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Review Article

Proteomics: an evolving technology in Laboratory Medicine

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ABSTRACT: The rapid developments in both genomics and proteomics will allow scientists to define the molecular pathways in normal and diseased cells. With these models, researchers will have the ability to predict previously unknown interactions and verify such predictions experimentally. Novel proteins, cellular functions, and pathways will also be unravelled. It is hoped that understanding the connections between cellular pathways and the ability to identify their associated biomarkers will greatly reduce the suffering and loss of life due to diseases.

KEY WORDS: Proteomics; Laboratory medicine

INTRODUCTION

Completion of the Human Genome Project was a pivotal first step to revolutionize medicine in the 21st century[1]. The completed human genome was found to contain between 30,000 and 35,000 genes, far less than the 100,000 genes predicted when the project commenced in the mid-1990s[2]. Subsequently it was found that one gene can produce more than one protein, each with a different functional capability. The generation of multiple proteins from a single gene can occur as a result of alternate splicing where a single DNA template can produce several different messenger RNAs, each of which is then used to make different proteins[3]. In addition, the protein may undergo modification by cellular processes after it is created (termed post-translational modification). The result is that one gene can produce as many as 1,000 different proteins. On average, however, a gene produces five to ten different proteins[4].

Genomics is the systematic use of information on the expression, regulation and structural association of genes. It is used in genetic analysis, measurement of gene expression and determination of gene function[5]. As genomics has proven inadequate to predict the structure and dynamic properties of all proteins, a new field of protein study termed proteomics has developed. This is the large-scale study of protein expression, structure and function. It aims to correlate the structural and functional diversity of proteins with underlying biological processes, including disease processes[6]. Proteomics has created opportunities to identify, investigate and target proteins that are differentially expressed in health and disease. Clinical medicine is poised to benefit enormously with the potential to develop better diagnostic and prognostic tests, to identify new therapeutic targets and ultimately to allow patient-individualized therapy. Finding the protein or proteins (biomarker) associated with a disease or adverse event will lead to a much earlier identification of disease, potentially prior to the onset of symptoms[7].

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TECHNOLOGIES

The first high resolution protein separations were achieved by two-dimensional gel electrophoresis in 1975; this was followed by the first computerised 2-D gel image analysis platform to quantify changes in 2-D gel protein spot levels[8]. However there was a lack of useful tools to identify proteins of interest. Furthermore, the lack of reproducibility hindered the expansion of the technique until the introduction of immobilized pH gradients (IPGs) in the 80s. This has coincided with the development of mass spectrometry ionization techniques for peptides, allowing protein identification and characterization on a large scale[9]. However, it was not until the mid-90s that mass spectrometry became a mainstream technique for protein identification, mostly replacing Edman sequencing[10].

Currently, there is no diagnostic amplification technique for proteins as there is for amplifying genes. It is therefore not possible to make copies of proteins that are present in very small amounts. Another challenge is that amino acids are very small, ranging from 7 to 24 atoms, which represents an immense scientific challenge to amplify precisely. Hence, proteins are studied using a synergistic combination of electrophoresis, mass spectrometry, multidimensional liquid chromatography and bioinformatics[11].

2D gel electrophoresis is a suitable technique for asking, "Where do differences arise amongst the proteins in two similar samples?" For example, closely matched samples from diseased and healthy cells can be compared. Differences in protein abundance or covalent modification (e.g. phosphorylation, glycosylation and acylation) can provide important clues to the pathogenesis, progress and treatment of a disease. Once a protein has been isolated and digested, the mass spectrometer (ICP-MS, MALDI-TOF) is a suitable tool for asking, "What is this protein?", "Which residues are modified?", and "What is the modification?" By taking 3-D pictures of proteins, X-ray Tomography allows researchers to see biomolecules in their cellular context. Tomograms provide insights into the conformation and flexibility of functional targets and their environment (**Table 1**).

Table 1: Technologies used in proteomics

Technology	Uses
2-D Gel electrophoresis	Used to identify low abundance proteins in complex biological samples such as blood, urine and oral fluid.
Tandem mass spectrometry	Used to separate ions based on a sample's electronic mass, to study inborn errors of metabolism and metabolic profiles, and to identify therapeutic drugs, drugs of abuse, disease markers and toxic compounds.
Mass spectrometry MALDI-TOF (Matrix Assisted Laser Desorption Ionisation-Time Of Flight)	Deals with thermolabile, non-volatile organic compounds and those of high molecular mass. It is used in for the analysis of proteins, peptides, glycoproteins, oligosaccharides and oligonucleotides.
ICP-MS (Inductively Coupled Plasma-Mass Spectrometry)	Involves the formation of gas containing electrons, ions and neutral particles from Argon gas. Technology is used for ultrasensitive quantification of proteins and peptides down to low attomole range.
X-ray Tomography	Used to determine the location of labelled proteins or protein complexes in an intact cell. Frequently correlated with images of cells from light based microscopes.
Microarray 'chips'	These are matrix-support surfaces for binding selected proteins and allowing high-throughput screening for disease associated proteins.
Other methods: 1. Affinity chromatography 2. Yeast two hybrid techniques 3. Fluorescence Resonance Energy Transfer (FRET) 4. Surface Plasmon Resonance (SPR)	These methods are used for detection of drug-protein, hormone-protein, protein-protein, DNA-protein, carbohydrate-protein, and lipid-protein interactions.

CLINICAL APPLICATIONS

The potential applications of proteomics in the laboratory revolve around: identifying components of the proteome; comparing the expression of proteins between normal and diseased organs at certain stages of disease; bioinformational analysis to determine how proteins interact with each other in vivo; identification and characterization of proteins post-translationally; study the structure and function of protein complexes to understand the organization of cells at the molecular level.
The goal of clinical proteomics and molecular medicine is to assist in the study of characterization of the cellular components and cellular networks to be used in the understanding of the pathology of disease process, diagnosis and patient management. The translational nature of this technology provides unique challenges and boundless opportunities that promise to transform the way disease is diagnosed, treated and managed[12]. It has many clinical applications including the following:

- Translational pathology and immunohistochemistry applied to protein biomarkers in tissue
- Bioinformatics tools including pattern recognition, artificial intelligence and computer learning algorithms
- Biomarker discovery and validation from clinical samples
- Signal transduction pathways profiling in clinical tissue samples
- Discovery of new drug targets from clinical samples
- Use of proteomic technologies in the drug development pipeline
- Use of proteomic technologies to monitor prognosis, therapeutic end points, toxicity and efficacy
- Clinical trials using proteomic monitoring

Some of the major areas in which clinical proteomics are utilized include cancer, cardiovascular disease, Alzheimer's disease, infectious diseases, infertility, obstetrics and immune rejection following transplantation[13].

TARGETED MODALITIES OF PROTEOMIC CLINICAL APPLICATIONS

Diagnosis of infectious diseases: Tuberculosis (TB) affects millions of people around the globe with many drug-resistant Mycobacterium tuberculosis strains spreading worldwide[14]. Among the communicable diseases, TB is the second leading cause of death worldwide after HIV-AIDS, killing nearly two million people each year. More than 50% of TB cases occur in the largest Asian countries (India, China, Indonesia, Bangladesh, Philippines and Pakistan). Sub-Saharan Africa has the highest incidence rate (approximately 300/100,000 population/year). Even though TB has declined steadily in Western Europe and North America, the global TB burden appears on the rise, especially in the former Soviet Union, Eastern Europe, and Africa[15].

A serum or saliva-based screening test that could detect pre-clinical infection would allow early treatment, potentially reducing transmission, and have widespread application. Proteomic techniques have identified proteins secreted in vitro by common clinical isolates. Two of these (rRv3369 and rRv3874) have shown great potential as serodiagnostic antigens, with sensitivity of 60%–74% and specificity of 96%–97% in clinical studies. These proteins are potential candidates for a kit-based serum screening test.

Diagnosis of Severe Acute Respiratory Syndrome: The pathogenesis of severe acute respiratory syndrome (SARS) is not well understood, and a specific diagnostic method is critical for the management and control of this disease. Proteomic analysis of sera from patients with SARS has identified potential biomarkers. These are truncated forms of α (1)-Antitrypsin, which were consistently found in higher concentrations in the sera of SARS patients compared with healthy controls. These markers may prove useful as diagnostic tools and therapeutic targets. Moreover, studies of the protein structure of the SARS virus may reveal potential vaccine targets[16].

PROTEOMIC AND CANCER

Many studies using proteomic techniques have been performed on biomarkers to investigate potentials of early cancer diagnosis[17].

Ovarian Cancer: Ovarian cancer represents the sixth most commonly diagnosed cancer among women in the world, and causes more deaths per year than any other cancer of the female reproductive system. Ovarian cancer is more common in Northern European and North American countries. Ovarian cancer is a major focus of early biomarker discovery because it is usually diagnosed at an advanced stage with a median five-year survival rate of about 20 percent[18]. To evaluate the potential use of proteomics as a diagnostic tool, a group of researchers from the National Cancer Institute (NCI) in Bethesda, MD, collected

serum from 50 ovarian cancer patients and 50 controls and used a computer algorithm to search for the protein patterns that distinguished cancer cells from non-cancer cells. They have shown that with a set of blinded serum samples, the test pattern correctly identified all 50 patients with cancer, and was able to discriminate them from 63 out of 66 patients without cancer or had benign disease. Using the same approach, two other groups reported similar results[19,20].

Prostate cancer: The worldwide incidence of prostate cancer (PCa) ranks third among cancers in men. The highest incidence of prostate cancer in the world is found in American black men, who have approximately a 9.8% lifetime risk of developing this cancer compared to the 8% lifetime risk for American white men. The Japanese and mainland Chinese populations have the lowest rates of prostate cancer[21]. Since the advent of prostate specific antigen (PSA) screening, a significant number of men have had a PSA test performed and this has led to a significant increase in the number of diagnosed cases[22]. However, the PSA lacks sensitivity and therefore, evaluating multiple proteins will be essential to establishing signature proteomic patterns that distinguish cancer from non-cancer as well as identify all genetic subtypes of the cancer and their biological activity.

In one study, proteomic analysis of prostate cancer patients versus healthy controls was carried out by looking for differences in protein patterns between the two groups. Using blood samples from 167 prostate cancer patients, 77 patients with benign prostate hyperplasia and 82 healthy men, protein patterns developed as a classification system had correctly classified 96 percent of the samples as either prostate cancer or non-cancer (benign prostate hyperplasia/healthy men)[23]. A further proteomic approach is to determine whether the changes in specific phosphoproteins believed to be involved in cellular signalling events and cancer progression in prostate cancer patients have been speculated to serve as a biomarker of early disease[24].

Breast Cancer: Breast cancer is the most common malignancy among women in the Western world and constitutes 18% of all cancers in women[25]. Traditional prognostic factors include the axillary lymph node status, the tumor size, the nuclear grade and the histologic grade. Interest in novel prognostic markers is based on the fact that a significant number of patients with early-stage breast cancer harbour microscopic metastasis at the time of diagnosis. It is now well established that adjuvant systemic therapy improves survival in patients with early-stage breast cancer. Recent technical advances in mass spectrometry, such as matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and its variant surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF MS), have enabled high-throughput proteome analysis[26,27].

A multitude of molecules involved in breast cancer biology have been studied as potential prognostic markers. In one study a combination of three candidate proteins in the blood were found to be useful in discriminating between 169 patients at various stages of breast cancer compared to women with benign breast disease and healthy controls[28]. In other studies, nipple aspirate fluid was used to identify tumor marker candidates[29]. Proteomic analysis of breast nipple aspirate fluid (NAF) holds promise as a non-invasive, low cost method to identify markers of breast cancer. These protein molecules when secreted, they represent the final processed form of the marker protein, which makes proteomic analyses less ambiguous to provide clues to changes in protein translational rates, post-translational modification, sequestration, and degradation that lead to disease. Many of the proteins that have been identified in the NAF proteome could potentially be markers of disease, including ras-related protein; metastasis-associated protein; BCL2, which has been implicated in the suppression of cell death; CD5, which is reported to play a role in the inhibition of apoptosis; retinol-binding protein, which has recently been shown to suppress breast cancer cell survival and has been shown to be down-regulated in a subset of breast cancer; clusterin, which has been associated with cell death and apoptosis; and transferrin, which has been assigned a role in cell proliferation[30].

Bladder Cancer: Bladder cancer incidence varies widely throughout the world. Belgium and Italy, , have the highest recorded incidence rates in Europe (42.5/100,000 and 41/100,000 population respectively), much more than in the United States with an incidence of 24.1/100,000 and an estimated 61,160 newly diagnosed cases in 2007. However,, cancer registries in Slovenia, Croatia, and Switzerland have reported even lower European bladder cancer incidence (10.1/100,000, 11.7/100,000 and 12.0/100,000 respectively) with the lowest

rates found in Asian and South American countries. Bladder cancer affects men four times more often than women. The risk of bladder cancer increases with age with over 70 percent of people diagnosed are older than 65 years[31,32].

Biological characteristics of urothelial carcinomas range from benign, superficial, low-grade, non-life threatening, papillary lesions, that respond well to resection and adjuvant treatment but are prone to recurrence to highly invasive malignant carcinomas with grave outcome.

Several laboratories have successfully demonstrated that specific protein patterns can be detected from tumor tissue and these could discriminate adequately between diseased and healthy tissue. In the case of bladder cancer, proteomics analysis has identified several keratin proteins that are expressed in different amounts as the disease progresses from the early transitional epithelium stage to full blown squamous cell carcinoma. The measurement of keratin levels in bladder cancer biopsies can therefore be used to monitor the progression of the disease. Another protein, psoriasin, is found in the urine of bladder cancer patients and can be used as an early diagnostic marker for the disease. The study and utilization of these novel markers support the notion that proteomics, but not DNA arrays, can be used in cancer diagnosis. Urine, in common with most bodily fluids, contains proteins but no RNA[33,34].

PROTEOMICS AND THERAPEUTICS

Drug Resistance

Drug resistance represents a major clinical obstacle in the management of many infectious diseases, and, in many cases, the mechanism is unknown. Genetic and protein-sequence data for many microorganisms is now available and provides tools for understanding their resistance to drugs and for identifying novel agents for treating drug-resistant disease, such as azole resistance in Candida albicans which has been linked with differential expression of proteins such as Erg10p, a protein involved in the ergosterol biosynthesis pathway. This has been shown as a potential drug target for the treatment of resistant disease[35].

Chloroquine resistance: Chloroquine has been one of the most successful drugs used to treat malaria but has been rendered virtually ineffective in many parts of the world by the widespread emergence of chloroquine resistance. Proteomics technologies are playing a major role in identifying potential therapeutic targets in Plasmodium species, as well as host-pathogen interactions and protein-drug interactions. Advances to date include the identification of differences between Plasmodium species, identification of immune targets for vaccination and immune protection, better understanding of the cellular target(s) of chloroquine and the mechanisms of chloroquine resistance[36].

Development of new Therapeutic Agents

Proteomics as an evolving science is expected to have a major impact on drug development in the near future. It has been shown that some proteins which are differentially expressed by microorganisms, and that differ primarily in thier tertiary structure from related proteins in the host have now become potential therapeutic drug targets. These can be tested against commercially available libraries of chemical agents to identify lead compounds - compounds with in vitro activity that can be used to target these protein markers and to represent potential new therapies. Exploitation of these scientific findings could assist to develop improved therapeutic agents to challenge the complexity of various clinical entities.

FUTURE DEVELOPMENTS

At present, it is fairly premature to utilize many of the newly discovered biomarkers using proteomics analyses as screening or diagnostic tools. However, these exploratory studies point to the promise of proteomics as an investigatory tool to be used to screen or diagnose many disease entities using newly discovered biomarkers.

Applied research in medical diagnostics is being developed and continues on several metabolic, inherited, infectious and malignant disease entities with construction of proteomic maps of many serum and body fluids biomarkers.

These include amniotic fluid biomarkers which are being studied for the complex determination of fetal pathology biomarkers. Moreover, based on dynamics of specific biomarkers' alterations, special attention has been given to elucidation of the pathogenesis and the etiology of female infertility, and of recurrent miscarriages with the elaboration of clinical algorithms for the management of these conditions

Further research is needed to examine specific features of posttranslational modification of

peptide hormones that could as markers of some pathological processes such as colorectal and breast cancer, to assist with the determination of pharmacogenetic approaches to target and manage these conditions

Moreover, continuous utilization of genomics and proteomics to study and examine biomarkers to screen and diagnose malignant tumors, has lead to and continues to discover and evaluate many of the markers for the so-called "silent tumors" of bone, ovaries, pancreas and others. In particular, the determination of proto-oncogene-encoded proteins such as myc, src, fos, jun, myb, fms, and raf-1. This is being carried out as an aid to screening for the presence of these cancers.

Further development underway is the construction of proteomic maps of malignant tumor tissues to be used for diagnostic purposes, and to aid an early diagnosis and treatment optimization.

Additionally, several studies utilizing proteomics modalities and techniques is now targeting many common disease processes such as the diagnosis of chronic hepatitis, whereby, scientific research have continued to reveal the protein biomarkers of serum Hepatitis B and Hepatitis C viruses to aid in the diagnosis and the control of therapeutic efficacy[37,38].

Validation of these new tests in large clinical trials is necessary prior to implementing proteomics techniques and patterns routinely in clinical use as tools for early disease detection. It is anticipated that databases of several proteins from tissues, cells and body fluids in health and disease shall soon be available. These will link multiple parameters such as expression of specific proteins and cellular pathways and proliferation with genetic data and disease states that can be used for accurate and rapid diagnosis[39].

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