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Research Article

PROTEOMICS: AN EMERGING TOOL FOR MOLECULAR UNDERSTANDING AND APPLIED BIOSCIENCES

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ABSTRACT

Proteomics makes it possible to comprehend the complete protein complement of a cell, tissue, or organism under carefully regulated conditions, including its composition, structure, function, and connections. Stress or pharmacological side effects can change the pattern of a protein, causing it to either be present or absent or to fluctuate slowly in quantity. Numerous biological fields, including as industry, pharmaceuticals, food microbiology, medicine, and drug discovery, use proteomics. The identification of biomarkers, monitoring of drug effects on patients, and in-vitro and in-vivo proteome analysis of drug-treated individuals demonstrate the close relationship between proteomics advancements and applications and drug development. A snapshot of cellular activity and physiological processes can be obtained from changes in the proteome. Exploring the degree to which the environment, transcriptome, and genome impact the variability of the proteome under particular conditions across time has been made possible by the collecting of quantitative multi-layered omics data on different individuals over predetermined time intervals. Large, repeatable datasets are produced by contemporary quantitative, highly parallel, targeted proteomic techniques, which have unrealized promise for the identification, validation, and discovery of biomarkers. This review's goal is to provide an overview of the developments in targeted proteomics techniques for measuring the cellular proteome and discuss how to use this information to enhance the techniques now employed for precision medicine and biomarker evaluation.

Keywords: Industry, Pharmaceuticals, Food microbiology, Medicine, Drug discovery.

INTRODUCTION

New pharmaceutical approaches, biomarker discoveries, and advances in the life sciences have all resulted from a better understanding of disease biology. Growing understanding of inter-individual variation in response to clinical care has led to a recent surge in the demand for personalized medical care. Examining the connections between DNA, RNA, proteins, and eventually between the molecular composition and illness phenotypes is essential to comprehending disease biology. According to the fundamental rules of biology, a gene's coding sequence defines its mRNA output, and the mRNA sequence further controls the amino acid order in the polypeptides that arise from a protein. For a variety of reasons, many functional protein variants may arise from a specific mRNA or gene locus. Involving protein modification in modules and alternative splicing. While transcript abundance in a human cell can vary from two to three orders of magnitude, protein abundance can reach ten orders of magnitude (N. Leigh Anderson *et al.,* 2003, Yansheng Liu *et al*., 2016). All proteins expressed in a cell at any given moment, including protein isoforms, co-, and post-translational modified (PTM) forms, are included in the proteome, the time- and cell-specific protein complement of the genome. For several reasons, it is far more difficult to analyze the proteome than the genome. The proteome is extremely dynamic and complex since it is constantly reacting to external factors, including other cells, nutritional status, temperature, and drug therapy, to mention a few. The genome of a cell, on the other hand, is constant, almost the same for all the cells in an organ or organism, and

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consistent across species. Thus, the proteome is not fixed. Therefore, any proteome investigation is a "snap-shot." Proteome analysis is further complicated by the dynamic range of protein expression, which can vary by up to 7–12 orders of magnitude, while DNA fluctuates by just 5 orders of magnitude (Shao-En Ong *et al*., 2001; Kislinger T *et al*.,2003; O'Donovan C *et al*., 2001).

Protein study is considered the most difficult and complex topic of analytical chemistry since it is difficult to fully understand the many structural and physicochemical properties of proteins. However, the field of proteomics has experienced tremendous growth and development (Zhou H *et al*., 2011). The most extensively studied method for identifying and detecting a particular protein in a biological process is quantitative proteomics, which measures the protein in several situations that show significant differences in protein abundance among defined proteomes (Elliott MH *et al*., 2009). Numerous quantitative proteome analysis methods have been employed in recent years, taking into consideration a number of obstacles, challenges, advantages, and disadvantages (Zamanian-Azodi *et al*., 2013).

The specific protein can be identified and quantified using liquid chromatography and tandem mass spectrometry analysis, which works similarly to label-free quantification. Spectra ascribed to the peptides that comprise a particular protein can be counted using mass spectrometry frequencies and signal intensities. Depending on the objectives of the study, protein post-translational modification (PTM) is another significant area of proteomics research. A sample should be created utilizing both qualitative and quantitative techniques in order for the PTM methodology to yield a satisfactory outcome. (Reinders J *et al*., 2007). Proteome analysis offers a far better understanding of an organism because genomics can give an approximate estimate of protein expression. Since most proteins cooperate with one another and proteomics identifies which proteins interact, it is often considered the most advanced stage in the study of biological systems. This is due to the fact that while the genome of an organism remains relatively stable, the overall profile of protein expression is continuously altering in response to time and macro and microenvironmental factors (Petricoin III EF *et al*., 2002).

DIFFERENT CATEGORIES OF PROTEOMICS ARE DISTINGUISHED BY THE PROTEIN RESPONSE

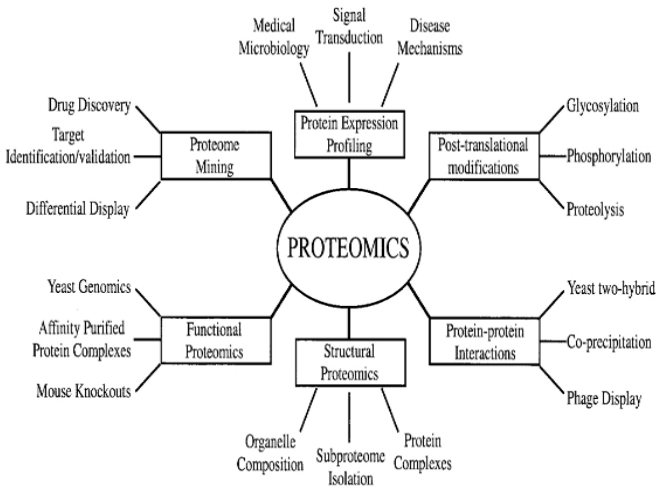

Figure 1. Types of proteomics and their applications to biology.

Source: Microbiol Mol Biol Rev. 2002 Mar;66(1):39–63. Doi:10.1128/MMBR.66.1.39-63.2002

Structural proteomics

Structural proteomics can help understand the three-dimensional shape and structural complexity of functional proteins, give detailed information about the structure and function of protein complexes found in a given cell or organism, and predict the structure of a protein when its amino acid sequence is determined directly from the gene or by sequencing using a technique called homology modelling. NMR spectroscopy and X-ray crystallography were the main techniques used to determine structure (Okayama S *et al*., 2000).

Expression proteomics

It contrasts the qualitative and quantitative expression of total proteins in two different circumstances (normal cells and treated or sick cells) and looks at the patterns of protein expression in aberrant cells. 2-D gel electrophoresis and mass spectrometry (MS) were used to evaluate changes in protein expression. Determining the changes in these

proteins' expression will offer crucial information about the disease's molecular biology and can be utilized as clinical markers or therapeutic targets (Banks RE *et al*., 2000; Hinsby AM *et al*., 2003).

Functional proteomics

This identifies the interacting protein partners, demonstrates how proteins build bigger complexes, and establishes the roles of proteins. The biological role of an unknown protein whose partners are a member of a certain protein complex involved in a given mechanism can be determined using functional proteomics (Gavin AC *et al*., 2002; Ho Y *et al*., 2002).

Proteomics in pharmaceutical industry

A person's proteome consists of over 100,000 proteins, and 10,000 genes influence the expression of specific cell types. Furthermore, the makeup of a proteome can vary widely, and phenotypes can develop in an infinite number of ways (Wilkins MR *et al*., 2013). Genetics has established gene function and has also been used to understand the molecular origins of disease and to infer protein function (Collins FS *et al*., 2001). Environmental stimuli that modify the parameters of expression, such as stress or medications, can lead to changes in the protein pattern, the presence or absence of a protein, or gradual oscillations in its abundance. Proteomics techniques are necessary for analyzing and comparing protein expression profiles in order to ascertain the quantitative protein expression profile of a cell, organism, or tissue under carefully specified circumstances. The fundamental reasons of the observed variations in protein patterns can be linked with the aid of proteomics analysis.

Proteomics in drug discovery

Finding novel medications is a complex process that includes a number of processes, including functional, chemical, and clinical proteomics-based approaches. Patient care and therapy are now included in the proteome's application in drug discovery (Collins FS *et al*., 2001). 2-DE cannot be used in drug discovery because it cannot distinguish between the membrane-associated proteins that make up around half of important therapeutic targets. Moreover, 2-DE cannot identify low-abundance proteins (Drews J, 2000). In drug discovery proteomics, it is essential to ascertain the behaviour and interactions of proteins in mixtures. Furthermore, the methods must to be able to identify low-abundance proteins and how they operate. Consequently, other methods, including as protein-chip and MS, have been used to identify and segregate phage proteins. Other methods, like as two-hybrid assays and activity-oriented assays, can also be used for this (Burbaum J, 2002). Rats with Alzheimer's disease were treated with Lavandula angustifolia using 2D-PAGE-MALDI-TOF/TOF (Zali H *et al*., 2015).

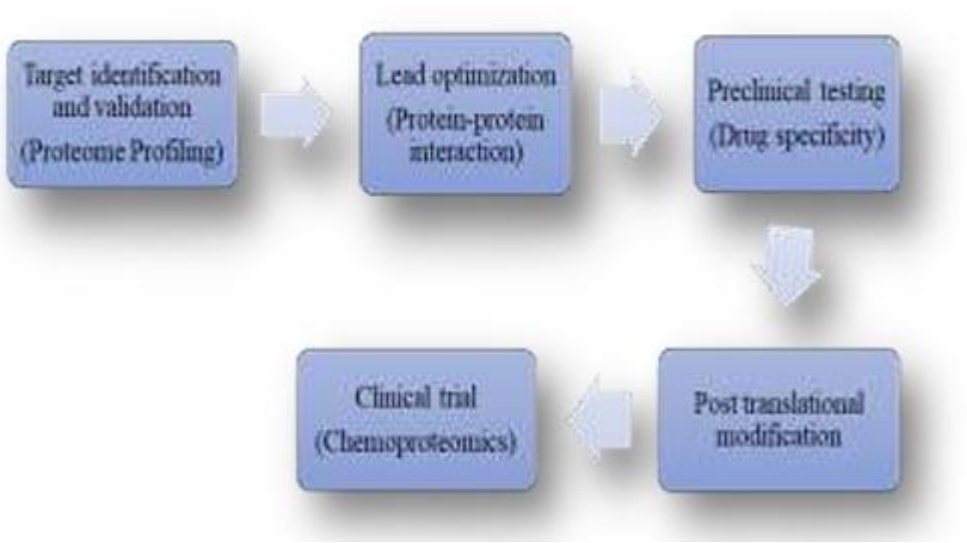

Figure 2. Stages of drug discovery

Source: Application and development of proteomics in biopharmaceutical industry; July 2021. DOI:10.2015/IJIRMF.2455.0620/202107008

Proteomics in biomarkers

Generally speaking, a biomarker is a protein or biochemical indicator associated with the disease that is used in the clinic to detect or track the disease's activity, diagnosis, and progression. It can also be used to guide biological target treatment or assess the therapeutic response (Poste G, 2011; Anderson D *et al*., 2014). In clinical settings, cancer biomarkers are employed to monitor the course of disease and the effectiveness of treatment (Goossens N *et al*., 2015).

A detectable substance introduced into an organism to investigate organ function or other facets of health is known as a biomarker in medicine. In medicine, prostate-specific antigen, or PSA, is a frequently used biomarker.

However, because biomarker tracking is expensive and time-consuming, malignancies are usually detected at advanced stages, when patients have a poor prognosis and few therapeutic options. Additionally, combining many platforms of once-proteome data and developing a higher throughput analysis technique are essential prerequisites for early detection (Zamanian-Azodi M *et al*., 2013). Since protein expression alters in biological processes under illness settings, tracking altered proteins in tissue, blood, urine, or other biological materials can offer disease indicators (He QY *et al*., 2003).

In molecular medicine, proteomics technology has been widely applied, especially in the identification of biomarkers. Through the analysis of global protein profile in bodily fluids, proteomics can identify important disease-specific biomarkers. By comparing the protein expression profiles of healthy and sick samples, proteomics expression enables the identification of biomarkers. 2D-PAGE, the most straightforward method used in biomarker discovery, examines the protein patterns of healthy and diseased materials, including tumor tissues and body fluids (Gam L-H *et al*., 2012). Blood offers several benefits over the other samples in Figure 2, making it a suitable fluid to use for the discovery of biomarkers in human disease. However, there are drawbacks to using plasma proteomics for biomarker discovery, including (i) the wide dynamic range of plasma proteins, (ii) the limited quantity of priceless biomarkers in plasma, and (iii) the variety of patients' body fluids and tissues (Rifai N *et al*., 2006). The following challenges in the biomarker verification process in plasma proteome cannot be addressed by the current single method, despite advancements in proteomic methodologies. The most popular methods for finding biomarkers for a variety of diseases are surface-enhanced laser desorption/ionization (SELDI)/protein chip and 2D-PAGE/MALDI-TOF. Due to its high-throughput applications and ease of use, SELDI-MS is utilized for biomarker detection in a variety of illnesses (Huang F *et al*., 2009)

ANALYTICAL TECHNIQUES OF PROTEOMICS

Two-dimensional polyacrylamide gel electrophoresis

For almost thirty years, 2-D PAGE has proven a reliable and efficient method for protein separation based on mass and charge. It can separate thousands of different proteins in a single gel. High-resolution 2-D PAGE can resolve up to 10,000 protein spots per gel. Stains such as Coomassie blue, silver, SYPRO Ruby, and Deep Purple can be used to see the proteins (Lauber W.M. *et al*., 2001). The proteins are separated by mass in the second dimension and by charge (isoelectric point) in the first. During isoelectric focusing, proteins move down a pH gradient until they reach a pH where they have no net charge. The most common proteins are arranged vertically according to size and horizontally according to isoelectric point. Unfortunately, 2-D PAGE is a time-consuming and labor-intensive process. It implies some inherent limitations, especially with regard to hydrophobic and alkaline proteins, which are usually underrepresented in 2-D PAGE (Nilsson C.L *et al*., 2000).

Liquid chromatography

LC is a method that separates compounds within a sample in order to identify and quantify them. Proteomics is finding increasing usage in research because it may be used to analyze large, delicate macromolecules. Thanks to advancements in ionization methods and apparatus, LC and MS combined have developed into a powerful tool for the characterization and identification of peptides and proteins in a complex mixture (Chen G *et al*., 2007). LC is a method that separates compounds within a sample in order to identify and quantify them. Proteomics is finding increasing usage in research because it may be used to analyze large, delicate macromolecules. Thanks to advancements in ionization methods and apparatus, LC and MS combined have developed into a powerful tool for the characterization and identification of peptides and proteins in a complex mixture (Florens L *et al*., 2006).

LC is a powerful technique that may be used to investigate large and delicate biomolecules and extract proteins from complex mixtures. To determine which peptides are present in the mixture, it can be employed in combination with MS (Chen G *et al*., 2007). LC can help scientists understand the mechanics behind cancer and discover novel biomarkers by changing proteins. For example, some studies use LC-MS/MS to rapidly assess congenital adrenal hyperplasia using dried filter-paper blood samples (Lai CC *et al*., 2001).

Ion exchange chromatography

IEC is used to purify proteins according to their charges. Proteins can be separated according to their charge nature using this methodology, which is different from other approaches. The charge that the molecule of interest accepts can be readily used by altering the buffer's pH. The IEC approach is resilient to fluctuating buffer conditions and is reasonably priced (Jungbauer A *et al*., 2009).

Size exclusion chromatography

SEC can be used to separate different compounds according to their size (hydrodynamic volume), which is based on how well they enter the stationary phase's pores. However, this strategy is less successful than other proteomics methods (Lecchi P *et al*., 2003).

Affinity chromatography

Affinity chromatography separates proteins according to their interactions with an immobilized ligand. Proteins in both 2-DE and non-2-DE are less complicated thanks to affinity chromatography (Marouga R *et al*., 2005).

Mass spectrometry

Mass spectrometry is the most efficient analytical technique for rapidly enabling protein sequencing (Martin DB *et al*., 2001). The molecular weight of a protein can

also be ascertained using it. Protein molecules are ionized using this technique, and their mass is calculated using mass-to-charge ratios. The three main components of a mass spectrometer are an analyzer, an ion source, and a detector. MALDI and ESI are the ionization methods (Krishnankutty R *et al*., 2016). The peptides are mixed with a chemical matrix and spotted using MALDI onto a metal multiwall microliter plate to form a crystal lattice. The energy is transferred to the samples by the matrix molecules after they have absorbed it. The peptide ions are then discovered using a mass analyzer. MALDI helps determine the m/z value by producing primarily singly charged ions (Smith L *et al*.,2006). In ESI, the power of the protein sample is activated to create charged droplets that enhance the production of gaseous ions, which are then analyzed with a mass analyzer (Lahm HW *et al*., 2000). The benefits of ESI include its great reproducibility and strong flexibility in combining multiple MS categories. Moreover, ESI can be fixed by quadruple, time-of-flight (TOF)-MS, ion traps, and Fourier transform ion cyclotron resonance. However, the limitations of ESI include the incapacity to be applied to molecular imaging, the requirement for a large number of samples, and the multiple peaks produced by the multiple charged ions that contribute to the complexity of MS/MS spectra (Chiou SH *et al*., 2011).

CONCLUSION

The best technique for isolating and detecting proteins is PAGE (Ramakrishnan S *et al*., 1996). For separation, 1-DE and 2-DE can both be used. Furthermore, 2D-DIGE and SDS-PAGE are 2D versions that are used in gel electrophoresis (Kostanski LK *et al*., 2003). One-dimensional gel electrophoresis: 1-DE can be used to separate proteins with molecular weights between 10 kDa and 300 kDa. It uses SDS, a detergent that denatures secondary and non-disulfide-linked tertiary structures, to combine them with a negative charge proportional to their volume. As a result, molecular weights can be ascertained (Chen ZT *et al*., 2015). SDS-PAGE can be used for molecular weight determination for unknown proteins, protein purification testing, and sample purity checks (Brunelle JL *et al*., 2014). Two-dimensional gel electrophoresis: 2-DE identifies proteins better than 1-DE because protein molecules differ in their molecular weight and isoelectric point (Chen ZT *et al*., 2015). Compared to 1-DE, the resolution is improved by splitting the protein into two dimensions. Protein separation in 1-DE is based on net charge; in 2-DE, it is based on molecular mass and isoelectric point. Several protein types can be identified with this method, including phosphorylation and PTMs. 2-DE can be used to identify specific proteins that arise from different proteolysis processes and alternative mRNA splicing (Lewis TS *et al*., 2000). 2-DE has many applications, including cell map proteomics and protein expression profiling. Using protein expression profiling, healthy and diseased tissues can be compared. 2-DE protein mapping is useful for microorganisms, biological organelles, and protein complexes (Jung E *et al*., 2000; Rappsilber J *et al*., 2000; Benner P, 1996). Proteins can be categorized using 2-DE, and the database can be created via the Internet (Appel RD *et al*., 1999). However, low molecular weight proteins, as well as the limits of size and isoelectric point separation, cannot be detected by 2-DE (Moseley MA, 2001).

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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