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Clinical Applications of Proteomics: Another Look

Raji Sundararajan[1,2]*

[1]SoET, Purdue University, West Lafayette, USA

[2]CBST, Vellore Institute of Technology, Vellore, India

Abstract

Proteomics is the study of the large set of proteins expressed by an organelle, a cell, a tissue, a liquid at a given time and condition. Clinical proteomics is the application of proteomics for clinical samples, such as blood, urine, serum and other. Since protein is the basic, fundamental unit of cell functions, its characteristics and pathways, study of clinical proteomics is of practical interest, relevance, and importance. Clinical proteomics is mostly applied in cancer samples and studies on cardiac, COVID-19, and other disease are also on the way, leading to the identification of novel biomarkers and targets, enabling better clinical treatment to the patients. Clinical proteomics also enables personalized/precision medicine by studying the characteristic attributes of the patient under concern. Of course, application of proteomics to clinical practice also comes with its challenges on standardization of the sample collection (such as amount, time duration, etc.), validation of the results, and other. In this mini-review, the various clinical applications of proteomics are discussed, to illustrate the potential of this powerful technique. The challenges and potential future needs are also indicated.

Keywords: Clinical proteomics; Cancer; Biomarker; Mass spectrometry

Introduction

Proteomics is the fast, powerful study of the whole proteome (sum of all protein profiles) of a cell, biofluid, tissue, organ or a part of these. The results provide an information-rich landscape of differentially expressed (up and down regulated) proteins and their modulations of two or more samples of interest, under specific conditions [1-5]. Proteomics could be used to identify Posttranslational Modifications (PTMs), expression levels, metabolites, metabolic flux, enzymatic activity, targeted biomarkers, discovery analysis, differentiate heterogeneous mixture, serial analysis of patient samples, pharmacokinetics, and therapeutic monitoring of proteins [2]. High throughput LC-MS/MS Proteomics technology has the speed, throughput, robustness, and sensitivity than other technologies [5]. Clinical applications of proteomics are a powerful tool to unravel the molecular mechanisms of clinical samples including blood, serum, urine and tissues of various diseases, such as cancer, cardiovascular disorders, COVID-19, and other. High-throughput molecular spectrometry analysis of human plasma/serum proteomes is emerging as a promising technique for identifying distinct protein profiles in cancer patients. Proteomic patterning of serum was recently developed for the early detection of ovarian cancer [3]. Label-free, quantitative, high throughput Mass Spectrometry (MS)-based proteomics enables system-level analysis of thousands of proteins in complex biological samples [6-8]. Bioinformatic analysis of the expressed proteins and their known and predicted molecular interactions can be used to determine functional roles of each expressed protein. Modern proteomics provides us opportunity to discover new proteins and pathways involved in the cellular signaling network, as ultimately cells are the basic fundamental building blocks of our bodies. Biological systems have to be explained in terms of the activity, regulation, and modifications of proteins, and the ubiquitous occurrence of PTM. It is immensely useful to identify proteins involved in the diseases and could be used for tailoring the treatment(s). It also offers identification of novel biomarkers and targets due to proteins altered by the disease. Proteomics involves study and analysis of 1000s of proteins at one time, allowing to the specific proteins expressed as a function of diseases cellular functions or interactions [4]. The biomarkers identified as a result of proteomics studies will have higher sensitivity and specificity, due to the multiplexed panels of altered proteins of clinical samples, highly suitable for multifactorial disease, like cancer. Many of these molecules, including essential proteins and most transcription factors among the abundance of proteins, ranging from <50 to >10^6 molecules per cell, are present at such low levels that are not readily detectable by other techniques. The diverse chemical nature of proteins makes the development of globally applicable proteomic assays very challenging [9]. Proteomics enables not only the study of 1000s of proteins, but also their cellular components, such as protein

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Correspondence:
Raji Sundararajan, SoET, Purdue University, West Lafayette, IN-47907-USA,
E-mail: raji@purdue.edu

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containing complexes, cell parts, organelle, and etc. Gene ontology enrichment of these proteins and their pathways furnish biological processes, molecular functions, and cellular components, subcellular components, that throw light on regulation, transcription, molecular transduction, receptor, translational and transport activities and more [6-8]. In this review, the various clinical applications of proteomics are discussed, to illustrate the potential of this powerful technique. The challenges and potential future needs are also indicated.

Cancer Applications

Tissue-based proteomic strategies have been applied to the study of many cancer types, including prostate, breast, melanoma, lung, ovarian, and oropharyngeal carcinoma [10]. Tissue global proteomic studies can be further extended to the investigation of PTMs. Yang et al. [11] studied six primary lung Squamous Cell Carcinoma (SqCC) and eleven Adenocarcinoma (ADC) tumor tissue samples of non-small cell lung cancer, using MS-proteomics (and glycoproteomics). They identified over 6000 global proteins. The up and down regulated proteins revealed several critical pathways that were activated in SqCC and ADC tumor tissues. Using 96 frozen breast cancer samples of the conventional five types, Bouchal et al. [12] performed SWATH-MS. Their results indicated over 4,400 proteins, of which, 2,842 had consistent quantification, the majority of which were involved in well-established breast cancer pathways. These were obtained by performing proteomic analysis using an extensive spectral library containing the reference spectra for 28,233 prototypic peptides and their modified variants. Mittal et al. [7] identified thousands of proteins using the state-of-the-art bioinformatics techniques in their label-free, quantitative, high throughput LC-MS/MS, tandem mass spectroscopy-based proteomics studies of MDA-MB-231, human triple negative breast cancer cells treated with 1200 V/cm, 100 µs, 8 pulses at 1 HZ and cisplatin, curcumin and turmeric nanoparticle respectively [6-8]. The curcumin proteomics results indicated the presence of 1,456 proteins, of which 453 proteins were modulated (up/down regulated). These included kinases, heat shock proteins, transcription factors, structural proteins, and metabolic enzymes. Similar results were also obtained in the case of cisplatin and turmeric nanoparticles. These in vitro studies show the promise and potential of future preclinical and clinical studies, leading to every day clinical practices. Protein kinases are critical players in signal transduction pathways, and the dysregulation of these pathways is an intriguing area of study in cancer [13]. Latest MS instruments and phosphopeptide enrichment methods enable the large-scale detection and quantification of thousands of phosphorylation sites. Phosphoproteomic studies in clinical samples allow the identification of aberrantly activated kinases and their downstream substrates, serving as potential therapeutic targets. Many studies have shown important roles in understanding the molecular mechanism of cancers governed by phosphorylation-mediated pathways [14,15]. Zagorac et al. [16] performed label-free quantitative analysis of the Triple Negative Breast Cancer (TNBC) phosphoproteome and compared relapsed and non-relapsed patients. In total, 34 patient samples were lysed, digested and enriched for phosphopeptides. Label free single-shot runs resulted in the detection of more than 10,000 phosphosites, corresponding to 2,643 phosphoproteins. The analysis showed 159 phosphosites to have increased phosphorylation status. Thus, proteomics studies are useful to treat TNBC, the unmet need among the various breast cancer subtypes. MS-based proteomics analysis was also used to demonstrate the inter-tumor heterogeneity across different breast cancer subtypes compared to healthy controls, which enhanced the precision and accuracy in discovery and validation of candidate protein markers [17]. Protein expression of Hepatocellular Carcinoma (HCC) patients using serum auto-antibodies indicated cross-reactivity with proteins from the patient's tumor [4]. Three proteins-HSP70, peroxidoxin, and Manganese-Superoxide Dismutase (Mn-SOD) were identified to be overexpressed. These could serve as potential markers for HCC. Proteomics analysis of lung cancer patients had shown that Napsin A protein was only expressed in patients with primary lung adenocarcinoma [4]. This has been used as a potential biomarker to differentiate the primary form of lung adenocarcinoma from its metastatic form. Proteomics information is essential to classify the functional subtypes and stages of the breast cancer, to decipher its tumorigenesis mechanisms, c behavior and aggressiveness, to predict recurrence, to assess and reduce the drug resistance, to choose and monitor the most appropriate breast cancer treatment and its efficacy [17]. The office of cancer clinical proteomic research, in the Division of Cancer treatment & Diagnosis of NCI is a national effort to accelerate the understanding of the molecular basis of cancer through the applications of large-scale proteome and genome analysis (Proteogenomics) [18].

COVID-19 Applications

Shen et al. [19] performed the proteomic (and metabolic) profiling of sera from 46 COVID-19 patients and 53 control individuals, using a machine learning model. The study revealed the characteristic proteins and metabolic changes in the sera of severe COVID-19 patients. They identified molecular changes in the sera of COVID-19 patients, compared to other group, implicating dysregulation of macrophage, platelet degranulation, complement system pathways, and massive metabolic suppression. There were 93 proteins with differential expression in severe COVID-19 patient sera. 204 metabolites in COVID-19 patient sera correlate with disease severity and the various pathways analysis highlighted metabolic and immune dysregulation in COVID-19 patients. These could be used as potential blood biomarkers for severity evaluation in the clinics. These molecular changes could be used for therapy development in COVID-19 patients. Demichev et al. [20] also reported a proteomic survival predictor for COVID-19 patients in intensive care.

Cardiac Application

Proteomics can be a key tool for the prognosis of cardiac allograft rejection [4]. 13 out of 100 overexpressed proteins were identified to have cardiac tissue specificity in post-transplant endomyocardial biopsies. Of those 13, two proteins (alpha-beta-crystallin and tropomyosin) could be measured in patient's serum presenting cardiac rejection after three months. Thus, the power of proteomics in the clinic could enable biomarker discovery, useful for future patent treatments. Proteomics enables a more thorough investigation into molecular mechanisms underlying cardiovascular disease, facilitating identification of both quantitative (number of modified proteins) and qualitative (the nature of their modification) details [21]. It helps analyze organ and tissues at the subcellular, and molecular levels have revealed dynamic, complex, and subtle intracellular processes associated with heart and vascular disease.

Human Body Fluid Proteomics

Less or non-invasive body fluids, representative of a tumor or tissue or other, such as blood, urine form suitable samples for proteomics study. In clinics, blood is the most widely used human body fluid in disease diagnosis, prognosis and treatment outcomes

[10]. Blood consists of cellular components and the liquid component (plasma). Blood is tested for various plasma proteins via enzymatic assays or antibody-based immunoassays. The vast abundance of 22 proteins, constituting 99% of protein content have a dynamic range of mg/mL to pg/mL. This could mask salient, low abundant potential disease biomarker proteins of clinical significance. Advances in MS-based proteomic detection technology and sample preparation have helped to partially overcome these issues.

Circulating Tumor Cells (CTC) are tumor cells, shed from the primary tumor into the circulatory system and can lead to metastasis in different organs, provide a unique source of potential biomarkers. The occurrence of CTCs in blood is very small, only one CTC/mL. Different approaches, including that approved by FDA are used to isolate and detect the epithelial-based CTCs in breast cancer and CRC metastatic studies [10]. Advances in MS-based analysis, especially recent single cell proteomics approaches could potentially provide proteome-wide insights of these CTCs to identify novel protein markers for their detection and insights into tumour heterogeneity, cancer progression and treatment outcomes. Isolating minute amounts of these cells is a challenging task and all the methods mentioned above come with limitations such as leukocyte crosslinking, which leads to contamination of the target CTC proteome. Recent advancements in moving toward single-cell proteomics will improve coverage of the CTC proteome in future studies.

Urine is another commonly sampled human body fluid, because of its large volume and ease of non-invasive collection [10]. Compared to blood, it has a narrower dynamic range, and is less prone to proteolytic degradation allowing for more stable storage over longer periods of time. Over 6,000 proteins in normal subjects have been identified in urine, using advanced LC–MS instrumentation, leading to the analysis of urinary biomarkers. The challenges associated with urinary proteomics studies include inter-patient variability, as urine protein concentrations depend on kidney filtration and reabsorption performance, which greatly fluctuates within a population. Secondly, intra-patient variability needs further characterization because urinary protein concentrations are affected by the time of day, exercise, diet and age. Leng et al. [22] studied the urine proteomics of 167 healthy individuals and created the reference levels, of over 1,500 proteins. They indicate that this study provided a proof-of-principle concept for the use of urine proteome for health monitoring and disease screening using the physiological and pathological changes. More work needs to be performed in this area.

In addition to blood and urine, there are other human body fluids that could potentially be used for biomarker discovery. Lassman et al. [9] identified two biomarkers, SME1-1, and SME-2 from the cerebrospinal fluid from post-mortem diagnosed Alzheimer's disease. Some of these non-conventional fluid samples are rich sources of organ-specific proteins due to their close proximity, but need invasive sample collection, and hence they may not be as applicable for routine clinical practices, such as early cancer detection or longitudinal monitoring of cancer progression-yet.

Summary

Effective and economical applications of proteomics for clinical practice could help patients all over the world, immensely. Cancer biomarkers have transformed current practices in the oncology clinic. Mass spectrometry has the potential that enables increasingly comprehensive insights into changes of the proteome to advance clinical medicine. Label-free, quantitative, high throughput, tandem mass spectrometry-based proteomic studies in multi-centers, with standardized protocols, with high specificity and selectivity of proteins enable identification of novel pathways and biomarkers that enhance the quality of patient treatment that are affordable and efficacious, in cancer and other diseases. Design, development, standardization, and validation of clinical-friendly assays that take into account of the heterogeneity and other complexities involved in the tumors and other diseases could address the global challenges of engineering better biomarkers, and hence better medicines and treatments.

References

1. Husi H, Albalat A. Proteomics, in handbook of pharmacogenomics and stratified medicine. Sandhosh Padmanabhan, editor. Elsevier. 2014.
2. Duarte TT, Spencer CT. Personalized proteomics: The future of precision medicine. Proetomics. 2016;4(4):29.
3. Nicole VM. Clinical proteomics: Present and future prospects. Clin Biochem Rev. 2006;27:99-116.
4. Dominguez DC, Lopes R, Torres ML. Proteomics: Clinical applications. Clin Lab Sci. 2007;20(4):245-8.
5. Lassman ME. The clinical utility of mass spectrometry based protein assays. Clin Chim Acta. 2016;459:155-61.
6. Mittal L, Aryal UK, Camarillo IG, Ferreira RM, Sundararajan R. Quantitative proteomic analysis of enhanced cellular effects of electrochemotherapy with cisplatin in triple-negative breast cancer cells. Sci Rep. 2019;9(1):19124.
7. Mittal L, Camarillo IG, Varadarajan GS, Srinivasan H, Aryal UK, Sundararajan R. High-throughput, label-free quantitative proteomics studies of the anticancer effects of electrical pulses with turmeric silver nanoparticles: An *in vitro* model study. Sci Rep. 2020;10(1):7258.
8. Mittal L, Aryal UK, Camarillo IG, Vishak R, Sundararajan R. Effective electrochemotherapy with curcumin in MDA-MB-231 human, triple negative breast cancer cells: A global proteomics study. Bioelectrochemistry. 2020;131:107350.
9. Ghaemmaghaml S. Global analysis of protein expression in yeast. Nature. 2003;425(6959):737-41.
10. Macklin A, Khan S, Kislinger T. Recent advances in mass spectrometry based clinical proteomics: Applications to cancer research. Clin Proteomics. 2020;17:17.
11. Yang S. Protein signatures of molecular pathways in Non-Small Cell Lung Carcinoma (NSCLC): Comparison of glycoproteomics and global proteomics. Clin Proteomics. 2017;14:31.
12. Bouchal P. Breast cancer classification based on proteotypes obtained by SWATH mass spectroscopy. Cell Rep. 2019;28(3).
13. Brognard J, Hunter T. Protein kinase signaling networks in cancer. Curr Opin Genet Dev. 2011;21(1).
14. Wei W. Single cell phosphoproteomics resolves adaptive signaling dynamics and informs targeted combination therapy in glioblastoma. Cancer Cell. 2016;29(4).
15. Mertins P. Reproducible workflow for multiplexed deep-scale proteome and phosphoproteome analysis of tumor tissues by liquid chromatography-mass spectrometry. Nat Protoc. 2018;13(7).
16. Zagorac I. *In vivo* phosphoproteomics reveals kinase activity profiles that predict treatment outcome in triple negative breast cancer. Nat Commun. 2018;9(1).
17. Neagu AN. Proteomics and its applications in breast cancer. Am J Cancer

Res. 2021;11(9):4006-49.

18. OCCPR: A Leader in Cancer Proteomics and Proteogenomics.

19. Shen B. Proteomic and metabolic characterization of COVID-19 patient sera. Cell. 2020;82.

20. Demichev V. A proteomic survival predictor for COVID-19 patients in intensive care. PLOS Digital Health. 2022.

21. Arrell DK, Neverova I, Van Eyk JE. Cardiovascular proteomics: Evolution and potential. Circ Res. 2021;88(8)763-73.

22. Leng W, Ni X, Sun C, Lu T, Malovannaya A, Jung SY, et al. Proof-of-concept workflow for establishing reference intervals of human urine proteome for monitoring physiological and pathological changes. EBioMedicine. 2017;18:300-310.