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Review Article

The Relevance of Proteomics in Medical Laboratory Diagnosis

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Abstract

Proteomics research is an emerging field of biological, medical and analytical research in recent times. This is due to its high impact on the understanding of various diseases and contribution to treatment planning. Proteins are the functional output of the cell and therefore are expected to provide the most relevant information for molecular understanding of (patho) physiological processes. Proteomics is a huge scale that studies different functions of proteins, structures of proteins, roles behind the appearance of specific proteins and the principal of each protein. Medical researchers and scientists have applied the applications and techniques of proteomics approach to identify biomarkers, which has helped in diseases diagnosis, prognosis, and treatment. Samples been used in the diagnosis are; pleural effusion, saliva, blood, urine, tissue, and tissue interstitial fluid (TIF) .The goal of proteomics in medical laboratory diagnosis aims to determine the causal relationship between changes of proteins in body fluids and clinical phenotypes, so as to identify target molecules, that play key roles in the pathogenesis of diseases, eventually leading to the development of new treatment protocols. Early detection of these diseases can help patients to get early treatment and the quality of patients' life would be better. Also, having as much as possible of proteomic data in order to help patients with these types of diseases to be early diagnosed in the future.

Keywords: Relevance, proteomics, medical laboratory diagnosis

INTRODUCTION

Molecular diagnosis is moving beyond genomics to proteomics. In order to understand proteomics, understanding what proteome is very necessary. According to the American Medical Association (AMA) and the Office of Cancer Clinical Proteomics Research at National Cancer Institute, the term proteome was taken from two words; protein and genome, so "prote-" was taken from protein and "-ome" from genome. Therefore, proteomes are proteins that are expressed by completely different genomes, and alternative cells[1].

Proteomics, the study of the protein is very important as a result of proteins represent the particular practical molecules within the cell. Importantly, genetics provides vital insights into our understanding of cell signaling; specifically the quantitative identification of super molecule loading and biological insights of living thing vesicles[2].

Proteomics is a multifaceted approach to study all proteins in a biological system and is being employed progressively by clinical researchers to spot biomarkers of disease. The application of mass spectrometry to the study of diseases will ultimately lead to the identification of biomarkers. These biomarkers are applied to several areas of patient care including disease prediction, diagnosis, staging, monitoring, and the assessment of drug efficacy[3].

Proteomics may be a key tool in health analysis as a result of it's created doable systematic analysis of tons of super molecules in clinical samples with the promise of discovering new protein biomarkers for various illness conditions[4].

Proteomics approach in Medical Laboratory diagnosis can be used to identify all proteins in particular sample, elucidate extra elements of organic chemistry pathway(s), or analyze post-translational modifications at little or massive scale [3]. This research work will describe the potential impact of clinical proteomics, discovery, validation workflows and their limitations; several examples of proteomic-based applications; and finally multiple innovative approaches that are gaining in popularity in the field of medical laboratory diagnosis.

WHY PROTEOMICS?

Proteomics usually provides US a stronger understanding of Associate in nursing organism than genetics. Proteins, not genes, are responsible for the phenotype of cells. The level of complexness ensuing from co- and post-translational modification events will solely be dissected and understood through qualitative and quantitative studies at the extent of the useful proteins themselves. Finally, protein degradation rate plays an important role in protein content[5]. Particularly, when interpretation of protein expression takes into account the dynamics of this expression in specific biological contexts[6].

KEY QUESTIONS PROTEOMICS CAN ANSWER

Broadly speaking, proteomic research provides a global view of the processes underlying healthy and unhealthy cellular processes at the super molecule level[7]. To do this, every proteomic study usually focuses on one or a lot of the subsequent aspects of a target organism's proteome at a time to slowly build on existing knowledge.

The key questions proteomics can answer include;

S/N	KEY QUESTIONS	ANSWERS
1).	**Protein identification**	Which proteins are normally expressed in a particular cell type, tissue or organism as a whole, or which proteins are differentially expressed?
2).	**Protein quantification**	Measures total ("steady-state") protein abundance, as well as investigating the rate of protein turnover (i.e., how quickly proteins cycle between being produced and undergoing degradation).
3).	**Protein localization**	Where a protein is expressed and/or accumulates is just as crucial to protein function as the temporal order of expression, as cellular localization controls that molecular interaction partners and targets are offered.
4).	**Post-translational modifications**	Post-translational modifications will have an effect on super molecule activation, localization, stability, interactions and signal transduction among alternative super molecule characteristics, thereby adding a major layer of biological complexity.
5).	**Functional proteomics**	This area of proteomics is focused on identifying the biological functions of specific individual proteins, classes of proteins (e.g., kinases) or whole protein interaction networks.
6).	**Protein-protein interactions**	Investigates how proteins interact with each other, which proteins interact, and when and where they interact.

TYPES OF PROTEOMICS

Based on the protein response under stress conditions proteomics are classified into different groups as follows;

Expression proteomics

Expression proteomics is used to study the qualitative and quantitative expression of total proteins under two different conditions; like the normal cell and treated or diseased cell which can be compared to know the super molecule that's answerable for the strain or unhealthy state or the super molecule that's expressed because of illness[8].

Structural proteomics

Structural genetic science Structural genetic science offers elaborated info concerning the structure and performance of super molecule complexes gift in a very specific cellular organ. It is possible to identify all the proteins present in a complex system such as membranes and cell organelles and to characterise all the super molecule interactions which will be doable between these super molecules and protein complexes. Different technologies like X-ray natural philosophy and magnetic resonance spectrum analysis were primarily used for structure determination[9].

Functional proteomics

Functional genetics analyses and evaluates macromolecule activity and protein-protein interactions[8]. Functional genetics explains understanding the super molecule functions additionally as noncommittal molecular mechanisms within the cell then depend on the identification of the interacting protein partners. Functional proteomics can be applied to understand signaling mechanisms involving pathological conditions such as cancer, myocardial disease, and brain damage [10].

PROTEOMICS APPROACH TO IDENTIFY RELIABLE BIOMARKERS

This approach is necessary to identify reliable biomarkers for the diagnosis of different diseases from different types of samples. After collecting the samples from patients; types of samples include blood samples, tissue samples, tissue interstitial fluid samples, saliva and urine samples[11]. There are 3 main steps in proteomic analysis so as to spot a biomarker in an exceedingly specific illness. These steps include;

(1). Extraction and separation of proteins,
(2). Identification of proteins, and
(3). Verification of proteins[12].

Collection, pre-treatment and preparation of the samples

First, collecting different types of samples from a group of patients. So, obtaining from every patient a sample of serous membrane effusion, saliva, blood, urine, tissue, and tissue extracellular fluid (TIF). Secondly, pre-treatment and preparation of samples depends on the character of the sample such as; tissue samples pre-treatment and preparation are different from blood samples pre-treatment and preparation. Therefore, using different lists buffers, saline's, and different digestive processes are required from sample to sample. Also, the environments and conditions that are required to save the samples helpful for the tests are different[13].

Extraction and separation of proteins

There square measure many genetics techniques to extract and separate proteins involving: 2-DE, LCM, and 2D-DIGE[14].

DE (two-dimensional electrophoresis) is the most common used proteomics tool in proteomics research because it has the ability to compare the amount of proteins similarly as showing the isoforms of those proteins on an equivalent actual gel[12].

So, throughout this method, separation of proteins would be supported two principles; first, in 2-DE, separation of proteins would be primarily based mostly on isoelectric points, then; two-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDSPAGE) would separate proteins according to the molecular weights of these proteins[12].

LCM (Laser Capture Micro dissection) - is a technique that is used in proteomics researches to extract the exact needed cells from a sample. So, by exploitation least common multiple, researchers make sure that the isolation of those isolated cells is clean from other cells of the sample similarly as keeping the molecular characteristics of the cells from being changed. This technique is able to detect signals of proteins that have low presence by diluting the signals of these proteins. Integration between LCM and 2-DE gives a powerful technique that is used by proteomics researches in order to look for new targets and biomarkers[15].

D-DIGE (Two-dimensional gel electrophoresis - DIGE in genetics researches provides reliable results as a result of the labeling of the proteins which gives different signals of different fluorescence showing various specific proteins[12].

1-DE and 2-DE techniques are considered as powerful proteomic techniques in order to extract and separate proteins. 1-DE separates proteins reckoning on these proteins charge whereas 2-DE separates proteins reckoning on these proteins relative molecular mass. However, these techniques have some limitations in proteins separation[12].

Identification of proteins

There are several proteomics techniques to identify proteins involving; Tissue array and mass spectrometry (MS)[12,16].

Tissue array isn't the tool that is preferred by genetic science researchers as a result of it identifies Associate in nursing awfully massive vary of proteins in quick methodology. Thus, it causes a challenge and tough work to settle on and prove the simplest target. On the other hand, MS save that time and make the work easier than tissue array does[17].

Because of the accuracy and sensitivity of mass measurements, MS became the foremost vital basic technique that is wont to establish proteins in genetics approach particularly within the application of growth marker identification[16]. There are many types of MS, however only a few type of MS that are used in proteomics approach in order to identify biological markers. Types of proteomics-based mass spectrometry that are used to identify biological markers include:
1). LC-MS/MS,
2). SELDI-TOF/MS,
3).Two dimensional gel electrophoresis–mass spectrometry (2-DE/MS),
4). MALDI-TOF/MS[18].

LC–MS/MS may be a smart technique to identify biomarkers in genetic science researches. This technique allows for identifying several proteins at the same time, and for this reason these techniques are called high-throughput[14]. However, this system has some disadvantages such as; incompletion of proteins digestion, and facing some issue in peptides action separation. LC–MS/MS has high selectivity as well as high sensitivity that enable it to analyses complex biological substances even if in low amounts. However, LC–MS/MS generally needs sample preparation process in order to remove unwanted materials for clear analysis[14].

SELDI-TOF/MS is one among the foremost vital techniques to spot reliable biomarkers in genetics researches. In SELDI-TOF/MS, there is chromatographic surface that can bind to a specific part of the wanted protein and the rest will be removed away [14,19]. So, this specific a part of super molecule would bind thereupon surface supported electricity interaction, absorption, or organic chemistry affinity. Also, during this technique, there are unit totally different chips area units employed in order to isolate proteins from the whole sample. SELDI-TOF/MS has two advantages including;

testing proteins that are not digested, and it analyses rapidly. Also, this technique allows for identifying several proteins at the same time, and for this reason these techniques are called high throughput[14].

Moreover, SELDI-TOF/MS is high selectivity as well as high sensitivity that enable it to analyses complex biological substances even if in low amounts more than other techniques of proteomics requirements[14,19]. However, SELDI-TOF/MS has some disadvantages including; results reliability of this system is poor because of many various chips area unit used, and noise. Moreover, this technique isn't very correct as various proteomics-based spectroscopy analysis techniques as well because it can't be appropriate for proteins that have massive relative molecular mass[14, 19].

Two dimensional gel electrophoresis–mass spectrometry (2-DE/MS)

The most important advantage of 2-DE/MS is the availability in a lot of laboratories. In fact, this system is simple and easy to use. Moreover, it's usually use in separation and identification of proteins as a result of it's high throughput that establish and separate a large range of proteins in just one occasion on one gel. This technique permits genetics researchers to observe any modification that will occur throughout the wellness process which as a result of the searching with antibodies which will be performed on transfer blots. However, 2-DE/MS has some disadvantages that it takes terribly very long time and it cannot take care of proteins that have terribly low molecular weights. Also, it has limitations in repeatability as well as limitations in reproducibility[14].

MALDI-TOF/MS (Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight) is the most powerful technique in proteomics researches to identify proteins. It is the most appropriate MS technique for analyzing proteins that have high molecular weight[19]. As any technique, MALDI-TOF/MS has some blessings and a few disadvantages. MALDI-TOF/MS is high outturn as a result of it permits for distinctive many proteins at an equivalent time. Also, this system is simple to use and has high property similarly as high sensitivity.

Disadvantages of this system include: limitations in development, mass window vary; preparation of sample is variable and totally different advantageous peaks for identical samplesSSS[20].

So, MS is that the best technique for this purpose as a result of it's correct and sensitive[18].

Verification of proteins

There are several proteomics techniques to verify proteins involving; ELISA, and Western blot[12,14].

ELISA is a powerful technique that is used in proteomics researches in order to verify biomarkers[14]. However, ELISA is better for proteins verification because it is a rapid sensitive technique, and it is very accurate it quantifies the level of targeted proteins. ELISA has antigens, antibodies, similarly as capture antibodies. In this technique, there is a hard surface that has been attached with capture antibodies in order to bind the biomarker onto that hard surface[14].

Western blot is another powerful technique that's employed in genetics researches so as to verify biomarkers. In this technique, polyclonal antibodies or being antibodies are a unit used for detective work proteins. So, in western blot, proteins would be separated by using SDS-PAGE to get an idea about molecular weight of separated proteins and their isoforms[14].

TECHNIQUES EMPLOYED IN PROTEOMICS

Low-throughput methods

Antibody-based methods

Techniques such as ELISA (enzyme-linked immunosorbent assay) and western blotting rely on the provision of antibodies targeted toward specific proteins or epitopes to spot proteins and quantify their expression levels.

The ELISA is highly sensitive immunoassay and widely used for diagnostic purpose. Western blotting is an important and powerful technique for detection of low abundance proteins that involve the separation of proteins

Using electrophoresis, transfer onto nitrocellulose membrane and the precise detection of a target protein by enzyme-conjugated antibodies. Western blotting is a dominant tool for antigen detection from various microorganisms and is quite helpful in diagnosis of infectious diseases[21].

Gel-based methods

Two-dimensional gel ionophoresis (2DE or 2D-PAGE), the primary proteomic technique developed, uses an electrical current to separate proteins during a gel supported their charge (1st dimension) and mass (2nd dimension). Differential gel ionophoresis (DIGE) could be a changed style of 2DE that uses totally different fluorescent dyes to allow the simultaneous comparison of two to three protein samples on the same gel. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DE) and two-dimensional differential gel ionophoresis (2D-DIGE) techniques area unit used for separation of complicated super molecule samples [22].These gel-based methods are used to separate proteins before further analysis as well as for relative expression profiling[23].

Chromatography-based methods

Chromatography-based methods can be used to separate and purify proteins from complex biological mixtures such as cell lysates. For example, ion-exchange chromatography separates proteins based on charge, size. Exclusion chromatography separates proteins based on their molecular size and affinity chromatography employs reversible

interactions between specific affinity ligands and their target proteins (e.g. the utilization of lections for purifying immunoglobulin M and IgA molecules). These ways are often accustomed purify and establish proteins of interest, additionally on prepare proteins for more analysis by e. g., downstream MS[23].

High-throughput methods:

With the support of high-throughput technologies, a huge volume of proteomics data is collected. Bioinformatics databases area unit established to handle huge amount of information and its storage. Various bioinformatics tools are developed for 3D structure prediction, protein domain and motif analysis, rapid analysis of protein–protein interaction and data analysis of MS[24].

Analytical, functional and reverse-phase microarrays

Protein microarrays apply tiny amounts of sample to a "chip" for analysis (this is sometimes in the form of a glass slide with a chemically modified surface). Specific antibodies can be immobilized to the chip surface and used to capture target proteins in a complex sample. This is termed Associate in nursing analytical super molecule microarray, and these sorts of microarray are a unit used to live the expression levels and binding affinities of proteins during a sample[25].

Functional macromolecule microarrays square measure accustomed characterize macromolecule functions like protein–RNA interactions and enzyme-substrate turnover. Functional Micro array represented the study of several interactive patterns such as protein-DNA, RNA with protein, in between protein, affinitive with drugs, lipid and protein and enzyme substrate association as they are made of purified protein.

Primary application of functional microarray was to test substrate specificity of protein kinase[25].

In a reverse-phase super molecule microarray, proteins from e.g., healthy vs. diseased tissues or untreated vs. treated cells are bound to the chip, and the chip is then probed with antibodies against the target proteins. The reverse phase approach was assessed for quantification of phosphoprotein and other carcinoma disease associated protein in non-small lung cancer cell lines approaches. It examines apoptosis, DNA damage and involving various signaling pathways[26].

Mass spectrometry-based proteomics

There are several "gel-free" methods for separating proteins, including isotope-coded affinity tag (ICAT), stable isotope labelling with amino acids in cell culture (SILAC) and isobaric tags for relative and absolute quantitation (iTRAQ). These approaches leave each quantitation and comparative/differential genetics. Isotope-coded affinity tag (ICAT) labelling, stable isotope labelling with amino acids in cell culture (SILAC) and isobaric tag for relative and absolute quantitation (iTRAQ) techniques have recently developed for quantitative proteomic[27]. There are different, less quantitative techniques like four-dimensional macromolecule identification technology (MudPIT), which supply the benefits of being quicker and simpler. Other gel-free, chromatographic techniques for protein separation include gas chromatography (GC) and liquid chromatography (LC)[23].

GENERAL APPLICATIONS OF PROTEOMICS

The applications of proteomics are incredibly numerous and varied. The table below lists just some of these applications and provides links to examples of studies using these approaches.

S/N	PROTEOMICS APPLICATION	DESCRIPTION AND EXAMPLES
1).	Personalized medicine	Tailoring disease treatment to each patient based on their genetic and epigenetic makeup, thus on improve affectivity and scale back adverse effects. While genetic science and transcriptomics are the most focus of such studies to this point, genetics knowledge can seemingly add an additional dimension for patient-specific management.
2).	Biomarker discovery	Identification of protein markers for e.g., the diagnosis and prognosis of glioblastoma, and evaluating patients' response to therapeutic interventions such as stem cell transplantation
3).	Drug discovery and development	Identifying potential drug targets, examining the drug ability of selected super molecule targets, and developing medicine geared toward candidate therapeutic super molecule targets (e.g., for hepatocellular carcinoma).
4).	Systems biology	System-wide investigations of disease pathways and host–pathogen interactions to identify potential biomarkers and therapeutic targets; system-wide investigations of drug action, toxicity, resistance and efficacy.
5).	Agriculture	Investigations of plant–pathogen interactions, crop engineering for enlarged resilience to e.g., flooding, drought and other environmental stresses.
6).	Food science	Food safety and quality control, allergen detection and improving the nutritional value of foods.
7).	Paleoproteomics	The study of ancient proteins to further our understanding

		of evolution and archeology.

CLINICAL BENEFITS OF PROTEOMICS IN MEDICAL LABORATORY DIAGNOSIS

The application of proteomics in the diagnosis and treatment of bronchial asthma

Body fluids including blood (serum), alveolar lavage fluids, sputum, and saliva make up the majority of the samples currently used to conduct proteomic analysis in bronchial asthma patients. Serum is the most commonly used and is also the most important body fluid sample in asthma research. Chung *et al.* developed a method for analysing serum potential biomarkers in patients with bronchial asthma[28]. Unlike other diseases (e.g., cancer), the goal of proteomics in patients with bronchial asthma is not to develop a new method for asthma diagnosis through analysis of body fluids; instead, it aims to determine the causal relationship between changes of proteins in body fluids and clinical phenotypes, so as to identify target molecules that play key roles in the pathogenesis of asthma, eventually leading to the development of new treatment protocols.

Oncology

Oncology refers study of Tumor cells. Tumor metastasis, is the process spread of cancer from one organ to another non-adjacent organ cause death in patients[28] The major challenge in medicine is to describe the molecular and cellular mechanisms underlying tumor metastasis, analyses the protein expressions correlated to the metastatic process which help to understand the mechanism of metastasis and thus facilitate the development of strategies for the therapeutic interventions and clinical management of cancer. Proteomics is a systematic research; the main aim of this research is to characterize the super molecule expressions, functions of tumor cells and wide employed in biomarker discovery[11].

Cancer Proteomics

Cancer is a class of complex diseases in which the cells in a specific tissue (or) organ are no longer fully responsive to the signals that regulate cellular differentiation, survival, proliferation and death. The diagnosis and classification of cancer are based on the cellular morphology and histological architecture[11]. However, the applied strategies area units subjective and liable to variable interpretations, and patients with an analogous histopathology, cancer staging, and coverings program, have incontestable variable clinical outcomes. Therefore the diagnostic tools for cancers have evolved from histology to methods such as genomic testing and chromosome karyotype analyses. Gene expression profiles can be used as unique molecular signatures to facilitate diagnosis, classify histopathologically similar tumors into biologically distinct subtypes and identify patients with a high risk for occurrence and poor survival.

Proteomics in Heart Diseases

Heart failure arising from systemic or specific heart-muscle diseases is one of the leading causes of morbidity and mortality (Heart Disease and Stroke Statistics, 2004 Update) .The pathogenesis of the cardiac dysfunction remains largely unknown. In the last decade the quantification and characterization of changes in the proteome of cardiac myositis at the onset of disease, have provided insight regarding the holistic response of injured tissue. A number of biomarkers have been established in clinical practice and reflects the decompensated hemodynamic status, myocardial strain, acute global stress response and other organ-specific injuries[29].

Proteomics in Neurodegenerative Disease

Neurodegenerative diseases are a particularly devastating class of disorders that are characterized by neuronal impairment that leads to neuronal cell death. Diagnosis necessitates a brain diagnostic test or PM sample, though several periodic cases have a typical clinical image. Treatments aimed at inhibiting the neurodegenerative processes only focus on symptom management[30] and are a unit seemingly to be only if the treatment is initiated as early as possible.

Proteomics in Parkinson's disease (PD)

In 1984, Harrington and colleagues showed for the first time, evidence that the super molecule expression pattern within the CSF of palladium patients differs from healthy controls. The first unbiased profile of the human SNpc identified 44 proteins nine of which were significantly differentially expressed in PD compared with controls tissues[31].

Met proteomics

The human gastro-intestinal (GI) tract contains a vast and diverse microbial ecosystem: the micro biome that has co-evolved with our species in terms of our metabolic and nutritional requirements[32] and is essential for human health[33]. The GI micro biota perform a large number of important functions that define the physiology of the host, such as immune system maturation conditioning and response the intestinal response to epithelial cell injury and xenobiotic and energy metabolism[34].

Proteomics in Alzheimer's disease (AD)

Several proteins that are involved in inflammation and the complement system are significantly up regulated in patients with AD; chemokine's and chemokine receptors are also up regulated in AD brain cells and contributed to plaque-associated inflammation and neurodegeneration processes. The increased levels of CSF chemokine ligand 2 (CCL2), which plays a significant role in the inflammatory processes in AD, correlate positively with the level of p-tau[35]. Consequently, CCL2 may serve as a potential biomarker to monitor the progression of the pathologic state of AD.

CONCLUSION

Based on the above findings, the present review was concluded that the applications for proteomics are relevant to all of the biological process and provides a means to utilise the expressed protein data in a more effective way. Proteomics is a key tool in health research because it has made possible systematic analysis of hundreds of proteins in clinical samples with the promise of discovering new protein biomarkers for different disease conditions.

Proteomics, the study of the protein is very important as a result of proteins represent the particular practical molecules within the cell. Importantly, genetics provides vital insights into our understanding of cell signaling; specifically the quantitative identification of super molecule loading and biological insights of living thing vesicles.

Clinical proteomics aims to identify proteins involved in pathological processes and to evaluate changes in protein expression during illness. Moreover, clinical proteomics offers technical capabilities to develop biomarkers for diagnosis and therapeutic interventions.

The diagnosis of various disease states such as cancers and cardiomyopathies is currently based on the detection of single proteins such as troponin, prostate specific antigen (PSA), CA-125, and others. Proteomic analysis examines thousands of proteins at one time allowing the detection of specific protein patterns expressed as a consequence of abnormal cellular function or cellular interactions.

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