Advancing mass spectrometry-based clinical proteomics in Saudi Arabia

Establishing a Saudi Proteomics Society

Abdul A. Peer-Zada, *MSc, PhD,* ***Mohammed H. Al-Qahtani,*** *MSc, PhD.*

ABSTRACT

تشكل التطورات السريعة في مجال تقنية التحليل البروتيومي الكمي المعتمدة على قياس الطيف المتعدد أملاً كبيراً في عملية البحث عن العلامات الحيوية البروتينية والتي تفيد سريرياً في عملية الكشف المبكر عن المرض، وتشخيصه، ومعرفة نتائجه المستقبلية، ورصد مدى الاستجابة للعلاج. يساهم علم البروتيوميات أيضاً في إيجاد الأهداف الجديدة للعقاقير، والكشف عن المسارات الجزيئية المرتبطة بالمرض. وبالرغم من تطبيق علم البروتيوميات السريري على نطاق واسع في الأبحاث المتعلقة بالأمراض عالمياً، إلا أنه لا يزال هناك نقصاً في بيانات البروتيوميات الكمية بالمملكة العربية السعودية والمتعلقة بالأمراض الشائعة في المجتمع السعودي، ولذلك سوف نناقش في هذا المقال بعضاً من جوانب البروتيوميات السريرية، وسنقوم أيضاً بتلخيص منظورنا ورؤيتنا للموضوع في المملكة العربية السعودية. نهدف في هذا المقال إلى إدخال مفهوم مجتمع البروتيوميات السعودي الوطني في المملكة العربية السعودية، وذلك كجزء من البرنامج المتقدم للبحث و التطوير.

Rapid developments in the field of mass spectrometry-based quantitative proteomic technologies holds great promise in the search for clinically useful protein biomarkers for early detection, diagnosis, and prognosis of disease in general, and for monitoring response to therapy. Proteomics may contribute to finding novel drug targets and unravel molecular pathways associated with disease. Despite the application of 'clinical proteomics' to a wide spectrum of disease research globally, however, there is lack of data with regard to quantitative proteomics in the Kingdom of Saudi Arabia (KSA) for the diseases common to the Saudi population. In this review therefore, we will discuss some aspects of clinical proteomics with regard to the occurrence of common diseases, and outline our perspectives and vision in the context of KSA. Furthermore, we aim to introduce the concept of a national Saudi Proteomics Society in the Kingdom as part of an advanced research and development program.

Saudi Med J 2011; Vol. 32 (3): 225-235

From the Center of Excellence for Genomic and Medicine Research (Peer-Zada), King Fahad Medical Research Center, and the Department of Applied Medical Technology (Al-Qahtani), King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

Address correspondence and reprint request to: Dr. Abdul A. Peer-Zada, Center of Excellence for Genomic and Medicine Research, King Fahad Medical Research Center, Jeddah, Kingdom of Saudi Arabia. Tel. +966 (2) 6401000 Ext. 25484. Fax. +966 (2) 6952521. E-mail: apeerzada@kau.edu.sa

The study of proteins at the level of global expression profiling, post-translational modifications (PTMs), protein-protein interactions, protein subcellular localization, and protein structure is termed 'proteomics'.[1-4] The term 'proteome' was initially coined by Marc Wilkins in 1995 who described it as protein complement expressed by the genome.[5,6] Extending the scope of proteomics to human health and disease with an aim towards improved diagnostics, prognostics, and biomarker discovery for future therapeutics is commonly referred to as "clinical proteomics". For the past decade there has been tremendous advancement in clinical proteomics technologies with the development of more quantitative methodologies in the search for clinically useful protein biomarkers for early detection, diagnosis, and prognosis of cancer in general, and for monitoring response to therapy. However, with the availability of

Disclosure. This study was supported by the King Abdullah City for Science and Technology (KACST), Jeddah, Kingdom of Saudi Arabia.

only a few clinical biomarkers and specific diagnostic assays, the need for novel biomarkers in many areas of healthcare is increasingly becoming essential. Without reliable biomarkers, diseases such as cancer, other chronic malignancies, and genetic disorders are difficult to detect early, and thereby could affect the therapeutic response in patients. In this respect, Anderson et al[7] argued that clinical biomarker(s) will have substantial impact on the patient well-being and financial viability of healthcare systems worldwide.

Substantial literature supporting the application of mass spectrometry (MS) proteomics in relation to disease does exist. The MS-based protein expression studies have been reported for various human malignancies, such as with hematologic disorders in leukemia, lymphoma, and chronic myeloproliferative neoplasms, with solid cancers in breast,[8-10] colorectal,[11-13] ovarian, bladder, and prostate,[14-17] in addition to other endometrial and non-endometrial cancers, such as stomach,[18] and lung.[19,20] Despite this, no comprehensive MS-based proteomic studies have been performed in the Kingdom of Saudi Arabia (KSA) for diseases that are common to the Kingdom. Thus, a need for clinical MS-proteomics research in KSA can be envisaged not only to establish advanced technology platforms as part of the 'Research and Development' program, and bring KSA on an international forefront of medical research, but also for general research purposes, to understand disease pathogenesis that may help finally in identifying novel drug targets.

The purpose of this review is to highlight not only the importance of MS-based clinical proteomics research in KSA, but also to introduce the concept of forming a 'Saudi Proteomics Society (SPS)', which will form a vital platform for international collaboration in the field of proteomics. In order to grasp the whole concept with an aim to bring it to practice, it will on one hand, require a description of the use of MS-based proteomics in understanding the pathogenesis of the disease in general worldwide, and on the other hand, will be essential to pinpoint some of the common diseases in KSA, for which no proteomic data are available but have been studied elsewhere from a proteomics perspective. While we will discuss these issues one by one, this review article by no means covers the whole spectrum of proteomics application or its limitations in disease, which have been discussed elsewhere.[21-31]

Proteomics and MS. Two sides of the same coin. Proteins are derived in a complex genotype-phenotype information loop and are regulated under tight transcriptional, post-transcriptional, and translational control (**Figure 1**). Proteins are the functional read-outs of any biological process, and therefore their study through a high throughput approach that directly addresses protein function, such as proteomics, becomes essential. Proteomics is not a single technique or a straight-forward approach, but involves a number of complex and interdependent methodologies and platforms, some of which are described for simplicity (**Figure 2**). Conventional proteomics can be classified as 'top-down',[32] or 'bottom up',[33] and may or may not involve the use of isoelectric focusing (IEF) techniques.[34] In the IEF technique, proteins are separated based on their charge in an electric field when proteins stop migration at the point of their zero net charge (their isoelectric points [pI]). The IEF in the first dimension is followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension that will separate proteins based on their molecular weight (referred to as 2-dimensional gel electrophoresis or 2D-E).[35,36] Alternatively, the IEF technique can be avoided, and instead liquid chromatography (LC) prior to SDS-PAGE[37] is used. Each approach has advantages, as well as limitations, and the choice depends upon many factors, including the question being addressed.

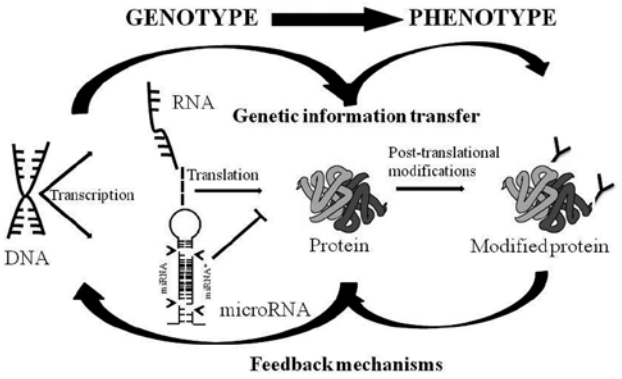

Figure 1 - Genotype-phenotype information loop: proteins that give rise to the phenotype are generated in a sequential and controlled manner as a result of transcription and translation via a genetic information transfer from DNA to RNA to protein. The proteins in turn, are modified by post-translational-modifications, and are controlled by feedback mechanisms and small RNA molecules called microRNAs.

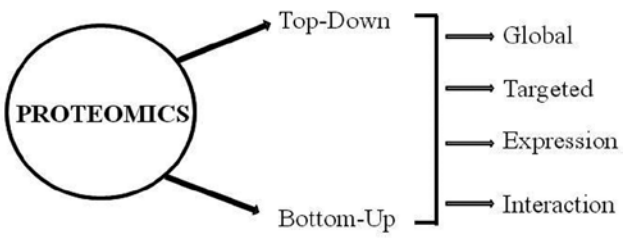

Figure 2 - Various proteomics platforms useful in clinical research: conventional proteomics can be broadly classified into 'top down' and 'bottom up', which may involve expression, interaction, targeted, or global proteomics.

For instance, in case of expression profiling experiments for a particular disease, the 'IEF-based' approach may be most suitable, whereas in the case of complex protein mixtures, an approach that avoids IEF will be a method of choice. With the advent of quantitative MS methodologies, such as the use of labeling strategies based on stable isotope labeling of amino acids in culture (SILAC),[38] the 'bottom up' approach is widely used in recent times. Despite this, 2D-E still remains the basic method for all the proteomics laboratories worldwide. Depending upon the experimental settings, both the 'top down' and the 'bottom up' approaches can be further subdivided into: global proteomics[39] that will include the study and analysis of all proteins present in a cell, tissue, or organism;[40-42] targeted proteomics with an aim to reduce complexity of the proteome will include more specific questions in a sense to characterize a well defined subproteome, for example, phosphoproteins or PTMs;[43-46] expression proteomics that will involve qualitative and quantitative comparison of samples using 2DE, 2D-difference gel electrophoresis (DIGE), LC, and LC-MS/MS using isotope labels (SILAC, and so forth), antibody array, and nucleotide microarrays;[1,38] interaction proteomics that will attempt to analyze protein-protein associations by isolating complexes, and define partners by LC-MS/MS (affinity pull-downs using antibody or tags), or identify complexes of 1D gels.[37,47,48]

One of the major developments that revolutionized the field of proteomics is MS, in particular the development of "soft" ionization methods-matrix assisted laser desorption ionization (MALDI), and electro-spray ionization (ESI).[49] The mass spectrometer can be considered analogous to a weighing balance for biomolecules. The MS is an analytical tool that operates under vacuum and is used for measuring the molecular mass of a sample, such as biomolecules with an accuracy of 0.01% of the total molecular mass of the sample, and organic molecules with an accuracy of 5 parts per million (ppm).[50,51] In general, mass spectrometers consist of 3 fundamental parts, namely the ionization source, the analyzer, and the detector under complete data system control (**Figure 3**). It is the ability of the mass spectrometer to rapidly identify proteins that makes it unique. The main approach to mass spectrometric protein identification are the 'peptide-mass mapping'.[52] In a series of steps, spectra are generated when the analyte undergoes ionization and fragmentation resulting in ions with different charge to mass (m/z) ratios, and detected by the detector. The spectra are analyzed by software-based database search resulting in the identification of proteins.

Applications of mass spectrometry. The MS is a global technique and therefore, has diverse applications in the biotechnology, pharmaceutical, clinical, and energy sector. With regard to clinical settings, the proteomic-pattern diagnostics in serum using surface-enhanced laser desorption ionization-time of flight (SELDI-TOF) to identify ovarian cancer cases,[53] can be considered one of the first applications of MS in this direction. This

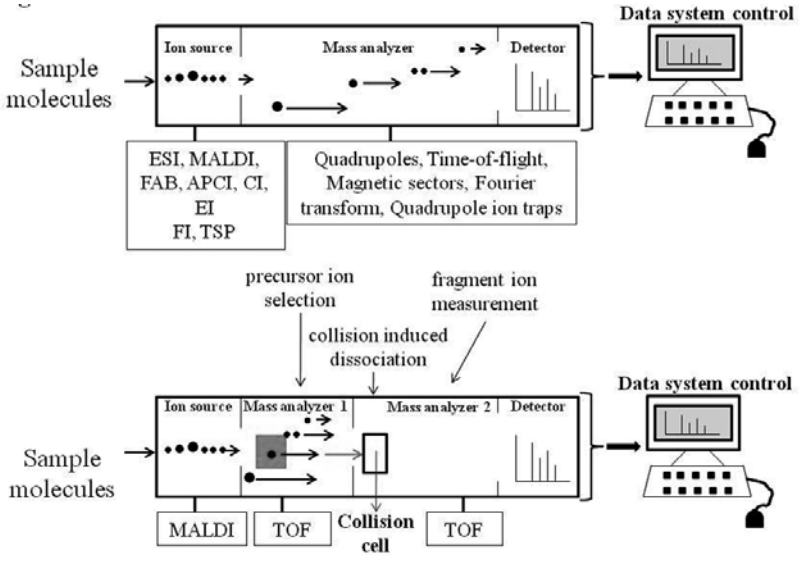

Figure 3 - Parts of the mass-spectrometer and tandem mass spectrometry (MS) instrument. Most popular analyzer configurations for "2-dimensional" MS are shown. The MS has one mass analyzer (top) and the tandem MS has 2 mass analyzers (bottom). Q-time of flight (TOF) and TOF-TOF are real tandem instruments. Various ion sources are shown. ESI - electrospray ionization, MALDI - matrix assisted laser desorption ionization, FAB - fast atom bombardment, APCI - atmospheric pressure chemical ionization, CI - chemical ionization, EI - electron impact, FI - field ionization, TSP - thermospray

was followed by major developments in fractionation of complex patient material in addition to advances in MS instrumentation that transformed into the use of LC and MS in metabolite identification studies in modern drug discovery, and biotransformations.[54-56] Other advances include MS applications in profiling, and imaging proteins directly in tissue sections in diagnostic pathology,[57] as well as development of quantitative MS strategies based on labeling, or label free approaches.[58,59] The MS proteomics has found versatile applications in genomic research, new born screening, serum and plasma biomarker discovery, stem cell research, PTM identification, and micro ribonucleic acid target identification. In summary, proteomics in conjunction with quantitative tandem MS, has and in the future, will profoundly improve our ability to understand protein pathways and systems biology, and certainly keeps alive our hope of identifying real disease biomarkers to be targeted for therapy.

Having described various functional proteomic platforms, let us briefly review the application of these platforms for diseases common in KSA.

Common diseases and their occurrence in KSA. The incidence of cancer and other genetic and metabolic disorders is high in KSA. According to the year 2008 Tumor Registry published by King Faisal Specialist Hospital and Research Center, Riyadh in June 2010,[60] and the Saudi Cancer Incidence Report 2005 published by the Ministry of Health KSA in 2009,[61] breast cancer (mostly in women), leukemia, non-Hodgkin's lymphoma, thyroid cancer, brain tumor, colorectal and bladder cancer (mostly in men) constitute some top cancers in adults in the Kingdom. On the other hand, in addition to leukemia and lymphoma, malignancies of brain and central nervous system, bone and cartilage, and soft tissue malignancies are common in children.[61] This is compounded by the fact that the distribution of these common malignancies has significantly risen over the past 30 years in the Kingdom despite advances in medical technology and treatment. A number of factors can be attributed to this increasing trend. First, modern diagnostic tools and better screening practices adopted by physicians, such as analysis of chromosomal abnormalities using advanced cytogenetic tools (CGH arrays, chromosomal banding, and so forth), immunophenotyping using multi-color flow-cytometry with specific markers, and others, such as nuclear magnetic resonance and MS make these amenable for the early detection of a malignancy/abnormality. Second, the prevalence of unidentified risk factors, which could be demographic-specific for cancer in concurrence with advanced screening techniques may in turn, be important contributing factors in the detection of new cancers. Third, globalization with a vast amount of knowledge transfer has led in general, to increased awareness of the need to seek early and routine medical check-ups. This is particularly true in KSA, where the proportion of female cancer patients has dramatically increased over the past decades (from 27% in 1975 to 50% in 1995, and to 54% in 2005),[60,61] a trend that is attributed to awareness among women to seek routine clinical examinations. It is important to pinpoint the observation that the number of cancer patients during a 5-year period from 2002-2005 in most cases is reduced compared to periods before, which may be an indication of improved health care, and better treatment modalities in KSA. However, the number of cancer patients showed an upward trend in other types of cancers, such as colorectal cancers, indicating that we still need more work to eradicate cancer.[60,61]

Other diseases common in KSA include inherited genetic disorders, especially metabolic disorders (IMDs), such as autosomal recessive disorders, sickle cell anemia and thalassemia, lysosomal storage disease, Niemann Pick disease, and other unique syndromes, such as Sanjad Sakati and Al-Aqeel-Sewairi syndromes.[62-67] The inherited metabolic disorders are relatively common in children in KSA, and this would require special attention since it can have a tremendous impact on the society as a whole, as well as on the economy. These inherited genetic disorders are thought to be a result of the consanguineous nature of the Middle Eastern culture including KSA, where 60-70% of the marriages are known to be first cousin marriages. Thus, understanding metabolic response to physio- or non-physiological stimuli for the improvement of disease identification would be an essential step in this direction. The developing field of 'metabolomics' can make it possible, for example, by quantifying intermediate metabolites in biological fluids using high resolution MS.[54,55,66,68-71] From the medical perspective, while significant progress has been achieved in diagnosis, prognosis, and treatment of various diseases, such as breast cancer and leukemia, most of them still remain incurable. These analyses underscore the need for early detection, and strengthen our motives and efforts in trying to find new ways of preventing, or reducing the occurrence of common malignancies found in KSA. Combining better application of existing knowledge with advanced research to improve prevention, early detection, and treatment will be needed to sustain and extend this progress in the future. As one way forward in this direction, global MS-based proteomics has found significant expansion and advancement, with respect to its clinical applications in identifying biomarkers and putative drug targets in disease relevant processes,[2,23,72-87] and also as a diagnostic tool.[53,88-91] Thus, we strongly support and envisage research in this area by the incorporation of tandem MS-based

proteomics platforms at institutions of medical and scientific importance, in order to pave the way for improved diagnostics, prognostics, and therapy for patients visiting the Saudi hospitals.

MS-based proteomic studies in 'common diseases': global view. Breast cancer. It may be important to remind the readers here that breast cancer is the most common malignancy in KSA, for which extensive proteome profiles are lacking. In this disease, the mortality rate is high with a poor outcome owing to the accumulation of malignant cells in the regional lymph nodes having the potential for distant metastases, preferentially in the bone, lung, and liver.[10,92] Bini et al[93] reported 32 protein spots from whole biopsy fragments, that were indicative of epithelial neoplasia using protein expression profiles in human breast ductal carcinoma and histologically normal tissue. Jacquameir et al,[9] monitored the expression of 26 selected proteins in more than 1,600 cancer samples from 552 consecutive patients with early breast cancer, and identified relevant clusters of coexpressed proteins and clusters of tumors with a significant correlation to 5-year metastasis-free survival. The authors conclude that protein expression profiling may be a clinically useful approach to assess breast cancer heterogeneity and prognosis in stage I, II, or III disease.[9] Using SELDI-TOF MS-based protein chip arrays, Goncalves et al[94] performed protein profiling of human breast cell lines (BCL), and classified the 2 groups of BCLs corresponding to "luminal-like" cell lines and to "basal-like" cell lines. They further demonstrated the clinical relevance of one of the proteins S100A9 in its association with basal subtypes, as well as with its poor prognosis value, clearly indicating the potential of profiling technologies to identify biomarkers of diagnostic and prognostic significance.

Leukemia (myeloid and lymphoid). Leukemia is a cancer that derives from blood cells that incorrectly respond to normal cues to grow and develop. Leukemia is not a single disease, but a group of neoplasms with diverse genetic abnormalities and variable responses to treatment. Depending upon the lineage, that is, affected leukemia can be very broadly classified into myeloid and lymphoid.[95] Acute myeloid leukemia or AML is a disease that is characterized by a block in the normal process of myeloid differentiation, thereby leading to the accumulation of immature cells termed blasts.[96,97] The French-American-British, or FAB, classification has been the standard system used to classify acute leukemias. The AML is divided into 8 major FAB subtypes (M0-M7), which are defined by morphology and immunophenotype.[98,99] In a study by Cui et al,[61] cases of FAB classified acute leukemia were resolved, and analyzed by both MALDI-TOF-MS and tandem (ESI-MS/MS to successfully obtain distinct protein profiles characteristic of each group.[100,101] However, the choice of therapy often depends upon the specific cytogenetic abnormality found in the leukemic blasts rather than their morphology, or degree of differentiation. More than half of the AML patients display detectable and usually single cytogenetic abnormalities.[98] Using MS-based proteomics in 42 cytogenetically different AML patient samples Balkhi et al,[102] reported that cytogenetically risk groups differ not only in their proteome, but also in the levels of PTMs. Cui et al,[103] also applied the proteomic approach to the identification of proteins that commonly elicit a humoral response in acute leukemia. Using MS for protein identification, the authors detected autoantibodies against some proteins in the sera of AL patients suggesting a possibility for development of a serum-based assay for AL screening and diagnosis. Dr. Behre's laboratory[37,48,102,105,106] extensively used MS-based proteomic technology to understand the molecular events underlying AML, and we successfully applied it to the analysis of target proteins of the fusion proteins involved in the pathogenesis of AML (for example, promyelocytic leukemia-retinoic acid receptor alpha),[104] to the analysis of target proteins and protein-protein interactions (for example, CCAAT enhancing binding protein alpha),[37,48,105,106] and also provided a novel approach to leukemia classification based on post-translational modifications of specific proteins.[102] Although these studies have clearly laid the foundation for new thinking in the pathogenesis of leukemia, future studies should use larger cohort of patient samples and advanced quantitative MS, such as the use of labeling strategies based on SILAC, if putative biomarkers are to be identified and validated.[58,107,108]

Chronic lymphocytic leukemia or CLL is a disease characterized by defects in apoptotic pathways, resulting in a prolonged life span of the precursor and fully transformed antigen-experienced mature B-lymphocytes in the blood, secondary lymphoid tissues, and bone marrow (BM) in vivo.[109,110] Voss et al,[111] demonstrated a correlation of large-scale protein expression profiles with clinical parameters such as patient survival in CLL. Boyd et al,[112] reported the use of plasma-membrane-based proteomic analysis in CLL to identify potential B-cell-specific proteins whereas, Cochran et al,[113] analyzed the proteome of mutated and unmutated CLL. Recently, Su et al[114] reported LC-MS based profiling of histones in CLL.

Lymphoma. Mantle cell lymphoma (MCL), a type of non-Hodgkin's lymphoma is characterized by malignant transformation of the mantle zone cells surrounding the germinal centers and by the t(11;14)(q31;q32) translocation resulting in the up-regulation of cyclin D1 is a B cell disease with a poor prognosis, and a median survival time of approximately 3-5 years.[115] The patients

have malignant cell invasion of spleen, bone marrow, and particularly the gastrointestinal tract.[116,117] The 2D gel electrophoresis has been used to compare the protein profiles of lymph node tissue in MCL patients and normal control samples.[118] A protein (antibody) microarray study compared proteins in CD19+ purified B cells obtained from normal tonsils and histologically confirmed MCL patients.[119] Miguet et al,[120] and Boyd et al,[77] recently reported the proteomic approach based on MS analysis of plasma membrane microparticles in MCL, as well as in CLL and small cell lymphoma. In the case of follicular lymphoma, Gulmann et al[121] reported prognostic factors belonging to apoptotic pathways using laser capture microdissection and reverse-phase protein microarrays.

Thyroid cancer. Thyroid cancer, an endocrine neoplasm is derived from thyroid epithelial cells displaying diverse neoplastic phenotypes, including benign follicular adenomas, well-differentiated papillary and follicular carcinomas, and aggressive anaplastic carcinomas.[122,123] Brown et al[124] utilized DIGE coupled to MS to uncover biomarkers in pooled protein extracts from patients with papillary thyroid carcinoma (PTC). Giusti et al,[125] performed a comparative proteome analysis to examine the global changes of fine needle aspiration fluid protein patterns of 2 variants of malignant PTC (classical variant PTC [cPTC, and tall cell variant [TCV] PTC) with respect to the controls. Gorla et al[126] performed a proteomic-multiplexed analysis of the phosphotyrosine signaling in 2 human medullary thyroid carcinoma cell lines.

Ovarian cancer. Ovarian cancer is an epithelial cancer in women associated with metastases and invasion of the peritoneal cavity and ascites.[127] Ovarian cancer is one of the first cancers, for which proteomic patterns in serum that distinguish neoplastic from non-neoplastic disease within the ovary were described.[53] Other proteomics studies for biomarker identification analyzed serum, ovarian tumor tissue, or secreted, or cell surface proteins of tumor cells.[76,128-133] Serum proteomic expression profiles analyzed in 153 patients with invasive epithelial ovarian cancer, 42 with other ovarian cancers, 166 with benign pelvic masses, and 142 healthy women that revealed 3 biomarkers as apolipoprotein A1, a truncated form of transthyretin and a cleavage fragment of inter-trypsin inhibitor heavy chain H4 were reported by Zhang et al.[134]

Hepatocellular carcinoma. Proteome analysis of hepatocellular carcinoma using 2D-PAGE and MALDI-TOF-MS is reported.[135,136]

Neurodegenerative diseases. Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and prion diseases are disorders caused by the deterioration of certain nerve cells, for which diagnosis is made primarily on clinical grounds including neuropsychological testing, limited laboratory tests, and brain imaging.[137] However, there are no cure for these disorders making biomarker identification essential. Thus, MS based protein profiling is being applied in this direction.[137-141]

Other diseases. There are certain other diseases in KSA that represents a major public health concern and need utmost attention. One of them is osteoporosis, a bone disease mainly characterized by low mineral bone density (BMD). Different techniques like BMD testing, optical and electrochemical testing have been studied.[142] Kubota et al[143] demonstrated proteome analysis of secreted proteins during osteoclast differentiation. Osteoclasts are the cells that express factors responsible for the modulation of bone resorption and bone remodeling. Prahalad et al[144] employed ProteinChip technology to generate protein profiles from sera of mice treated with parathyroid hormone, which has variable effects on bone in osteoporosis patients. Deng et al[145] reported comparative proteomic study of circulating monocytes (CNMC) in Chinese premenopausal females with extremely discordant BMD. Despite this, comprehensive studies using tandem MS in osteoporosis research are clearly lacking in order to understand the molecular pathways underlying the disease that could be used in early detection of the disease.

In summary, while significant progress has been achieved worldwide with regard to MS-based proteomics research, we still face enormous challenges in KSA, given the lack of basic proteomics infrastructure and the research outcome. However, recent developments are encouraging, and provide a ray of hope for all researchers in this direction. This is in part, due to enhanced research funding during the period of the current government under the leadership of King Abdullah Al-Saud. What is now required on the part of researchers are consistent and dedicated efforts to build up a research environment with collaborative platforms in respective fields. Team efforts at the national level is the way forward to achieve success at the international level.

Introducing the SPS. With the achievement in 2001 of a major milestone in genomics that culminated in obtaining the human genome sequence, the need for establishing the Human Proteome Organization (HUPO, www.hupo.org) was recognized. Since then, proteomics is not only playing a major role in elucidating the functional role of many novel genes and their products, but also in understanding genotype-phenotype relationship in normal cellular processes and disease. The HUPO as an international consortium to promote proteomics research has championed the cause of launching a number of scientific initiatives with the help of international collaboration. A number

of proteomics network or societies from different regions and countries are currently a part of this wide international network, and contribute one way or another to the scientific initiatives launched by HUPO such as brain, liver, plasma, and stem cell proteome initiatives. In the Middle East, Iran is the only nation to have a proteomics society affiliated with the HUPO, at present.

In the context of KSA, MS-based proteome profiles of diseases common to the Kingdom, or data on evaluation of multi-parametric metabolites quantitatively in biological fluids such as serum, urine, blood, cerebrospinal fluid (CSF) in Saudi patients are clearly lacking. It may be important to mention here that proteomic profiling studies, or Saudi biomarker studies from blood in a massive scale-cohort, or even determining the levels of proteins in Saudi subjects and how they may differ from populations in other countries could be highly valuable studies for biomarker discovery, in addition to obtaining a comprehensive proteomic database. The assumption that specific proteomic research on Saudi patient material will likely differ from populations in other countries is not surprising, given the fact that significant differences are observed at the genomic and protein level when the comparison is made between Saudi and non-Saudi populations by our colleagues at the Center of Excellence for Genomic and Medicine Research (CEGMR) recently.[146,147] Biofluid proteomic profiling studies for obtaining individualized diagnostic fingerprints can add another dimension to proteomics research in the Kingdom. Thus, in order to identify specific signatures, if any, associated with a specific disease that could serve as prognostic or diagnostic markers in the Saudi population, and in the process for discovering protein biomarkers, 'proteome profiles,' and research in other aspects of clinical proteomics, such as population studies for the development of early blood tests from multicenter studies using MS needs to be performed. We need to move forward with innovative approaches in this area to benefit not only the academia, but also the industrial sector as well. This can be possible only under the umbrella of committed research teams with an interdisciplinary approach that will combine innovative application of existing knowledge with advanced research to improve prevention, early detection, and treatment in the Kingdom. Consequently, we propose the idea of forming a 'Saudi Proteomics Society (SPS)'. The aim of such a society would be initially to establish and enforce clinically useful proteomics projects at the multicenter level, gather all proteomics data from national research centers, and organize it in a national database. Affiliations with international consortiums, such as HUPO and other international collaborations are envisioned in the process. We strongly believe that KSA can be a vital part of international consortiums with regard to initiating population-based proteomics studies in Saudi Arabia, known to be of a diverse ethnicity. Strong commitment on the part of researchers and extensive collaborations within these multicenter studies will enable us to succeed for the better human cause. In the future, a need to establish such advanced technologies and curricula at higher education levels including at the level of universities and research institutions of medical importance in KSA, not only to foster knowledge for human cause, but also for improving health standards in Saudi society as well, is desirable. It will also be fruitful to incorporate proteomics in the national curriculum so as to encourage and generate interest among students opting for research in the future in this area. In fact, advanced technology programs of the King Abdullah Center for Science and Technology (KACST) implemented by the Ministry of Higher Education for KSA is a welcome step towards this goal, and must be put in place and practiced to incorporate such technologies, which may benefit the society in one way or another.

In conclusion, rapid developments in the field of MS-based quantitative proteomic technologies hold great promise in the search for disease biomarkers and thus, proteomics is considered a valuable tool towards understanding biological processes for early detection, diagnosis, and prognosis of cancer in general, and for monitoring response to therapy. Given highly diverse applications of MS-proteomics, there is a need for an organized effort to develop an infrastructure in proteomics that will help us unravel mechanisms associated with the disease, and advance our efforts in combating diseases in the Kingdom, where proteomics research is under-represented. To achieve astounding success in this direction, it is imperative to perform multicenter studies with a concerted effort on the part of researchers, physicians, and other collaborating partners.

Acknowledgment. *The authors gratefully acknowledge Dr. Adeel G. Chaudhary for his kind help and generous etiquette regarding his contribution. We would like to acknowledge Dr. Farid Ahmed for his valuable discussions, and Ms. Shireen Hussein for her kind efforts and help. We apologize to authors whose contributions to the proteomics field could not be cited due to space limitations. We would like to acknowledge King Abdullah City for Science and Technology, Jeddah, Kingdom of Saudi Arabia for promoting and funding mass spectrometry-based proteomics research in the Kingdom.*

References

1. Hanash SM, Madoz-Gurpide J, Misek DE. Identification of novel targets for cancer therapy using expression proteomics. ***Leukemia*** 2002; 16: 478-485.
2. Hanash S. Disease proteomics. ***Nature*** 2003; 422: 226-232.

3. Latterich M, Abramovitz M, Leyland-Jones B. Proteomics: new technologies and clinical applications. ***Eur J Cancer*** 2008; 44: 2737-2741.
4. Pieroni E, de la Fuente van Bentem S, Mancosu G, Capobianco E, Hirt H, de la Fuente A. Protein networking: insights into global functional organization of proteomes. ***Proteomics*** 2008; 8: 799-816.
5. Pasquali C, Frutiger S, Wilkins MR, Hughes GJ, Appel RD, Bairoch A, et al. Two-dimensional gel electrophoresis of Escherichia coli homogenates: the Escherichia coli SWISS-2DPAGE database. ***Electrophoresis*** 1996; 17: 547-555.
6. Wilkins MR, Pasquali C, Appel RD, Ou K, Golaz O, Sanchez JC, et al. From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. ***Biotechnology (N Y)*** 1996; 14: 61-65.
7. Anderson NL, Anderson NG, Pearson TW, Borchers CH, Paulovich AG, Patterson SD, et al. A human proteome detection and quantitation project. ***Mol Cell Proteomics*** 2009; 8: 883-886.
8. Laronga C, Drake RR. Proteomic approach to breast cancer. ***Cancer Control*** 2007; 14: 360-368.
9. Jacquemier J, Ginestier C, Rougemont J, Bardou VJ, Charafe-Jauffret E, Geneix J, et al. Protein expression profiling identifies subclasses of breast cancer and predicts prognosis. ***Cancer Res*** 2005; 65: 767-779.
10. Leth-Larsen R, Lund R, Hansen HV, Laenkholm AV, Tarin D, Jensen ON, et al. Metastasis-related plasma membrane proteins of human breast cancer cells identified by comparative quantitative mass spectrometry. ***Mol Cell Proteomics*** 2009; 8: 1436-1449.
11. Rho JH, Qin S, Wang JY, Roehrl MH. Proteomic expression analysis of surgical human colorectal cancer tissues: up-regulation of PSB7, PRDX1, and SRP9 and hypoxic adaptation in cancer. ***J Proteome Res*** 2008; 7: 2959-2972.
12. Gulmann C, Sheehan KM, Conroy RM, Wulfkuhle JD, Espina V, Mullarkey MJ, et al. Quantitative cell signalling analysis reveals down-regulation of MAPK pathway activation in colorectal cancer. ***J Pathol*** 2009; 218: 514-519.
13. Alaiya AA, Roblick UJ, Franzén B, Bruch HP, Auer G. Protein expression profiling in human lung, breast, bladder, renal, colorectal and ovarian cancers. ***J Chromatogr B Analyt Technol Biomed Life Sci*** 2003; 787: 207-222.
14. Wright ME, Han DK, Aebersold R. Mass spectrometry-based expression profiling of clinical prostate cancer. ***Mol Cell Proteomics*** 2005; 4: 545-554.
15. Grubb RL, Deng J, Pinto PA, Mohler JL, Chinnaiyan A, Rubin M, et al. Pathway biomarker profiling of localized and metastatic human prostate cancer reveal metastatic and prognostic signatures. ***J Proteome Res*** 2009; 8: 3044-3054.
16. Liu AY, Zhang H, Sorensen CM, Diamond DL. Analysis of prostate cancer by proteomics using tissue specimens. ***J Urol*** 2005; 173: 73-78.
17. Matharoo-Ball B, Ball G, Rees R. Clinical proteomics: discovery of cancer biomarkers using mass spectrometry and bioinformatics approaches--a prostate cancer perspective. ***Vaccine*** 2007; 25 (Suppl 2): S110-S121.
18. Tomlinson AJ, Hincapie M, Morris GE, Chicz RM. Global proteome analysis of a human gastric carcinoma. ***Electrophoresis*** 2002; 23: 3233-3240.
19. Gharib TG, Chen G, Huang CC, Misek DE, Iannettoni MD, Hanash SM, et al. Genomic and proteomic analyses of vascular endothelial growth factor and insulin-like growth factor-binding protein 3 in lung adenocarcinomas. ***Clin Lung Cancer*** 2004; 5: 307-312.
20. VanMeter AJ, Rodriguez AS, Bowman ED, Jen J, Harris CC, Deng J, et al. Laser capture microdissection and protein microarray analysis of human non-small cell lung cancer: differential epidermal growth factor receptor (EGPR) phosphorylation events associated with mutated EGFR compared with wild type. ***Mol Cell Proteomics*** 2008; 7: 1902-1924.
21. Taylor AD, Hancock WS, Hincapie M, Taniguchi N, Hanash SM. Towards an integrated proteomic and glycomic approach to finding cancer biomarkers. ***Genome Med*** 2009; 1: 57.
22. Alaiya A, Al-Mohanna M, Linder S. Clinical cancer proteomics: promises and pitfalls. ***J Proteome Res*** 2005; 4: 1213-1222.
23. Hanash S. HUPO initiatives relevant to clinical proteomics. ***Mol Cell Proteomics*** 2004; 3: 298-301.
24. Hanash S, Celis JE. The Human Proteome Organization: a mission to advance proteome knowledge. ***Mol Cell Proteomics*** 2002; 1: 413-414.
25. Liotta LA, Petricoin EF 3rd. The promise of proteomics. ***Clin Adv Hematol Oncol*** 2003; 1: 460-462.
26. Mann M. Mass tool for diagnosis. ***Nature*** 2002; 418: 731-732.
27. Petricoin EF, Liotta LA. Clinical applications of proteomics. ***J Nutr*** 2003; 133 (Suppl 7): S2476-S2484.
28. Verrills NM. Clinical proteomics: present and future prospects. ***Clin Biochem Rev*** 2006; 27: 99-116.
29. Geiger T, Cox J, Ostasiewicz P, Wisniewski JR, Mann M. Super-SILAC mix for quantitative proteomics of human tumor tissue. ***Nat Methods*** 2010; 7: 383-385.
30. Walther TC, Mann M. Mass spectrometry-based proteomics in cell biology. ***J Cell Biol*** 2010; 190: 491-500.
31. Macek B, Mann M, Olsen JV. Global and site-specific quantitative phosphoproteomics: principles and applications. ***Annu Rev Pharmacol Toxicol*** 2009; 49: 199-221.
32. Han X, Jin M, Breuker K, McLafferty FW. Extending top-down mass spectrometry to proteins with masses greater than 200 kilodaltons. ***Science*** 2006; 314: 109-112.
33. Hüttenhain R, Hess S. A combined top-down and bottom-up MS approach for the characterization of hemoglobin variants in Rhesus monkeys. ***Proteomics*** 2010; 10: 3657-3668.
34. Weiss W, Görg A. High-resolution two-dimensional electrophoresis. ***Methods Mol Biol*** 2009; 564: 13-32.
35. Görg A, Weiss W, Dunn MJ. Current two-dimensional electrophoresis technology for proteomics. ***Proteomics*** 2004; 4: 3665-3685.
36. Smith R. Two-dimensional electrophoresis: an overview. ***Methods Mol Biol*** 2009; 519: 1-16.
37. Zada AA, Pulikkan JA, Bararia D, Geletu M, Trivedi AK, Balkhi MY, et al. Proteomic discovery of Max as a novel interacting partner of C/EBPalpha: a Myc/Max/Mad link. ***Leukemia*** 2006; 20: 2137-2146.
38. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, et al. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. ***Mol Cell Proteomics*** 2002; 1: 376-386.
39. Hanash SM. Global profiling of gene expression in cancer using genomics and proteomics. ***Curr Opin Mol Ther*** 2001; 3: 538-545.
40. Anderson NL, Anderson NG. Proteome and proteomics: new technologies, new concepts, and new words. ***Electrophoresis*** 1998; 19: 1853-1861.
41. Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. ***Mol Cell Proteomics*** 2002; 1: 845-867.

42. Pandey A, Mann M. Proteomics to study genes and genomes. ***Nature*** 2000; 405: 837-846.
43. de la Fuente van Bentem S, Mentzen WI, de la Fuente A, Hirt H. Towards functional phosphoproteomics by mapping differential phosphorylation events in signaling networks. ***Proteomics*** 2008; 8: 4453-4465.
44. Gruhler A, Olsen JV, Mohammed S, Mortensen P, Faergeman NJ, Mann M, et al. Quantitative phosphoproteomics applied to the yeast pheromone signaling pathway. ***Mol Cell Proteomics*** 2005; 4: 310-327.
45. Mann M, Jensen ON. Proteomic analysis of post- translational modifications. ***Nat Biotechnol*** 2003; 21: 255-261.
46. Ping P, Vondriska TM, Creighton CJ, Gandhi TK, Yang Z, Menon R, et al. A functional annotation of subproteomes in human plasma. ***Proteomics*** 2005; 5: 3506-3519.
47. Wepf A, Glatter T, Schmidt A, Aebersold R, Gstaiger M. Quantitative interaction proteomics using mass spectrometry. ***Nat Methods*** 2009; 6: 203-205.
48. Bararia D, Trivedi AK, Zada AA, Greif PA, Mulaw MA, Christopeit M, et al. Proteomic identification of the MYST domain histone acetyltransferase TIP60 (HTATIP) as a co-activator of the myeloid transcription factor C/EBPalpha. ***Leukemia*** 2008; 22: 800-807.
49. Rappsilber J, Ishihama Y, Mann M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. ***Anal Chem*** 2003; 75: 663-670.
50. Ashcroft AE. Recent developments in electrospray ionisation mass spectrometry: noncovalently bound protein complexes. ***Nat Prod Rep*** 2005; 22: 452-464.
51. Dass C. Mass spectrometry and hyphenated techniques in neuropeptide research. Jerzy Silberring and Rolf Ekman, John Wiley & Sons, Inc., New York, NY, USA, 10158ISBN 0-471-35493-7, 2002, Hardcover, $925, pp. 558. ***J Am Soc Mass Spectrom*** 2003; 14: 81-82.
52. Lasso G, Matthiesen R. Computational methods for analysis of two-dimensional gels. ***Methods Mol Biol*** 2010; 593: 231-262.
53. Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, et al. Use of proteomic patterns in serum to identify ovarian cancer. ***Lancet*** 2002; 359: 572-577.
54. Tolonen A, Turpeinen M, Pelkonen O. Liquid chromatography-mass spectrometry in in vitro drug metabolite screening. ***Drug Discov Today*** 2009; 14: 120-133.
55. Tiller PR, Yu S, Castro-Perez J, Fillgrove KL, Baillie TA. High-throughput, accurate mass liquid chromatography/tandem mass spectrometry on a quadrupole time-of-flight system as a 'first-line' approach for metabolite identification studies. ***Rapid Commun Mass Spectrom*** 2008; 22: 1053-1061.
56. Anari MR, Sanchez RI, Bakhtiar R, Franklin RB, Baillie TA. Integration of knowledge-based metabolic predictions with liquid chromatography data-dependent tandem mass spectrometry for drug metabolism studies: application to studies on the biotransformation of indinavir. ***Anal Chem*** 2004; 76: 823-832.
57. Caldwell RL, Caprioli RM. Tissue profiling by mass spectrometry: a review of methodology and applications. ***Mol Cell Proteomics*** 2005; 4: 394-401.
58. Mann M. Functional and quantitative proteomics using SILAC. ***Nat Rev Mol Cell Biol*** 2006; 7: 952-958.
59. Haqqani AS, Kelly JF, Stanimirovic DB. Quantitative protein profiling by mass spectrometry using label-free proteomics. ***Methods Mol Biol*** 2008; 439: 241-256.
60. Haya S Al-Eid. Cancer Incidence Report Saudi Arabia 2005. Riyadh (Kingdom of Saudi Arabia): Ministry of Health Saudi Cancer Registry; 2009.
61. Te OB, Al-Qahtani F, Urcia JC. 2008 Tumor Registry Annual Report. Riyadh (Kingdom of Saudi Arabia): King Faisal Specialist Hospital and Research Center (KFSHRC); 2010.
62. Al-Ali AK, Al-Mustafa ZH, Al-Madan M, Qaw F, Al-Ateeq S. Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in the Eastern Province of Saudi Arabia. ***Clin Chem Lab Med*** 2002; 40: 814-816.
63. Al-Aqeel AI. Al-Aqeel Sewairi syndrome, a new autosomal recessive disorder with multicentric osteolysis, nodulosis and arthropathy. The first genetic defect of matrix metalloproteinase 2 gene. ***Saudi Med J*** 2005; 26: 24-30.
64. Al-Dirbashi OY, Abu-Amero KK, Alswaid AF, Hoffmann GF, Al-Qahtani K, Rashed MS. LC-MS/MS determination of dibasic amino acids for the diagnosis of cystinuria. Application in a family affected by a novel splice-acceptor site mutation in the SLC7A9 gene. ***J Inherit Metab Dis*** 2007; 30: 611.
65. Hellani A, Schuchman EH, Al-Odaib A, Al Aqueel A, Jaroudi K, Ozand P et al. Preimplantation genetic diagnosis for Niemann-Pick disease type B. ***Prenat Diagn*** 2004; 24: 943-948.
66. Rashed MS. Clinical applications of tandem mass spectrometry: ten years of diagnosis and screening for inherited metabolic diseases. ***J Chromatogr B Biomed Sci Appl*** 2001; 758: 27-48.
67. Rashed MS, Al-Ahaidib LY, Al-Dirbashi OY, Al Amoudi M, Al-Sayed MM, Rahbeeni Z, et al. Tandem mass spectrometric assay of succinylacetone in urine for the diagnosis of hepatorenal tyrosinemia. ***Anal Biochem*** 2005; 339: 310-317.
68. Petsalo A, Turpeinen M, Tolonen A. Identification of bupropion urinary metabolites by liquid chromatography/mass spectrometry. ***Rapid Commun Mass Spectrom*** 2007; 21: 2547-2554.
69. Rousu T, Pelkonen O, Tolonen A. Rapid detection and characterization of reactive drug metabolites in vitro using several isotope-labeled trapping agents and ultra-performance liquid chromatography/time-of-flight mass spectrometry. ***Rapid Commun Mass Spectrom*** 2009; 23: 843-855.
70. Denkert C, Budczies J, Kind T, Weichert W, Tablack P, Sehouli J, et al. Mass spectrometry-based metabolic profiling reveals different metabolite patterns in invasive ovarian carcinomas and ovarian borderline tumors. ***Cancer Res*** 2006; 66: 10795-10804.
71. Ideker T, Thorsson V, Ranish JA, Christmas R, Buhler J, Eng JK, et al. Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. ***Science*** 2001; 292: 929-934.
72. Schenk S, Schoenhals GJ, de Souza G, Mann M. A high confidence, manually validated human blood plasma protein reference set. ***BMC Med Genomics*** 2008; 1: 41.
73. Aebersold R, Mann M. Mass spectrometry-based proteomics. ***Nature*** 2003; 422: 198-207.
74. Ardekani AM, Liotta LA, Petricoin EF 3rd. Clinical potential of proteomics in the diagnosis of ovarian cancer. ***Expert Rev Mol Diagn*** 2002; 2: 312-320.
75. Baker CL, Kettenbach AN, Loros JJ, Gerber SA, Dunlap JC. Quantitative proteomics reveals a dynamic interactome and phase-specific phosphorylation in the Neurospora circadian clock. ***Mol Cell*** 2009; 34: 354-363.
76. Bengtsson S, Krogh M, Szigyarto CA, Uhlen M, Schedvins K, Silfverswärd C et al. Large-scale proteomics analysis of human ovarian cancer for biomarkers. ***J Proteome Res*** 2007; 6: 1440-1450.

77. Boyd RS, Jukes-Jones R, Walewska R, Brown D, Dyer MJ, Cain K. Protein profiling of plasma membranes defines aberrant signaling pathways in mantle cell lymphoma. ***Mol Cell Proteomics*** 2009; 8: 1501-1515.
78. Brehme M, Hantschel O, Colinge J, Kaupe I, Planyavsky M, Köcher T, et al. Charting the molecular network of the drug target Bcr-Abl. ***Proc Natl Acad Sci U S A*** 2009; 106: 7414-7419.
79. Calvo KR, Liotta LA, Petricoin EF 3rd. Clinical proteomics: from biomarker discovery and cell signaling profiles to individualized personal therapy. ***Biosci Rep*** 2005; 25: 107-125.
80. Cristea IM, Gaskell SJ, Whetton AD. Proteomics techniques and their application to hematology. ***Blood*** 2004; 103; 3624-3634.
81. Espina V, Dettloff KA, Cowherd S, Petricoin EF 3rd, Liotta LA. Use of proteomic analysis to monitor responses to biological therapies. ***Expert Opin Biol Ther*** 2004; 4: 83-93.
82. Hanash SM, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. ***Nature*** 2008; 452: 571-579.
83. Johann DJ Jr, McGuigan MD, Patel AR, Tomov S, Ross S, Conrads TP, et al. Clinical proteomics and biomarker discovery. ***Ann N Y Acad Sci*** 2004; 1022: 295-305.
84. Liao H, Wu J, Kuhn E, Chin W, Chang B, Jones MD, et al. Use of mass spectrometry to identify protein biomarkers of disease severity in the synovial fluid and serum of patients with rheumatoid arthritis. ***Arthritis Rheum*** 2004; 50: 3792-3803.
85. McGregor E, Dunn MJ. Proteomics of the heart: unraveling disease. ***Circ Res*** 2006; 98: 309-321.
86. Pitteri SJ, Faca VM, Kelly-Spratt KS, Kasarda AE, Wang H, Zhang Q, et al. Plasma proteome profiling of a mouse model of breast cancer identifies a set of up-regulated proteins in common with human breast cancer cells. ***J Proteome Res*** 2008; 7: 1481-1489.
87. Pitteri SJ, Hanash SM. Proteomic approaches for cancer biomarker discovery in plasma. ***Expert Rev Proteomics*** 2007; 4: 589-590.
88. Petricoin EF, Liotta LA. Mass spectrometry-based diagnostics: the upcoming revolution in disease detection. ***Clin Chem*** 2003; 49: 533-534.
89. Petricoin EF, Liotta LA. SELDI-TOF-based serum proteomic pattern diagnostics for early detection of cancer. ***Curr Opin Biotechnol*** 2004; 15: 24-30.
90. Petricoin EF, Liotta LA. Proteomic approaches in cancer risk and response assessment. ***Trends Mol Med*** 2004; 10: 59-64.
91. Petricoin EF, Ornstein DK, Liotta LA. Clinical proteomics: Applications for prostate cancer biomarker discovery and detection. ***Urol Oncol*** 2004; 22: 322-328.
92. Zhang DH, Tai LK, Wong LL, Sethi SK, Koay ES. Proteomics of breast cancer: enhanced expression of cytokeratin19 in human epidermal growth factor receptor type 2 positive breast tumors. ***Proteomics*** 2005; 5: 1797-1805.
93. Bini L, Magi B, Marzocchi B, Arcuri F, Tripodi S, Cintorino M, et al. Protein expression profiles in human breast ductal carcinoma and histologically normal tissue. ***Electrophoresis*** 1997; 18: 2832-2841.
94. Goncalves A, Charafe-Jauffret E, Bertucci F, Audebert S, Toiron Y, Esterni B, et al. Protein profiling of human breast tumor cells identifies novel biomarkers associated with molecular subtypes. ***Mol Cell Proteomics*** 2008; 7: 1420-1433.
95. Tenen DG. Disruption of differentiation in human cancer: AML shows the way. ***Nat Rev Cancer*** 2003; 3: 89-101.
96. Bacher U, Schnittger S, Haferlach T. Molecular genetics in acute myeloid leukemia. ***Curr Opin Oncol*** 2010; 22: 646-655.
97. Rubnitz JE, Gibson B, Smith FO. Acute myeloid leukemia ***Hematol Oncol Clin North Am*** 2010; 24: 35-63.
98. Vardiman JW. The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues: an overview with emphasis on the myeloid neoplasms. ***Chem Biol Interact*** 2009; 184: 16-20.
99. Yin CC, Medeiros LJ, Bueso-Ramos CE. Recent advances in the diagnosis and classification of myeloid neoplasms-- comments on the 2008 WHO classification. ***Int J Lab Hematol*** 2010; 32: 461-476.
100. Cui JW, Wang J, He K, Jin BF, Wang HX, Li W, et al. Proteomic analysis of human acute leukemia cells: insight into their classification. ***Clin Cancer Res*** 2004; 10: 6887-6896.
101. Cui JW, Wang J, He K, Jin BF, Wang HX, Li W, et al. Two-dimensional electrophoresis protein profiling as an analytical tool for human acute leukemia classification. ***Electrophoresis*** 2005; 26: 268-279.
102. Balkhi MY, Trivedi AK, Geletu M, Christopeit M, Bohlander SK, Behre HM et al. Proteomics of acute myeloid leukaemia: Cytogenetic risk groups differ specifically in their proteome, interactome and post-translational protein modifications. ***Oncogene*** 2006; 25: 7041-7058.
103. Cui JW, Li WH, Wang J, Li AL, Li HY, Wang HX, et al. Proteomics-based identification of human acute leukemia antigens that induce humoral immune response. ***Mol Cell Proteomics*** 2005; 4: 1718-1724.
104. Zada AA, Geletu MH, Pulikkan JA, Müller-Tidow C, Reddy VA, Christopeit M, et al. Proteomic analysis of acute promyelocytic leukemia: PML-RARalpha leads to decreased phosphorylation of OP18 at serine 63. ***Proteomics*** 2006; 6: 5705-5719.
105. Geletu M, Balkhi MY, Peer Zada AA, Christopeit M, Pulikkan JA, Trivedi AK, et al. Target proteins of C/EBPalphap30 in AML: C/EBPalphap30 enhances sumoylation of C/EBPalphap42 via up-regulation of Ubc9. ***Blood*** 2007; 110: 3301-3309.
106. Trivedi AK, Bararia D, Christopeit M, Peerzada AA, Singh SM, Kieser A, et al. Proteomic identification of C/EBP-DBD multiprotein complex: JNK1 activates stem cell regulator C/EBPalpha by inhibiting its ubiquitination. ***Oncogene*** 2007; 26: 1789-1801.
107. Hanke S, Besir H, Oesterhelt D, Mann M. Absolute SILAC for accurate quantitation of proteins in complex mixtures down to the attomole level. ***J Proteome Res*** 2008; 7: 1118-1130.
108. Graumann J, Hubner NC, Kim JB, Ko K, Moser M, Kumar C, et al. Stable isotope labeling by amino acids in cell culture (SILAC) and proteome quantitation of mouse embryonic stem cells to a depth of 5,111 proteins. ***Mol Cell Proteomics*** 2008; 7: 672-683.
109. Tam CS, Keating MJ. Chemoimmunotherapy of chronic lymphocytic leukemia. ***Nat Rev Clin Oncol*** 2010; 7: 521-532.
110. Hallek M, Pflug N. State of the art treatment of chronic lymphocytic leukaemia. ***Blood Rev*** 2011; 25: 1-9.
111. Voss T, Ahorn H, Haberl P, Dohner H, Wilgenbus K. Correlation of clinical data with proteomics profiles in 24 patients with B-cell chronic lymphocytic leukemia. ***Int J Cancer*** 2001; 91: 180-186.
112. Boyd RS, Adam PJ, Patel S, Loader JA, Berry J, Redpath NT, et al. Proteomic analysis of the cell-surface membrane in chronic lymphocytic leukemia: identification of two novel proteins, BCNP1 and MIG2B. ***Leukemia*** 2003; 17: 1605-1612.

113. Cochran DA, Evans CA, Blinco D, Burthem J, Stevenson FK, Gaskell SJ, et al. Proteomic analysis of chronic lymphocytic leukemia subtypes with mutated or unmutated Ig V(H) genes. ***Mol Cell Proteomics*** 2003; 2: 1331-1341.
114. Su X, Lucas DM, Zhang L, Xu H, Zabrouskov V, Davis ME, et al. Validation of an LC-MS based approach for profiling histones in chronic lymphocytic leukemia. ***Proteomics*** 2009; 9: 1197-1206.
115. Diefenbach CS, O'Connor OA. Mantle cell lymphoma in relapse: the role of emerging new drugs. ***Curr Opin Oncol*** 2010; 22: 419-423.
116. Williams ME, Connors JM, Dreyling MH, Gascoyne RD, Kahl BS, Leonard JP, et al. Mantle cell lymphoma: report of the 2010 Mantle Cell Lymphoma Consortium Workshop. ***Leuk Lymphoma*** 2010 Dec 6.
117. Aukema SM, Siebert R, Schuuring E, van Imhoff GW, Kluin-Nelemans H, Boerma EJ, et al. Double hit B-cell lymphomas. ***Blood*** 2010 Nov 30.
118. Antonucci F, Chilosi M, Parolini C, Hamdan M, Astner H, Righetti PG, et al. Two-dimensional molecular profiling of mantle cell lymphoma. ***Electrophoresis*** 2003; 24: 2376-8235.
119. Ghobrial IM, McCormick DJ, Kaufmann SH, Leontovich AA, Loegering DA, Dai NT, et al. Proteomic analysis of mantle-cell lymphoma by protein microarray. ***Blood*** 2005; 105: 3722-3730.
120. Miguet L, Béchade G, Fornecker L, Zink E, Felden C, Gervais C, et al. Proteomic analysis of malignant B-cell derived microparticles reveals CD148 as a potentially useful antigenic biomarker for mantle cell lymphoma diagnosis. ***J Proteome Res*** 2009; 8: 3346-3354.
121. Gulmann C, Espina V, Petricoin E 3rd, Longo DL, Santi M, Knutsen, T et al. Proteomic analysis of apoptotic pathways reveals prognostic factors in follicular lymphoma. ***Clin Cancer Res*** 2005; 11: 5847-5855.
122. Bongarzone I, Pierotti MA. The molecular basis of thyroid epithelial tumorigenesis. ***Tumori*** 2003; 89: 514-516.
123. Gimm O. Thyroid cancer. ***Cancer Lett*** 2001; 163: 143-156.
124. Brown LM, Helmke SM, Hunsucker SW, Netea-Maier RT, Chiang SA, Heinz DE, et al. Quantitative and qualitative differences in protein expression between papillary thyroid carcinoma and normal thyroid tissue. ***Mol Carcinog*** 2006; 45: 613-626.
125. Giusti L, Iacconi P, Ciregia F, Giannaccini G, Donatini GL, Basolo F, et al. Fine-needle aspiration of thyroid nodules: proteomic analysis to identify cancer biomarkers. ***J Proteome Res*** 2008; 7: 4079-4088.
126. Gorla L, Mondellini P, Cuccuru G, Miccichè F, Cassinelli G, Cremona M, et al. Proteomics study of medullary thyroid carcinomas expressing RET germ-line mutations: identification of new signaling elements. ***Mol Carcinog*** 2009; 48: 220-231.
127. Naora H, Montell DJ. Ovarian cancer metastasis: integrating insights from disparate model organisms. ***Nat Rev Cancer*** 2005; 5: 355-366.
128. Faca VM, Hanash SM. In-depth proteomics to define the cell surface and secretome of ovarian cancer cells and processes of protein shedding. ***Cancer Res*** 2009; 69: 728-730.
129. Faca VM, Ventura AP, Fitzgibbon MP, Pereira-Faça SR, Pitteri SJ, Green AE, et al. Proteomic analysis of ovarian cancer cells reveals dynamic processes of protein secretion and shedding of extra-cellular domains. ***PLoS One*** 2008; 3: 2425.
130. Nishizuka S, Chen ST, Gwadry FG, Alexander J, Major SM, Scherf U, et al. Diagnostic markers that distinguish colon and ovarian adenocarcinomas: identification by genomic, proteomic, and tissue array profiling. ***Cancer Res*** 2003; 63: 5243-5250.
131. Jones MB, Krutzsch H, Shu H, Zhao Y, Liotta LA, Kohn EC, et al. Proteomic analysis and identification of new biomarkers and therapeutic targets for invasive ovarian cancer. ***Proteomics*** 2002; 2: 76-84.
132. Ma X, Fan L, Meng Y, Hou Z, Mao YD, Wang W, et al. Proteomic analysis of human ovaries from normal and polycystic ovarian syndrome. ***Mol Hum Reprod*** 2007; 13: 527-535.
133. An HJ, Kim DS, Park YK, Kim SK, Choi YP, Kang S, et al. Comparative proteomics of ovarian epithelial tumors. ***J Proteome Res*** 2006; 5; 1082-1090.
134. Zhang Z, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. ***Cancer Res*** 2004; 64; 5882-5890.
135. Lim SO, Park SJ, Kim W, Park SG, Kim HJ, Kim YI, et al. Proteome analysis of hepatocellular carcinoma. ***Biochem Biophys Res Commun*** 2002; 291: 1031-1037.
136. Salvi A, Bongarzone I, Miccichè F, Arici B, Barlati S, De Petro G, et al. Proteomic identification of LASP-1 down-regulation after RNAi urokinase silencing in human hepatocellular carcinoma cells. ***Neoplasia*** 2009; 11: 207-219.
137. Wei X, Li L. Mass spectrometry-based proteomics and peptidomics for biomarker discovery in neurodegenerative diseases. ***Int J Clin Exp Pathol*** 2009; 2: 132-148.
138. Nielsen PA, Olsen JV, Podtelejnikov AV, Andersen JR, Mann M, Wisniewski JR, et al. Proteomic mapping of brain plasma membrane proteins. ***Mol Cell Proteomics*** 2005; 4: 402-408.
139. Kashem MA, Sarker R, Des Etages H, Machaalani R, King N, McGregor IS, et al. Comparative proteomics in the corpus callosal sub-regions of postmortem human brain. ***Neurochem Int*** 2009; 55: 483-490.
140. Hwang H, Zhang J, Chung KA, Leverenz JB, Zabetian CP, Peskind ER, et al. Glycoproteomics in neurodegenerative diseases. ***Mass Spectrom Rev*** 2010; 29: 79-125.
141. Murray J, Capaldi RA. Screening for the metabolic basis of neurodegeneration: developing a focused proteomic approach. ***Ann N Y Acad Sci*** 2008; 1147: 348-357.
142. Liu YJ, Shen H, Xiao P, Xiong DH, Li LH, Recker RR, et al. Molecular genetic studies of gene identification for osteoporosis: a 2004 update. ***J Bone Miner Res*** 2006: 21: 1511-1535.
143. Kubota K, Wakabayashi K, Matsuoka T. Proteome analysis of secreted proteins during osteoclast differentiation using two different methods: two-dimensional electrophoresis and isotope-coded affinity tags analysis with two-dimensional chromatography. ***Proteomics*** 2003; 3: 616-626.
144. Prahalad AK, Hickey RJ, Huang J, Hoelz DJ, Dobrolecki L, Murthy S, et al. Serum proteome profiles identifies parathyroid hormone physiologic response. ***Proteomics*** 2006; 6: 3482-3493.
145. Deng FY, Liu YZ, Li LM, Jiang C, Wu S, Chen Y, et al. Proteomic analysis of circulating monocytes in Chinese premenopausal females with extremely discordant bone mineral density. ***Proteomics*** 2008; 8: 4259-4272.
146. Buhmeida A, Al-Maghrabi J, Merdad A, Al-Thubaity F, Chaudhary A, Gari M, et al. Nuclear morphometry in prognostication of breast cancer in Saudi Arabian patients: comparison with European and African breast cancer. ***Anticancer Res*** 2010; 30: 2185-2191.
147. Al-Khattabi H, Kelany A, Buhmeida A, Al-Maghrabi J, Lari S, Chaudhary A, et al. Evaluation of HER-2/neu gene amplification by fluorescence in situ hybridization and immunohistochemistry in saudi female breast cancer. ***Anticancer Res*** 2010; 30: 4081-4088.