JPP JOURNAL OF PROTEINS AND PROTEOMICS
2(1), January-June 2011, pp. 55-64

# UNPRECEDENTED INSIGHTS OF CANCER BY PROTEOMICS PROFILING

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***Abstract:*** Although cancer research has come a long way since the early days of molecular biology, cancer still remains one of the leading causes of death in the world. With the increased knowledge over the past decades, high-throughput technologies in the area of cancer research have opened new avenues for better diagnosis and treatment of human cancer. One of which, the quantitative assessment of protein levels in malignancies with the application of proteomics profiling techniques plays a major role for comprehensive understanding of cancer and turns out to be very crucial for diagnostic and therapeutic promise in clinic. Following the dynamic alterations of cellular proteins by comparing protein content of two samples (healthy vs. diseased or treated vs. untreated or metastatic vs. non-metastatic or sensitive vs. resistant) in malignancy provide an excellent tool in diagnosis, treatment and prediction of clinical outcomes. In this paper, importance of proteomics profiling in cancer research, its clinical uses for the treatment and diagnosis of various cancer types, the role of proteins in cancer as a biomarker and drug target are reviewed.

***Keywords:*** Cancer, Proteomics Profiling, Biomarker, Drug Target

## 1. Introduction

Cancer is a set of diseases characterized by the unregulated, abnormal growth of cells leading to invasion of surrounding tissues and spreads to other parts of the body by a process named as metastasis, via lymph or blood. Although there are multiple risk factors including genetic (biological) factors, age, declining immune system, exposure to chemicals and/or radiation and virus, environment and lifestyle factors are known to have a major impact in constituting the cancer. Relevant research demonstrates that of all cancer-related deaths, nearly 5-10% are due to genetic factors, whereas the rest of it, nearly 90-95%, are due to lifestyle and environment factors (Anand et al., 2008, Irigaray *et al.*, 2007).

Although the number of research groups dealing with the biological basis of cancer formation, progression and new treatment options increase in number with the expansion of knowledge in the field of cancer research, unfortunately, cancer still remains one of the leading causes of death after the heart diseases worldwide (Jemal *et al.*, 2007). The significant challenge of this field includes unsolved molecular and cellular mechanisms of cancer for the explanation of its formation, progression and metastasis, and also specific signaling pathways which are responsible for the communication of cancerous cells for certain cancer types. An additional important challenge is the unknown proteins associated with cancer development and drug targets for the different stages of tumor improvement, which is believed to have a key function. Therefore, the discovery and utilization of advanced technologies requires the cooperation of various multidisciplinary research areas for better diagnosis and treatment options of human cancer (Boyle and Levin, 2008).

Proteomic approaches are one of the ways in complement to genomics for the study of mainly cells undergoing malignant transformation during cancer. Specifically, the role of proteins in signaling pathways and the connection between protein structure and its function with developing protein separation (One-dimensional-gel electrophoresis, 1-DE or Two dimensional-gel

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electrophoresis, 2-DE or Liquid Chromatography, LC) (O'Farrell, 1975, Shi *et al.*, 2004) and proteomics profiling techniques need to be utilized during cancer investigations. In other words, in order to obtain an extensive identification of proteins in cells, proteomics in fact has the potential to address all questions on many aspects of biological events as a rapidly expanding discipline (Ahram, 2007, Graves and Haystead, 2002, James, 1997, Lane, 2005, Molloy and Witzmann, 2002, Pandey and Mann, 2000, Tyers and Mann, 2003). One of the applications of proteomics is proteomics profiling; an independent, emerging sub-specialty of it which has opened a new era to provide unprecedented insight into biological events by comparing the total protein mixtures of two samples (can be healthy and diseased or treated and untreated or metastatic and non-metastatic or sensitive and resistant). This is very crucial for diagnostic and therapeutic promise in clinic to follow the dynamic alterations of cellular proteins (Aebersold and Mann, 2003, Graves and Haystead, 2002, Lane, 2005, Wilkins *et al.*, 1996).

In the light of all available information, proteomics profiling techniques are vital to understand the characteristics of various cancers by discovering new biomarkers. The ability to cure some type of cancer directly depends on detecting it at its earliest stage. Therefore, the identification of sensitive and effective biomarker proteins is critical in the battle against cancer (Hutter and Sinha, 2001, Lohr and Faissner, 2004, Wulfkuhle *et al.*, 2003). Furthermore, we can also benefit from proteins within the cell as drug targets, for the development of a novel therapeutic assessment. In the case of identifying new targets for anti-cancer drugs and facilitating the evaluation of their action, toxicity and resistance in clinical trials, proteomics profiling might provide new insights into the interactions between a drug and the protein content of a cancer cell.

## 2. Proteomics Profiling in Cancer Research

In order to understand what kind of alterations occur at the protein level of a diseased state differently from healthy one, proteomics profiling emerged as a key technology lately. This method basically deals with identifying the changes in protein patterns by comparing two status and has a wide variety of applicability ranging from diseased vs. healthy, treated vs. untreated, sensitive vs. resistant to metastatic vs. non-metastatic (Bantscheff *et al.*, 2007, Şanlı-Mohamed *et al.*, 2011). The significance and usage of this technique in cancer research are increasing day by day. Innumerable current investigations as regards the causes of several types of cancer prove that there are close relationship between cancer formation and alteration of protein structure which results in loss of protein functions (Dzikiewicz-Krawczyk, 2008, Hall *et al.*, 2004, Horn *et al.*, 2006, Lee *et al.*, 2010, Mitsudomi *et al.*, 2000, Morton *et al.*, 2008, Rakha *et al.*, 2008, Schmitt *et al.*, 2002, Shahin *et al.*, 2000, Sørensen et al., 1993, Strimpakos *et al.*, 2009, Triantafyllou *et al.*, 2008). It is well known that protein alterations are common characteristics of cancer cells, when compared with normal counterparts, and have recently been indicated to have a significant role in the maintenance of malignancies. The most commonly occurring loss of function through deletion and/or mutation belongs to p53 tumor suppressor protein, which is the most frequently changing protein in human cancer (Acha-Sagredo *et al.*, 2009, Kim *et al.*, 2009, Pastor, 2011). Mutations and/or deletions in the p53 protein are observed in greater than 50% of all human malignancies (Hainaut and Hollstein, 2000, Harris, 1996, Hollstein *et al.*, 1991). Going by the name of guardian of the genome, p53 is a potent transcription factor that has the ability to activate the target genes causing cell cycle arrest, apoptosis and senescence (Choisy-Rossi and Yonish-Rouach, 1998, El-Deiry, 2001, Levine, 1997, Schlereth *et al.*, 2010, Symonds *et al.*, 1994, Vogelstein *et al.*, 2000, Vousden and Lu, 2002). And also it takes an active role in every multicellular organism by regulating cell growth, autophagy and inhibiting the angiogenesis (Mason and Rathmell, 2010, Rubio *et al.*, 2002). Apart from p53, there are some other proteins which are very useful to distinguish the healthy state from the diseased state for some certain cancer types, for instance; BCR/ABL (Rudolph *et al.*, 2005), ABL1/NUP214 (Graux al., 2004) is important for Acute Lymphoblastic Leukemia (ALL), PML/RARα (Melnick and Licht, 1999),

CEBPA (Smith *et al.*, 2004), RUNX1/RUNX1T1 (Jang *et al.*, 2010) for Acute Myelogenous Leukemia (AML), ZAP-70 (Wiestner *et al.*, 2003) for Chronic Lymphocytic Leukemia (CLL) and MDM2 (Fuchs *et al.*, 1998) for Chronic Myelogenous Leukemia (CML).

Taking into consideration every aspect of the importance of proteomics profiling on cancer research in terms of discovering new biomarker for the early diagnosis of a cancer, assessing the prognosis and providing potential targets for the development of new cancer therapies, we can easily pronounce that proteomics profiling is of great value for clinicians. Therefore, most scientists recently have focused on this area and today's technological developments have opened a new era for analyzing the changes in protein profiles. The stages of proteomics profiling technique used in most of the cancer-related research are fundamentally represented in Figure 1. So far, many high-throughput protein identification and characterization methods developed for proteomics have been applied to proteomics profiling. Mass spectrometry (Ahram, 2007, Gavin *et al.*, 2006, Washburn *et al.*, 2001), protein microarray (MacBeath and Schreiber, 2000, Zhu *et al.*, 2001) and high performance liquid chromatography-laser induced fluorescence (HPLC-LIF) technique are (Bhat *et al.*, 2010, Vidyasagar *et al.*, 2009) the most widely and efficiently used ones. Although the analysis of important proteins in biological systems are important, there are some drawnbacks of proteomics profiling due to the techniques used (Aebersold and Mann, 2003, Washburn *et al.*, 2001, Wolters *et al.*, 2001). Almost all the techniques used in the area are labor intensive, time consuming for analysis of proteins and lacks sensivity to detect low abundance proteins (Issaq and Veenstra, 2008, Sydor and Nock, 2003). Recently, the advantages and disadvantages of the techniques used for proteomics profiling have been summarized by Şanlı-Mohamed *et al.* (2011).

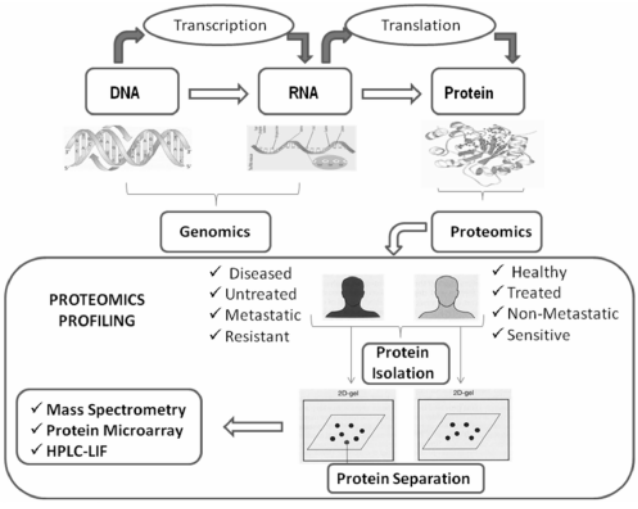



**Figure 1: Understanding of Proteomics Profiling Techniques**

## 3. Biological Sources in Cancer Research

Proteomics profiling technique basically depends on identifying the protein profile changes by comparing total proteins of two different samples as mentioned earlier. These samples can be obtained from several biological sources to compare the protein contents efficiently. Cancer cell line, human tissue and body fluids are available as a biological source for studying the proteome in cancer (Chen and Yates, 2007).

Cancer cell lines are largely in use in cancer-related research, especially in *in vitro* cancer progression studies, which present many advantages. One of the positive aspects of the samples is that they are both renewable and limitless self-replicating sources which provide large quantities of biological samples for proteomics profiling technique. Another advantage is the predisposition of cancer cell line towards the gene manipulation. An additional benefit of them is their low cost, because they are easily acquired by using cell culture methods. Combination of all the benefits resulted in tremendous amount of cell line utilized clinical research. A report listed differentially expressed proteins in ovarian cancer cell lines which are either sensitive or resistant to cisplatin, a chemotherapeutic agent that is widely used against ovarian cancers in clinic (Stewart *et al.*, 2006). A total number of 121 proteins were found to be varying between sensitive and resistant cells; some of which are overexpressed in sensitive cells while others are overexpressed in resistance. In another research, coupling of 2D gel electrophoresis with mass spectrometry revealed several proteins differentially expressed upon oxaliplatin treatment in various colon cancer cell

lines (Yao et al., 2009). This report found different numbers of proteins in three different lines (HT29, SW620 and LoVo), 21 of which are overlapping. Those proteins were known to be important in numerous aspects of cell cycle control including apoptosis, signal transduction and metabolism. Although there are many advantages of cell lines in the usage of proteomics profiling, of course they are not without drawbacks. The most significant disadvantage is the possibility of subpopulation formation on account of the phenotypic alterations in cell culture (Osborne *et al*., 1987).

Human tissues are another biological source which can be applied to cancer investigations by the method of proteomics profiling. The evaluation of changing protein levels lets broaden our horizon for both disease progression and new therapeutic options. Actually, human tissue have the high physiological/pathological relevance as a positive feature; unfortunately, the study of human tissues are associated with higher cost as compared to cancer cell line and also they have limited availability. Reyzer and Caprioli (2005) have postulated that the direct analysis of tissue sections in cancer research by MALDI mass spectrometry holds tremendous potential for biomarker discovery. In the another research, the direct profiling of proteins present in tissue sections for several organs of the mouse has been accomplished using matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) (Chaurand *et al*., 1999). As a result of the study, the unique protein profiles measured from these tissue blots showed differences from strain to strain of the mouse, with genetically similar strains having very similar patterns.

One of the most studied biological samples in cancer research is body fluids, such as urine, saliva and plasma, since they have advantages like low cost, easy availability from patient, high physiological and pathological relevance. For instance, while Roelofsen *et al.* (2007) has used surface enhanced laser desorption/ionization time of flight mass spectrometry for proteomics profiling of urine as an interesting source of biomarkers, comprehensive analysis and identification of the proteomic content in human whole and ductal saliva have been performed by Hu *et al.* (2007) for cancer detection and surveillance of oral health. Plasma as a body fluid, especially, represents an attractive source for cancer biomarker discovery and early cancer prediction. However, characterizing the proteome of that fluid presents significant challenges due to extreme complexity and large dynamic range in protein concentrations compared to cell lines and tissues for the study of clinical research. (Lee *et al*., 2006).

## 4. The role of Proteins in Cancer Research

Until recent years, cancer research have mainly and extensively targeted DNA and/or mRNA as genomic approaches. In fact, the actual active protein produced may not be results of mRNA expression levels (Anderson and Seilhamer, 1997, Martin and Nelson, 2001) since mRNAs are not always translated into protein (Lane, 2005). Hence, in the area of cancer research, the data provided from the genomic studies should be supported with the data obtained from the studies associated with proteins (Hanash, 2003). As an accepted task, proteomics profiling is often considered as the following stage of genomics in the study of biological systems. It would be very beneficial to investigate protein profiles and understand the dynamic alterations of cellular proteins for diagnostic as biomarkers and for therapeutics as drug targets in cancer research. The following section describes the utilization of proteins with proteomics profiling in the area of cancer biology as biomarkers and drug targets.

### *4a. Proteins as Biomarkers*

As a molecular indicator of biological status of malignancies during cancer, biomarkers are the molecules often produced by the cancer itself or the host system in response to the cancer. The importance of their discovery and utilization arises from the purpose of early detection of cancer, diagnosis, prognosis, response to anticancer therapies and/or cancer recurrence. Since survival rates depend on the early detection of the disease for most cancers, discovering novel early cancer-specific biomarkers in complex biological samples may improve diagnosis and monitoring of various disease states of almost all

types of cancer. So far, there is no specific biomarker in clinic that objectively measure and evaluate as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. In fact, it is well known that discovery of biomarkers in the area of cancer is not an easy task to cope with.

In an attempt to find novel biomarkers for various cancer types, proteins represent the primary source of diagnostic markers in body fluids and tissues. Understanding the proteome, the structure and function of each protein and the complexities of protein-protein interactions by protein expression profiles as a subspecialty of proteomic technologies is believed to be critical for developing the most effective biomarkers and techniques in cancer research. Thus, the discovery of new biomarkers can make it possible to discriminate healthy and malignant cells more accurately, follow each stage of cancer precisely, predict clinical response of a medicine applied to cancer patients specifically.

The study of correlation between the proteins expressed in healthy versus diseased state can reveal biochemical networks at the protein level which may be responsible for the development of the particular cancer type and could be thus helpful in terms of identification of the biomarker proteins characteristic of this cancer type (He and Chiu, 2003). Implementing such an approach on serum proteins achieved the discrimination of prostate cancer from benign prostate hyperplasia, both of which are manifested by the upregulation of prostate-specific antigen, by evaluating several proteins to establish a signature for the malignancy (Adam *et al.*, 2002). Similar achievements were obtained in other proteomic studies conducted on different tumor models including hepatocellular carcinoma (Poon *et al.* 2003) and esophageal squamous cell cancer (Zhou *et al.*, 2002). Li and colleagues (Li et al. 2002) used a proteomics profiling approach to identify serum biomarkers to detect breast cancer and found three discriminatory biomarkers with high sensitivity (93%) and specificity (91%).

Up to date, proteomics profiling was extensively applied in a number of clinical studies to detect early stage of cancer that determines the survival rate in most cases. For example, serum proteomics profiling in ovarian cancer patients recently has demonstrated a big hope for the early detection of the disease with 100% sensitivity and 95% specificity by identifying candidate proteins associated with that type of cancer as biomarkers (Kozak *et al.*, 2005, Petricoin *et al.*, 2002, Zhang *et al.*, 2006). Prostate cancer is another decently studied cancer by proteomics profiling to discover protein biomarkers for the early stage of the disease. As the results of extensive studies, Pca-24 protein is reported to be utilized potentially as a biomarker of prostate cancer (Zheng *et al.*, 2003). Similar studies have been performed for colorectal cancer and three potential proteins were identified as biomarkers of this disease especially in the detection of early stage (Chen and Yates, 2004).

The detection of protein alterations with the current proteomics profiling methods might be more valuable for some types of cancers compared to others. Pancreatic cancer is a wonderful example to this situation because this cancer is a uniformly lethal disease that is difficult to diagnose at early stage and even more difficult to cure. Therefore, an extensive study has been focused on for the finding of pancreatic cancer biomarkers by proteomics profiling and so far, two proteins could be used as biomarker for malignant pancreatic cancer patient with 78% sensitivity and 97% specificity (Honda *et al.*, 2005, Koopmann *et al.*, 2004). Recent progress and challenges for applying proteomics profiling for biomarker discovery in pancreatic cancer are discussed in details by Chen *et al.* (2005).

Proteomics profiling also can be utilized for invasive and non-invasive cancers by discriminating two different protein expression profiles to identify specific proteins as biomarkers for invasive type. For this purpose, Jones et al. (2002) has studied ovarian cancer and found out a group of proteins including FK506 binding protein, glyoxalase I as biomarkers of invasive ovarian cancer. Sometimes clinical response of a medicine in cancer patients are predicted through a protein marker as a result of proteomics profiling. In the case of multiple myeloma cancer, the clinical response was studied with bortezomib and two proteins, apoC-I and apoC-I', were

predicted as potential response biomarkers of multiple myeloma cancer (Hsieh *et al*., 2009).

### *4b. Proteins as Drug Targets*

Since most of biochemical and cellular events that promote tumor growth are enacted by proteins, one of the most promising developments in the area of cancer research is coming from the study of proteins as the identification of potential new drugs for the treatment of malignant diseases. In fact, many pharmaceutical companies are lately focused on developing new molecular therapies in the cost of protein utilization as effective, target specific, and individually tailored molecules to treat cancer. The use of proteomics profiling to investigate the possible drug targets in cancer treatment not only provides the identification of possible anti-cancer compounds but also provides relevant information regarding the molecular mechanism behind drug efficacy.

All things considered, Verrills *et al.* (2006) recently has demonstrated the importance and novel role of cytoskeletal proteins in vincristine resistant leukemia emphasizing that these proteins may be important potential target in the treatment of leukemia. Similarly, for the sake of the most appropriate treatment of chronic myelogenous leukaemia (CML), the characteristics of the BCR-ABL fusion protein and its biological function were shown to be possible anti-cancer drug target which then made it possible to develop a very specific inhibitor (STI-571) in CML therapy (Druker, 2002).

The fundementals of new drug-discovery for a disease, especially in cancer, relies on finding some types of compounds which can inactivate proteins involved in disease. To do so, the structural features of the target protein which is implicated in a malignancy should be known in details so that specific molecules called drugs (usually inhibitors) can be designed to interfere the action of the protein. For this purpose, drugs in cancer are targeted towards specific signaling pathways responsible for tumor growth and metastasis and target specific antigen, growth factor, receptor or other molecule in the signaling pathway of interest. For example, erlotinib is specifically designed to target the epithelial growth factor receptor (EGFR) expressed in many solid tumors (Kim and Murren, 2002). Another drug, bevacizumab, directed against the vascular endothelial growth factor (VEGF) was approved for the treatment of patients with metastatic carcinoma of the colon and rectum (Giantonio, 2006). The success in developing tratuzumab to target HER-2 in human mammary carcinoma is a good example of specific protein targeting as a therapy (Nabholtz *et al*., 2002). A new anti-cancer drug, trastuzumab, shows remarkable effectiveness in which proteins were designed to target other proteins (Pegram *et al*., 2004). This concept in cancer therapy open a new era because until recent years, commonly used anti-cancer drugs were non-selective and most of the time they influence non-specific processes rather than tumor-specific targets. The utilization of proteomics profiling in ovarian (Jones *et al*., 2002), prostate (Nelson *et al*., 2000) and breast cancer (Celis *et al*., 2004) revealed cancer-specific proteins that might prove to be new therapeutic targets in the area of cancer. Especially, as genetic differences among individuals are found, in recent years, researchers expect to use proteomics profiling techniques to develop personalized drugs that are more effective for the individual.

## Conclusion

Accumulating scientific literature with increased knowedge, it is pertinent that proteins have been quested to be utilized as important molecules in the area of cancer research. Changes in the protein profiles of cells under different circumcances provide a wide variety of critical knowledge. As a challenging and rapidly developing technology, proteomics profiling allows us to find out cancer associated protein patterns to assist better understanding of disease mechanism, enable early detection, provide fruitful diagnosis, monitor the disease progression and improve long-term survival of patients. Although proteomics profiling is still at its early stage and not fully utilized clinicaly in the area of cancer, the efforts so far have been productive and the results are encouraging. As a conclusion, protein players having role in malignancies are not confined to the ones discussed in this review. There are many others identified to be responsible for the malignant transformation, but without any doubt, there are lots of proteins whose mysteries

are unsolved and to do so the proteomics profiling approach is very promising, but more investigation is needed in cancer research.

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