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Review Article

Proteomic Studies: An Examination of Proteomics Transformations from 2008 to 2018

Kamal F Alblwei* and Talal H Alshehri

Department of Pathology and Laboratory Medicine, Security Forces Hospital Program, Saudi Arabia

***Corresponding Author:** Kamal F Alblwei, Department of Pathology and Laboratory Medicine, Security Forces Hospital Program, Saudi Arabia.

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Abstract

Entitled 'The Emergence of Proteomics in 1996 and Its Ongoing Evolution,' this research paper offers a novel synthesis of prior research, presenting innovative perspectives and providing fresh insights into the subject matter. This study explores the dynamic field of proteomics, which has witnessed significant progress from 2008 to 2018. Researchers emphasis on a specific period is motivated by the profound and valuable transformation that transpired within the domain of Proteomics during that time. These advancements encompass various techniques used to analyze proteomics. Notably, breakthroughs and enhancements have been achieved, with a spotlight on the early cancer detection capabilities of SELDI-TOF-MS and its utility in identifying biomarkers for various diseases. While proteomics has made substantial strides, it remains ripe for further improvement. The report will discuss separation technologies, including 2DE, and the transition to more sophisticated methods such as advanced chromatography techniques, like 1D LC-MS/MS. Furthermore, the paper will touch upon the application of proteomics in related fields, such as cancer research. Ultimately, it will provide recommendations for future advancements to enrich the existing knowledge on proteomic techniques.

Keywords: Proteomics; Mass Spectrometry; Cancer; SELDI-TOF-MS; 2D Gel Electrophoresis

Abbreviations

NAF: Nipple Aspirate Fluid; HPV: Human Papilloma Virus; HPIDB: Host-Pathogen Interactions Data Base; ND: Neurodegenerative Disease; HRMS: High-Resolution Mass Spectrometry; QC: Quality Control

Introduction

Marc Wilkins and his colleagues coined the term "proteomics" to characterize the comprehensive study of protein complements on a large scale [1]. This report aims to examine the progress achieved in the field of proteomics and the resulting impacts on the discipline. It will provide a comparative analysis of the techniques, offering insights into how they have evolved from their 2008 state to a more advanced stage by 2018.Initially, the paper will emphasize the advancements in proteomic technology. Subsequently, it will provide a concise overview of sample separation methods. Finally, it will delve into the utilization of proteomic techniques within the realm of cancer research.

Advances in proteomic technology

Mass spectrometry

SELDI-TOF-MS

In 2008, SELDI-TOF MS played a crucial role in the identification of protein biomarkers within Nipple Aspirate Fluid (NAF), facilitating the differentiation between normal and abnormal cells. Through this approach, researchers discovered eight protein

biomarkers capable of distinguishing between cancerous and non-cancerous cells. This method exhibited a sensitivity of 63%, specificity of 89%, and an overall accuracy of 76%. Studies during this period suggested the need for further research to develop more advanced techniques that could enhance the specificity and selectivity of target biomarkers. Additionally, they recommended investigations into methods for identifying biomarkers present in low abundance. Over time, this method has undergone improvements, enabling the early detection of diseases like cancer. Furthermore, it has expanded its applicability to the detection of various diseases, including autoimmune and infectious diseases.

MALD-IMS

A decade ago, MALD-IMS played a pivotal role in delineating the spatial distribution of diverse biological compounds within tissue samples [2]. While it demanded substantial effort, this approach facilitated the identification of various compounds and evolved into a significant imaging tool for biochemical research. Presently, it continues to be utilized for the discovery of biomarkers in heterogeneous tumors sharing comparable histological characteristics and exhibiting a moderate response to chemotherapy and radiotherapy [3]. It transforms 2-D MS data into a format suitable for advanced analysis [4].

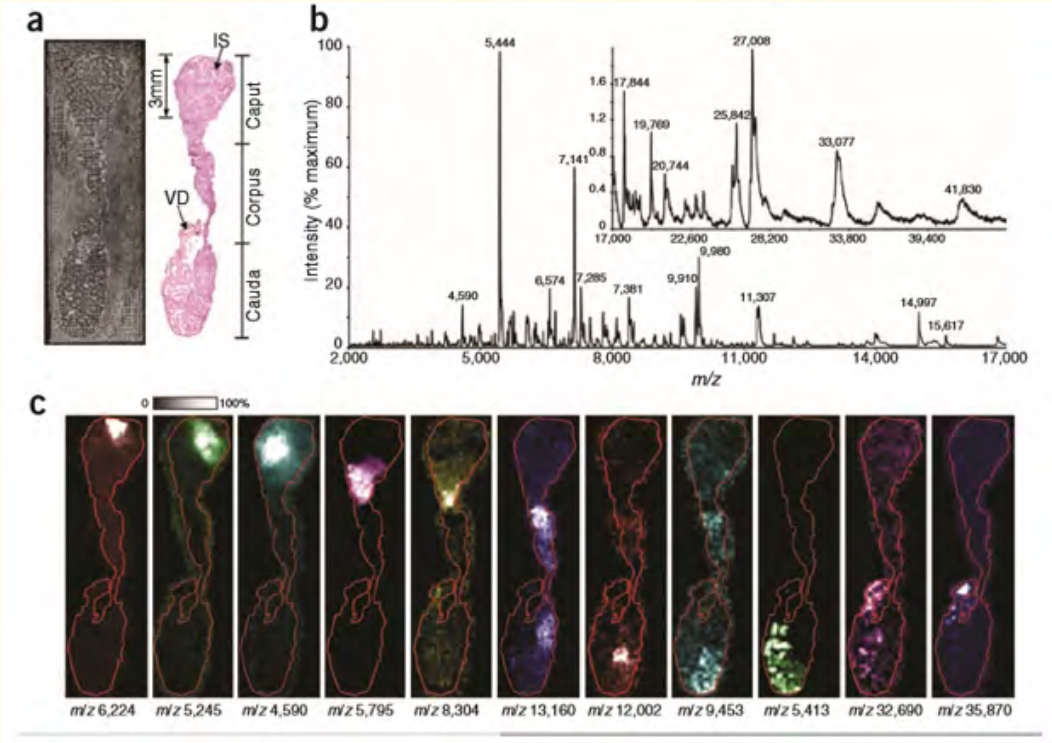

Figure 1: MALDI-IMS being employed to analyse 12-µm of mouse epididymis (a) Photomicrographs (b) Average mass spectrum (c) False-colour ion images of selected protein.

iTRAQ

iTRAQ played a significant role as a vital quantification technique in proteomic investigations. Notably, it was highly valuable in elucidating the molecular mechanisms of natural killer cells [5]. Presently, scientists depend on this approach for research management, thereby guaranteeing the accuracy of data collected in proteomic studies, ultimately enhancing the reliability of findings and data. Nowadays, iTRAQ can simultaneously assess eight samples and has the capacity to identify proteins with low abundance, such as the Human Papilloma Virus (HPV) in cervical samples [6].

Separation methods

In proteomics, researchers utilize two primary methods for compound separation, namely 2D electrophoresis and chromatography. The outcomes of these methods significantly rely on a well-devised strategy and the appropriate handling of samples. This includes the selection of suitable instruments, as well as the correct type and quantity of the sample. Furthermore, the techniques employed and the data processing methods employed can exert a substantial impact on the results of the experiment.

2DE

A decade ago, 2D electrophoresis demonstrated notable effectiveness, offering a high degree of separation resolution and the ability to preserve the gel spot of interest for subsequent experiments [7]. Furthermore, it had the capacity to separate a multitude of proteins and aid in the identification of their post-translational and co-translational modifications. There was an assumption that this method would endure for years. However, 2D electrophoresis has undergone enhancements, including the use of carrier ampholytes, the development of immobilized pH gradient techniques, the creation of IPG acrylamide strips, and advanced optimization through the labeling of samples with fluorophores. Today, the advanced version of 2D electrophoresis can identify proteins with high quality and reproducibility. Substantial improvements have been made in sample preparation and purification techniques. Additionally, enhancements have been implemented in isoelectric focusing and the storage of immobilized pH gradient strips [8]. 2D-DIGE stands out as a more robust separation technique, surpassing the performance of 2DE. It excels in its ability to identify low-abundance proteins that serve as biomarkers for diseases such as cancer, yielding superior results [6]. efore initiating the electrophoresis process, this method allows for the covalent labeling of three samples on a single gel, enabling a direct comparison [6].

Chromatography

Several multidimensional chromatographic methods have effectively filled the voids left by the limitations of 2DE [9]. For example, they can aid in the identification of proteins with hydrophobic properties and those that are present in low quantities.

LC-MS/MS

These techniques play a crucial role in shotgun proteomics, particularly when conducting large-scale analyses. This is attributed to the substantial throughput and sensitivity they offer. However, a notable challenge of this approach is dealing with the intricacy of the samples. Presently, multidimensional liquid chromatography, which is a more sensitive method, has been the subject of extensive research aimed at preserving its technical advancements [10]. In the present day, it's feasible to employ (1D) LC-MS/MS for the analysis of large sample volumes with high throughput, eliminating the necessity for prior fractionation [11]. This approach enables the examination of a vast array of multiprotein complexes in a single analysis.

Developments in proteomic applications

Proteomic applications

Proteomic research has observed significant progress throughout the years. Notably, advancements in mass spectrometry have facilitated a more in-depth analysis of proteins and their profiles. A particular focus has been placed on the identification of biomarkers associated with oncoproteins, which has had a noteworthy impact on the clinical management and prognosis of cancer [12]. As an example, Mass Spectrometry played a crucial role in research related to Melanoma, a type of skin cancer characterized by a high prevalence and resistance to treatment in its advanced phases [13]. The condition also exhibits a substantial portion of cases with regression and infiltration by T lymphocytes, making it a promising candidate for immunotherapy treatments. Cytokines play a crucial role in immune response regulation and intercellular communication within tumors. With this understanding, researchers manipulate cytokines to modulate immune responses, harnessing their diagnostic and prognostic potential. Cytokine production serves as an indicator of the effects of immunotherapies. There have been notable advancements in proteomic techniques used for the detection and quantification of cytokines in melanoma research [2]. Numerous methodologies have been developed to aid in this research, including mass spectrometry, immunoassays, multiplex assays, planar techniques, and highly sensitive methods. Over the past decade, there has been a notable advancement in multiplex immunoassays, allowing the simultaneous quantification

of multiple analytes in a single measurement. This is primarily driven by the recognition that a single cytokine is insufficient as a disease biomarker. Multiplex platforms are essential because the interactions between cells and molecules are intricate, and multiple regulatory processes result in various observations. Simultaneously measuring multiple analytes not only saves time and resources but also reduces sample requirements. Examples of multiplex immunoassays include antibody arrays, with bead-based arrays and planar arrays being common forms. In proteomic studies, 2DE was a commonly used method, but it had limitations, including low sensitivity. These limitations were addressed with the development of 2D-DIGE, which offers higher throughput, user-friendliness, reproducibility, and precise quantification of protein expression [2]. In the present day, proteomics is advancing towards customizing cancer treatment for individual patients. For example, breast cancer proteomic data has been derived through mass spectrometry. This breakthrough is transforming the field of biological research, enabling more efficient large-scale studies on patient groups [14]. By combining genomics and proteomic approaches, tailoring breast cancer treatment to each individual represents a clear path towards optimized and definitive solutions [15].

A proteomics approach to chracterising tick salivary secretions

Shortly before 2008, in 2007, researchers initiated a quest to investigate the proteome of tick salivary proteins. They established a database for the examination of proteins found in tick saliva, with the aim of developing preventive measures against ticks and the diseases associated with them, leveraging the knowledge of salivary proteins [6]. A decade later, they successfully developed a tick vaccine by utilizing data from the Host-Pathogen Interactions Database (HPIDB) to categorize the ticks [16].

Emerging techniques for ultrasensitive detection of secreted cytokines

Enhancements in proteomic technology have facilitated the creation of highly sensitive methods capable of efficiently scrutinizing analytes present in minute quantities [17-19]. Certain of these highly sensitive methods are designed for the detection of proteins at extremely low levels in blood and serum, which proves crucial in the early identification of conditions such as neurodegenerative diseases (ND), cardiovascular disorders, and various forms of cancer [20]. Highly sensitive techniques contribute to enhancing sensitivity, selectivity, and ease of use [21]. Additionally, they aid in diminishing matrix effects and the requirement for extensive sample volumes [2]. In addition to depending on traditional detection approaches such as chemiluminescence and fluorescence, emerging technologies make use of electrochemical and optical methods [2,22].

A proteomics approach to study the plants

A decade ago, investigations into the “cherry tomato pericarp” commenced. The analysis involved the separation of proteins using 2D gel techniques and their subsequent examination through either MALDI/TOF-MS or LC-MS [23]. The researchers successfully identified the importance of proteomic variances during the various growth stages of the tomato fruit. They compared these variances with changes in mRNA and metabolite profiles. Initially, the primary techniques utilized in these studies included 2DE in combination with MS. However, as the research progressed, other techniques gained prominence, mostly involving gel-free separation methods. Additionally, subsequent proteomic methods like MudPIT, DIGE, iTRAQ, and SILAC also became widely adopted [24]. Substantial enhancements were subsequently implemented in data acquisition, experiment design, and data analysis methods.

A decade later, the investigation into the proteomic expression of tomato fruit achieved its zenith. Researchers were able to discern abundant proteins, allowing the identification of skin and flesh tissues in developing tomatoes. This contributed valuable new insights into the plant’s genome utilization. Furthermore, the study expanded its knowledge of the transcriptome and metabolome through the application of high-throughput shotgun proteomics techniques. Methods like 2D chromatography and High-Resolution Mass Spectrometry (HRMS) were among those employed [25].

Statistics and bioinformatics in proteomic studies

Data analysis is critically important in proteomic studies as it helps with interpretation of experiments in proteomic studies. A decade ago, scientists had attained descent progress in statistical methods. However, there was still the need to bring various experts such as statistician, mathematicians and biologists together for joint studies [26]. For instance, preprocessing step approach discarded spectra from MS. MALDI-TOF, for instance showed

low technical quality in terms of spectra alignment, baseline subtraction, normalisation and peak detection [27]. Moreover, at around this time there was computerised analysis of 2D gel. Suggestion were made at this time to improve the image processing algorithms [28]. Additional suggestions were put forward, including the incorporation of q values alongside the initial use of p values to determine the achievement of a significant threshold. This adjustment was proposed due to the tendency of p values to frequently yield erroneous positive results. Recommendations were also made to enhance the development of a strong experimental design that permits thorough statistical analysis [29].

A decade later, significant enhancements in image processing emerged as Marczyk introduced an advanced spot detection technique for 2D gel electrophoresis, greatly improving the process [30]. Numerous platforms for Quality Control (QC) metrics have been introduced, with the primary emphasis on assessing the performance and precision of analytical results [31]. The utilization of these sophisticated computational methods has increased the reliability of proteomics studies, clearing the path for the clinical utilization of proteomics. Presently, there are suggestions for the creation of a computational tool to evaluate publicly available data, enabling future studies to benefit from automated quality control services.

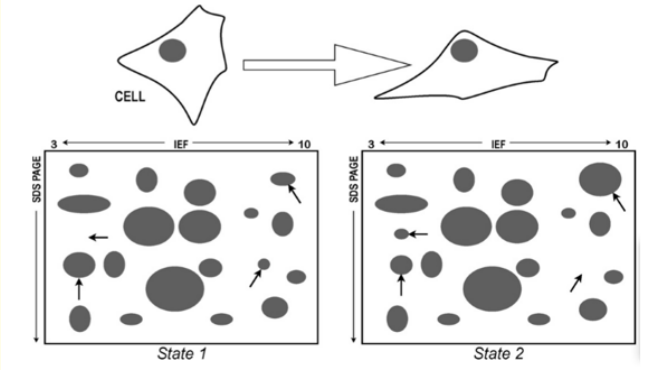

Figure 2: 2D gel illustration of the various stages of a proteome up and down regulated genes. Quantitative and qualitative changes marked with arrows.

Conclusion

The diverse array of proteomic techniques has expanded the horizons for protein research. While there's a significant push to adopt new proteomic methods, the older ones remain in practice. Additionally, techniques that were seldom utilized a decade ago have become commonplace. Researchers consistently uncover new findings that are anticipated to reshape the landscape of proteomic studies in the years to come. These advancements in proteomic research have found practical use in a range of fields, including cancer research, early disease detection, vaccine development, and the deeper understanding of plant biology at the molecular level.

An excellent illustration of this progress is the advancement of methods for detecting conditions such as melanoma and breast cancer. For instance, Mass Spectrometry has undergone development and has been integrated with other approaches to enable the early detection of cancer. New, highly sensitive techniques have emerged, enabling the analysis of minute quantities of analytes and aiding in the early diagnosis of diseases due to their enhanced sensitivity, precision, and simplicity. Presently, liquid chromatography and mass spectrometry enable the identification and quantification of samples without the need for prior fractionation, as well as the analysis of a multitude of multiprotein complexes in a single assessment. Notwithstanding these achievements, there is still substantial work required to enhance quality control methodologies.

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Conflict of Interest Statement

There is no conflict of interest according to the author.

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