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ORIGINAL PAPER

Applications of SELDI-TOF technology in cancer biomarkers discovery

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Abstract

Cancers represent a major class of morbidity and the primary cause of death in the segment of active population. Improvement of therapeutically outcomes depends on more precise and earlier diagnostics, and biomarkers discovery and validation are considered the key for this, and also an essential tool in drug discovery.

Omics technologies are intensely involved in biomarkers research. SELDI-TOF mass spectrometry combines flexibility, resolving power and sensitivity with high throughput potential, generating a suitable proteomic platform for research and clinical applications.

SELDI platforms have been used to identify protein biomarkers in a variety of cancers, resulting in promising diagnostic strategies. Outcomes of SELDI technology in protein profiling of different samples prove its resources and reliability in biomarkers discovery, and recommend it as a major clinical tool of the future. Significant results obtained in pancreatic, colon, cervical, breast and brain tumors have been described. Relevant proteomic signatures could be identified in different sample types, setting up potential diagnostics and/or prognostics instruments for various cancers. Success in validation has already been achieved in some cases, yet the overall conclusion is that SELDI technology represents one component of a broader spectrum of versatile investigation technologies that may finally lead to setting up a more "clinical friendly" set of dedicated diagnostics tools.

Key words: SELDI-TOF, biomarkers, proteomics, mass spectrometry, early detection, cancer
Running title: **SELDI-TOF in cancer**

Introduction

Cancer represents a major challenge for the health systems throughout the world. At global level, it ranks third among causes of death, and in the western world is the second cause of death, after cardiovascular diseases. Unfortunately, it is the first death cause for the active population segment, namely for the 45-65 years age segment (1).

WHO estimations pointed out that in 2020 there will be an incidence of 16 million cancer cases at global level. Cancer has a death toll of seven million per year worldwide, which represents 12.5% (2).

Early detection and treatment improvement are key issues for all types of cancer. Non-invasive tests for early detection are suitable for blood or other types of body fluids; however, cancer classification are often achieved using tissues samples (tumoral and peritumoral), as they represent a source of more reliable discrimination between different types and subtypes of cancer. Therefore, protein biomarkers detected in blood or tissues may play important roles in cancer detection, monitoring and treatment (3).

Because of the lack of early detection methods and of the deficiency in specific and sensitive biomarkers, both scientists and clinicians are moving towards using proteomics as a mean to discover and validate new biomarkers or pattern of biomarkers. The new discovered

biomarkers have to prove better specificity and sensitivity than the existing ones and furthermore improve our understanding in cancer initiation and progression (2).

Therefore, it is now necessary to identify new tumor associated serum proteins with a clear advantage over the markers that have been previously assessed in clinical settings. These new biomarkers can be used clinically as markers to detect early cancer and to monitor therapy or recurrence of disease (4).

It is commonly agreed upon the characteristics of a suitable cancer biomarker that is generally a substance found in an altered amount in the body, implying its presence associated to a certain type of cancer. Unfortunately, the development of tumor associated serum protein biomarkers over the past few decades has not been effective for diagnosing primary cancer.

There is a considerable pressure for the discovery of new disease-related biomarkers. Biomarkers are quantitative measures of biological effects that provide informative links between mechanism of action and clinical effectiveness. They can provide new insights into a drug mechanism of action, metabolism, efficacy and safety, and into disease mechanisms and disease course (5).

Furthermore, the identification of diagnostic biomarkers will be essential for improved early intervention in disease, and will be a key technology in the development of more focused drug prescribing. However, the vision will be achieved if there is the right approach to optimization of biomarker investment, performance and application. This is a deliverable core of translational medicine research (6).

Multiple investigative instruments have been "launched" in the space of biomarker discovery. Emerging **omics** technologies are among the best suited to approach the domain.

Genomics

Genomic analysis is one of the most intensively exploited "instrument" in biomarkers discovery; it is deciphering cancer biomarkers by a large array of distinct, "analyte oriented" approaches, that include DNA sequencing, RNA expression profiling, epigenetics, microRNA (7).

DNA sequencing is frequently used to identify changes in candidate genes. The new generation of sequencing technologies has accelerated the discovery process of DNA sequence variants with clinical significance. The detection of DNA sequence versions, or small insertions or deletions in genes are now entering the clinical practice. Oncogenic DNA sequence variants became diagnostic biomarkers providing specific therapeutic targets. Large scale sequencing of candidate genes provides new biomarkers for diagnosis and prognosis of cancers. Somatic DNA sequence analysis has recently expanded to genome-wide high-performance DNA sequencing leading to whole genome analysis of DNA sequence variations in tumors (8, 9, 10).

The ***mRNA expression*** is used to study gene expression profiles as relevant biomarkers for stage or specific prognosis classification of cancer patients. Gene expression profiles containing panel of mRNAs can be more effective than standard methods for patient stratification such as histopathology biomarkers. Biomarker discovery usually starts with large scale gene profiling (on microarrays) with a technological progression for clinical application to simple microarrays with fewer probes or multiplexed quantitative RT-PCR. There are numerous software approaches to pathways analysis for a given gene expression dataset. Those that weight the impact of the particular genes provide additional guidance to the identification of shorter lists of biomarkers (11, 12).

Epigenetic modifications are the most recent candidate biomarkers; groups of CpG methylation events in promoters are easily detected by PCR-sequencing of DNA from cells or tumors, or by methylation specific PCR techniques. DNA sequencing is providing whole genome approaches to discover novel epigenetic changes at DNA methylation level. Aberrant

promoter methylation has been found in growth suppressive genes in human tumorigenesis (13, 14).

Due to their small size, ***MicroRNAs*** (miRNAs) have an elevated potential as biomarkers, since they are easily quantified in normal and cancer tissues as well as body fluids. However, this field is still in a very early stage, thus no miRNA biomarkers are in clinical use (15, 16).

Proteomics

Protein biomarkers show a high potential of conversion into clinical diagnostic tests. Often, they are identified in basic science studies of cancer cells as over-expressed proteins. Cancer-specific alterations in proteins may occur at the level of protein abundance or post-translational protein modification such as glycosilation or phosphorylation (7). There are a number of direct approaches to identify cancer specific changes in proteins including abundance or post-translational modifications.

Proteomic changes in cancer can be identified by **two-dimensional gel electrophoresis**. In this technique different visualization approaches may be applied: radioactive labeling, covalent attachment of fluorescent tags and silver staining. Significant enhancements seem to be provided by the recent development of DIGE, which combines the ease and sensitivity of detection with the simultaneous processing of samples and controls.

Mass spectrometric analysis can represent a stand-alone approach for biomarker discovery, or a second stage, for post-electrophoretic sequence identification. 2DE-MALDI platforms already have a long history in biomarkers discovery (16), and they still represent a major player.

Proteomic mass spectroscopy - based platforms have provided the ability to identify large numbers of novel proteins with the potential to be biomarkers. These studies are being performed in various biological matrices such as cultures of normal and tumor cells or human clinical samples (serum, plasma, urine, cerebrospinal fluid and saliva). Novel biomarker discovery technologies are developed using quantitative isotopic labeling, improved mass spectroscopy algorithms and improved sample preparation (17). Isotopic labeling is exploited for relative quantification of proteins by MALDI-TOF or LC-MS/MS (Liquid chromatography-mass spectrometry/mass spectrometry) high-resolution mass spectrometry (18, 19).

New methods for rapid identification of both known and unknown proteins are poorly developed. Matrix-assisted laser desorption and ionization with time-of-flight detection mass spectrometry (MALDI-TOF MS) and surface-enhanced laser desorption and ionization with time-of-flight spectrometry (SELDI-TOF) are two of the methods being currently used. MALDI techniques immobilize protein/peptide samples in an energy absorbing matrix (chemical) on a chip or plate. The entire repertoire of proteins in the sample interacts with the matrix from which a selected subset of proteins is bound to, a function of the composition of the selected matrix. MALDI analysis is well suited for resolution of proteins <20 kDa, the low molecular weight proteome. It proves relevant resolving power for peptide fragments, in association with bioinformatics tools, while recent systems are also able to perform de novo sequencing.

Conversely, SELDI technology uses selective surfaces for binding a subset of proteins based on absorption, partition, electrostatic interaction or affinity chromatography on a solid-phase protein chip surface (20, 21). In SELDI, like in MALDI, the protein-bound chip is pulsed with laser energy causing proteins or protein fragments to ionize and fly from the chip surface down a vacuum tube to the detector plate. The time of flight is affected by the mass of the particle and the charge it bears (m/z ratio). The detector plate records the intensity of the signal at a given m/z value, and a spectrum is generated. The different peaks in the spectrum correspond to different m/z protein species. This data stream of information can be coupled

with data streams from a series of test subjects and complex bioinformatics to define discriminants for cancer detection.

Microarrays - multianalyte protein detection in complex matrices is approached using highly parallel immunoassays on antibody microarrays that resemble highly parallel ELISAs. There are a few approaches for this type of biomarker discovery tool (22, 23). The protein analytes can be measured in serum, plasma, or urine for non-invasive biomarker assays or in extracts of tumor tissue for evaluation of prognostic biomarkers or potential therapeutic targets (23).

Biomarker research is considered a key to understand the mechanism of the disease, optimization of diagnostics and patient monitoring, as well as an important tool in new drug discovery and validation. It addresses multiple classes of analytes, relies on emergent laboratory and information technologies; although still novel as field of research, it already divides in a "core" of basic research approach that closely relates to systems biology and a set of clinical, translational applications where validation and robustness appear to be the keywords.

The purpose of this paper is to present SELDI mass spectrometry as state of the art technology for cancer biomarkers discovery.

SELDI-TOF – an overview

SELDI-TOF MS is a novel approach in proteomics, which was introduced by Hutchens T.W. and Yip T.T (24). SELDI-TOF MS allows for integral protein profiling from a variety of complex biological materials such as serum, blood, plasma, tissue, urine, saliva, cell lysis products with limited sample preparation.

The system is most effective when profiling low molecular weight proteins (<20 kDa). SELDI-TOF MS is more sensitive, faster and requires smaller amounts of sample than other proteomics methods.

The SELDI-TOF MS technology consists of three major components: the ProteinChip Array, the reader, and the software.

The ProteinChip Array uses surfaces designed to select proteins from crude extracts according to general or specific protein properties. Surfaces can be treated with biochemical agents, such as antibodies or other ligands, and thus can be designed to interact specifically with unique target proteins, while the chemically treated surfaces retain whole classes of proteins (25).

The procedure for detecting protein biomarkers follows few steps. A small amount of the complex biological sample is applied directly to ProteinChip arrays. Proteins bind to chemical or biological "docking sites" on the ProteinChip surface through selective interaction. Unbound proteins and buffer contaminants are washed away, eliminating sample's "noise". Energy absorbing molecule (EAM) solution is applied, producing aggregates of protein with multiple EAM units.

The ProteinChip SELDI reader uses a nitrogen laser to desorb and ionize the sample. Photon absorption induces EAM ionization; ionized EAMs are transferring charge with protein. Electrostatic interaction generally prevents multiple charging of proteins and also induces separation of charged EAMs from the charged protein. In short, the laser energy induces both protein ionization and a change of state from the solid, crystalline phase into the mobile phase. The charged analyte can move very rapidly ("fly"), upon application of a voltage differential. TOF MS region of the instrument measures the mass-to-charge ratio (m/z) of each protein, based on its velocity through an ion chamber.

Protein clusters are defined based on molecular weight, and intensity variation is compared to normalized spectra, generating reports on relative distributions.

The versatility of SELDI-TOF MS approach for protein analysis has been demonstrated by several published applications.

The unlimited type of samples and the unique surface chemistries of the arrays allow the researcher to exploit chemical and biochemical characteristics of protein families, to capture and retain proteins on the surface, and to analyze the captured proteins by TOF MS or MS/MS directly on the array.

A specific pattern can be distinguished using statistical programs such as Biomarker Patterns Software and artificial neural network (ANN) algorithm.

Proteomic pattern analysis by SELDI-TOF-MS is one of the most promising new approaches for the discovery and identification of potential biomarkers for various diseases (26).

Applications of SELDI proteomics in oncology

One of the important goals of oncology is to develop biomarkers that can be identified through simple, less invasive methods proving the potential to identify cancer risk, possibility to improve early diagnostic, display utility in accurate grading and treatment monitoring (27).

The clinical investigations using SELDI-TOF-MS for different types of cancer e.g., pancreatic, colorectal, cervical, breast, brain, and several infectious diseases revealed high sensitivities and specificities for diagnostic (28).

Several different proteomic methods have been described in literature (29). While it is commonly agreed that no single method is suitable for biomarker discovery, a suitable combination of methods needs to be chosen depending on the type of material to be analyzed, the genre of disease and previous knowledge of the putative markers. The proteomic technologies appear to form the mainstay of the serum proteomics and several poor outcome types of cancers would benefit from these approaches.

Pancreatic cancer

Pancreatic cancer has a very poor prognosis, fact that is partially due to its diagnosis at late clinical stages of the disease, the tumor's aggressive nature and limited response to therapy.

Quantitative proteomic profiling of serum samples to identify differentially expressed proteins represents a very promising approach for improving the outcome of this disease and was the subject of various studies (30, 31).

Several laboratories have demonstrated the feasibility of using SELDI mass spectrometry for the diagnosis and detection of pancreatic cancer. The survival rate of pancreatic cancer patients is the lowest among those with common solid tumors, and early detection is one of the most feasible means of improving outcomes (32, 33).

Honda K. et al. compared plasma proteomes between pancreatic cancer patients and sex- and age-matched healthy controls using SELDI coupled with hybrid quadruple time-of-flight mass spectrometry (32). A discriminating proteomic pattern was extracted from a training cohort (71 pancreatic cancer patients and 71 healthy controls) using a support vector machine learning algorithm and was applied to two validation cohorts. A set of four mass peaks was identified at 8766, 17272, 28080, and 14779 m/z, whose mean intensities differed significantly (Mann-Whitney U test, $p < 0.01$), accurately discriminating cancer patients from healthy controls in the training cohort sensitivity of 97.2%, specificity of 94.4%, and area under the curve value of 0.978.

The SELDI study of Ehmann M. et al., comparing 96 serum samples from PAC patients and 96 healthy controls, was in concordance with the results of Honda K. et al. (32, 34). The protein peaks at 8700, 17270, and 28090 m/z were very close to the masses identified previously in the study of Honda K. et al. (32). Therefore two independent groups that analyzed different samples with SELDI-TOF-MS achieved similar results. The

independent identification of the same protein cluster as tumor signature confirms SELDI-TOF analysis as a robust method for serum profiling.

Ge C. et al. have been able to establish a serum protein pattern model for screening pancreatic cancer (35). The resulting profiles when comparing serum from cancer and normal patients were analyzed with the Biomarker Wizard system, to establish a model using the Biomarker Pattern system software. A double-blind test was used to determine the sensitivity and specificity of the model. A group of 4 biomarkers (relative molecular weights 5705, 4935, 5318, 3243 Da) was selected to set up a decision tree to produce the classification model to effectively screen pancreatic cancer patients. The results yielded a sensitivity of 100%, specificity of 97.4%, ROC (Receiver operating characteristic) area of 99.7%. A double-blind test used to challenge the model resulted in a sensitivity of 88.9% and a specificity of 89.5%.

Another study was aimed to demonstrate that SELDI profiling of serum is significantly better than the current standard serum biomarker CA19–9 distinguishing patients with pancreatic cancer from those with pancreatitis and from healthy controls. The superiority of SELDI was evident over multiple serum fractions and multiple array types (36).

In a preliminary study performed on pancreatic cancer, we attempted to establish the protein profile and to apply proteomic technologies in identification of biomarkers for early detection of cancer and possible therapeutic targets.

Our data were obtained using SELDI-TOF MS proteomic technology on serum samples from 3 groups - pancreatic cancer (PDAC), pancreatitis (PAN) and healthy subjects (CTRL). The samples were analyzed on Q10 ProteinChip Arrays (strong anion exchanger). Data acquisition and processing were achieved on SELDI-TOF MS Personal Edition, using the ProteinChip Data Manager Software 3.0.7. Cluster analysis led to the selection of 44 clusters; two distinct subsets of clusters could be extracted: a first subset of 17 signatures differentiates PDAC patients vs. normal, and a second subset of 7 signatures, with good relevance between PDAC and pancreatitis.

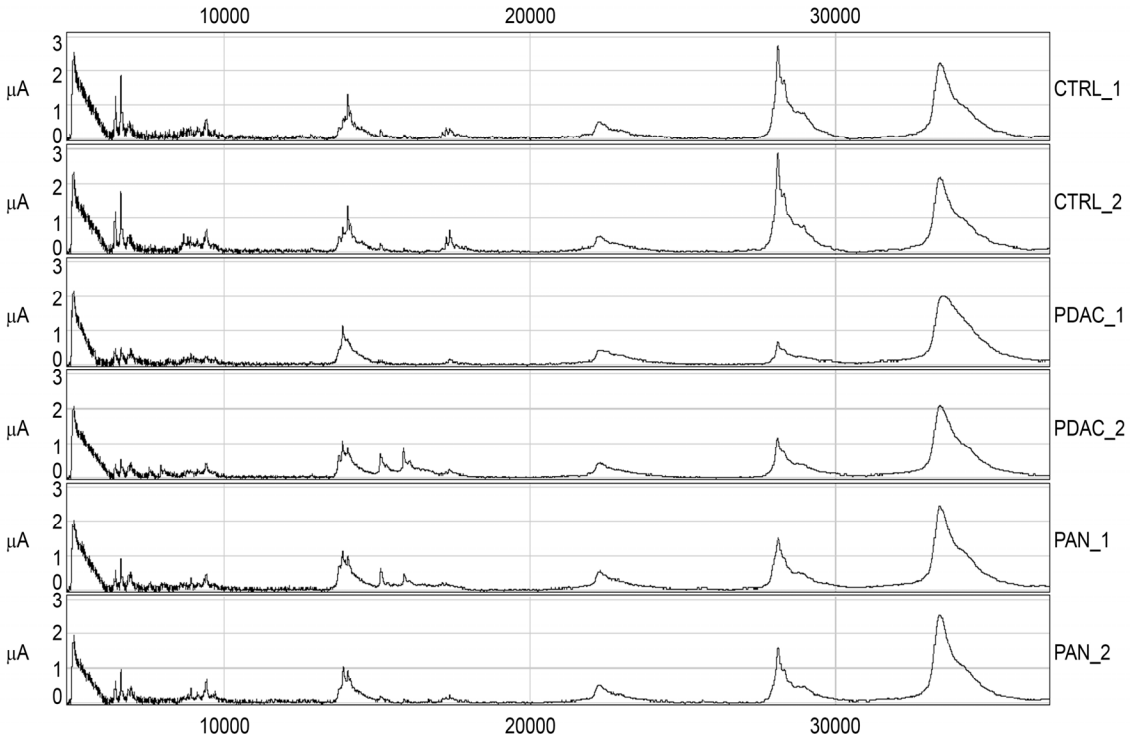

Figure 1. Representative spectra of the selected patients and healthy individuals (pancreatic cancer (PDAC), pancreatitis (PAN) and healthy subjects (CTRL))

Colorectal cancer

Colorectal cancer (CRC) stands as the third cause of death among cancers, affecting almost equally male and female population. It has an elevated aggressiveness and a high potential of metastasis.

The therapeutical outcome is considerably influenced by early approaches, as well as of adequacy of therapy, thus, early detection, precise classification and clear definition of patient variables are considered of severe importance. The importance of early/non-invasive biomarkers detection in colorectal cancer was proven by the use of screening programmes based on the fecal occult blood test that have resulted in a significant reduction in mortality caused by colorectal cancer (37, 38). Attempts on other serum biomarkers - MMP-9, complement C3a des-arg and α-defensins (39) or other proteomics studies seeking characteristic diagnostic signatures (40) had limited success, but demonstrated sensitivities and specificities superior to CEA.

SELDI technology was applied for CRC biomarkers discovery studies, on biological materials such as blood/serum, urine, feces or tissue samples. Several reports have been made of differential expression of the same m/z values in colorectal cancer, even though different chip surfaces were used.

In a double study on serum proteome profiling for CRC's, Engwegen J.Y. et al. applied a classification tree strategy for the identification of biomarkers. Using a CM10 chip, out of a group of 16 proteins with modified expression, the best candidate biomarkers were selected for the m/z peaks proteins 3100, 3300, 4500, 6000 and 28000 Da, with sensitivities ranging from 77.8 to 89.5% and specificities ranging 73.3-89.9% (41).

Yu J.K. et al (42) reported a 3329 and 6669 Da proteins to be differentially expressed on a hydrophobic chip surface, that were not selected in the final diagnostic pattern. In the same study, a 4477 Da protein, which was a classifier in the final pattern, was also up-regulated in colorectal cancer patients.

In another study, IMAC chips were used for serum protein profiling, leading to a protein pattern that provided reliable discrimination on a second blind group. Masked analysis of an independent set of serum samples showed the diagnostic pattern could differentiate patients with different stages of colorectal cancer from healthy subjects with a sensitivity of 95.00 % and specificity of 94.87 % (43).

Proteomic changes occur in and can be detected at early stages, such as Duke stages A, B. Due to proteolytic activities, such as those of MMPs, peptides can be present primarily in blood, but their detection is affected by the presence of major proteins, such as albumin or gamma-globulins. Renal excretion is one major pathway for their clearance, therefore urine offers improved detection of peptides, due to the lack of dominant proteins.

Ward D.G. et al. performed a study on urine samples, using a combination of SELDI (IMAC chips) and MALDI technology and 19 peaks were finally sorted from an initial group of 100 low mass (1.5-20 kDa) and 35 high mass (20-200 kDa) peaks. These were found significantly different in patients, and offered 78% sensitivity and 87% specificity in discriminating patients from controls (39). Four peaks – with m/z values of 6086, 11750, 11960, 39480 and 53760 Da, were proved to have a very good correlation with cancer in early stages. Expression of hepcidin by colon cancer cells was demonstrated by Ward et al. using MALDI-TOF and SELDI-TOF MS in urine samples harvested from colon cancer patients and in surgical resection specimens. Hepcidin 20 (m/z = 2195) and hepcidin 25 (m/z = 2793) were proved to correlate with disease T stage (44).

Cervical cancer

Cervical cancer is the third most common form of cancer affecting women over 25 years. Human papilloma virus (HPV) has been shown to play a major role in cervical its development (45).

Melle C. et al. et al. used ProteinChip technology to analyze pure micro-dissected tumor cells with and without HPV infection to detect discriminating protein profiles which elucidated the infection activated pathways (46). In this investigation, 18 proteins from tumor tissues have been identified by peptide fingerprint mapping and SELDI MS after separation by 2-DE. This study, investigating for the first time proteomic changes in micro-dissected HPV infected tumor tissue, provides an indication on the oncogenic potential of viruses. The SELDI measurements of all tissue samples detected up to 373 peaks in the 2.5–200 kDa interval, with normalized intensities.

Wong et al. used SELDI analysis to differentiate cervical cancer from non-cancer patients on tissue were applied to WCX2 ProteinChip arrays. A training set was used to develop a classification scoring system and a blind test set was used to evaluate this scoring systems ability to distinguish cervical cancer from non-cancer samples. Using this model, a sensitivity of 87%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 86% for the test population were obtained. Seven proteins were down-regulated in cervical cancer cells compared to normal cervical epithelial cells (47). These findings suggest that SELDI mass spectrometry may play a role in distinguishing cervical cancer from normal cervical cells (48).

SELDI-MS has also been successfully used for the detection of differentially expressed proteins in the serum of patients with cervical cancer. SELDI-TOF MS was utilized to detect the serum proteomic patterns in patients with early stage cervical squamous cell carcinoma, patients with cervical intraepithelial neoplasia III (CIN III), and healthy women (control) (49).

Xia T. et al. have identified a total of 122 protein peaks at the molecular range of 1,5 to 20 kD, among which 19 ones were significantly different between invasive cervical squamous cell carcinomas and controls ($p<0,001$). A diagnostic model consisting of 2 protein peaks at 3977 and 5807 m/z was established. Its specificity was 83.78% and its sensitivity was 97.29%. A sensitivity of 94.44% and a specificity of 94.44% in a large-scale blind test were obtained. The diagnostic model consisting of 2 protein peaks at 3977 and 5807 m/z can discriminate cervical cancer patients from healthy women (49).

In another study, on weak cationic chips, fifty-two differentially expressed proteins were detected in the serum of cervical cancer patients ($p<10^{-5}$), among which 6 proteins with mass/charge ratio of 4173.77, 5903.09, 6087.12, 10716.9, 6109.61 and 3397.41, respectively, showed lowered expression in the serum of cervical cancer patients. Two diagnostic models for cervical cancer were generated using a specific software: one based on the 4173.77 (m/z) protein, with the diagnostic specificity of 96% and sensitivity of 75% for cervical cancer and the other based on 3 proteins (5335.81, 7562.99, and 9287.89 (m/z)) with specificity of 91.67% and sensitivity of 96 % (50).

Breast cancer

Breast cancer represents a major burden, since it represents about one third of the cancer cases in women, and the second cause of death in female population, after lung cancer.

Early detection is considered essential in reducing mortality in breast cancer, and protein profiling could contribute to distinguishing high risk patients, and orient personalized therapies (51).

As well in this type of cancer, SELDI-TOF MS has been used to investigate a broad range of biological samples: tissue (52) plasma (53), serum (54), nipple aspirate (55) and ductal lavage fluid (56).

Therefore, Gast M.C. et al. used SELDI technology to analyze serum and tissue samples, on IMAC30-Ni chips and Q10 chips, on breast cancer patients. Three relevant peaks were identified in serum, while tissue samples resulted in 14 cancer related peaks (57). The

same authors reported on a larger number of cases the use of SELDI-technology associated to a "decision tree" strategy; a signature of 14 serum proteins was defined as high discriminating power diagnostic tool (58).

Several SELDI-TOF MS peaks (not structurally identified) were reported to differentiate between serum or plasma of breast cancer patients and/or healthy controls (52, 53).

Tissue protein profiling is reflecting the earliest changes determined by the genetic mutations leading to breast cancer, thus, the concentration of potential biomarkers is highest in tumor and its microenvironment (59). However, tissue sampling is highly invasive, thus limiting the amount of protein profiling studies performed so far (60). SELDI-TOF analysis of tumor tissue lysates revealed peaks significantly associated with lymph node status or cancer subtype (61, 62).

Brain tumors

The prognosis of patients with glioblastoma, the most malignant adult glial brain tumor, remains poor in spite of the advances in treatment procedures, including surgical resection, irradiation and chemotherapy.

As well in this cancer pathology serum biomarkers have the potential to revolutionize cancer diagnosis, grading, prognostic and treatment response monitoring. The major applications of serum biomarkers are diagnosis and accurate grading of tumor (63).

Although challenging, proteomic profiling by SELDI-TOF-MS has recently also become one of the most important areas in human brain tumor research.

Petrik V. et al, identified serum biomarkers that improve survival prediction in patients with brain tumors using SELDI-TOF MS. One peak, identified as the B-chain of α2-Heremans-Schmid glycoprotein (AHSG), was less prominent in accordance with increasing tumor grade (64).

In another study using weak cation-exchange chips a total of 140 serum samples from brain tumors patients were analyzed. Seven serum biomarkers were significantly deregulated in tumors group compared to the normal control group. Among them, four were up-regulated and three were down-regulated. A sensitivity of 84.6% and a selectivity of 84.6% were achieved to discriminate tumors from controls (65).

Intensity of two peaks, m/z 7567 and 15115, were significantly higher in low grade compared with high grade astrocytoma. Using the combination of SELDI-TOF MS with an artificial neural network (ANN) algorithm, the screening and evaluation of protein biomarkers for the differentiation between malignant and benign brain tumors was improved (66).

Melanoma

Malignant cutaneous melanoma is the most serious form of skin cancers and originates from the melanocytes of the epidermis. The incidence of melanoma is increasing steadily at a rate of 5% per year throughout the world. Mortality is also increasing, but at a slower rate than the increase in incidence (67, 68).

In melanoma, several serum markers have been evaluated, but they are only available as prognostic variables, to monitor response to therapy and to detect recurrence (68).

Proteomics analysis has been proposed as a novel tool that would lead to the discovery of potential new tumor markers.

SELDI-TOF MS profiling of serum proteins, coupled with univariate and multivariate statistical data analyses, could effectively discriminate between melanoma patients and controls, using a proteomic pattern to investigate the feasibility of diagnosing melanoma before metastases occurrence. Multiprotein classifier was tested in an independent validation set of melanoma and non-cancer serum samples patients, maintained in a good diagnostic accuracy of 98.1% (sensitivity 96.7%, specificity 100%), and 100% stage I/II classification assignment (67).

Lakomy R. et al. indicated that it is hard to identify any significant differences in protein profiles or laboratory parameters in the predefined chemo-sensitive or chemo-resistant groups of patients. They investigated human blood serum samples from patients with metastatic malignant melanoma treated with palliative chemotherapy. The analysis was performed by SELDI-TOF MS (69).

Wilson L.L. et al. compared serum proteomic profile from patients with stage I and II melanoma that developed the diseases or had no recurrence in order to identify prognostic markers. Weak cationic exchange chromatography (WCX2), metal binding and IMAC3-Cu protein chips were used for SELDI analysis. Results showed a sensitivity of 72% and a specificity of 75% for the prediction of recurrence. Data of this first clinical pilot study were not analyzed using relevant bioinformatics classification algorithms, and results were not validated in a blinded population set (70).

Conclusions

The deployment of validated biomarkers will result in improvement of clinical study designs in more suitably defined populations. The application of biomarkers will translate into benefits such as:

- Improvement of early diagnostics and prognosis
- Increasing the probability of therapeutic programme success and reduction of treatment cycles, matching patients with therapy;
- Faster optimization of therapy;
- Improved compliance with therapy;
- Reduced complications of disease and less therapy related adverse effects;
- More efficient drug development;
- More efficient healthcare delivery;
- Reduced social healthcare burden.

SELDI-TOF technologies have proved significant advantages as a “first line” approach in biomarker discovery, due to its relative simplicity and its high-throughput potential, yet it needs back-up from other proteomics instruments (such as 2DE and MALDI-TOF/TOF) in order to produce a clear identification and validation of proposed biomarkers.

Relevant proteomic signatures could be identified in different sample types, setting up potential diagnostics and/or prognostics instruments for various cancers. Success in validation has already been achieved is some cases, yet the overall conclusion is that SELDI technology represents one component of a broader spectrum of versatile investigation technologies that may finally lead to setting up a more “clinical friendly” set of dedicated diagnostics tools.

The use of proteomics in biomarker discovery is one of the major options, and individual platforms or platform combinations are often used. SELDI-TOF is often doubled by MALDI-TOF in biomarker discovery studies, the latter being applied to provide “in depth” biomarker identification.

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References

1. National Vital Statistics Report 50 (15) 2002.
2. L.C. WHELAN, K.A.R. POWER, D.T MCDOWELL, J. KENNEDY, W.M. GALLAGHER**,** Applications of SELDI-MS technology in oncology, Molecular Diagnosis. *J. Cell. Mol. Med*., 12 (5A), 1535-1547 (2008).
3. M. LU, K. F. FAULL, J.P. WHITELEGGE, J. HE, D. SHEN, R.E. SAXTON, H.R. CHANG, Proteomics and Mass Spectrometry for Cancer Biomarker Discovery. *Biomarker Insights*, 2, 347–360 (2007).
4. V. SEIBERT, M.P. EBERT, T. BUSCHMANN, Advances in clinical cancer proteomics: SELDI-ToF-mass spectrometry and biomarker discovery. *Briefings in functional genomics and proteomics*. 4(1), 16–26 (2005).
5. The Innovative Medicines Initiative (IMI)*, Strategic Research Agenda "Creating Biomedical R&D Leadership for Europe to Benefit Patients and Society"* http://www.efpia.org/4_pos/SRA.pdf, 40 (2006).
6. A.J. ATKINSON , W.A. COLBURN , V.G. DEGRUTTOLA, D.L. DEMETS, G.J. DOWNING, D.F. HOTH, J.A. OATES, C.C. PECK, R.T. SCHOOLEY, B.A. SPILKER, J. WOODCOCK, S.L. ZEGER**,** Biomarkers and Surrogate Endpoints: Preferred Definitions and Conceptual Framework. *Clinical Pharm. & Therapeutics,* 69(3), 89–95 (2001).
7. M.A. TAINSKY**,** Genomic and proteomic biomarkers for cancer: a multitude of opportunities, *Biochim Biophys Acta,* 1796(2), 176-93 *(*2009).
8. T.J. LEY, E.R. MARDIS, L. DING, B. FULTON, M.D. MCLELLAN, K. CHEN, D. DOOLING, B.H. DUNFORD-SHORE, S. MCGRATH, M. HICKENBOTHAM, L. COOK, R. ABBOTT, D.E. LARSON, D.C. KOBOLDT, C. POHL, S. SMITH, A. HAWKINS, S. ABBOTT, D. LOCKE, L.W. HILLIER, T. MINER, L. FULTON, V. MAGRINI, T. WYLIE, J. GLASSCOCK, J. CONYERS, N. SANDER, X. SHI, J.R. OSBORNE, P. MINX, D. GORDON, A. CHINWALLA, Y. ZHAO, R.E. RIES, J.E. PAYTON, P. WESTERVELT, M.H. TOMASSON , M. WATSON, J. BATY, J. IVANOVICH, S. HEATH, W.D. SHANNON, R. NAGARAJAN, M.J. WALTER, D.C. LINK, T.A. GRAUBERT, J.F. DIPERSIO, R.K. WILSON, DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome**.** *Nature*. 456(7218), 66–72 (2008).
9. L. DING, G. GETZ, D.A. WHEELER, E.R. MARDIS, M.D. MCLELLAN, K. CIBULSKIS, C. SOUGNEZ, H. GREULICH, D.M. MUZNY, M.B. MORGAN, L. FULTON, R.S. FULTON, Q. ZHANG, M.C. WENDL, M.S. LAWRENCE, D.E. LARSON, K. CHEN, D.J. DOOLING, A. SABO, A.C. HAWES, H. SHEN, S.N. JHANGIANI, L.R. LEWIS, O. HALL, Y. ZHU, T. MATHEW, Y. REN, J. YAO, S.E. SCHERER, K. CLERC, G.A. METCALF, B. NG, A. MILOSAVLJEVIC, M.L. GONZALEZ-GARAY, J.R. OSBORN, R. MEYER, X. SHI, Y. TANG, D.C. KOBOLDT, L. LIN, R. ABBOTT, T.L. MINER, C. POHL, G. FEWELL, C. HAIPEK, H. SCHMIDT, B.H. DUNFORD-SHORE, A. KRAJA, S.D. CROSBY, C.S. SAWYER, T. VICKERY, S. SANDER, J. ROBINSON, W. WINCKLER, J. BALDWIN, L.R. CHIRIEAC, A. DUTT, T. FENNELL, M. HANNA, B.E. JOHNSON, R.C. ONOFRIO, R.K. THOMAS, G. TONON, B.A. WEIR, X. ZHAO, L. ZIAUGRA, M.C. ZODY, T. GIORDANO, M.B. ORRINGER, J.A. ROTH, M.R. SPITZ, I. WISTUBA, B. OZENBERGER, P.J. GOOD, A.C. CHANG, D.G. BEER, M.A. WATSON, M. LADANYI, S. BRODERICK, A. YOSHIZAWA, W.D. TRAVIS, W. PAO, M.A. PROVINCE, G.M. WEINSTOCK, H.E. VARMUS, S.B. GABRIEL, E.S. LANDER, R.A. GIBBS, M. MEYERSON, R.K. WILSON, Somatic mutations affect key pathways in lung adenocarcinoma*. Nature*. 455, 1069–1075 (2008).
10. R.J. LEARY, J.C. LIN, J. CUMMINS, S. BOCA, L.D. WOOD, D.W. PARSONS, S. JONES, T. SJÖBLOM, B.H. PARK, R. PARSONS, J. WILLIS, D. DAWSON, J.K. WILLSON, T. NIKOLSKAYA, Y. NIKOLSKY, L. KOPELOVICH, N. PAPADOPOULOS, L.A. PENNACCHIO, T.L. WANG, S.D. MARKOWITZ, G. PARMIGIANI, K.W. KINZLER, B. VOGELSTEIN, E. VELCULESCU, Integrated analysis of homozygous deletions, focal amplifications, and sequence alterations in breast and colorectal cancers*. Proc. Natl. Acad. Sci. U. S. A.*, 105, 16224–16229 (2008).
11. S. OKUDA, T. YAMADA, M.A. HAMAJIMA, M. ITOH, T. KATAYAMA, P. BORK, S. GOTO, M. KANEHISA. KEGG Atlas mapping for global analysis of metabolic pathways. *Nucleic Acids Res*. 36, W423 – 6 (Web Server issue) (2008).
12. I. SEGOTA, N. BARTONICEK, K. VLAHOVICEK, MADNet: microarray database network web server, Nucleic. *Acids. Res*, 36, W332–W335 (Web Server issue) (2008).
13. M.O. HOQUE, Q. FENG, P. TOURE, A. DEM, C.W. CRITCHLOW, S.E. HAWES, T. WOOD, C. JERONIMO, ROSENBAUM E, J. STERN, M. YU, B. TRINK, KIVIAT NB, D. SIDRANSKY, Detection of aberrant methylation of four genes in plasma DNA for the detection of breast cancer*, J. Clin. Oncol.* 24, 4262–4269, (2006).
14. Y. MURAKI, K. BANNO, M. YANOKURA, Y. KOBAYASHI, M. KAWAGUCHI, H. NOMURA, A. HIRASAWA, N. SUSUMU, D. AOKI, Epigenetic DNA hypermethylation: clinical applications in endometrial cancer. *Oncol Rep.,* 22(5), 967-72 (2009).

15. C.H. LAWRIE, S. GAL, H.M. DUNLOP, B. PUSHKARAN, A.P. LIGGINS, K. PULFORD, A.H. BANHAM, F. PEZZELLA, J. BOULTWOOD, J.S. WAINSCOAT, HATTON CS, A.L. HARRIS, Detection of elevated levels of tumor-associated microRNAs in serum of patients with diffuse large B-cell lymphoma*, Br. J. Haematol. ,* 141, 672–675 (2008).
16. M. ROESSLER, W. ROLLINGER, L. MANTOVANI-ENDL, M.L. HAGMANN, S. PALME, P. BERNDT, A.M. ENGEL, M. PFEFFER, J. KARL, H. BODENMÜLLER, J. RÜSCHOFF, T. HENKEL, G. ROHR, S. ROSSOL, W. RÖSCH, H. LANGEN, W. ZOLG, M. TACKE, Identification of PSME3 as a novel serum tumor marker for colorectal cancer by combining two-dimensional polyacrylamide gel electrophoresis with a strictly mass spectrometry-based approach for data analysis**.** *Mol. Cell. Proteomics,* 5, 2092–2101, (2006).
17. J. MARTOSELLA, N. ZOLOTARJOVA, H. LIU, G. NICOL, BE BOYES, Reversed-phase high performance liquid chromatographic prefractionation of immunodepleted human serum proteins to enhance mass spectrometry identification of lowerabundant proteins. *J. Proteome. Res.,* 4, 1522–1537, (2005).
18. V. FACA, M. CORAM, D. PHANSTIEL, V. GLUKHOVA, Q. ZHANG, M. FITZGIBBON, M. MCINTOSH, S. HANASH, Quantitative analysis of acrylamide labeled serum proteins by LC-MS/MS. *J. Proteome. Res.***,** 5, 2009–2018, (2006).
19. V.P. ANDREEV, L. LI, T. REJTAR, Q. LI, J.G. FERRY, B.L. KARGER, New algorithm for 15N/14N quantitation with LC-ESI-MS using an LTQ-FT mass spectrometer. *J. Proteome Res.*, 52039–2045, (2006).
20. M. MERCHANT, S.R. WEINBERGER, Recent advancements in surface enhanced laser desorption/ionization-time of flight-mass spectrometry. *Electrophoresis*, 21, 1164–1177,(2000).
21. E.F. PETRICOIN, K.C. ZOON, E.C. KOHN, J.C. BARRETT, L.A. LIOTTA, Clinical proteomics: translating benchside promise into bedside reality*, Nat Rev Drug Discov.*, 1, 683–695 (2002).
22. B.B. HAAB, Antibody arrays in cancer research. *Molecular & Cellular Proteomics. 4,* 377-383 (2005).
23. M. SANCHEZ-CARBAYO, N.D. SOCCI, J.J. LOZANO, B.B. HAAB, C. CORDON-CARDO, Profiling bladder cancer using targeted antibody arrays. *Am. J. Pathol.* 168, 93–103 (2006).
24. T.W. HUTCHENS, T.T. YIP, New desorption strategies for the mass spectrometric analysis of macromolecules. *Rapid Commun Mass Spectrom*.7, 576–580 (1993).
25. H.J. ISSAQ, D.T. VEENSTRA, P.T. CONRADS, D. FELSCHOW, The SELDI-TOF MS Approach to Proteomics: Protein Profiling and Biomarker Identification. *Biochemical and Biophysical Research Communications*. 292, 587–592 (2002).
26. J.A.P. BONS, D. DE BOER, M.P. VAN DIEIJEN-VISSER, W KWH WODZIG, Standardization of calibration and quality control using surface enhanced laser desorption ionization-time of flight-mass spectrometry. *Clinica Chimica Acta*, 366, 249 – 256 (2006).
27. K. SOMASUNDARAM, M. B. NIJAGUNA, D. M. KUMAR**,** Serum proteomics of glioma: methods and applications. *Expert Rev. Mol. Diagn*., 9(7), 695–707 (2009).
28. M. NEAGU, C. CONSTANTIN, G. MANDA, D. POPESCU, V. UNGUREANU, D. TATU, M. PANA, D.L. RADU, A. BULMAN, K. VORGIAS, O. TSITSILONIS, W. VOELTER, SELDI –ToF-MS for prothymosin – alpha detection as bacterial infection apoptosis marker –Nato SfP Project. *Medimond - Monduzzi Editore*, www.monduzzi.com - pg. 419 - 422, (2009).
29. L.F. STEEL, B.B. HABB, S.M. HANASH**,** Methods of comparative proteomic profiling for disease diagnostics. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* 815(1–2), 275–284 (2005).
30. C. PISTOL TĂNASE, Caveolin -1 – A Marker For Pancreatic Cancer Diagnosis. *Expert review of molecular diagnostics*, 8(4), 395-404 (2008).
31. C. PISTOL-TĂNASE, E. RADUCAN, S.O. DIMA, L. ALBULESCU, A. IONITA, M. POROSCHIANU., L.M.. CRUCERU, E. CODOREAN, T.M. NEAGU, I. POPESCU, Assessment of soluble angiogenic markers in pancreatic cancer. Biomarkers in Medicine, 2(5), 447-455 (2008).
32. K. HONDA, Y. HAYASHIDA, T. UMAKI, T. OKUSAKA, T. KOSUGE, S. KIKUCHI, M. ENDO, A. TSUCHIDA, T. AOKI, T. ITOI, F. MORIYASU, S. HIROHASHI, T. YAMADA, Possible Detection of Pancreatic Cancer by Plasma Protein Profiling*. Cancer Res*, 65: (22), 10613 -10622 (2005).
33. C. PISTOL TĂNASE, M. NEAGU, R. ALBULESCU, E. CODOREAN, S.O. DIMA, Biomarkers in the diagnosis and early detection of pancreatic cancer. *Expert Opinion On Medical Diagnostics***,** 3 (5), 533-546 (2009).
34. M. EHMANN, K. FELIX, D. HARTMANN, M. SCHNOLZER, M. NEES, S. VORDERWULBECKE, R. BOGUMIL, M.W. BUCHLER, H. FRIESS**,** Identification of Potential Markers for the Detection of Pancreatic Cancer Through Comparative Serum Protein Expression Profiling*. Pancreas*., 34, 205-214 (2007).
35. C. GE, N. MA, D. YAO, F. LUAN, C. HU, Y. LI, Y. LIU, A Serum Biomarker Model to Diagnose Pancreatic Cancer Using Proteomic Fingerprint Technology. *Chin J Clin Oncol*., 5, 200-205 (2008).
36. J. KOOPMANN, Z. ZHANG, N. WHITE, J. ROSENZWEIG, N. FEDARKO, S. JAGANNATH,. M.I. CANTO, C. J YEO, D.W. CHAN, M. GOGGINS, Serum Diagnosis of Pancreatic Adenocarcinoma Using Surface-Enhanced Laser Desorption and Ionization Mass Spectrometry. *Clinical Cancer Research*, 10, 860–868 (2004).

37. P. HEWITSON, P. GLASZIOU, L. IRWIG, B. TOWLER, E. WATSON, Screening for colorectal cancer using the faecal occult blood test. *Hemoccult, Cochrane Database Systematic Reviews.*, 1-25 (2007).
38. M. MOSHKOWITZ, N. ARBER, Emerging technologies in colorectal cancer screening. *Surg Oncol Clin N Am*, 14(4), 723-746 (2005).
39. D.G. WARD, S. NYANGOMA, H. JOY, E. HAMILTON, W. WEI, C. TSELEPIS, N. STEVEN, M.J.O. WAKELAM, P.J. JOHNSON, T. ISMAIL, A. MARTIN, Proteomic profiling of urine for the detection of colon cancer, *Proteome Science*, 6(1), 19-34 (2008).
40. D.G. WARD, N. SUGGETT, Y. CHENG, W. WEI, H. JOHNSON, L.J. BILLINGHAM, T. ISMAIL, M.J.O. WAKELAM, P.J. JOHNSON, A. MARTIN, Identification of serum biomarkers for colon cancer by proteomic analysis, *British Journal of Cancer*, 94(12), 1898-1905 (2006).
41. J.Y.M.N. ENGWEGEN, A.C.T.M. DEPLA, M.E. SMITS, A. CATS, H. TUYNMAN, H.A. VAN HEUKELEM , P. SNEL, W. MEULEMAN, L.F. WESSELS, J.H.M. SCHELLENS, J.H. BEIJNEN, Detection of Colorectal Cancer by Serum and Tissue Protein Profiling: A Prospective Study in a Population at Risk. *Biomarker Insights*, 3, 375–385 (2008).
42. J.K.YU, Y.D. CHEN, S. ZHENG, An integrated approach to the detection of colorectal cancer utilizing proteomics and bioinformatics. *World J. Gastroenterol.,* 10(21), 3127–31(2004).
43. X.P. LIU, J. SHEN, Z.F. LI, L. YAN, J. GU, A serum proteomic pattern for the detection of colorectal adenocarcinoma using surface enhanced laser desorption and ionization mass spectrometry, *Cancer Invest.*, 24(8), 747–53 (2006).
44. D.G. WARD, K. ROBERTS, M.J. BROOKES, H. JOY, A. MARTIN, T. ISMAIL, R. SPYCHAL, T. IQBAL, C. TSELEPIS, Increased hepcidin expression in colorectal carcinogenesis. *World J Gastroenterol.,* 14(9), 1339-45 (2008).
45. L.C. WHELAN, K.A.R. POWER, D.T. MCDOWELL, J. KENNEDY, W.M. GALLAGHER, Applications of SELDI-MS technology in oncology. *J. Cell. Mol. Med,* 12(5):1535-1547(2008).
46. C. MELLE, G. ERNST, R. WINKLER, B. SCHIMMEL, J.P. KLUSSMANN, C. WITTEKINDT, O. GUNTINAS-LICHIUS, F. VON EGGELING, Proteomic analysis of human papillomavirus-related oral squamous cell carcinoma: Identification of thioredoxin and epidermal-fatty acid binding protein as upregulated protein markers in microdissected tumor tissue. *Proteomics,* 9, 2193–2201(2009).
47. Y.F.WONG, T.H. CHEUNG, K.W.K. LO., V.W. WANG., C.S. CHAN, T.B. NG, T.K.H. CHUNG, S.C. MOK, Protein profiling of cervical cancer by protein-biochips: proteomic scoring to discriminate cervical cancer from normal cervix.*Cancer Letters,* 211(2*)*, 227-234 (2004).
48. X. ZHU, J. LV, L. YU, X. ZHU, J. WU, S. ZOU, S. JIANG**,** Proteomic identification of differentially-expressed proteins in squamous cervical cancer. *Gynecologic Oncology***,** 112(1), 248-256 (2009).
49. T. XIA , Z.G. ZHENG , Y. GAO , H.Z. MOU , S.H. XU , P. ZHANG , J.Q. ZHU **,** Application of SELDI-TOF serum proteome profiling in cervical squamous cell carcinoma. *Ai Zheng,* 27(3), 279-82 (2008).
50. S.N. CAI, G.B. LIU, X.H. GUO, J.G. CHEN, J. ZHANG, Y.L. WU, W.J. LIANG, R.C. LUO**,** Proteinchip-flight mass spectrometry for screening differentially expressed proteins in the serum of cervical cancer patients. *Nan Fang Yi Ke Da Xue Xue Bao,* 29(1), 32-5 (2009).
51. E.F. PETRICOIN, K.C. ZOON, E.C. KOHN, Clinical proteomics: translating benchside promise into bedside reality, *Nat Rev Drug Discov***,** 1, 683–695 (2002).
52. W.E. GRIZZLE, O.J. SEMMES, W.L. BIGBEE, The need from review and understanding of SELDI/MALDI mass spectroscopy data prior to analysis, *Cancer Inform,* 1, 86–97 (2005).
53. D.M. GOOD, V. THONGBOONKERD, J. NOVAK, J.P. SCHANSTRA, J.J. COON, A. DOMINICZAK, H. MISCHAK, Body fluid proteomics for biomarker discovery: lessons from the past hold the key to success in the future, *J Proteome Res.*, 6, 4549–4555 (2007).
54. A.K. CALLESEN, W. VACH, P.E. JORGENSEN, S. COLD, Q. TAN, R. DEPONT CHRISTENSEN, O. MOGENSEN, T.A. KRUSE, O.N. JENSEN, J.S. MADSEN, Combined experimental and statistical strategy for mass spectrometry based serum protein profiling for diagnosis of breast cancer: a case-control study, *J Proteome Res.*, 7, 1419–1426 (2008).
55. M.E. NOO, A. DEELDER, W.M. VAN DER WERFF, A. OZALP, B. MERTENS, R. TOLLENAAR, MALDITOF serum protein profiling for the detection of breast cancer, *Onkologie***,** 29, 501–506 (2006).
56. C. LARONGA, S. BECKER, P. WATSON, B. GREGORY, L. CAZARES, H. LYNCH, R.R. PERRY, G.L. WRIGHT JR, R.R. DRAKE, O.J. SEMMES, SELDI-TOF serum profiling for prognostic and diagnostic classification of breast cancers, *Dis Markers.*, 19, 229–238 (2003).
57. M.C. GAST, E.J. VAN DULKEN, T.K. VAN LOENEN, F. KINGMA-VEGTER, J. WESTERGA, C.C. FLOHIL, J.C. KNOL, C.R. JIMENEZ, C.H. VAN GILS, L.F. WESSELS, J.H. SCHELLENS, J.H. BEIJNEN, Detection of breast cancer by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry tissue and serum protein profiling, *Int J Biol Markers.*, 24(3), 130-41(2009).

58. M.C. GAST, C.H. VAN GILS, L.F. WESSELS, N. HARRIS, J.M. BONFRER, E.J. RUTGERS, J.H. SCHELLENS, J.H. BEIJNEN, Serum protein profiling for diagnosis of breast cancer using SELDI-TOF MS, *Oncol Rep*., 22(1), 205-13 (2009).
59. R.J. SIMPSON, O.K. BERNHARD, D.W. GREENING, R.L. MORITZ , Proteomics - driven cancer biomarker discovery: looking to the future**,** *Curr Opin Chem Biol.*,12, 72–77 (2008).
60. M.C. GAST, J.H. SCHELLENS, J.H. BEIJNEN, Clinical proteomics in breast cancer: a review, *Breast Cancer Res Treat.,* 116, 17–29 (2009).
61. T. NAKAGAWA, S.K. HUANG, S.R. MARTINEZ, A.N. TRAN, D. ELASHOFF, X. YE, R.R. TURNER, A.E. GIULIANO, D.S. HOON, Proteomic profiling of primary breast cancer predicts axillary lymph node metastasis, *Cancer Res.*, *66*, 11825–11830 (2006).
62. F. TRAUB, H. FEIST, H.H. KREIPE, A. PICH, SELDI-MS-based expression profiling of ductal invasive and lobular invasive human breast carcinomas, *Pathol Res Pract,* 201, 763–770 (2005).
63. K. SOMASUNDARAM, M.B. NIJAGUNA, D.M. KUMAR, Serum proteomics of glioma: methods and applications, *Expert Rev. Mol. Diagn.*, 9(7), 695–707 (2009).
64. V. PETRIK, S. SAADOUN, A. LOOSEMORE, J. HOBBS, K.S. OPSTAD, J. SHELDON, E. TARELLI, F.A. HOWE, B. BELL, M.C. PAPADOPOULOS, Serum α2-HS Glycoprotein Predicts Survival in Patients with Glioblastoma, Clinical Chemistry, 54(4), 713–722 (2008).
65. H. ZHANG, G. WU, H. TU, Discovery of serum biomarkers in astrocytoma by SELDI–TOF MS and proteinchip technology*, J. Neurooncol.*, 84(3), 315–332 (2007).
66. J. LIU, S. ZHENG, J. YU, J. ZHANG, Z. CHEN, Serum protein fingerprinting coupled with artificial neural network distinguishes glioma from healthy population or brain benign tumor, *Journal of Zhejiang University SCIENCE*, 6B(1), 4-10 (2005).
67. J. CARON, A. MANGÉ, B. GUILLOT, J. SOLASSOL, Highly sensitive detection of melanoma based on serum proteomic profiling, *J Cancer Res Clin Oncol*, 135, 1257–1264 (2009).
68. M. NEAGU, C. CONSTANTIN, G. MANDA, I. MARGARITESCU, Biomarkers of metastatic melanoma, *Biomarkers in Medicine,* 3(1), 71-89 (2009)
69. R. LAKOMY, K. GREPLOVA, R. PILNY, E. BUDINSKA, D. VALIK, A. POPRACH, R. NEMECEK, R. VYZULA, Profiles of low-molecular proteome spectrum obtained through SELDI-TOF mass spectrometry in the sera of patients with metastatic malignant melanoma:pilot study, *Klin Onkol*., 22(5), 228-32 (2009).
70. L.L. WILSON, L. TRAN, D.L. MORTON, D.S. HOON, Detection of differentially expressed proteins in early-stage melanoma patients using SELDI-TOF mass spectrometry*, John Wayne Cancer Institute Ann N Y Acad Sci*, 1022, 317-22 (2004).