. lipoproteins and aminoacids) [27].

CANCER BIOMARKERS:

Proteomics provided enormous data-gathering capabilities by developing several clinical biomarkers from serum, plasma, urine, tissues and other biological samples. Serum proteome profile is used to monitor the development and progression of Ovarian and colon cancers. Several biomarkers associated with different types of Cancers were identified that include psoriasin for bladder. Squamous cell carcinoma, prostate cancer antigen-1 and calgranulin B for prostate cancer, combination of five novel proteins and seven protein clusters for transitional cell carcinoma, defensing for bladder cancer and matrix metallo proteinases, 2,-4,fibronectin and their fragments for general cancers [28-32].

CARDIOVASCULAR BIOMARKERS:

Troponin B-type, natriuretic peptide and C-reactive proteins are increasingly moving towards more productive clinical use in Cardiac diseases. Directed proteomic approaches identified a set of 177 candidate biomarker proteins associated with cardiovascular diseases. Although more than 1000 proteins were detected in plasma or serum, a paradoxical decline occurred in the number of new protein markers approved for diagnostic use in clinical laboratories [28].

NEUROLOGICAL BIOMARKERS

Clinical proteomics, used for discovery of biomarkers in blood and cerebrospinal fluid, reflected central pathogenic processes of Alzheimer's and Parkinson's diseases. Protein sequence analysis identified a 13.4-kDa protein species as cystatin C and a 4.8-kDa protein species as a peptic fragment of the neurosecretory protein VGF. Detection of new biomarkers in brain proteome further strengthened diagnosis and provided useful information in drug trials in mouse models. Alzheimer's disease (AD) is an invariably fatal neurodegenerative disorder with no effective treatment or definitive antemortem diagnostic test. Little is known about the changes in the brain preceding or accompanying initiation of the disease. Understanding the biological processes, which occur during AD onset and/or progression, will improve the diagnosis and treatment of the disease. However, the expressions of proteins involved in synaptic plasticity are selectively altered in the brain of cases at high risk to develop AD dementia [33, 34].

HIV BIOMARKER

From the analysis of the sera from HIV-infected individuals with or without concurrent infection through SELDI-TOF-MS analysis followed by weak cation exchange chromatography and one-dimensional electrophoresis, it is found that the proteins gelsolin and pre albumin are differentially expressed. The use of integrated proteomic platform to assess cerebrospinal fluid protein profiles from 50 HIV-1 seropositive Hispanic women, gave information that macrophage capping protein L-plastin and Cu/Zn superoxide dismutase (Cu/Zn SOD) were only detected in CSF.

Urine proteomic analysis of HIV-infected children with renal disease proved that high urine levels of ß2-microglobulin and retinol-binding protein is due to the presence of tubular injury. Elevated urine levels of iron and

the iron-related proteins, transferrin, hemopexin, haptoglobin, lactoferrin, and neutrophil gelatinase-associated lipocalin were also found in children with HIV-nephropathy (HIVAN) and HIV-associated hemolytic uremic syndrome (HIV-HUS) [28, 35-38].

TARGET IDENTIFICATION

Proteomics provides a protein profile of a cell or tissue that can be used to compare a healthy state with a diseased one for protein differences in the search for drugs or drug targets. This is one of the most important applications of proteomics; it has the unique added advantage that bodily fluids can be used for profiling. Identification of diagnostic markers for disease states such as cancer by a differential proteome profiling of diseased and normal tissue is a subset of target identification [22].

TARGET VALIDATION & TOXICOLOGY

Proteomics has an application as an assay for the potential utility of drug candidates. This can be achieved by a comparative analysis of reference protein profiles from normal or disease states with profiles after drug treatment. Proteomic technologies can also be integrated with combinatorial chemistry to evaluate comparative structure–activity relationships of drug analogs. These applications could accelerate identification and optimization of lead candidates for clinical development.

A variant of target validation is the use of proteomics to study the toxicity of drugs. A comparison of the protein profiles from normal tissue or tissue treated with the known toxic agent might give an indication of the drug's toxicity. There is no substitute for dealing directly with proteins when studying Protein–protein interactions and complexes. The combination of high-sensitivity Bio-core affinity purification and MS analysis have shown to be an ideal combination for identification of specific protein–protein interactions at nano gram levels.

CHEMICAL WARFARE

Medical research on the effects of chemical warfare agents (CWAs) has been going on for nearly 100 years, yet these agents continue to pose a serious threat to deployed military forces and civilian populations. CWAs are extremely toxic, relatively inexpensive, and easy to produce, making them a legitimate weapon of choice for terrorist organizations. While the mechanisms of action for many CWAs have been known for years, questions about their molecular effects following acute and chronic exposure remain largely unanswered. Global approaches that can pinpoint which cellular pathways are altered in response to CWAs and characterize long-term toxicity have not been widely used. Fortunately, innovations in genomics and proteomics technologies now allow for thousands of genes and proteins to be identied and subsequently quantied in a single experiment. Advanced bioinformatics software can also help decipher large-scale changes observed, leading to mapping of signaling pathways, functional characterization, and identication of potential therapeutic targets [39].

PROTEOMICS AND DRUG DISCOVERY

Proteins are the principal targets of drug discovery. Most of the large pharmaceutical companies now have a proteomics-oriented biotech or academic partner or have started their own proteomics division. Common applications of proteomics in the drug industry include target identication and validation, identication of efficacy and toxicity biomarkers from readily accessible biological fluids, and investigations into mechanisms of drug action or toxicity. Target identication and validation involves identifying proteins whose expression levels or activities change in disease states. These proteins may serve as potential therapeutic targets or may be used to classify patients for clinical trials.

If a certain protein is implicated in a disease, its 3D structure provides the information to design drugs to interfere with the action of the protein. A molecule that fits into the active site of an enzyme, but cannot be released by the enzyme, will inactivate the enzyme. This is the basis of new drug-discovery tools, which aim to find new drugs to inactivate proteins involved in disease. As genetic differences among individuals are found, researchers expect to use these techniques to develop personalized drugs that are more effective for the individual.

The practice of proteomics ranges from the identication of thousands of proteins in a particular model system, to the detailed analysis of the 3D structure, possible modifications/ isoforms, and function of a single protein. In addition to the hope that proteomic technologies can help achieve higher drug development success rates, the recent emphasis on developing disease-modifying compounds makes proteomic analysis of disease etiology and progression of critical importance.

Once a target protein has been validated, the task of identifying chemical compounds that can appropriately modulate the target can also be aided by proteomic techniques: namely structural proteomics. The knowledge of the crystal structure of the protein facilitates the production of suitable drug candidates, as it can be directly fed into virtual screening software that will select fragments or compounds that will bind the protein surface of interest. Such virtual screening is valuable not only to the selection of a group of chemical entities which will "hit" the protein target, but as a screen for structure-activity relationships among those agents to optimize the lead compound [40]. Thus proteomics study helps in identifying new drug targets and optimizing the choice of lead compound candidates by more closely predicting their success or failure [3].

CHALLENGES INVOLVED IN PROTEOMICS

Many thousand types of proteins are present in our body as functional agents. Proteomics therefore generates much larger datasets requiring more resources to handle and analyze data effectively [10]. One of the important problems in the study of proteins is the maintenance of secondary and tertiary structure during the study, which can be denatured by the action of enzymes, heat, light, or by aggressive mixing. The cost is also a precluding factor for the wide spread use of proteomics in clinical laboratory. Most proteomics technologies use complex instrumentation, critical computing power, and expensive consumables.

Proteins cannot be amplified like DNA, therefore less abundant species are more difficult to detect. Albumins together with another 10 proteins may constitute 90% of total proteins in body. Low abundant proteins like cytokines constitute a very small fraction (1-5 pg/ml). The removal of high abundant proteins from plasma or serum is the prerequisite for conducting more detailed proteomics studies on low abundant proteins. However, many potentially important biomarkers may be lost in this process due to non-specific binding or the co-removal of proteins/peptides intrinsically bound to the high abundant carrier proteins.

In addition to the above mentioned factors, several limitations are there in development of proteomics as a biomarker. The major challenge is the discrimination of changes due to inter individual variation, experimental back ground noise in protein profiling and post-translational modifications. Despite intensive researches, a very limited number of plasma proteins have been validated as biomarkers for disease [41].

TRENDS IN PROTEOMICS

The technologies in proteomics are developing tremendously. The applications of proteomics which were facing so many challenges have become popular nowadays. Differential Gel Electrophoresis has become popular due to the advancement in the separation of proteins. With the emergence of new staining reagents, like Pro-Q Diamond dye, gel electrophoresis has also achieved new developments [42]. Databases like SWISS-2DPAGE, World-2DPAGE Portal, and 2DWG Meta-Database are augmenting the research works taking place in proteomics by facilitating protein separation.

Changes in the mass spectroscopy components have also made proteomics research easier. Metabolic labeling like Stable Isotope Labeling by Amino acids in Cell culture, SILAC along with the hybrid linear ion trap Fourier transform ion cyclotron resonance (LTQ-FTICR) increased the accuracy of protein identification. SILAC-combined HPLC/MS/MS technology has developed into one of the most effective techniques in current proteomic quantifications. Chemical labeling like isotope coded affinity tags (ICAT) had been applied to the analysis of whole-cell protein expression changes. Isobaric tags for relative and absolute quantification (iTRAQ) is an isotope-labeled technology for labeling the N-terminal and lysine residues in amino acid after digestion.

Label-free methods for quantization of proteins are also used in proteomics. Label-free method is based on the MS spectral counts or the peak intensity to analyze the protein changes in different samples. Label-free strategy combined with MALDI-Q-TOF and LTQ-FT MS are used to analyze the major residues of the human EGF receptor, such as protein dynamics, coverage, sequence identification and new phosphorylated sites.

Reagents like fluorescent isotope-coded affinity tag (FCAT), metal-coded affinity tags (MeCAT) and software, like MaxQuant, are used to facilitate quantitative proteomics and provide relevant biological information for protein function studies. Absolute quantification (AQUA) of proteins allowed us to learn the nature and function of proteins in the network. Thus trend in the proteomics widens the possibilities of proteomics research and its application[42-45]. Various trends in the field of proteomics are explained in the table 1

Table 1 The developments that occurred in the various areas of proteomics like 2-DE, Mass Spectroscopy, Databases & Software

TOOLS	NAME	APPLICATIONS
Instruments	**Electrophoresis**	
	2-Dimensional Electrophoresis	To separate the proteins from a group of proteins.
	Differential Gel Electrophoresis	More accurate in separating proteins.
	Mass Spectroscopy	
	Fourier transform ion cyclotron resonance (LTQ-FTICR), MALDI-Q-TOF, LTQ-FT MS	Accuracy in Finding out the exact mass.
Softwares	MaxQuant	Software specialized on the quantitative proteomics.
	Scaffold	For accurate protein identification.
	MassQC	Helps to achieve more reproducible LC-MS/MS experiments
Databases	SWISS-2DPAGE, World-2DPAGE Portal, 2DWG Meta-Database	Helps in Comparing and confirming the specific protein by Protein ID.
	Matrix Science - Mascot	Helps in Finding out the accurate mass of the protein.
	Pro-Q Diamond	Helps in Electrophoresis.
Kits	Stable Isotope Labeling by Amino acids in Cell culture(SILAC),Isotope coded affinity tags (ICAT), Enzyme-catalyzed ^{18}O isotope labeling, Isobaric tags for relative and absolute quantification (iTRAQ)	Labeling of protein to enhance the proteomics study. Helps in finding accurate mass of the protein by combining with mass spectroscopy.

CONCLUSION

The discoveries and breakthroughs in proteomics in the last decade have already provided new and exciting information in this field. Gene expression microarrays are now being used as test cases for speeding up the drug discovery and development process. The technologies presented are having a considerable impact on biomarker identification and drug discovery. Although proteomics is being promoted as a separate industry, it is in fact a set of technologies, which are being increasingly used in combination with genomic technologies in the post-genomic era. There is a growing realization of the importance of proteomics in the life sciences industry. This is evident by the increasing investment in this area by various pharmaceutical and genomic companies. This increased attention and investment should fuel the continued and rapid advancement of proteomics technology.

REFERENCES

1. Marc RW, Christian P, Ron DA, Keli O, Olivier G, Sanchez JC *et al.* From Proteins to Proteomes: Large Scale Protein Identification by Two-Dimensional Electrophoresis and Amino Acid Analysis. Nat Biotechnol. 1996;14(1):61–65.

2. Jeffery CJ. Moonlighting proteins: complications and implications for proteomics research. DDT: TARGETS. 2004;3(2):71-78.

3. Cunningham MJ. Genomics and Proteomics: The new millennium of drug discovery and development. J Pharmacol Toxicol Methods. 2000;44:291-300.

4. Brenner C, Duccan D. Oncogenomics, molecular approaches to cancer. John Wiley & Sons;Hoboken,New Jersey,2004.p.128-131.

5. Hamacher M, Marcus K, Stuhler K, Hall AV, Warscheid B, Meyer HE. Proteomics in Drug Research. 28th vol. Wiley-VCH;Weinheim,2006.p.57-80.

6. Rabilloud, T, Valette C, Lawrence JJ. Sample application by in-gel rehydration improves the resolution of two-dimensional electrophoresis with immobilized pH gradients in the first dimension. Electrophoresis. 1994;15:1552–1558.

7. Sanchez JC *et al.* Improved and simplified in-gel sample application using re-swelling of dry immobilized pH gradients. Electrophoresis. 1997;18:324–327.

8. Rabilloud T *et al.* Improvement of the solubilization of proteins in two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis. 1997;18:307–316.

9. Molloy MP *et al.* Extraction of membrane proteins by differential solubilization for separation using two-dimensional gel electrophoresis. Electrophoresis. 1998;19:837–844.

10. Alpantaki K, Tsiridis E, Pape HC, Giannoudis PV. Application of clinical proteomics in diagnosis and management of trauma patients. Injury. 2007;38:263-267.

11. Henzel WJ *et al.* Identifying proteins from two-dimensional gels by molecular mass searching of peptide fragments in protein sequence databases. Proc Natl Acad Sci 1993;90:5011–5015.

12. James P *et al.* Protein Identification by Mass Profile Fingerprinting. Biochem Biophys Res Commun. 1993;195:58–64.

13. Mann M, Hojrup P, Roepstorff P. Sequence tag identification of intact proteins by matching tanden mass spectral data against sequence data bases. Biol Mass Spectrum. 1993;22:338–345.

14. Pappin D, Hojrup P, Bleasby A. Rapid identification of proteins by peptide-mass fingerprinting. Curr Biol. 1993:3:327–332.

15. Yates JR *et al.* Primer on Medical Genomics Part IV: Expression Proteomics. Anal Biochem. 1993;214:397–408.

16. Hunt DF *et al.* Protein sequencing by tandem mass spectrometry. Proc Natl Acad Sci. 1986;83:6233–6237.

17. Hunt DF *et al.* Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. Science. 1992;255:1261–1263.

18. Eng JK, McCormack AL, Yates JR. An Approach to Correlate Tandem Mass Spectral Data of Peptides with Amino Acid Sequences in a Protein Database. J Am Soc Mass Spectrum. 1994;5:976-989.

19. Mann M. Wilm M. Finding Protein Sequences Using PROWL. Anal Chem. 1994;66:4390–4399.

20. Wilm M, Mann M. Mass Spectrometric Sequencing of Proteins from Silver-Stained Polyacrylamide Gels Anal Chem. 1996;68:1–8.

21. Davis MT *et al.* Method for structural characterization of biological moieties through HPLC separation. Anal Chem. 1995;67:4549–4556.

22. Wang JH, Hewick RM. Proteomics in drug discovery. DDT. 1999;4(3):129-133.

23. Klamp CW. CE with MS detection: a rapidly developing hyphenated technique. Electrophoresis. 2009;30:S83–S91.

24. Desiderioa C, Rossettib DV, Iavaroneb F, Messanac I, Castagnola M. Capillary electrophoresis–mass spectrometry: Recent trends in clinical proteomics. J Pharm Biomed Anal. 2010;53:1161-1169.

25. Petricoin EF. Clinical applications of proteomics. J Nutr. 2003;133(7):S2476-S2484.

26. Jenne A, Tebbe A, Schaab C, Godl K, Kaminski M, Muller S. Drug profiling and biomarker discovery using mass spectrometry-based proteomics technologies. J Biotechnol. 2010;08:S9.

27. Silberring J, Ciborowski P. Biomarker discovery and clinical proteomics. Trac-Trend Anal Chem. 2010;29(2):128-140.

28. Sinha A, Singh C, Parmar D, Singh MP. Proteomics in clinical interventions: Achievements and limitations in biomarker development. Life Sci. 2007;80:1345–1354.

29. Tieneke BM, Visser S, Brakenhoff RH, Leemans CR, Heck AJR, Slijper M. Protein biomarker discovery for head and neck cancer. J Proteomics. 2010;73:1790-1803.

30. Ball BM, Graham B, Robert R. Clinical proteomics: Discovery of cancer biomarkers using mass spectrometry

and bioinformatics approaches-A prostate cancer perspective. Vaccine. 2007;25S:B110–B121.

31. Makridakis M, Vlahou A. Secretome proteomics for discovery of cancer biomarkers. J Proteomics. 2010;73:2291-2305.

32. Hays JL, Kim G, Giuroiu I, Kohn EC. Proteomics and ovarian cancer: Integrating proteomics information into clinical care. J Proteomics. 2010;73:1864-1872.

33. Hoa L, Sharma N, Blackman L, Festa E, Reddy G, Pasinetti GM. From proteomics to biomarker discovery in Alzheimer's disease. Brain Res Rev. 2005;48:360– 369.

34. German DC, Garner HR, Arrastia RD, Nandi A, Gurnani P, Moore C *et al.* Clinical proteomics to diagnose Alzheimer's disease. Bomarkers. 2005:Suppl1:S14.

35. Laspiur JP, Anderson ER, Ciborowski P, Wojna V, Rozek W, Duan F *et al.* CSF proteomic fingerprints for HIV-associated cognitive impairment. J Neuroimmunol. 2007;192:157–170

36. Pinheiro FV, Pimentel VC, Moresco RN, Moretto MB. Evaluation of cerebrospinal fluid adenosine deaminase activity in HIV-seropositive subjects and its association with lactate dehydrogenase and protein levels. Biomed Pharmacother. 2004;64:302-305.

37. Kreuter A, Jesse M, , Potthoff A, Brockmeyer NH, Gambichler T, Stucker M. Expression of proliferative biomarkers in anal intra epithelial neoplasia of HIV-positive men. J Am Acad Dermatol 2010;63:490-498.

38. Zhang L, Zhang X, Ma Q, Zhou H. Host Proteome Research in HIV Infection. Geno Prot Bioinfo. 2010;8(1):1-9.

39. Everley PA, Dillman JF. Genomics and proteomics in chemical warfare agent research: Recent studies and future applications. Toxicol Lett. 2010;198:297-303.

40. Walgren JL, Thompson DC. Application of proteomic technologies in the drug development process. Toxicol Lett. 2004;149:377–385.

41. Cho WCS. Proteomics Technologies and Challenges. Geno Prot Bioinfo. 2007;5(2):77-84.

42. Jin-Lei Z, Kai Z, Xi-Wen H, Yu-Kui Z. New Developments of Quantitative Mass Spectrometry-based Proteomics. Chin J Anal Chem. 2010;38(3):434-441.

43. Jain KK. Proteomics: new technologies and their applications. DDT. 2001;6(9):457-459.

44. Zhang J, McCombie G, Guenat C, Knochenmuss R. FT-ICR mass spectrometry in the drug discovery process. DDT. 2005;10(9):635-642.

45. Hager JW. Q TRAP™ mass spectrometer technology for proteomics applications. DDT: Targ. 2004;3(2):S31-S36.