PROTEOMICS

A Promising Approach for Cancer Research

Edited by

SHAFAT ALI, SABHIYA MAJID, AND MUNEEB U. REHMAN

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Contents

CHAPTER 1

Cancer proteomics: An overview

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1. Introduction

Proteins are polymers of amino acids and act as building blocks of living organisms. They differ in their function, stability and 3D structures and are treated as final products of many genes. The complete sequencing of the genomes of different organisms, including humans, are very well known. Nonetheless, the functions of most of the genes remain unclear. The availability of this novel unexplored ocean of data has led some scientists to designate the 21st century as the postgenomic or proteomic era.

The term proteome means the total set of proteins coded by a genome. Proteomics includes the analysis of total protein composition of a cell or organism. It is essential to display the level along with the function of proteins. Proteomic data are extremely beneficial for classifying cells and tissues having diseases and for understanding various biological mechanisms. The structure, interactions, and functions of all cellular proteins in an organism can be identified by using methods of assessment. This field includes technologies that are used to extract essential biological information on molecules from serum and tissues and these biomolecules act as biomarkers, helping clinicians and scientists to unravel dynamic biological systems, especially in cancer patients (Posadas, Simpkins, Liotta, MacDonald, & Kohn, 2005). A biomarker is an essential bioindicator to diagnose cancer, or the status and development of the physiological state of a cell at a particular time; biomarkers represent an effective tool for the assessment of cancer status and can be used in evaluating the potential and safety of novel

Proteomics
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therapeutic agents (Cho, 2007). Therefore a complete understanding of the developing field of proteomics will provide knowledge required for recognition of these bioindicators and targeting of specific pathways, and may improve the healthcare system.

2. Definition and goals of proteomics

Kiernan defined proteomics as the use of quantitative protein-level assessment of gene expression to illustrate biological processes (e.g., disease processes and drug effects) and decipher the mechanisms of gene expression control (Kiernan, 2008). Genomic sequencing revealed that the human genome comprises over 30,000 unique genes, which might give rise to 100,000 different protein products. These amazing numbers have shifted the attention of young biotechnologists from the characterization of individual genes or proteins, to methods that are able to rapidly monitor all the possible proteins present in a living organism (Patricelli, 2002). Proteomics does not only deal with all the proteins in any given cells, but it also includes other isoforms of proteins and amendments, the interactions among them, the conformational explanations of proteins and their higher-order complexes, and also includes postgenomic data (Tyers & Mann, 2003). The field of proteomics has appeared with the goals of developing and applying methods for the global assessment of protein expression and function (Aebersold & Cravatt, 2002). The recent aims of proteomic research are more diverse and directed toward the systematic determination of a wide range of properties of proteins in different physiological and pathological conditions (Patterson & Aebersold, 2003). It is predicted that the production of impactive procedures for the quick and parallel examination of proteins will increase the functionalization of biomolecules and hence enable the discovery of new biomarkers and therapeutic targets for the examination and treatment of human diseases, as well as increase our mechanistic understanding of the functioning of biological systems (Aebersold & Cravatt, 2002).

2.1 Need for proteomics

The purpose of functional genomics is to allow the assessment of alterations in gene expression in response to different investigational conditions (Klein & Thongboonkerd, 2004). When a specific gene is transcribed and translated, at which rate and under which standard conditions and which functional end products have remained unexplored from the genomic sequencing studies (Pardanani, Wieben, Spelsberg, & Tefferi, 2002). This kind of assessment

plays an important role in studying physiology and pathophysiology of different diseases (Patricelli, 2002). Attention should be given to gene expression and functions of the proteins the genes encode, once the initial stage of genomic sequencing and gene discovery is completed (Pardanani et al., 2002). Nevertheless, instead of the expected numerous advancements in bioinformatics, it has been hard to predict genes precisely from genomic data (Klein & Thongboonkerd, 2004). Some of the restrictions of genomic examination are inability to provide complete information on cellular, subcellular, and intercellular functions, in which proteins, not genes, carry out the functions (Patterson & Aebersold, 2003) and there is not a particular association between genes and the protein complements or proteome of a cell (Pandey & Mann, 2000). Moreover, various genes are pseudogenes that do not show their expression in the cells (Klein & Thongboonkerd, 2004). Generally, it is predicted that assessments taken under consideration at the proteomic levels are essential, due to the following reasons.

1. There is poor association between mRNA (messenger ribonucleic acid) at large and the corresponding protein level.
    - There is no strong association between mRNA (messenger ribonucleic acid) richness and consistent protein levels. This signifies that concentration of proteins cannot be simply predicted from corresponding mRNA level.
    - Some mRNA molecules are noncoding and do not produce any protein product.
    - In some primary mRNA transcripts alternative splicing occurs, so the protein derived from the gene might have multiple isomeric forms.
    - Almost all eukaryotic organisms' proteins undergo posttranslational modifications and these modifications have functional significance.
    - The translocation of proteins from target site to the site of their action cannot always be subtracted from the data sequence.
    - Proteins degrade with each other and highly vary in stability.
    - The information present in the data sequences cannot determine the function of a protein.

3. Methods of protein measurement and biomarker identification

Different technologies have been developed to recognize proteomic assessment (Pierce, Fakhari, Works, Pierce, & Clancy, 2007). Proteomic data are extremely beneficial in classification of cells and tissues in diseased condition

and also in understanding mechanisms of biological systems (Kenyon et al., 2002). Scientists have identified the structure, interactions, and functions of all proteins in cells and organisms by applying various methods and techniques for the assessment of proteins. The aim of proteomics is to define the nature of proteins and their expression level in cells using protein detection assessment. At a clinical level, protein samples can be examined using various procedures, including mass spectrometry, 2-dimensional polyacrylamide gel electrophoresis (2D PAGE), and protein assays in order to compare the variability of protein structures (Resing & Ahn, 2005).

3.1 2D PAGE

2D PAGE is a common technology used for the examination of structural variability among different proteins. During this process, proteins are first denatured, processed, and separated based on their isoelectric point in the first dimension. The mixture is then separated through another gel matrix, i.e., the second dimension on the basis of their molecular weight. Particular protein spots become stained, which are identified by cutting and dividing them into separate fragments. The fragments are examined by high-power mass spectrometry. The digested protein fragments are then identified by matching with a reference protein database (Patricelli, 2002).

3.2 Mass spectrometry

The most exact and reliable method for assessment of a sample from a patient is mass spectrometry. This method is used for proteins in a substance according to their mass and charge (Gu & Chen, 2005). Matrix-assisted laser desorption ionization-time of flight detection mass spectrometry (MALDI-TOF) and surface-enhanced spectrometry (SELDI-TOF) are two common molecular methods used for the detection of proteins. MALDI technology immobilizes the protein by absorbing the matrix energy (chemical) on a chip or plate. The whole of the collected protein sample interacts with the matrix from which a selected subset of proteins is bound, a function of the composition of the selected matrix. In the SELDI technique, selective surfaces are used for binding with a subset of protein according to the absorption, partition, electrostatic interaction, or affinity chromatography on a solid-phase protein chip surface (Posadas et al., 2005). Proteomic techniques that use mass spectrometry include the following:

1. Protein–protein interactions
2. Posttranslational modifications
3. Structural proteomics

4. Protein quantification or differential modifications
5. Protein identification

All these techniques are commonly used in research laboratories for the isolation and identification of proteins in order to characterize them, so as to help clinicians differentiate between healthy and diseased patients (Dowling & Sheehan, 2006).

3.3 Protein arrays

A protein microarray is a portion of nitrocellulose coated glass slide on which various molecules of proteins are bound at different locations (Shruthi and Palani Vinodhkumar, 2016). Protein arrays use antibodies of a known affinity and specification, printed on the surface of aptamers. This method permits examination of the biochemical activities of thousands of proteins (Bertone & Snyder, 2005). Previous literature about the molecules is required to use this method for determination of biomarkers. One feature of the protein array method that differentiates it from DNA microarrays is its potential to detect a particular isoform of a protein that could be important in the diagnosis of a disease and its pathogenesis (Resing & Ahn, 2005). Since discovery of these particular bioindicators, they have been used by clinicians to differentiate between healthy and diseased patients. The obtained information can be helpful for clinicians in disease diagnosis and also to monitor the response of a therapy prescribed to a patient (Lim et al., 2009).

3.4 Protein bioinformatics

Experimental studies revealed that laboratory studies must be followed by computer-aided confirmation using specific software. Moreover, software packages for the examination of electrophoretic separation and bioinformatics tools must be developed to validate the data. Some of the data is available on the internet along with links to various sites provided in the expert analysis system (ExPASy) proteomics server (Lim et al., 2009). This software permits identification and characterization ranging from calculation of fundamental physicochemical properties up to the prediction of posttranslational modifications and three-dimensional structures (Lim et al., 2009).

4. Biomedical applications

Despite progress in understanding the molecular basis of different diseases, large gaps still exist in our understanding of the pathogenesis of a disease and the development of efficient approaches for the initial diagnosis and

treatment strategies. Recent advancement in proteomics is due to its role in the diagnosis of various diseases and possible method of treatment in order to combat the pathogenesis of that particular disease (Hanash, 2003). The field of proteomic assessment deals with the study of selected proteins and determining their function in biology and pathology; hence this field might be applied for diagnosis and to search for appropriate treatments (Posadas et al., 2005). It is well understood that proteomics can differentiate between the qualitative and quantitative characteristics of proteins. These applications have been used in different areas of biomedicine, ranging from interpretation of molecular pathogenesis of a disease to optimization of a drug and its characterization against that particular disease, and the discovery of effective diagnostic tools (Tambor et al., 2010).

4.1 Proteomics and cancer

Cancer is a multifactorial disease related to an irregular cellular signaling network that controls the behavior of cells with regard to multiplication and programmed cell death. It is caused by genetic, genomic, and epigenetic changes at the cellular or tissue level (Zhang et al., 2009). In 2020 16 million new cancer cases were diagnosed worldwide. In 2005, among the total 58 million deaths worldwide, 7.6 million (13%) were due to cancer. And the numbers are continually increasing: it is estimated that in 2015 9 million deaths occurred due to cancer, and if the problem remains unresolved it may reach 11.4 million by 2030 (Cho, 2007).

Deaths due to cancer do not occur due to absence of therapies, but rather because of the late-stage diagnoses (Veenstra, Prieto, & Conrads, 2004). Prevention, initial detection, and early interference are the main aims of oncologists and cancer biologists (Posadas et al., 2005). If genes are considered as the master controller of cellular behavior, proteins are the effectors, and the expression of proteins and their activities display the health of a cell or a diseased patient at the molecular level. In the case of cancer, the expressed protein leads to the growth of tumor, invasion, metastases, communication with neighboring cells, and the effects of therapy (Zenner, 2017). Unfolding of the changes in protein network signaling, such as the cell cycle network in cancer, helps to identify the molecular mechanisms of carcinogenesis, development of cancer, and metastasis and hence recognizes the features of signaling network marking specific for various cancers and specific cancer subtypes. Changes occurring in the signaling network are accumulated at every stage of carcinogenesis due to the genetic, epigenetic, and environmental changes as studied in models of carcinogenesis (Zhang et al., 2009).

Oncoproteomics is a subcategory of proteomics that deals with the study of proteins and their association in a cancer cell using proteomic technologies. There is great interest in implementing proteomic techniques to foster an improved understanding of cancer pathogenesis and to develop new tumor-specific biomarkers for diagnosis and detection at an early stage by using a proteomic representative of samples. Oncoproteomics has the potential to revolutionize the clinical practice of cancer diagnosis and screening based on proteomics platforms as a complement to histopathogenesis; personalized selection of combinatorial therapy that will target the whole cancer-specific protein network; real-time evaluation of potency and toxic manifestations of therapy; and rational modulation of therapy according to the changes in the cancer network linked with prediction and drug resistance (Cho, 2007). Currently, the tests available for cancer screening have low sensitivity and specificity when used for the screening of ordinary people, so there is no clear distinction between benign and malignant tumors. The emerging field of oncoproteomics has created hope for the discovery of novel and effective biomarkers for cancer screening, early diagnosis, and response to therapy. Like normal cells, many cancer cells use multiple redundant intracellular signals to certify the maintenance and viability of functions essential to their survival. Hence, cellular signaling integral to cell function, existence, propagation, and receptor expression is a probable target for therapeutic intervention. Clinicians should recommend adjunct therapy of molecular agents and others according to the proteomic profile of the patient (Cho, 2007).

4.2 Early diagnosis of cancer

Conversion of cancer into malignancy involves changes in the expression of proteins along with subsequent clonal multiplication of the modified cells. These modifications can be assessed at the protein level qualitatively as well as quantitatively. Protein signs in cancer give important information that can be helpful for efficient diagnosis, prediction, and effects of therapy (Veenstra et al., 2004). A principal task in proteomic research is to restrict the function of cellular proteins that has been taken into consideration. Moreover, proteins associated with the homeostasis, metabolic activities and structure of the cell are abundantly present in cells and are approximately 10,000- to 100,000-fold abundant than proteins associated with the signaling network of a particular cell. Hence, detection and quantification of the proteins involved in cellular signaling are more difficult (Zhang et al., 2009). The capability of characterizing proteins in biological fluids like serum, plasma,

nipple aspirate fluid, and urine using proteomic technology has reached a level where hundreds of types can be recognized in a rapid screening, yielding a higher probability for identification of required biomarkers of cancer (Cho, 2007; Chung, Levy, Chaurand, & Carbone, 2007). Among the various types of bodily fluids, human plasma is not only a key clinical specimen, but also is the largest and deepest version of the human proteome. The human plasma proteome has played a revolutionary role in cancer diagnosis and testing of therapies; moreover it has given birth to new challenges in proteomics, such as detection at an earlier stage, disease prognosis, and the associated disciplines that must be addressed (Anderson & Anderson, 2002). Studies associated with the identification of novel antigens or markers for identification, prediction, or testing of a therapy, or with molecules and processes involved in carcinogenesis, have emerged at an intense rate. Currently, markers for the detection of tumors are based on protein-based methods; such methods date back to the 1800s when an abnormal urinary precipitate was examined for the detection of multiple myeloma (Bence Jones protein) in order to produce tumor-specific antibodies against the epithelial cancer cell lines. Genetic markers identified by cytogenetic approaches or by detection of mutations are also used at the clinical level; however, various changes must also be taken into consideration during the detection of carcinogenesis and its investigation, such as the fact that alterations in the expression of proto-oncogenes might not be associated with visible genetic lesions (Banks et al., 2000). Biomarkers were first revealed by conventional approaches such as protein distillation, enzyme-linked immunosorbent assay (ELISA), Western blot, and gel electrophoresis. However, these are not very specific or sensitive methods, so newer and more progressive methods were found. Recently used proteomic analyzers for the discovery of biomarkers include 2D PAGE, mass spectrometry, MALDI, electrospray ionization, and SELDI-TOF. The essential stage of the biomarker discovery process is the authentication stage, which requires a clinical assay to be developed and widely tested on thousands of clinical samples (Tambor et al., 2010; Zhang et al., 2009), along with processing of the derived samples (Veenstra et al., 2004).

4.3 Determining tumor aggressiveness

Clinically, it is a fact that nearly all tumors metastasize and/or develop without the use of therapeutic agents; however, some tumors grow gradually and do not undergo metastasis. Despite the great variation in tumor behavior, cancer is treated as if it were a single disease. Due to this monolithic practice, a number of patients are needlessly exposed to destructive therapies

that can be life threatening. Similarly, some patients are suffering from a life-threatening disease that, if it had been diagnosed at an early stage, might have been easily treated over a short time. Although proteomic techniques are not used to separate cancers according to clinical aggressiveness, hence diagnostic methods should be designed to explore the signaling pathways that govern aggressiveness of tumor (Zenner, 2017).

4.4 Individualized therapy

The recent developments made in proteomics have opened new horizons for the discovery of cancer-associated biomarkers. With the arrival of "proteomic technologies such as the development of quantitative proteomic methods, high resolution, high speed, high throughput, high-sensitivity mass spectrometry and proteinchip, as well as advanced bioinformatics for data handling and interpretation" (Cho, 2007), there is the potential of discovering efficient biomarkers to evaluate the treatment and management of cancer. Molecular bioindicators can be used in different tumors to guide the therapeutic proteomic system using lysates or collected antibodies developed that have the potential to regulate the activation of various or efficiently well-recognized kinases within a particular tumor. Documentation of kinases that drive the growth or aggressiveness of specific tumors could be used against targeted specific kinase inhibitors as a therapy for the treatment of tumors. Mass spectrometry can be used to recognize prognostic markers of tumor metastatic potential. Thus proper identification of tumor behavior may allow effective evaluation of cancer treatments (Zenner, 2017).

5. Mechanisms of proteomic changes in cancer

Even though the causes of various types of cancer are not clearly understood, it is known that cancer is caused by both genetic and environmental factors. Genetic alterations include mutations, variations in number of copies, chromosomal aberrations, and alternative splicing. One of the possible mechanisms for the proteomic differences in cancer is global aneuploidy, which means an imbalance in chromosomal quantity (Donnelly & Storchová, 2014). Aneuploidic cells undergo proteotoxic stress due to the defect in proteostasis, and in the latter stages the state of dynamic equilibrium, during which synthesis of proteins and their correct folding occurs, becomes unbalanced, with degradation of proteins. Thus faulty proteostasis leads to proteotoxic stress, cellular dysfunction, and also pathogenesis (Balch, Morimoto, Dillin, & Kelly, 2008). Current research has focused on the

unexplored mechanisms associated with aneuploidy, proteotoxic stress, and irregular multiplication of cells and tumorigeneses (Donnelly & Storchová, 2014). However, this relationship is under consideration due to controversial remarks due to lack of explanation regarding the exact association. For example, an extra chromosome that leads to upregulation of gene expression and a hypothetically augmented generation of proteins is not essentially translated into a definite increase of circulatory proteins; meanwhile, there is a greater increase in protein degradation. It has been predicted that some proteins, such as different kinases and multimeric protein complexes, are required for the folding of cellular proteins and thus are more prone to misfolding than others.

These and other related examples were expansively reviewed by Donnelly and Storchová (2014). Recent findings that link aneuploidy, a faulty proteome, and progression of cancer are attracting attention because they provide for efficient treatment of aneuploid cancer cells using appropriate antineoplastic targeting agents to target the proteostatic machinery (Adams, 2004). A second efficient mechanism for proteomics changes in cancer biology is the result of a faulty structure of proteins and their functions. Mutations in cancer-related genes lead to production of structurally defective proteins. These defective proteins cause harmful effects by changing the protein stability and then these proteins become prone to degradation; this alters the function of proteins or changes the affinity regulating protein–protein interactions (Mosca, Céol, & Aloy, 2013). Changes at the genomic and proteomic levels in cancer could further proceed through the recent field of interactome profiling considering the network-centered method, which provides abundant data related to the representation of protein interactions and the effect of protein structures.

6. Cancer biomarker applications

6.1 Cancer heterogeneity

The recent concepts of cancer heterogeneity and biospecimen variables are objects of focus by some researchers as crucial challenges for proteomics and for other omics technologies. Current intratumoral heterogeneity has been investigated in invasive breast cancer, comparing biospecimens taken by intraoperative image-guided, core-needle biopsies to surgical biopsies taken from the center and the periphery of breast cancer. Proteomic techniques in this study have shown that, although most biomarkers investigated did not result in significant clarity of intratumoral heterogeneity, the protein

and phosphoprotein levels were influenced by the type of biospecimen and by preanalytic variables, such as surgical manipulation and the duration of cold ischemia (Meric-Bernstam et al., 2014). Current methods used to avoid the challenge of tumor heterogeneity and to extract useful data from formalin-fixed tissue blocks are matrix-assisted laser desorption ionization (MALDI) and imaging (MALDI imaging mass spectroscopy, MALDI-IMS). These are specific methods that permit proteomics-based studies to make patient-specific and cancer-specific data available for the discovery and categorization of cancerous tissues (Gustafsson et al., 2013). Moreover, researchers using MALDI-IMS investigations of specific cancer tissues are producing peptide standard datasets to simplify the identification of peptides for future advanced research on similar types of cancer. Nonetheless, various technical challenges are yet to be explored and resolved, such as small signal-to-noise ratio and lack of mass accuracy (Meding et al., 2013). Current studies regarding prostate cancer conducted by Shipitsin and coworkers developed a protocol for the simulation of biopsy "by tissue microarrays aiming at overstating prostate cancer tissue changes" that are frequent at the clinical level. Their method has developed into a beneficial model for the diagnosis of the aggressiveness of a cancer by reliable bioindicators, regardless of the differences in samples (Shipitsin et al., 2014).

6.2 Initial early detection of cancer

Detection of cancer at an early stage increases the chances of successful treatment; however, such early detection has become a challenge for the scientific community, due to the unavailability of blood biomarker protocols having sufficient sensitivity, accuracy, and specificity. Hori and Gambhir have developed a mathematical model concentrating on assessing the time at which ovarian cancer can be identified by quantifying the content of the cancer antigen 125 (CA 125) cut from the tumor during its growth. Instead of reporting the sensitivity of the CA 125 examination assay, it was shown that tumors are able to grow in an uncontrolled manner for 10 years and can attain a size of 2.5 cm or more prior to their detection. This mathematical method could provide similar results in other types of tumors; moreover, the model can be used virtually in any solid cancer and with related biomarkers (Hori & Gambhir, 2011). However, controversy has been raised due to the application of this method in other types of tumors and the type of conventions used for the calculation (Konforte & Diamandis, 2013). This example explains the uniqueness of a method to test the application of circulating biomarker assays in the early detection of cancer, its diagnosis, response to therapeutic agents, and monitoring.

7. Protocols for developing tumor biomarkers

Over a decade ago from the time of this writing, various groups of researchers formulated multistep processes to develop tumor biomarkers. Hammod and Taube's phased method is associated with the following steps/phases: the biomarker discovery, the development of an assay system, the progression of an assay system, the performance of preliminary examination for the biomarker's clinical potential, the standardization and evaluation of the biomarker's measurement assay, and ultimately the authentication of that assay for clinical utilization (Hammond & Taube, 2002). Despite the formal stepwise investigative criteria of this approach, preanalytical issues were not adequately addressed. Around the same time period, Pepe and coauthors suggested another approach that concentrated on the requirement for correct definition of the study aim and its outcomes, together with strict criteria for specimen selection, sample size calculation, and experimental methods (Pepe et al., 2001). After a number of years, the same research group designed a study for the development of tumor biomarkers, that the design presented would lead to high-quality research and improve the probability of obtaining a clinically promising biomarker ready for subsequent rigorous scrutiny (Pepe, Feng, Janes, Bossuyt, & Potter, 2008). Common issues that plagued the procedure of biomarker discovery research were claimed to be circumvented if this design, which is known as "nested case-control study design," was strictly implemented. This experimental design comprises identification and collection of potential leads and specimens for useful biomarkers to form a case–control study cohort that is associated with clinical implementation, and blind evaluation of the biomarkers in specimens obtained from arbitrarily chosen selected case and control cases (Pepe et al., 2008).

8. General guidelines for a good study design for biomarker discovery

To design a good quality study to discover cancer biomarkers, various features have to be accurately dealt with. The first step is careful planning and design of a research question followed by supportive and convincing evidence for its contribution and association with a clinical problem (Baldwin, 2004; Carr et al., 2014). A balanced choice of the most appropriate logical test methods for this research question is of equal significance. The performance features for such tests, including specificity, sensitivity, and positive and negative predictive power, should be suitable for the investigational

design and fully explained. Moreover, the confirmation and authentication approaches of the methods performed and strict and comprehensive explanations of the samples, nature, collection, and storage procedures have to be clearly defined. Descriptions of samples, their source, and details of the subject (age, gender, stage of disease, medication taken, and lifestyle) must be emphasized. In addition to this, cancerous tissues, selected biomarkers associated with research, and the process of sampling are of utmost importance so as to obtain reliable data. Similarly, the size of the sample for calculation is an important component of the study coherence and careful evaluation will help to exclude sample heterogeneity. Furthermore, procedures for the execution of experiments must follow basic and crucial protocols, including incorporation of proper blank(s), positive and negative control samples, and reference compound(s) during every experimental protocol. Description of the quality potential of instruments and their calibration are equally essential for the validation of protocols. Together, each step of this study design and execution needs clear explanation in sufficient detail to permit reproduction of the work and/or comparison of the data. Scientists are working diligently to standardize the protocols of proteomics-generated data for optimum use. Various data storehouses have been constructed, likewise Panorama (https://panoramaweb.org/) that, along with portals for proteomics assays associated with the targeting of cancer-related proteins and peptides, will help researchers to develop interest in the standardization of operational protocols for those assays, their quality, evaluation and validation of proofs for a particular protein or peptide (Carr et al., 2014).

9. Applications of proteomics research in various cancers

9.1 Types of cancers

In different types of cancers, the discovery of biomarkers may contribute to the following important applications: early analysis and projection and assessment of development stages of a disease, response and effectiveness of a therapy, and disease reappearance. High-throughput formulations of a hypothesis method have been explored for the hundreds to thousands of cancer-associated proteins (CAPs). This indicates that hundreds to thousands of efficient protein biomarkers have been reported in the literature and they require critical validation and authentication. After the proper validation, these potential molecules could be used in a wide range of clinical settings such as diagnosis, prognosis, staging, and classification of patients. This is an important prospect for translational cancer research (Ludwig & Weinstein, 2005;

Schiess, Wollscheid, & Aebersold, 2009). Typically, hypothesis testing has been conducted by using antibody-based methods, e.g., enzyme-linked immunosorbent assay (ELISA), but ELISAs have some limitations, such as high cost, time-consuming nature, and technical drawbacks due to their complexity, which create problems and hinder their use to validate a rapidly evolved list of potential cancer protein biomarkers (Whiteaker et al., 2011). These high-throughput hypothesis testing methods slow the process of scientific validation, so that these CAPs cannot be used in clinical practices. Hence, a persistent requirement for accurate, precise, and sensitive validation assays is the driving force in the current research horizons under process.

One potential track that has been devised is known as selected reaction monitoring (SRM) assay for protein targeting in proteomics. SRM assay is a recent tool developed and refined for various human CAPs that are functionally associated with cancer-driving mutations. These assays are used to detect the target proteins in the circulation or urine and have resulted in reproducible quantification across a large group of cancer patient samples. Hence, these assays have become important sources for increasing and planning for biomarker verification (Hüttenhain et al., 2012). Hence, different aspects shall be approached to address the issue of standardization and optimization of cancer biomarkers used in the study of preexamined and examined assay components.

9.2 Biomarkers for lung cancer detection

Serum biomarkers for the detection of lung cancer have been studied in mouse models and also in humans and data obtained correlated with each other in both species (Taguchi et al., 2011). These biomarkers include circulating levels of EGFR, SFTPB, and WFDC2 that significantly differ in lung cancer patients relative to control. However, exploration of biomarker(s) for other types of cancers having a screening ability when assessed in prediagnostic biological samples is an essential target, because it might have the ability to detect cancer during early screening (Taguchi et al., 2011). Regrettably, this target is yet to be achieved. Today integration of proteomic technologies and imaging tools is being used, which give potential outcomes for determination of detailed aspects of lung cancer pathogenesis at the molecular level. This experimental finding reveals information on the efficiency of anticancer drugs (Oh et al., 2014).

9.3 Breast cancer biomarkers

Proteomics methods used for the study of breast cancer have given progressive and improved results at diagnostic and therapeutic levels. A combined method involving in vitro and in vivo studies has been used for investigation of cultured breast cancer cell lines from well-defined breast cancer stages and was authenticated by using human breast cancer tissue. In this method the tumor stage-specific proteomic markers obtained from the in vitro study were authenticated on tissue microarrays. Transformed cells revealed that proteomic markers were characterized by loss of histoarchitecture and changes at the metabolic level in cells (Geiger, Madden, Gallagher, Cox, & Mann, 2012). Another current study reported that the plasma proteome in breast cancer also showed that the tumor microenvironment-derived proteins were associated with the number of innate physiologic processes like wound repair, immune response, and remodeling of tissues (Pitteri et al., 2011).

9.4 Ovarian cancer biomarkers and implications from proteomics

Previous studies have reported that ovarian cancer has become a serious health issue in females, especially during their reproductive stage, such that it is treated as one of the most deadly gynecological malignancies. Due to the fact that it is mostly asymptomatic in early stages, most of the cases are diagnosed at later stages after the metastasis of tumors. In addition to this, because of the low incidence of ovarian cancer, there is no well-designed screening biomarker for the diagnosis of ovarian cancer at a population level. In fact, due to the low incidence, screening tests for ovarian cancer must be very precise in order to be acceptable, and should have a positive predictive value (Leung, Diamandis, & Kulasingam, 2014). However, this state is not well-understood at a pathological level, and only a few years ago, after intense research efforts, it was reported that ovarian cancer is a heterogeneous class of reproductive disorder affecting common organs as confirmed at molecular level (Vaughan et al., 2011). Hence, the need of the day is to discover novel biomarkers to increase the early diagnosis of this dreadful disease.

During ovarian cancer, various biological processes become altered and the expression of abnormal molecules belonging to different biochemical processes, e.g., DNA, (mRNA), proteins (associated subfamilies such as glycosylated proteins, peptides, and autoantibodies), and metabolites, is

upregulated. The technological breakthroughs in the genomics and proteomics fields have helped us to understand the pathophysiology of the disease. Two markers, CA 125 and human epididymis protein 4 (HE4), have been approved by the US Food and Drug Administration (FDA) for monitoring treatment and diagnosing reappearance of ovarian cancer in patients (Leung et al., 2014). Various research groups worldwide are paying attention to the aspect of alteration in biological processes in ovarian cancer and exploring the potential molecular mediators as bioindicators or as therapeutic targets, by implementing proteomic tools. These tools include targets, besides the protein repertoire, of other related biochemical entities, for example, the glycosylated proteins (glycomics), the low molecular weight peptides (peptidomics), the metabolites (metabolomics), and the antitumor antibodies (immunoproteomics) (Leung et al., 2014). Mechref and colleagues reported on a major advancement in preanalytical separation methods and MS that permitted elevation of detailed characterization of the glycosylated proteins repertoire (the glycome) and specific cancer glycoproteins in different types of cancer like ovarian cancer (Mechref, Hu, Garcia, & Hussein, 2012). Even though the recognition and characterization of abnormal glycosylated proteins in biosamples are still technical challenges, current advancements in MALDI-MS and in the preexaminational enrichment approaches, such as peptide-*N*-glycosidase digestion and chromatographic separation, have enabled glycoproteomics techniques to be added to the list of cancer-specific glycoprotein biomarkers (Adamczyk, Tharmalingam, & Rudd, 2012; Alley et al., 2012; Kim et al., 2014). Glycosylation as a posttranslational modification is a heterogeneous, conformationally complex, broad spectrum, and cell- and protein-specific process. Thus, in assessment of cancer-specific glycans, scientists are facing technical restrictions and ambiguity in the biological interpretation. These technical limitations include the heterogeneity of the glycans from which glycoforms are collected and the isomers of each glycoprotein and the limited availability of proteomic technologies for the differentiation of these many forms and isomers. In addition to this, after the discovery of individual glycan biomarkers, highly accurate quantitative authentic methods having precise specificity for the glycan epitope are needed and must have better sensitivity. Trials have been conducted to develop such protocols, using lectin or antibody-capturing technology, but so far they lack scientific validation.

Scientists are facing the challenge of evaluating the biological consequences of these anomalous glycoprotein markers during cancer conditions. Considering the case of ovarian cancer, it is not clear whether the glycomic

profiles are unique in this type of cancer or rather are results of cancer-associated metabolic alterations. Thus deep investigation is required in this field to explore the diagnostic biomarkers of this type of cancer (Arnold, Saldova, Hamid, & Rudd, 2008).

The study of the entire metabolite's population in biospecimens, the metabolome, by MS-based evaluation methods has been progressively used to discover cancer biomarkers. Various biological fluids such as urine and serum or plasma are used to assess potential cancer biomarkers. Urine samples are preferably used for proteomics and associated technologies are used for the discovery of biomarkers over serum or plasma, because urine contains relatively minimal total protein content and it is noninvasive to collect urine samples. Urine also contains relatively lower concentration of free higher molecular mass proteins, which makes urine less complicated than the serum/plasma samples (Alfadda et al., 2014). Proteomics techniques like ultraperformance LC quadrupole time-of-flight MS (UPLCQ-TOF MS), hydrophilic interaction chromatography, and reversed-phase LC MS have potential to identify various metabolites in the urine of ovarian cancer patients in comparison to normal healthy subjects. Interestingly, certain metabolites are discriminatory between early and late clinical phases of those patients (Chen et al., 2012; Zhang et al., 2013). Currently, the metabolomics profiles of plasma samples derived from epithelia ovarian cancer (EOC), benign ovarian tumor (BOT), uterine fibroid, and healthy controls using UPLC are well known. Fifty-three metabolites were examined in this work as specific biomarkers for EOC. Again, these metabolites have the potential to differentiate between EOC and BOT and uterine fibroids, and early-stage from late-phase EOC. The critical assessment of the aberrant metabolites has recognized metabolic pathways that were associated with cancer, specifically those of phospholipids metabolism, tryptophan breakdown, and β-oxidation of fatty acid. These results clarify our understanding of ovarian cancer pathophysiology (Ke et al., 2015). However, instead of witnessing new developments in this field, a number of perplexing variables are still hindering the application of metabolomics for full clinical use. Technical limitations include the biases associated with preanalytical factors like sample collection and storage conditions. Associated biological restrictions involve the unbalanced nature of metabolites that might be transmuted during transition from the cancer site to the biospecimen collected, or even after collection. In addition, other challenging factors include the subject's age, smoking habits, sleep patterns, and lifestyle. Therefore standardized and robust procedures are required to remove such biases and to

permit assay precision (Leung et al., 2014). Ascites fluid has been studied as a source for proteomics and metabolomics potential biomarkers in ovarian cancer; it has benefits over plasma or serum due to its close immediacy to the site of the tumor. When malignant ascites was compared with cirrhosis ascites metabolomes, 41 metabolites were identified that differed significantly between pathologies.

A detailed assessment of these metabolites revealed that most of the cancer-specific metabolites belong to the signaling cascade. Likewise, proteomic examination detected various molecules that differentiate ovarian cancer from cirrhosis ascites. Remarkably, spliceosomal proteins and RNA are present in the ovarian cancerous ascites; moreover, some studies also reported that these molecules play an important role in intercellular communication among the cancerous cells (Shender et al., 2014). Research has shown that low-molecular weight proteomics or peptidomics can be used to study biosamples such as blood, urine, ascites, or tumor tissue, in order to recognize specific biomarkers for ovarian cancer (Bery, Leung, Smith, Diamandis, & Kulasingam, 2014; Smith et al., 2014; Xu et al., 2015). However, development of this method is in a beginning stage, and if this method is optimized, it may become a complementary conventional proteomic method due to its cancer-associated protease activity; however, the lack of an optimized method and vigorous quantifying authentic method is minimizing its global use.

During the last decade, it has been observed that a novel method must be designed for the discovery of cancer-specific biomarkers to target the identification of cancer-associated antitumor antibodies known as immunoproteomics (Cho-Chung, 2006; Katchman et al., 2017). However, like peptidomics, this method still lacks the suitable verified protocols needed before any application for the detection of potential biomarkers can be suggested. It has been stated that proteomic profiling of plasma is questionable due to the very dynamic range of protein contents, which makes it hard to detect a protein having minimal concentration. Despite this, scientists have turned their focus toward the more proximal biofluids like ovarian-tumor tissue interstitial fluid and have observed that these fluids are potential sources of biomarkers (Gortzak-Uzan et al., 2008). Nevertheless, biospecimens used clinically should be easily available and biospecimens generated through this method must be meticulously examined in biological fluids with clinical potential, such as serum, urine, or saliva, prior to their consideration as tumor-specific biomarkers (Hoskins et al., 2011).

Previous studies have reported that the screening protocols for ovarian cancer in normal subjects are lacking and are crucially needed due to the destructive effects of late-stage diagnosis. Moore and coauthors designed a composite immunoassay by combining CA 125 with a proteomic method, surface-enhanced laser desorption ionization time of flight MS (SELDI TOF MS), to evaluate and measure seven biomarkers (apolipoprotein A1, truncated transthyretin, transferrin, hepcidin, β-2-microglobulin, connective tissue activating protein III, and interalpha trypsin inhibitor heavy chain 4), in order to improve the specificity and sensitivity of detection of EOC at a preclinical level by using prediagnostic serum biospecimens (Moore et al., 2012). These research findings further revealed that combined postdiagnostic collected serum samples have higher sensitivity for the detection of ovarian cancer beyond CA 125 alone (Moore et al., 2006), whereas inclusion of these biomarkers with CA 125 does not increase the sensitivity toward the preclinical diagnosis (Moore et al., 2012). Therefore detection of a biomarker or a set of biomarkers required for the identification of ovarian cancer depends on the screening protocol.

Various screening and diagnostic methods have been developed; however, critical scrutiny revealed that there is a defect observed in the experimental design that disallowed its duplicability and hence attention has been given to the design of a method in which the data is reproducible (Duncan, 2012). Vast technological progress has been made in the field of traditional proteomics and its associated aspects, which has generated enormous amounts of data on ovarian cancer. However, optimized standardization and verification of these potential biomarkers, as individuals or in combination, are primary requirements before their introduction into clinical practices such as screening, diagnosis, prognosis, and monitoring for the response to treatment or recurrence. As shown in earlier studies, proteomics and associated technologies have made significant contributions in the recent past and may potentially continue to contribute in the future as well, in order to increase understanding of the biomarkers of ovarian cancer (Ding, Wendl, McMichael, & Raphael, 2014).

9.5 Ovarian cancer pathogenesis

Proteomic analysis has led to better understanding of the pathogenesis of ovarian cancer. Upregulation of specific signaling pathway molecules in ovarian cancer cells may be one mechanism that can lead to the development of ovarian cancer. The major signaling cascades that are altered during cancer pathogenesis include cancer cell differentiation, survival (proliferation

or apoptosis), migration, and metabolism, and the well-known signaling pathways are lysophosphatidic acid, the phosphatidylinositol 3-kinase, NF κB, MAPK, and the vascular endothelial growth factor signaling pathways (Longuespée et al., 2012; Toss et al., 2013). These results provided important information regarding the latent diagnostic and prognostic markers and their therapeutic targets for deep pharmacotherapeutic-associated ovarian cancer research. Moreover, a Gynecologic Oncology Group trial has generated potential data, such as a specific arrangement of glycans has been found unable to differentiate between epithelial ovarian cancer and low malignancy latent ovarian cancer patients from normal subjects. These individual glycan biomarkers showed higher sensitivity and specificity, which led to their in-depth verification before their introduction into the clinical field for early diagnosis of ovarian cancer (Kim et al., 2014).

9.6 Etiology

In ovarian cancer, whether irregular or genetic, the associated risk factors include mutations in BRCA1 and BRCA2 genes and in the DNA mismatch repair genes featuring the Lynch syndrome (Walsh et al., 2006). Proteomic techniques are used to detect these mutations through characterization. Proteomic profiling has revealed that malignant transformation of states having a higher risk of developing ovarian cancer, like ovarian endometriosis and pelvic inflammation, have major consequences during ovarian carcinogenesis (Fuseya et al., 2012). Awareness of heredity regarding gynecological tumors like breast and ovarian cancers is due to notable interest in screening of populations having higher risks of these malignancies. Well-developed cancer centers and institutes have developed a program that aims to formulate an interdisciplinary coordinated method to screen out women having higher incidence of breast and ovarian cancer, organize proper clinical care, update recommendations and guidelines, provide support to patients, and admit patients for research purposes and registries (Engel et al., 2012). Proteomic assessment is performed for samples collected during a surgical procedure of risk-reducing bilateral salpingooophorectomy (RRBSO) used for women falling in a higher risk category. LC/MS–MS and protein network database algorithms are used to examine the proteomic profiles showing pathological changes in these higher-risk women. Some years back, a high-throughput workflow for assessment of proteomes of pelvic tissues (peritoneal, fallopian tube, and ovarian surface epithelial samples collected at the time of this surgery) was discussed. The purpose of this method was to explore novel biomarkers

having prognostic or diagnostic value in pelvic tissues to detect precancerous and cancerous proteomic changes of high-risk toxic mutation carriers (Rungruang et al., 2010).

9.7 Ovarian cancer progression

The conversion of benign ovarian tissue into its initial malignant transformed condition is a crucial phase that should be widely explored in order to obtain a detailed profile; meanwhile, it has been previously stated that ovarian cancer does not have a good prognosis and has a very deadly clinical sequence. Proteomic techniques are involved in the subsequent progression of ovarian cancer through the evaluation of expression of different proteins in cancers at different clinical and pathological phases and also in healthy epithelial tissues. In an experimental study through 2D electrophoresis along with the MALDITOF/TOF technique, Li and coworkers detected 54 abnormally expressed proteins in critical ovarian cancers. Glia maturation factor beta (GMFB) is one among these abnormally expressed proteins; it was further examined in a large group of patients suffering from various stages of ovarian cancer, and expression of GMFB was found significantly higher in comparison to normal, benign, or borderline ovarian tissues. A statistically positive correlation was found between GMFB expression and FIGO staging of the tumor, and there was a relation between the expression of this protein and a weak disease-free survival and net survival, along with the multivariate assessment findings. All of these reveal that this protein is an independent analytical factor for disease-free survival and net survival has been observed in examined ovarian cancer subjects (Li et al., 2010).

Other researchers have made small changes in the experiment on different biospecimens, such as combining shotgun proteomics and SRM MS. Elschenbroich and colleagues conducted deep proteomic assessment of ovarian cancer ascites in comparison to ascites from benign ovarian tumors. They constructed an assessment pipeline system including discovery-based proteomics, bioinformatics, and targeted proteomics quantification of the identified biomarkers of cancer (Elschenbroich et al., 2011). Two analytic techniques were combined (2-DE and MS/MS) to examine the ovarian cancer tissues, interstitial fluid, and peritoneal effusion, in comparison to normal tissue and fluid from obtained surgical biospecimens (Cortesi et al., 2011). This comparative examination showed variance in the expression of six proteins associated with cell cycle progression, apoptosis, and signal transduction cascades. Calgranulin is one protein among these that showed significant overexpression in all pathogenic samples, and is a possible strong

diagnostic and/or prognostic biomarker. Previous studies have shown N-linked glycan structures and their expression gets modified by means of diagnostic signature in ovarian cancer subjects (Alley et al., 2012). A shotgun quantitative proteomic assessment of benign and malignant epithelial ovarian tumors in comparison to healthy tissue using iTRAQ technology combined with LCMALDI-TOF/TOF and LC-ESI-QTOF MS/MS was published recently. The PIK3K/Akt signaling cascade serves as a remarkable pathway, having the potential to differentiate the clinicopathologically various tissues (Waldemarson et al., 2012). However, some studies have reported that MS assessment of the secretome from ex vivo coculturing of ovarian cancer cells and peritoneal cells to identify biomarkers of proteomics from their communication was revealed to show the metastasizing nature of ovarian cancers. One of the biomarkers, namely Mucin 5AC, has proven to be a strong biomarker for the insensitivity of ovarian cancers, as expression of this protein becomes significantly increased in the ovarian-peritoneal cell coculture, in comparison to monoculture of each kind of cell (Musrap et al., 2014). Moreover, class III β-tubulin shows upregulation in expression within the ovarian tumor microenvironment and studies show a strong predictive potential and significant survival rate in patients treated with neoadjuvant chemotherapy.

9.8 Targets for therapeutic means

A specific histologic subgroup of ovarian cancer and clear ovarian cancer cells is known to have minimal survival rate in comparison to other kinds of ovarian cancers. Genomics and immunohistochemical experimental research studies revealed that the same types of genes and protein expression were observed in clear cellular cancers in other organs, particularly in the kidneys and uterus. Hence, it could be concluded that there is a requirement for some therapeutic strategy to treat this dreadful cancer histotype based on the expression of protein profiles, instead of their effect on the other organs (Zorn et al., 2005). In addition to this, Anglesio and colleagues have found that women having clear ovarian cancer cells display a positive response to sunitinib, which is a drug used for the treatment of renal cancers that has had successful results (Anglesio et al., 2011). Moreover, some protective prospects having anticancer therapeutic molecules targeting novel ovarian cancer cells have been evaluated using data obtained from different high-throughput techniques (Tan, Miller, & Kaye, 2013).

10. Can proteomics research findings in cancer be translated into clinically oriented research?

In the last few decades, numerous omics technologies having applicability in cancer research have been initiated and are now evolving consistently. This results of this research have now been transformed into genomics and proteomics cancer biomarkers. However, the ultimate result depends on the process of transformation of biomarkers into strong anticancer agents, affected by various factors. Wilhelm and coworkers studied an MS-based draft of human proteome, and they clearly evidenced upregulation of functional protein expression in association with particular types of cancer. For example, the protooncogene EGFR, discovered during the 1980s (Cohen, Fava, & Sawyer, 1982), is now showing higher expression in a particular manner in some cancerous tissues, like breast cancer. Beta-catenin, a member of the Wnt signaling pathway, has shown higher expression in colon cancer cells, where it participates in the progression of the malignancy (Wilhelm et al., 2014). These results and other research have generated an optimum source of information and created a platform, and by considering these data sources scientists might be able to effectively design a project to discover a novel anticancer therapeutic agent.

10.1 EGFR kinase inhibitors

Investigation of the mechanism of cancer in cells and the action of drugs has become a burning issue in proteomic cancer research. The outcomes of this research area have major clinical significance and have created the potential to move cancer proteomics from bench to bedside (Hanash & Taguchi, 2010). Cancer cell lines were developed by the National Cancer Institute (NCI) as a model system for various types of tissue and genetic diversity of human cancers, and evaluating the massive quantity of evidence derived from bioinformatics, Moghaddas and colleagues observed powerful cell line clusters based on the type of tissue. Hundreds of differentially expressed proteins were explained in this model system, all these acts as efficient biomarkers for various tumor properties. Further, when proteomic data were integrated with the publicly evaluated transcriptomic data for this model system, the researchers revealed consistency between mRNA and protein expression. These researchers were also able to explain that protein expression can be correlated to various FDA-approved anticancer drug responses, in aspects of drug sensitivity and resistance (Gholami et al., 2013). These anticancer drugs target different families of cellular protein kinases.

These proteins are an essential class of oncogenes and are key contributors in intracellular signaling; consequently, their differential expression can also cause functional dysregulation or, ultimately, tumorigenesis. Hence, kinases are essential anticancer therapeutic targets (Knapp et al., 2013). Moreover, EGFR kinase inhibitors erlotinib and lapatinib have been used against cancer. For the detection of markers for drug sensitivity (positive-effect size) or resistance (negative-effect size), proteomic methods in cancer cell lines using elastic net assessment are used (Wilhelm et al., 2014).

10.2 HSP90 inhibitors

Hsp90 is a molecular chaperone that is an important molecular tool for the correct folding, stability, and thus maintenance of the functions of various proteins. As such, it is part of a system that functions during physiological and pathological conditions (Wiech, Buchner, Zimmermann, & Jakob, 1992). It has been reported that cancer cells are resistant to chaperones, while they possess a particular prerequisite for the protein folding machinery components to process the extra proteins being produced. The significance of Hsp90 targeting in cancer therapy depends on the nature of its clients; meanwhile various proteins among these belong to the family of oncogenes, such as tyrosine kinases, transcription factors, and regulatory cell cycle proteins. Hence, inhibition of Hsp90 leads to the degradation of these proteins via proteasomal machinery. Therefore utilization of Hsp90 inhibitors to treat cancers has shown promising results in some types of solid tumors and hematological malignancies (Garcia-Carbonero, Carnero, & Paz-Ares, 2013).

11. Conclusion

The branch of proteomics has produced a group of technologies and analytical tools that are significantly contributing to the field of cancer diagnostics. These technologies have been found to be an effective way to identify advanced biomarkers for the initial detection of cancer, and have potential for serological screening. Proteomics has provided genomic-based strategies, which have provided additional information, but it also solves different problems in technical aspects, data collection, and its interference. For example, there is no technique equivalent to polymerase chain reaction for amplification of low-abundance proteins, so detection of various molecules from a cell is required. Some technological approaches, specifically separation of proteins and their analysis, are intrinsically skill-based and remain

difficult to automate. Separation techniques including capillary electrophoresis could be more amenable to automation, as to replace two-dimensional electrophoresis due to its superior resolving power. After the identification of proteins, bioinformatics plays an essential role in increasing the initial information on proteins and hence makes it a critical step, because mismanagement of data should be avoided to stop further mishaps.

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