American Journal of Cancer Science

http://ivyunion.org/index.php/ajcs/

Vo2. 2, Article ID 201300130, 18 pages

Review

Application of Proteomics in Cancer Study

Mona Zamanian-Azodi[1], Mostafa Rezaei-Tavirani[1]*, Amir Mortazavian[2], Reza Vafaee[1], Majid Rezaei-Tavirani[3], Hakimeh Zali[1], and Masoud Soheili- Kashani[4]

[1] Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Food Science and Technology, Shahid Beheshti University of Medical Sciences, Iran

[3] Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

[4]Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Cancer is one of the most malignant diseases in the world, accounting for 7.6 million deaths (around 13% of all deaths) in 2008 based on WHO reports. Early detection of cancer is vital due to its final control and prevention. Despite advances in diagnostic strategies, they have not the required sensitivity and specificity for prognosis. During the last decays, one of the most challenges for cancer research is to determine biological basis of this malignancy as a characteristic agents for an early-stage cancer. Understanding these agents requires molecular level examination of the disease followed by analysis of protein networks and their interactions in cells, signaling events among cancer cells, interactions among the cancer cells, and the tumor microenvironment. Proteomics as one of the modern areas of biochemistry holds great promise in cancer study. Inasmuch as, proteome reflects the real state of a cell, tissue or organism, it is expected to achieve more accurate tumor markers for disease diagnosis and therapeutic monitoring. In fact, the utility of this innovative large-scale proteome analyzer has shown significant prospective in biomarker discovery, patient monitoring, drug targeting and cell signaling; moreover, advances in the field of proteomics will provide new insight into the molecular complexity of the disease process, and enable the development of tools to help in treatment as well as in detection and prevention. In this review, proteomics approaches in cancer studies have been represented and discussed.

Keywords: Biomarker Discovery; Cancer; Proteomics; Diagnosis

Peer Reviewer: Kwok-Leung Cheung, MD, Division of Breast Surgery, University of Nottingham, United Kingdom; Avijit Majumdar, The University of Texas MD Anderson Cancer Center, United States, United States

Received: April 16, 2013; **Accepted**: July 13, 2013; **Published:** August 13, 2013

Competing Interests: The authors have declared that no competing interests exist.

***Correspondence to:** Mostafa Rezaei-Tavirani. Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. **Email:** tavirany@yahoo.com

1. Introduction

Cancer is one of the major life threatening diseases in 21 century. In 2008, about 7.6 million deaths (around 13% of all deaths) of cancer were reported. The gradual elimination of some other fatal diseases, combined with rising life expectancy, means that the risks of developing cancer are fluctuated slightly. The numbers of deaths from cancer worldwide are gradually rising, with an estimated 12 million deaths in 2030 [1]. Cancer has been a focus of biomedical studies for decades. The multigenic characteristic of cancer has led to progress in understanding of mechanisms of a specific disease phenotype [2]. Despite recent advances in the diagnosis and treatment of cancer, tumor cell progression and metastasis are the main cause of morbidity and mortality in cancer patients [3]. Most cancers are initially recognized either because signs or symptoms appear or through screening. Some tumors may not have any symptoms at all. In some certain cancers like gallbladder cancer, symptoms often do not start until the disease has reached its advanced stage. Consequently, more tools that are sensitive are required for early detection of diseases. Over the last 15 years, powerful high-throughput technologies, such as DNA microarrays, cDNA subtractions, and serial analysis of gene expression (SAGE), have been widely applied for identifying novel cancer related genes and for classifying cancers at the molecular level [4]. The cancer researchers are discovered varieties of biomarkers and therapeutic targets of different kinds of cancers recently. For instance, PSA is an appropriate diagnostic biomarker for Prostate cancer; furthermore, the most commonly used biomarker for ovarian cancer is CA125; nevertheless, malignancies are commonly detected at severe stages when patients have very poor prognosis and few treatment options that are mostly due to a high cost and time-consuming process in biomarker tracing. Thus, development of a better throughout analyzer method is a critical requirement for early detection, biomarker assay, and combination of the various platforms of oncoproteome data. Since proteome changes dynamically related to the state of an organism, it seems that proteomics as a high-throughput technique can uncover greater insight in to cancer biology rather than classical biochemistry, and statically methods such as genomics. It holds great promises for solving this matter by identifying biochemical evaluations of a disease process [5], and can be an accurate technology for cancer curing purposes. This high throughout scale provides new aspects for protein identification, quantization, fractionation, and enrichment to delve deeper into the oncoproteome in one single experiment. Cell lines, tissues, saliva and plasma/serum as the various sources of human samples are probed by a plethora of proteomics tools to discover novel biomarkers and elucidate mechanisms of tumor genesis [6]. Proteomics technologies and strategies are applied to determine therapy efficacy, identify novel drug targets, and ultimately develop personalized medicine for human malignancy [7, 8]. However, some limitations such as technical errors in the sensitivity of detecting low abundant biomarkers , probable systematic biases in the observed data, and biological heterogeneity manipulations are required to get improved in order to bring out the adequate cancer proteome mining [9, 10]. This article underlies the proteomics significant roles in cancer early detection and prevention from many perspectives.

2. Proteomics techniques and cancer

Proteomics contains two fundamental methods for protein characterization; first is proteins

separation by the tools such as two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) that was first introduced by P. H. O'Farrell, and the second is usage of mass spectrometry (MS) for protein identification purposes, which has greatly improved in accuracy and throughput recently [11]. The proteins separate according to their physicochemical properties and then the desired proteins considering their expression or function identify by MS techniques [12, 13]. Furthermore, surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) -MS is the backbone for serum or plasma analysis. Other methods including isotope-coded affinity tag technology, reverse-phase protein arrays, and antibody microarrays are emerging as alternative proteomic modus [14]. Data output from 2D-PAGE as a classical technique in proteomics is normally slow and analysis is limited to low-throughput means. This technology is not a rapid method for screen large sample numbers. In spite of these considerations, 2D-PAGE is still an efficient and common way to study several human cancers, both for expression and functional purposes. Expression proteomic studies are screening for differences in protein patterns between tumor and control samples. Many biological sources have been explored to generate valid comparisons for studying cancer proteome including cancer cell lines, human tissues and body fluids [15].

Overall, proteomics technologies can assist the development of cancer studies as follows [16]:

- Development of molecular detection (biomarker discovery) of cancer for diagnostic proposes.
- Proteomics provides a better understanding of molecular pathology of cancer (cell signaling).
- Drug targeting, facilitating integration of diagnostic and therapeutic aspect of cancer (personalized cancer care).
- Upgrade classification of cancer.
- Toxiproteomics; that could help in the development of safer therapies for cancer via identifying toxic effects of anticancer drugs at an early stage
- Patient monitoring

3. Molecular Detection (Prognostic and Biomarker Discovery) and Cell Signaling

A challenge in the treatment of cancer is the lack of early diagnostics. Since then, diagnosis is vital for prevention before its clinical demonstrations and its ultimate control, the molecular biology of cancer had been studied by different means such as proteomics that may make it possible to detect cancer at an early stage and arrange the treatment much more manageable. Although advances in conventional diagnostic strategies such a mammography and prostate specific antigen (PSA) testing have provided some specific improvement in detection of cancer, they still did not reach sensitivity and specify that are needed detecting early-stage disease. Most of the time, cancer is not detected and treated until cancer cell have already attack other tissues [17].

Cancer molecular pathway is complex and has a range of transcriptional and post-translational modified proteins. Besides, gene expression may change because of gene mutations or changes in environmental conditions and life style. Because many genes and several environmental factors are involved in cancer, mechanism of the development of several types of cancers is different. Consequently, by identifying these molecular pathways and molecular indicators called biomarkers, considerable improvement in cancer therapy may be achieved [18-20]. Indeed,

it is necessary that reproducibility and validation of these biomarkers address carefully, as their origin and identity. If these efforts are made, protein profiling can contribute to the better diagnosis of patients and the optimization of their treatment [21]. Some common cancer biomarkers and their related characteristic represent in table1.

To examining, the molecular changes that create these phenotypic and malignant changes, proteomic methods are now being used to study variations in protein expression, modifications, and enzyme activity [22, 23]. In addition to this, identifying key proteins and their changed regulatory role makes a new insight into the evolutionary process of tumor cells disclosing new functions and phenotypes [12, 15, 24]. The lack of confidence in using a particular single protein as a biomarker for a disease has led to the development of a panel of proteins as biomarkers instead of a single protein for certain diseases. It is shown that an increase in a combination of four proteins, such as leptin, prolactin, osteopontin, and insulin-like growth factor II, serves as a good indicator of ovarian cancer [25]. Another usage of proteomics is to elucidate the molecular mechanisms and signaling events that lead to cancer development [12].While genomic approaches have been used to establish the “blue-prints” of p53 signaling and its target genes, proteomics has become an essential tool to approve such information. Although the data obtained from functional genomics may explain more about p53 signaling, several other mechanisms involved are not gene mediated. In addition, information obtained from such study is limited, especially when post-transcriptional and post-translational modifications occur. The level of p53 modifies by many different products or proteins, which directly determine differential cellular functions and responses. Therefore, alliance of genomics and proteomics information with respect to one gene is important [26].

To examining, the molecular changes that create these phenotypic and malignant changes, proteomic methods are now being used to study variations in protein expression, modifications, and enzyme activity [22, 23]. In addition to this, identifying key proteins and their changed regulatory role makes a new insight into the evolutionary process of tumor cells disclosing new functions and phenotypes [12, 15, 24]. The lack of confidence in using a particular single protein as a biomarker for a disease has led to the development of a panel of proteins as biomarkers instead of a single protein for certain diseases. It is shown that an increase in a combination of four proteins, such as leptin, prolactin, osteopontin, and insulin-like growth factor II, serves as a good indicator of ovarian cancer [25]. Another usage of proteomics is to elucidate the molecular mechanisms and signaling events that lead to cancer development [12].While genomic approaches have been used to establish the “blue-prints” of p53 signaling and its target genes, proteomics has become an essential tool to approve such information. Although the data obtained from functional genomics may explain more about p53 signaling, several other mechanisms involved are not gene mediated. In addition, information obtained from such study is limited, especially when post-transcriptional and post-translational modifications occur. The level of p53 modifies by many different products or proteins, which directly determine differential cellular functions and responses. Therefore, alliance of genomics and proteomics information with respect to one gene is important [26].

Table1 some cancer biomarkers and their related information

Tumor markers	Related cancers	Non-cancer-related condition	Clinical usage	Source
AFP(Alpha-feto protein) [22, 23]	Liver, germ cell cancer of ovaries or testes	Also elevated during pregnancy	Help diagnose, monitor treatment, and determine recurrence	Blood
B2M (Beta-2 microglobulin) [24]	Multiple myeloma and lymphomas	Present in many other conditions, including Crohn's disease and hepatitis; often used to determine cause of renal failure	Determine prognosis	Blood
CA 15-3 (Cancer antigen 15-3) [25]	Breast cancer and others, including lung, ovarian	Also elevated in benign breast conditions; doctor can use CA 15-3 or CA 27.29 (two different assays for same marker)	Stage disease, monitor treatment, and determine recurrence	Blood
CA 19-9 (Cancer antigen 19-9) [26]	Pancreatic, sometimes colorectal and bile ducts	Also elevated in pancreatitis and inflammatory bowel disease	Stage disease, monitor treatment, and determine recurrence	Blood
CA-125 (Cancer antigen 125) [27]	Ovarian	Also elevated with endometriosis, some other benign diseases and conditions; not recommended as a general screen	Help diagnose, monitor treatment, and determine recurrence	Blood
Calcitonin [27]	Thyroid medullary carcinoma	Also elevated in pernicious anemia and thyroiditis	Help diagnose, monitor treatment, and determine recurrence	Blood
CEA (Carcino-embryonic antigen) [28]	Colorectal, lung, breast, thyroid, pancreatic, liver, cervix, and bladder	Elevated in other conditions such as hepatitis, COPD, colitis, pancreatitis, and in cigarette smokers	Monitor treatment and determine recurrence	Blood
Her-2/neu [29]	Breast	Oncogene that is present in multiple copies in 20-30% of invasive breast cancer	Determine prognosis and guide treatment	Tissue
PSA (Prostate specific antigen), total and free [30]	Prostate	Elevated in benign prostatic hyperplasia, prostatitis and with age	Screen for and help diagnose, monitor treatment, and determine recurrence	Blood
Thyroglobulin [31]	Thyroid	Used after thyroid is removed to evaluate treatment	Determine recurrence	Blood

4. Personalized Therapy via Molecular Targeting Strategies

Effective ways of treating cancer has been a great focus of biomedical investigation for decades. Cancer affects every patient and family in a different way. The most therapeutic challenges is to design a specific drug for each individual [2]. As proteins are the cause of diseases such as cancer in a way that, contribute to tumor formation, progression and metastasis, so knowledge of these individual molecules and deciphering signaling pathways can assist identify and characterize proteins involved in the disease and suggest combinatorial therapeutic strategies such as designing smart drugs [16]. One of the good examples of these molecules is PTK (Protein tyrosine Kinases) and other kinases that represent the feature of many cancers. These molecules placed in key positions in the signaling network; which are attractive targets for drug development such as inhibitors [27]. Drug targeting is a new developed way of treatment achieved by new molecular detection strategies such as proteomics. Thus, proteomics as a noteworthy technology can facilitate this way by the molecular highlighted methods [2, 28]. These methods aid to the identification of protein biomarkers, their modification, and altered metabolic pathways by comparison of the proteomes of normal cell and cell from a patient that leads to drug designing. In addition to this, bioinformatics is used for drug designing and in their final selection as a drug candidate, which is then used for biochemical and toxicological tests in animal model system and then in human before its approval by the FDA [29, 30]. Furthermore, knowledge of metabolic pathways and that of proteins interactions associated with bioinformatics tools facilitate the development of drugs in a cost-effective manner. In the future, high throughput screening (HTS) methods in a cost-effective manner will provide the interaction of possible chemicals as drugs with the target proteins rapidly. The use of combinatorial chemistry and the library of chemicals available on the database [31, 32] will aid this technique. In addition, as drug development is an expensive performance [32], proteomics is expected to decrease the expenses by increasing the number of target proteins used for the drug designed. Therefore, proteomics strategies make it feasible to translate basic science discoveries into the clinical application of personalized medicine and remedies [2].

5. Role of Proteomics in Cancer Classification

Recent reports signify that global proteomic approach may procreate the WHO disease classification including human cancers [33].Tumor classification is currently based on the idea of cell of origin. It is necessary that cancer classification affected by functional attribute of cancer cells. Inherently, all organs can produce various cancer forms as multiple cell types exist in these organs [34, 35]. The last two decades have witnessed the rise in molecular profiling which has already helped in better understanding of tumor development and identification cancer molecular classification. Current molecular classification systems are still dependent on morphologic variables. These classifications schemes use cell of origin as seen by light and electron microscopy [36, 37]. What is more, cancer classification schemes always reserve a group as unclassifiable that the subtypes are generated under the banner of a single, specific cell type of origin concept [38]. Therefore, the question is aroused that what model can serve unclassifiable cancers in a specific location. The integrated model of cancer classification presented here incorporates all morphology, cancer stem cell contributions, genetic, and functional attributes of cancer. Integrated cancer classification models could eliminate the unclassifiable cancers as used in current classifications.

Proteomics is one of the choices for classification of tumor origin and states, based on their molecular source [29, 30]. These molecules such as tyrosine kinases (PTKs) and their substrates are emerging as appropriate therapeutic targets and potential biomarkers for molecular classifications. The biological variability among patient samples as well as the huge dynamic range of biomarker concentrations is currently the major

challenges facing efforts to lessen diagnostic patterns that are unique to specific disease states. High-throughput profiling of the protein content of complex samples is detectable by recent advances in MS technology. For cancer classification, the protein samples from cancer patients relative to normal or from different cancer stages analyze through MS appliance and the MS patterns uses to build a diagnostic classifier. For example, lung cancers are traditionally classified into various subtypes on a histological basis. Adenocarcinoma and other histologists follow squamous lung cancer, the most common histological subtype. These various subtypes were classified based on 2D-PAGE [36] and MS profiles [39]. Future cancer treatments may be advanced by using an integrated model of cancer classification such as proteomics methods [40].

6. Toxicoproteomics

Toxicoproteomics is a new scientific method that combines proteomic technologies with bioinformatics. The emerging field of toxicoproteomics has been developed by quantitative and qualitative proteomics strategies and its increasing applications in toxicology study [41, 42]. This method seeks to identify important proteins and pathways in biological systems that are affected by toxicants, adverse chemical and environmental exposures using global protein expression technologies in mapping serum, plasma and other biofluid proteomes, and in parallel proteomic and transcriptomic studies toward understanding pathophysiology, and biomarker discovery of diseases including cancer [43-45]. Cancer is spread through any source of pollution namely through water pollution, air pollution and land pollution. A number of chemicals contaminations present in air, water, food and workplace are capable of inducing cancer. Many studies have discovered the link of various types of environmental pollution with the development of cancer. Although many of them have been classified as carcinogens according to United States of Environmental Protection Agency and International Agency for research on cancer; the understanding of their mechanism is still insufficient, and remained to identify [46]. In contrast to toxicogenomics, a discipline that determines genetic susceptibility of a particular individual following exposure to a carcinogenetic agent, toxicoproteomics allow the monitoring of the body's response to a specific toxicant. This advances supplies a means to identify and characterize mechanisms of action of toxicants in carcinogenesis. The current regulatory toxicological approach usually includes investigation of carcinogenicity, in generally lengthy (2 years) studies in rodents. This is especially true for detection of early protein biomarker signatures that precede neoplastic appearance [46]. Various examples exhibit the potential of proteomic approaches to reduce time and expense of traditional carcinogenicity testing. For instance, the liver carcinogen N-nitrosomorpholine (NNM) investigated in rats to identify potential early protein biomarker signatures indicative of the carcinogenic processes. Analysis was performed 18 weeks following treatment revealed significant up regulation of stress proteins, including caspase-8 precursor, vimentin, and Rho GDP dissociation inhibitor. Interestingly, the findings indicate that this treatment deregulates annexin A5 and fructose-1, 6-bisphosphatase. In addition, determining toxic effects of anticancer drugs at an early stage is useful for developing safer cancer therapies [47]. This finding may indicate their potential use as predictive biomarkers for early liver carcinogenicity [48].

7. Patient Monitoring

It is essential to know whether patients with malignant tumors are benefiting from the administered therapy or not. So, following initiation of treatment, serum probes can be observe for responding to therapy, screening and prediction of the therapeutic efficacy, as well as determining whether the tumor has developed resistance mechanisms that may need modification of treatment, that is called responder profiling. Multilabel detection coupled with high capture molecule density in immunosensors and arrays seems to be proficient of detecting a wide range of protein concentrations with sensitivity ranging into the sub pg mL^{-1} level.

Multilabel arrays can be designed to detect both high and ultralow abundance proteins in the same sample. Although, only a few of the newer ultrasensitive methods have been evaluated with real patient samples, which is key to launch clinical sensitivity and selectivity [49]. Proteomic technologies, such as serum protein pattern profiling, combined with protein microarray technologies, constitute a new paradigm for detecting disease and monitoring disease response to therapy [50]. Protein biomarkers such as CEA, CA 153, AFP,PTKs and PSA are useful for therapy monitoring, and it is reasonable that these biomarkers will be complemented by others in the future [6, 51]. As mentioned above, one of the best examples for monitoring treatment in patients with ovarian cancer is CA 125 [52].Response according to CA 125 occurred if there was either a 50% or a 75% reduction in CA 125 levels [53]. Following healing excision for primary cancer, it is now a common practice to follow-up patients at regular intervals. The main goal of this surveillance is to detect recurrent/metastatic disease at an early stage, the supposition being that the early detection of disease progression followed by the beginning of therapy, increase patient outcome compared to initiating therapy when the patient is symptomatic [44]. Finally, proteomics and genomics together are necessary for cancer patient management through the design and tracking of individualized therapy, and can possibly revolutionize cancer monitoring.

8. Proteomics Approach in Different Types of Cancer

8.1. Lung Cancer

Lung cancer is the most frequent type of cancer in the world [54]. There is a low overall 5-year survival rate, ranging from 10 % - 14 %. Early studies on lung cancer proteomics first published in the beginning of 1990s. These initial studies focused on the relationship between histopathological characteristics and 2D-PAGE reproducibility [55, 56]. A few years later, the first differentially expressed proteins in lung cancer were determined in small cell lung cancer (SCLC), including tubulin, heatshock proteins 73 and 90, lamin B, and proliferating cell nuclear antigen (PCNA). This study indicates for the first time that 2D-PAGE merge with protein identification was a noble approach to identify biomarkers in cancer [57]. After that, with improvements in MS technology, it was possible to identify about 20 potential biomarkers in lung cancer tissue [58].

A SELDI study in early stages in lung cancer and premalignant bronchial lesions analyzed LCM specimens of normal lung, atypical adenomatous hyperplasia and malignant tumors taken from patients participating in a screening program. Protein profiles were generated in each epithelial cell type and found to be in a great number reproducible in classifying populations at high risk for lung cancer [59]. Another study compared serum samples from lung cancer and healthy controls. Five protein peaks in a blinded test achieved a sensitivity of 87%, a specificity of 80%, and a positive predictive value of 92%. Sensitivity was even considerably better (91%) for detection of nonsmall cell lung cancers (NSCLC) [60]. Study of circulating autoantibodies in lung cancer patients was uncovered antibodies against annexins I and II, recoverin, protein gene product 9.5, and enolase [61]. Another comparative study between normal and non-small-cell lung cancer patients with the usage of Label-free quantitative liquid chromatography tandem mass spectrometry (1D-LC/MS/MS) shows that, 62 proteins were differentially expressed between non-small-cell lung cancer patients and normal controls which made it possible to distinguish non-small-cell lung cancer from the normal controls [62].

A combination of two dimensional gel electrophoresis LC-tandem mass spectrometry of A549 cells before and after green tea extract (GTE) exposure identified 14 protein spots that changed in expression (≥2 fold) after GTE treatment. These proteins are involved in calcium binding, cytoskeleton and motility, metabolism, detoxification or gene regulation. The result of the study demonstrates that GTE alters the levels of many proteins involved in growth, motility and apoptosis of A549 cells and their identification may

show the multiple anti-tumor activities of GTE [63]. Recent proteomic results have elucidated new aspects of derivation and validation a signature from the proteomic analysis of bronchial lesions that could predict the diagnosis of lung cancer. The possibility of having lung cancer based on the proteomic analysis of the bronchial specimens was characterized by an area under the receiver operating characteristic curve of 0.77 (95% CI 0.66–0.88) in this validation cohort. These results indicated that proteomic analysis of endobronchial lesions might facilitate the diagnosis of lung cancer and the monitoring of high-risk individuals for lung cancer in surveillance and chemoprevention trials [64].

8.2. Breast Cancer

Several proteomic technologies have been applied in different studies to discover biomarkers and molecular mechanisms associated with breast carcinoma, the most frequent cancer-related death in women which is accounted for 1.15million new cases and 410,000 deaths in 2002 [65-68]. For example, 2D-PAGE combined with MS analyzed changes in the proteome of infiltrating ductal carcinoma compared to normal breast tissue. Twenty-five differentially expressed proteins were identified comprising cell defense proteins, enzymes involved in glycolytic energy metabolism and homeostasis, protein folding and structural proteins, and proteins involved in cytoskeleton and cell motility. Further proteins were also mapped to establish a 2D-PAGE reference map of human breast cancer [69]. Another proteomic study, combining 2D-PAGE, MS, immunoblotting, and antibody arrays analyzed the proteome from adipose cells and interstitial fluid collected from mastectomy specimens of high-risk breast cancer patients to find factors present in the tumor microenvironment and responsible for tumor growth and development. A total of 359 unique proteins were diagnosed, including plentiful signaling molecules, hormones, cytokines, and growth factors involved in a variety of biological processes such as signal transduction and cell communication, energy metabolism, protein metabolism, cell growth and/or maintenance, immune response, transport, regulation of nucleobase, nucleoside, and nucleic acid metabolism, and apoptosis [61]. This proteomics study provided a unique phenotypic overview of tumor microenvironment in human epithelial cancer. Using SELDI-TOF, it was shown that combined measurement of serum complement component C3a (desArg) and a C-terminal-truncated form of C3a (desArg) considerably differentiates breast cancer patients from noncancerous controls [70].

In a confirmatory study on independent samples, C3a (desArg) appeared to lack specificity between patients with benign diseases [71]. This work could be partially validated in an independent prospective study where some peaks of interest could be detected, but the sensitivity for cancer detection was only between 33 and 45% [72]. SELDI-TOF was also applied to the analysis of breast ductal lavage and was start up to improve the potential of cytology [73]. Proteomic researches could also discredit putative data achieved with transcriptomic technologies; for instance, applying a proteomic approach complemented by immunohistochemical analysis showed that levels of expression of 14-3-3 sigma were alike with matched malignant and nonmalignant breast epithelial tissue. In addition to its biological features, the methodological relevance of this finding should be considered, since transcriptional expression of the sigma isoform of 14-3-3 is frequently impaired in human cancers, including breast, which has led to the suggestion that this protein might be involved in the neoplastic transformation of breast epithelial cells [74]. Another research indicates that, breast tumors lacking the estrogen receptor-α (ER-α) have increased incidence of resistance to therapy and poorer clinical prognosis. Comparative proteomic analysis of pooled tumors that were chosen base on being either ER-α-positive or ER-α-negative unexpectedly revealed differentially abundant [75] phosphorylated isoforms of the cytochrome b5-domain protein and progesterone receptor membrane component (PGRMC)1 [76] among these tumors. Two of three spots of PGRMC1 were more abundant in estrogen receptor negative tumors.

Thus, PGRMC1 phosphorylation may be play a role in the clinical differences that uphold breast tumors of differing ER status.

More studies by two-dimensional DIGE and mass spectrometry have resulted in the identification of ligand dependent multiprotein complex such as β-actin, myosins, and several proteins involved in actin filament organization and dynamics [77].

8.3. Colon Cancer

CRC (colorectal Cancer) is the second universal life threatening cancer in the world [78]. About 10 years ago, the first 2D-PAGE map of purified colorectal epithelial cells was published [79, 80]. At that time, it was possible to identify about 50 polypeptides, most of them by N-terminal sequencing since MS technology was only developing. In the meantime, expression proteomic researches were performed with cell lines, whole tissue biopsies, and purified epithelial cells of colorectal origin [81]. It was possible to synthesize translational studies results achieved in CRC in a quasimeta analysis out of 408 differentially expressed proteins, which 83% were found to be differentially expressed only in a single study, 16 proteins in 3 studies, 10 in 4 studies, 3 in 5 studies, and only a single protein in 8 studies. Confirmation at proteome level using large-scale transcriptomic studies was possible in only 25%. This proportion was higher (67%) for validating proteome results using transcriptomic methods. Clearly, reproducibility and overlap between published gene expression results at proteome and transcriptome level are low in human CRC. Essentially, the whole number of patients involved in the proteomic researches was only 11, a surprisingly low figure. Using SELDI technology, defensin isoforms were found to be elevated in serum from colon cancer patients and in protein extracts from CRC [82]. This result was approved by expression determinations of microarray data achieved from 283 tumors and normal tissues followed by serum analysis of colon cancer patients and controls by ELISA. This study profit a diagnostic sensitivity of 70% and specificity of 83% for α-defensin in colon cancer [83]. Despite the fact that, these figures appear too low for developing a screening test, this result is an appealing proof of concept for integrating tissue transcriptomic data with serum protein analysis as a means to discover serum biomarkers.

Another study in CRC tissue combined 2D-PAGE with SELDI-MS. This study clarified that PACAP protein, hnRNP A1, flavin reductase, calgizzarin, NDKB (NM23H2), cyclophilin A, and smooth muscle protein 22 showed considerably different levels. Subsequent immunohistochemical analysis of tissue distribution and subcellular localization of some of the differentially expressed proteins demonstrated alterations in subcellular protein distribution [84]. In another studies, a comparative proteomic study reveals that bacterial CpG motifs induce tumor cell autophagy *in Vitro* and *in Vivo*. These studies followed by two-dimensional gel electrophoresis and mass spectrometry identified numerous proteins modulated by bacterial CpG motifs, which many are related to autophagy including potential autophagic substrates. Besides, it was observed that, an increased glyceraldehyde-3-phosphate dehydrogenase expression, which has been shown to be sufficient to activate an autophagic process. Therefore, this study brings new insights on the effect of bacterial CpG motifs in tumor cells and may be useful for cancer therapy and more generally for gene therapy purposes in TLR9-positive tissues [84].

Recent proteomics based on one-dimensional gel electrophoresis coupled to nanoliquid chromatography tandem showed proteome differences between colon cancer stem cells and differentiated tumor cells. Out of a total data set of 3048 recognized proteins, 32 proteins were at least two fold up regulated in the colon cancer stem cells comparing with the differentiated cells. Pathway analysis showed that "cell death" regulation is remarkably different among the two cell types. Interestingly, one of the top-up-regulated proteins was BIRC6, which belongs to the class of inhibitor of apoptosis proteins. BIRC6 is an important mediator of cancer stem cell resistance against cisplatin and oxaliplatin. Targeting BIRC6, or other Inhibitors of apoptosis proteins, may help exterminate colon cancer stem cells. This study reveals that differentiation of colon cancer stem cells is accompanied by altered regulation of cell death pathways [84].

8.4. Gastric Cancer

Gastric cancer refers to cancer arising from any part of the stomach. It is the fourth most common malignant cancer worldwide [85, 86]. More than 990 000 cases come about yearly based on 2008 reports [85, 87]. Proteomics as a High-throughput molecular determination method represents promising in this field. The first proteomics analysis of gastric cancer was in 2001 which was about characterization of the differential protein expression associated with thermo resistance in human gastric carcinoma cell lines [88, 89]. Primary biomarker screening in gastric cancer performed using 2D-PAGE on purified gastric epithelial cells from gastrectomy specimens. In this study, 191 deferentially expressed proteins were identified by mass spectrometry. Overexpression of cathepsin B was detected in most cancer tissue samples. Elevated serum levels of cathepsin B were associated with a reduced survival rate, enabling the classification of some gastric cancer patients into a subgroup that should undergo aggressive therapy. Later on, other studies were performed based on biomarker discoveries. In one of these studies, NSP3, transgelin, prohibitin, heat shock protein (hsp) 27, and protein disulfide isomerase A3 proteins were indicated as over-expressed molecules in tumor tissue samples, when compared to normal tissue samples [90]. In another research, eight proteins, including 14-3-3 zeta, calcyclin, keratin, apolipoprotein A-1 precursor, proteasome activator complex subunit, nucleoside diphosphate kinase, nicotinamide *N*-methyltransferase, and pyridoxal kinase were detected as possible biomarkers [91]. Two other promising biomarker studies are characterization of pepsinogen C as a potential biomarker for gastric cancer using a histo-proteomic. For this aim, 74 cryostat sections of central gastric tumor, tumor margin, and normal gastric epithelium using protein chip arrays and SELDI-TOF MS studied. One peak was significantly down regulated in tumor tissue ($P = 1.43 \times 10^{-6}$) and identified as pepsinogen C. This signal was further characterized by immunohistochemistry [91, 92]. Last example is identification of IPO-38 as a novel serum biomarker both for diagnosis and prediction prognosis of gastric cancer by the use of MALDI-TOF/TOF mass spectrometer. A very recent comparative study was performed on gastric cancer (MKN45cell line) before and after exposing to Lavender aqueous extract. The results indicate that, among 1000 spots, more than 700 spots are imposed alternations in their expression levels. Of these proteins, expression of three cancer biomarkers, Annexin1, Anolase1 and HSP70 were suppressed by the extract [92].

8.5. Skin Cancer

Skin cancer is skin growth with differing causes and varying degrees of malignancy. It is a common disease in all European-derived populations and has shown rapid increases in incidence over the last century. Basal cell carcinoma is one of the most common types of non-melanoma skin cancers in human [93-95]. Investigations indicate that function of specific genes in skin cancer alters. These alterations affect conserved regulators of cellular proliferation and viability, including the Sonic Hedgehog, Ras/Raf, ARF/p53, p16INK4A/CDK4/Rb and NF-B pathways. New modalities designed to target these specific proteins may represent promising approaches to therapy of human skin cancers [96]. As one of these methods is proteomics, a wide variety of proteins profiles has been extensively constructed via this technology. However, the comprehensive proteomic profiling of the skin, is still far from complete. One of the first proteomics studies in skin cancer field was in 2000 [96]. This study compares the human epidermal stem cells with their differentiated daughters (transit amplifying cells). In 2003, in one study, six molecular chaperones, including HSP27, HSP60, HSP70, HSP84, ER60, and GRP78, were determined within the proteome map of the BALB/c abdominal, which belongs to the heat shock protein 90 family, was formerly identified as a tumor-specific transplantation antigen [97]. In another study, proteomics analysis was shown that, among 87 proteins 76 of them were determined with drastic difference in expression which seventh were identified by databases [98]. In the other study, anticancer effects of arbutin investigated on the protein expression profile of A375

cell line. MALDI-Q-TOF MS and MS/MS identified 26 differentially expressed proteins (7 up-regulated and 19 down-regulated proteins) after treatment with arbutin,. Of these proteins, there were 13 isoforms of six identical proteins. Moreover, revealed that interaction network of 14 differentially expressed proteins correlated with the downstream regulation of p53 tumor suppressor and cell apoptosis. In addition, three up-regulated proteins (14-3-3G, VDAC-1 and p53) and five down-regulated proteins (ENPL, ENOA, IMDH2, PRDX1 and VIME) in arbutin-treated A375 cells were validated by RT-PCR analysis. These proteins were found to play significant roles in the suppression of cancer growth [99].

8.6. Prostate Cancer

Prostate cancer rate is very high across the world; it is accounted for second male cancer deaths in the United States [100]. The need for other means of screening for this malignancy is prominent, due to shortcoming of the prostate-specific test for the early detection of prostate cancer antigen (PSA) [101]. One common treatment is androgen-deprivation therapy, which reduces symptoms in most patients. On the other hand, over time, patients develop tumors that are androgen-independent and finally fatal. First proteomics studies were around 2000 when David K. Ornstein et al. worked on analysis of laser capture microdissected (LCM) human prostate cancer and *in vitro* prostate cell lines. In this study normal and malignant epithelium from prostatectomy tissue specimens provided by LCM and the proteins analyzed by 2-D PAGE. Several proteins showed different expression including the well-known prostate biomarker, prostate-specific antigen (PSA), and intriguingly the remaining protein candidates were found to be at least as abundant as the PSA protein. The findings indicated that 2-D PAGE analysis of LCM-derived cells can introduces considerable alterations in protein expression associated with prostate cancer. Identification of these proteins provides new possibility for introducing novel biomarkers related to prostate cancer. These biomarkers can be used as diagnostic probes or therapeutic targets [102]. Two years later, proteomics pattern in serum was used as indicator to identify prostate cancer. A suitable pattern constructed by MS spectra with a bioinformatics tool for detection of prostate cancer. The proteomic pattern correctly predicted 36 (95%, 95% confidence interval [CI] = 82% to 99%) of 38 patients with prostate cancer, while 177 (78%, 95% CI = 72% to 83%) of 228 patients were correctly classified as having benign conditions. For men with marginally elevated PSA levels (4–10 ng/mL; n = 137), the specificity was 71%. If validated in future series, serum proteomic pattern diagnostics may be of value in deciding whether to perform a biopsy on a man with an elevated PSA level [103].

In 2005, Brian L. Hood *et al*. published a paper in which they studied about proteomics pattern of paraffin-embedded prostate cancer tissue. Mass spectral analysis of prostate cancer (PCa) and benign prostate hyperplasia (BPH) led to identification of more biomarkers as like prostatic acid phosphatase, and macrophage inhibitory cytokine-1 [104]. According to some recent proteomics studies, more proteins become up-regulated and or down-regulated in prostate cancer. ACAT1, BDH1, HMGCL, and OXCT1 are proteins that their expression is increased in the In Vitro studies [105]. Another study implies on the role of diethylstilbestrol (DES) action in prostate cancer inhibition and its proteins alteration. The 2D-DIGE analyses revealed DES-induced expression changes for 14 proteins (>1.3 fold; $P<0.05$) [106].

8.7. Renal Cancer

Renal cancer is the most deadly of urological malignancies. Molecular bases of this treatment-resistant neoplasm has been studied widely recently [107]. The first evaluation of renal carcinoma cancer (RCC) proteome was a comparison between normal renal and cancer type in 1997 in which 2-D PAGE was applied to determine normal and tumor kidney tissues in ten patients suffering from RCC. Among 2789 separated polypeptides, 43 of them were found through gel comparison, amino acid analysis, *N*-terminal sequencing, and/or immunodetection. Protein expression among normal

and tumor kidney tissues proved four polypeptides not present in RCC. One of them was identified as ubiquinol cytochrome c reductase (UQCR) and the second was mitochondrial NADH-ubiquinone oxido reductase complex I. Since the last biomarker determines role of mitochondrial abnormality in RCC it helps to candidate mitochondria as a drug target in RCC [108]. In one study in 2004, heat shock protein 27 over-expression was identified as a potential biomarker by 2-D PAGE separation, mass spectrometry, and Western blotting immunodetection methods. The result was also validated by immunohistochemistry on tissue sections [109].

Base on one recent proteomic study, expression levels of profilin-1 (Pfn1), 14–3-3 zeta/delta (14–3-3ζ), and galectin-1 (Gal-1) changed in RCC patients. In clustering analysis of changed expression proteins showed that protein expression profile for metastatic RCC in aggressive and non-aggressive RCC is different [110]. Another study investigated on validates diagnostic and prognostic serum markers using proteomic profiling which several peptides were identified as having independent prognostic but not diagnostic significance on multivariable analysis [111].. It seems that biomarker discovery and proteomic pattern have a key role in diagnostic and therapeutic aspects of RCC.

9. Conclusions

On the whole, each of the approaches in cancer study has its own strengths and weaknesses, especially with regard to the sensitivity and specificity of that method. Proteomics is not an exception; as disadvantages, it still suffers from several drawbacks; some of these pitfalls include the lack of the detection of low abundance proteins such as receptor, regulatory, and signal transduction proteins. In addition, basic proteins as well as the membrane proteins that represent 40% of all cellular proteins are hard to separate by proteomic methods. Some of these drawbacks can be solved by matching several techniques such as varieties of chromatography and electrophoresis in the multidimensional mode [12, 13]. However, proteomics is still the first choice technique to investigate major molecules relating to diseases such as cancer. What is expected from this sophisticated technology is that, not only detecting novel biomarkers and mapping biomarker panels related to the disease, but also by the aid of complicated bioinformatics make it as a outstanding high-throughput technique to determine molecular pathways and their interactions [25]. Therefore, it seems that, with aid of this marvelous achievement accompanied with other appropriate methods, approaching to cancer diagnosis and treatment may be accessible in the near future.

Acknowledgement

This paper has been resulted from the MSc thesis of Miss Mona Zamanian Azodi, and financially supported by Proteomics Research Center of Shahid Beheshti University of Medical Sciences.

References

1. Petersen PE. The world oral health report 2003: Continuous improvement of oral health in the 21st century–the approach of the who global oral health programme. ***Community Dentistry and oral epidemiology***. 2008, 31:3-24
2. John M. Koomen EBH, Gerold Bepler, Rebecca Sutphen, Elizabeth R. Remily-Wood, Kaaron Benson, Mohamad Hussein, Lori A. Hazlehurst, Timothy J. Yeatman, Lynne T. Hildreth, Thomas A. Sellers, Paul B. Jacobsen, David A. Fenstermacher and William S. Dalton. Proteomic contributions to personalized cancer care. ***MCP***. 2008, 11:1780-1794
3. Anderson NL AN. .Proteome and proteomics: New technologies, new concepts, and new words. ***Electrophoresis***. 1998, 19:1853–1861
4. Lakhani SR AA. Microarray and histopathological analysis of tumoursthe future and the past? ***Nat Rev Cancer***. 2001, 1:151–157
5. Donato P CF, Tranchida PQ, Dugo P, Mondello L,. Mass spectrometry detection in comprehensive liquid chromatography basic concepts, instrumental aspects, applications and trends. ***Mass Spectrom Rev***. 2012, 9:3166–3196
6. Schlegel MARaW. Proteomics in cancer. ***Advances in***

Clinical Chemistry. 2007, 44:103-142.

7. Emily I. Chen JRYI. Cancer proteomics by quantitative shotgun proteomics. ***molecular Oncology***. 2007:144-159
8. Blackstock WP WM. Proteomics: Quantitative and physical mapping of cellular protein. ***Trends Biotechnol***. 1999, 17:121-127
9. Zhang Z, Chan DW. Cancer proteomics: In pursuit of "true" biomarker discovery. ***Cancer Epidemiology Biomarkers & Prevention***. 2005, 14:2283-2286
10. Hanash S, Taguchi A. The grand challenge to decipher the cancer proteome. ***Nature reviews cancer***. 2010, 10:652-660
11. O'Farrell PHJ. High resolution two-dimensional electrophoresis of proteins. . ***Biol. Chem***. 1974, 250:4007–4021
12. Mishra NC. *Introduction to proteomics*. Canada: **Wiley**; 2010.
13. MRT. *Fundamental and methods in biophysics* Iran: **Andishe Zohoor**; 2012.
14. Mariana Guergova-Kuras IK, William Hempel, Nadège Tardieu, János,Kádas, Carole Malderez-Bloes, Anne Jullien, Yann Kieffer,Marina Hincapie, András,Guttman, Eszter Csánky, Balázs Dezső, Barry L. Karger and László Takács. Discovery of lung cancer biomarkers by profiling the plasma proteome with monoclonal antibody libraries. ***Mol Cell Proteomics***. 2011, 10
15. Nawin C Mishra. *Introduction to proteomics*. **Wiley**; 2010.
16. Sayed S. Daoud. *Cancer proteomics from bench to bed*. **Humana Press**; 2008.
17. Olsen JV, Blagoev, B., Gnad, F., Macek, B., Kumar, C., Mortensen, P., Mann, M. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. . ***Cell***. 2006, 127:635–648.
18. Liu Y, Patricelli, M.P., Cravatt, B.F. . Activity-based protein profiling: The serine hydrolases. ***Proc. Natl. Acad. Sci. USA***. 1999, 96:14694–14699.
19. Srinivas PR KB, Srivastava S. . Trends in biomarker research for cancer detection. 2001, 2:698-704.
20. : SD. Emerging molecular markers of cancer. . ***Nat Rev Cancer***. 2002, 2:210-219.
21. Engwegen JY, Gast M-CW, Schellens JH, Beijnen JH. Clinical proteomics: Searching for better tumour markers with seldi-tof mass spectrometry. ***Trends in Pharmacological Sciences***. 2006, 27:251-259
22. Albertson DG, Collins, C., McCormick, F., Gray, J.W. . Chromosome aberrations in solid tumors. ***Genet***. 2003, 34:369–376.
23. Birchmeier C, Birchmeier, W., Gherardi, E., Vande Woude, G.F. Met, . Metastasis, motility and more. ***Rev. Mol. Cell. Biol***. 2003, 4:915–925
24. Druker BJDAKAl. Imatinib as aparadigm of targeted therapies. ***J. Clin. Oncol***. 2003, 21:239–245
25. Blume-Jensen P, and Hunter, T. Oncogenic kinase signaling. ***Nature.*** 2001, 411:355–365.
26. Pawlik TM K. The evolving role of proteomics in the early detection of breast cancer. ***HM Int J Fertil Womens Med***. 2005, 50:212-216
27. Settleman UMaJ. Personalized cancer therapy with selective kinase inhibitors: An emerging paradigm in medical oncology. ***JCO***. 2009, 32:5650-5659
28. Bantscheff M, Scholten A, Heck AJR. Revealing promiscuous drug–target interactions by chemical proteomics. ***Drug Discovery Today***. 2009, 14:1021-1029
29. Semmes OJ MG, Ward M. J Application of mass spectrometry to the discovery of biomarkers for detection of prostate cancer. ***Cell Biochem***. 2006, 98:496–503
30. MA. L. Finding new drug targets in the 21st century. ***Drug Discov Today***. 2005, 10:1683–1687
31. Mishra NC. *Introduction to proteomics*. **Wiley**; 2010.
32. Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L, Geng J, Han Y, Li L, Li W, Hu G, Li J, Liu Z, Qi Q, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X, Cui P, Li X, Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Chen X, He J, Liu D, Tian W, Tian C, Xia H, Bao Q, Li G, Gao H, Cao T, Zhao W, Li P, Chen W, Zhang Y, Hu J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z, Chen R, Hao B, Zheng W, Chen S, Guo W, Tao M, Zhu L, Yuan L, Yang H. {a draft sequence of the rice genome (oryza sativa l. Ssp. Indica)}. ***Science***. 2002, 296:79-92
33. Idikio HA. Human cancer classification: A systems biology- based model integrating morphology, cancer stem cells, proteomics, and genomics. ***Journal of Cancer***. 2011, 2:107-115
34. Golub T SD, Tamayo P. . Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. ***Science***. 1999,

286:531-537

35. Stelow E S-LR, Bao F, and Garcia J, Klimstra D. American J Surgical Pathology Pancreatic acinar cell carcinomas with prominent ductal differentiation: Mixed acinar ductal carcinoma and mixed acinar endocrine carcinoma. . 2010, 34:510-518
36. Bild AH, Potti A, Nevins JR. Linking oncogenic pathways with therapeutic opportunities. ***Nature reviews cancer***. 2006, 6:735-741
37. Bair E, Tibshirani R. Semi-supervised methods to predict patient survival from gene expression data. ***PLoS biology***. 2004, 2:e108
38. Van De Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AAM, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ. A gene-expression signature as a predictor of survival in breast cancer. ***New England Journal of Medicine***. 2002, 347:1999-2009
39. Hirano T, Fujioka K, FranzÃ B. Relationship between ta01 and ta02 polypeptides associated with lung adenocarcinoma and histocytological features. ***British journal of cancer***. 1997, 75:978
40. George J, Singh R, Mahmood Z, Shukla Y. Toxicoproteomics: New paradigms in toxicology research. ***Toxicology mechanisms and methods***. 2010, 20:415-423
41. Wetmore BA, Merrick BA. Invited review: Toxicoproteomics: Proteomics applied to toxicology and pathology. ***Toxicologic pathology***. 2004, 32:619-642
42. Khodarahmi G, Jafari E, Hakimelahi G, Abedi D, Rahmani M, Hassanzadeh F. Synthesis of some new quinazolinone derivatives and evaluation of their antimicrobial activities. ***Iranian Journal of Pharmaceutical Research***. 2012, 11:789-797
43. George J, Shukla Y. Pesticides and cancer: Insights into toxicoproteomic-based findings. ***Journal of proteomics***. 2011,
44. Meyerson M, Carbone D. Genomic and proteomic profiling of lung cancers: Lung cancer classification in the age of targeted therapy. ***Journal of clinical oncology***. 2005, 23:3219-3226
45. Mehmood N, Zubair M, Rızwan K, Rasool N, Shahid M, Ahmad V. Antioxidant, antimicrobial and phytochemical analysis of cichorium intybus seeds extract and various organic fractions. ***Iranian Journal of Pharmaceutical Research***. 2012,
46. Rajapakse JC, Duan KB, Yeo WK. Proteomic cancer classification with mass spectrometry data. ***American Journal of PharmacoGenomics***. 2005, 5:281-292
47. Daoud SS. Cancer proteomics from bench to bed. 2008:59
48. Fella K, Glückmann M, Hellmann J, Karas M, Kramer PJ, Kröger M. Use of two‐dimensional gel electrophoresis in predictive toxicology: Identification of potential early protein biomarkers in chemically induced hepatocarcinogenesis. ***Proteomics***. 2005, 5:1914-1927
49. Kang MJ, Lee DY, Joo WA, Kim CW. Plasma protein level changes in waste incineration workers exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. ***Journal of proteome research***. 2005, 4:1248-1255
50. Ma Y, Visser L, Roelofsen H, de Vries M, Diepstra A, van Imhoff G, van der Wal T, Luinge M, Alvarez-Llamas G, Vos H. Proteomics analysis of hodgkin lymphoma: Identification of new players involved in the cross-talk between hrs cells and infiltrating lymphocytes. ***Blood***. 2008, 111:2339-2346
51. Ross JS, Fletcher JA, Bloom KJ, Linette GP, Stec J, Symmans WF, Pusztai L, Hortobagyi GN. Targeted therapy in breast cancer the her-2/neu gene and protein. ***Molecular & Cellular Proteomics***. 2004, 3:379-398
52. Di Leo A, Chan S, Paesmans M, Friedrichs K, Pinter T, Cocquyt V, Murray E, Bodrogi I, Walpole E, Lesperance B. Her-2/neu as a predictive marker in a population of advanced breast cancer patients randomly treated either with single-agent doxorubicin or single-agent docetaxel. ***Breast cancer research and treatment***. 2004, 86:197-206
53. Righetti PG CA, Antonucci F, Piubelli C, Cecconi D, Campostrini N, Rustichelli C, Antonioli P, Zanusso G, Monaco S, Lomas L, Boschetti E. Proteome analysis in the clinical chemistry laboratory: Myth or reality? ***Clin Chim Acta***. 2005, 357:123-139.
54. Franzén B, Iwabuchi H, Kato H, Lindholm J, Auer G. Two‐dimensional polyacrylamide gel electrophoresis of human lung cancer: Qualitative aspects of tissue preparation in relation to histopathology. ***Electrophoresis***. 1991, 12:509-515
55. Stewart BW, Kleihues P. *World cancer report*. **IARC press Lyon**; 2003.
56. Okuzawa K, Franzén B, Lindholm J, Linder S, Hirano T, Bergman T, Ebihara Y, Kato H, Auer G. Characterization of gene expression in clinical lung cancer materials by two‐dimensional polyacrylamide

gel electrophoresis. ***Electrophoresis***. 1994, 15:382-390

57. Bergman AC, Benjamin T, Alaiya A, Waltham M, Sakaguchi K, Franzén B, Linder S, Bergman T, Auer G, Appella E. Identification of gel‐separated tumor marker proteins by mass spectrometry. ***Electrophoresis***. 2000, 21:679-686
58. Yu JK, Chen YD, Zheng S. An integrated approach to the detection of colorectal cancer utilizing proteomics and bioinformatics. ***World Journal of Gastroenterology***. 2004, 10:3127-3131
59. MAURYA P, MELEADY P, DOWLING P, CLYNES M. Proteomic approaches for serum biomarker discovery in cancer. ***Anticancer research***. 2007, 27:1247-1255
60. Ueda K. Proteome analysis of autoantibodies in sera of patients with cancer. ***Rinsho Byori***. 2005, 53:437-445
61. Pan J, Chen HQ, Sun YH, Zhang JH, Luo XY. Comparative proteomic analysis of non-small-cell lung cancer and normal controls using serum label-free quantitative shotgun technology. ***Lung***. 2008, 186:255-261
62. Lu QY, Yang Y, Jin YS, Zhang ZF, Heber D, Li FP, Dubinett SM, Sondej MA, Loo JA, Rao JY. Effects of green tea extract on lung cancer a549 cells: Proteomic identification of proteins associated with cell migration. ***Proteomics***. 2009, 9:757-767
63. Rahman SMJ, Gonzalez AL, Li M, Seeley EH, Zimmerman LJ, Zhang XJ, Manier ML, Olson SJ, Shah RN, Miller AN. Lung cancer diagnosis from proteomic analysis of preinvasive lesions. ***Cancer research***. 2011, 71:3009-3017
64. Somiari RI, Somiari S, Russell S, Shriver CD. Proteomics of breast carcinoma. ***Journal of Chromatography B***. 2005, 815:215-225
65. Azodi MZ, Dolat E, Ardestani H, Mousavi M, Shadloo A. Breast cancer: Genetics, risk factors, molecular pathology and treatment. ***Journal of Paramedical Sciences (JPS) Winter***. 2013, 4:2008-4978
66. Luo Y, Zhang J, Liu Y, Shaw AC, Wang X, Wu S, Zeng X, Chen J, Gao Y, Zheng D. Comparative proteome analysis of breast cancer and normal breast. ***Molecular biotechnology***. 2005, 29:233-244
67. Safaei A, Rezaei-Tavirani M, Sobhi S, Akbari ME. Breast cancer biomarker discovery: Proteomics and genomics approaches. ***Iranian Journal of Cancer Prevention***. 2013, 6:45-53
68. Nwozo SO. Comparative study of biochemical and nutritional status of breast cancer patients on chemotherapy/radiotherapy in ibadan. ***American Journal of Cancer Science***. 2013, 2:51-60
69. Celis JE, Moreira JMA, Cabezón T, Gromov P, Friis E, Rank F, Gromova I. Identification of extracellular and intracellular signaling components of the mammary adipose tissue and its interstitial fluid in high risk breast cancer patients toward dissecting the molecular circuitry of epithelial-adipocyte stromal cell interactions. ***Molecular & Cellular Proteomics***. 2005, 4:492-522
70. Li J, Zhang Z, Rosenzweig J, Wang YY, Chan DW. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. ***Clinical chemistry***. 2002, 48:1296-1304
71. Mathelin C, Cromer A, Wendling C, Tomasetto C, Rio MC. Serum biomarkers for detection of breast cancers: A prospective study. ***Breast cancer research and treatment***. 2006, 96:83-90
72. Mendrinos S, Nolen JDL, Styblo T, Carlson G, Pohl J, Lewis M, Ritchie J. Cytologic findings and protein expression profiles associated with ductal carcinoma of the breast in ductal lavage specimens using surface‐enhanced laser desorption and ionization‐time of flight mass spectrometry. ***Cancer Cytopathology***. 2005, 105:178-183
73. Hong HY, Jeon WK, Bae EJ, Kim ST, Lee HJ, Kim SJ, Kim BC. 14-3-3 sigma and 14-3-3 zeta plays an opposite role in cell growth inhibition mediated by transforming growth factor-beta 1. ***Molecules and cells***. 2010, 29:305-309
74. Cahill MA. Progesterone receptor membrane component 1: An integrative review. ***The Journal of steroid biochemistry and molecular biology***. 2007, 105:16-36
75. Reymond MA, Sanchez JC, Hughes GJ, Günther K, Riese J, Tortola S, Peinado MA, Kirchner T, Hohenberger W, Hochstrasser DF. Standardized characterization of gene expression in human colorectal epithelium by two‐dimensional electrophoresis. ***Electrophoresis***. 1997, 18:2842-2848
76. Ambrosino C, Tarallo R, Bamundo A, Cuomo D, Franci G, Nassa G, Paris O, Ravo M, Giovane A, Zambrano N. Identification of a hormone-regulated dynamic nuclear actin network associated with estrogen receptor α in human breast cancer cell nuclei. ***Molecular & Cellular Proteomics***. 2010, 9:1352-1367
77. Reymond MA, Steinert R, Kähne T, Sagynaliev E, Allal AS, Lippert H. Expression and functional proteomics

studies in colorectal cancer. ***Pathology-Research and Practice***. 2004, 200:119-127

78. Safaei A, Sobhi S, Rezaei-Tavirani M, Zali MR. Genomic and epigenetic instability in colorectal cancer. ***Iranian Journal of Cancer Prevention***. 2013, 6:54-63
79. Sagynaliev E, Steinert R, Nestler G, Lippert H, Knoch M, Reymond MA. Web‐based data warehouse on gene expression in human colorectal cancer. ***Proteomics***. 2005, 5:3066-3078
80. Rezaie-Tavirani M, Fayazfar S, Heydari-Keshel S, Rezaee MB, Zamanian- Azodi M, Rezaei-Tavirani M, Khodarahmi R. Effect of essential oil of rosa damascena on human colon cancer cell line sw742. ***Gastroenterology and Hepatology From Bed to Bench***. In Press.
81. Albrethsen J, Bøgebo R, Gammeltoft S, Olsen J, Winther B, Raskov H. Upregulated expression of human neutrophil peptides 1, 2 and 3 (hnp 1-3) in colon cancer serum and tumours: A biomarker study. ***Bmc Cancer***. 2005, 5:8
82. Droin N, Hendra JB, Ducoroy P, Solary E. Human defensins as cancer biomarkers and antitumour molecules. ***Journal of proteomics***. 2009, 72:918-927
83. Bertin S, Samson M, Pons C, Guigonis JM, Gavelli A, Baquié P, Brossette N, Pagnotta S, Ricci JE, Pierrefite-Carle V. Comparative proteomics study reveals that bacterial cpg motifs induce tumor cell autophagy in vitro and in vivo. ***Molecular & Cellular Proteomics***. 2008, 7:2311-2322
84. Van Houdt WJ, Emmink BL, Pham TV, Piersma SR, Verheem A, Vries R, Fratantoni SA, Pronk A, Clevers H, Rinkes IHMB. Comparative proteomics of colon cancer stem cells and differentiated tumor cells identifies birc6 as a potential therapeutic target. ***Molecular & Cellular Proteomics***. 2011, 10
85. De Vries AC, Kuipers EJ. Epidemiology of premalignant gastric lesions: Implications for the development of screening and surveillance strategies. ***Helicobacter***. 2007, 12:22-31
86. Brenner H, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. ***Methods Mol Biol***. 2009, 472:467-477
87. Katanoda K, Yako-Suketomo H. Comparison of time trends in stomach cancer incidence (1973–2002) in asia, from cancer incidence in five continents, vols iv–ix. ***Japanese journal of clinical oncology***. 2009, 39:71-72
88. Jang JSJ, Cho HY, Lee YJ, Ha WS, Kim HW. The differential proteome profile of stomach cancer: Identification of the biomarker candidates. ***Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics***. 2004, 14:491-499
89. Sinha P, Poland J, Schnölzer M, Celis JE, Lage H. Characterization of the differential protein expression associated with thermoresistance in human gastric carcinoma cell lines. ***Electrophoresis***. 2001, 22:2990-3000
90. Melle C, Ernst G, Schimmel B, Bleul A, Kaufmann R, Hommann M, Richter KK, Daffner W, Settmacher U, Claussen U. Characterization of pepsinogen c as a potential biomarker for gastric cancer using a histo-proteomic approach. ***Journal of proteome research***. 2005, 4:1799-1804
91. Hao Y, Yu Y, Wang L, Yan M, Ji J, Qu Y, Zhang J, Liu B, Zhu Z. Ipo-38 is identified as a novel serum biomarker of gastric cancer based on clinical proteomics technology. ***Journal of proteome research***. 2008, 7:3668-3677
92. Zamanian-Azodi M, Rezaie-Tavirani M, Heydari-Kashal S, Kalantari S, Dailian S, Zali H. Proteomics analysis of mkn45 cell line before and after treatment with lavender aqueous extract. ***Gastroenterology and Hepatology from bed to bench***. 2011, 5
93. Tavirani MR, Jazi FR. Biomarkers detection of basal cell carcinoma.
94. Green CL, Khavari PA. *Targets for molecular therapy of skin cancer*. **Elsevier**; 2004, 14:63-69
95. Kasparian NA, McLoone JK, Meiser B. Skin cancer-related prevention and screening behaviors: A review of the literature. ***Journal of behavioral medicine***. 2009, 32:406-428
96. Franssen MEJ, Zeeuwen PLJM, Vierwinden G, Van De Kerkhof PCM, Schalkwijk J, Van Erp PEJ. Phenotypical and functional differences in germinative subpopulations derived from normal and psoriatic epidermis. ***Journal of investigative dermatology***. 2005, 124:373-383
97. Huang CM, Foster KW, DeSilva T, Zhang JF, Shi Z, Yusuf N, Van Kampen KR, Elmets CA, Tang DC. Comparative proteomic profiling of murine skin. ***Journal of investigative dermatology***. 2003, 121:51-64
98. Hamideh Moravej AZ, Hakimeh Zali, Mostafa Rezaei-Tavirani,Majid Rezaei- Tavirani, Ferdos Rastegar. Proteomics analysis of basal carcinoma via proteomics approaches. 2009, 69:137-141.

99. Cheng SL, Liu RH, Sheu JN, Chen ST, Sinchaikul S, Tsay GJ. Toxicogenomics of a375 human malignant melanoma cells treated with arbutin. ***Journal of biomedical science***. 2007, 14:87-105
100. Hoffman RM. Screening for prostate cancer. ***New England Journal of Medicine***. 2011, 365:2013-2019
101. Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giacherio D, Mehra R, Montie JE, Pienta KJ, Sanda MG, Kantoff PW, Rubin MA, Wei JT, Ghosh D, Chinnaiyan AM. Autoantibody signatures in prostate cancer. ***New England Journal of Medicine***. 2005, 353:1224-1235
102. Ornstein DK, Gillespie JW, Paweletz CP, Duray PH, Herring J, Vocke CD, Topalian SL, Bostwick DG, Linehan WM, Petricoin EF. Proteomic analysis of laser capture microdissected human prostate cancer and in vitro prostate cell lines. ***Electrophoresis***. 2000, 21:2235-2242
103. Petricoin EF, Ornstein DK, Paweletz CP, Ardekani A, Hackett PS, Hitt BA, Velassco A, Trucco C, Wiegand L, Wood K. Serum proteomic patterns for detection of prostate cancer. ***Journal of the National Cancer Institute***. 2002, 94:1576-1578
104. Hood BL, Darfler MM, Guiel TG, Furusato B, Lucas DA, Ringeisen BR, Sesterhenn IA, Conrads TP, Veenstra TD, Krizman DB. Proteomic analysis of formalin-fixed prostate cancer tissue. ***Molecular & Cellular Proteomics***. 2005, 4:1741-1753
105. Saraon P, Cretu D, Musrap N, Karagiannis GS, Batruch I, Drabovich AP, van der Kwast T, Mizokami A, Morrissey C, Jarvi K. Quantitative proteomics reveals that enzymes of the ketogenic pathway are associated with prostate cancer progression. ***Molecular & Cellular Proteomics***. 2013, 12:1589-1601
106. Bigot P, Mouzat K, Lebdai S, Bahut M, Benhabiles N, Tassin GC, Azzouzi A-R, Cussenot O. Quantitative proteomic determination of diethylstilbestrol action on prostate cancer. ***Asian journal of andrology***. 2013,
107. Laird A, O'Mahony FC, Nanda J, Riddick AC, O'Donnell M, Harrison DJ, Stewart GD. Differential expression of prognostic proteomic markers in primary tumour, venous tumour thrombus and metastatic renal cell cancer tissue and correlation with patient outcome. ***PloS one***. 2013, 8:e60483
108. Sarto C, Marocchi A, Sanchez J-C, Giannone D, Frutiger S, Golaz O, Wilkins MR, Doro G, Cappellano F, Hughes G, Hochstrasser DF, Mocarelli P. Renal cell carcinoma and normal kidney protein expression. ***Electrophoresis***. 1997, 18:599-604
109. Sarto C, Valsecchi C, Magni F, Tremolada L, Arizzi C, Cordani N, Casellato S, Doro G, Favini P, Perego RA, Raimondo F, Ferrero S, Mocarelli P, Galli-Kienle M. Expression of heat shock protein 27 in human renal cell carcinoma. ***PROTEOMICS***. 2004, 4:2252-2260
110. Masui O, White NM, DeSouza LV, Krakovska O, Matta A, Metias S, Khalil B, Romaschin AD, Honey RJ, Stewart R. Quantitative proteomic analysis in metastatic renal cell carcinoma reveals a unique set of proteins with potential prognostic significance. ***Molecular & Cellular Proteomics***. 2013, 12:132-144
111. Wood S, Rogers M, Cairns D, Paul A, Thompson D, Vasudev N, Selby P, Banks R. Association of serum amyloid a protein and peptide fragments with prognosis in renal cancer. ***British journal of cancer***. 2010, 103:101-111