Contributions of advanced proteomics technologies to cancer diagnosis

Sergio Ciordia, Vivian de los Ríos and Juan-Pablo Albar

Proteomics Facility. Centro Nacional de Biotecnología-CSIC. Universidad Autónoma. Madrid. Spain.

The ability of Medicine to effectively treat and cure cancer is directly dependent on their capability to detect cancers at their earliest stages. The advent of proteomics has brought with it the hope of discovering novel biomarkers in the early phases of tumorigenesis that can be used to diagnose diseases, predict susceptibility, and monitor progression. This discipline incorporates technologies that can be applied to complex biosystems such as serum and tissue in order to characterize the content of, and changes in, the proteome induced by physiological changes, benign or pathologic. These tools include 2-DE, 2D-DIGE, ICAT, protein arrays, MudPIT and mass spectrometries including SELDI-TOF. The application of these tools has assisted to uncover molecular mechanisms associated with cancer at the global level and may lead to new diagnostic tests and improvements in therapeutics. In this review these approaches are evaluated in the context of their contribution to cancer biomarker discovery. Particular attention is paid to the promising contribution of the ProteinChip/SELDI-TOF platform as a revolutionary approach in proteomic patterns analysis that can be applied at the bedside for discovering protein profiles that distinguish disease and disease-free states with high sensitivity and specificity. Understanding the basic concepts and tools used will illustrate how best to apply these technologies for patient benefit for the early cancer detection and improved patient care.

Key words: proteomics, cancer, biomarkers, profiling, SELDI-TOF.

Ciordia S, de los Ríos V, Albar JP. Contributions of advanced proteomics technologies to cancer diagnosis. Clin Transl Oncol. 2006;8(8):566-80.

INTRODUCTION

Large scale and high-throughput approaches

In last decade three main avenues have appear in the bioscience scenario for describing complex biological systems. The possibility to simultaneously analyze thousands of genes, transcripts and/or proteins by a cohort of new large scale and high-throughput technical platforms has produced a significative change in the way in which we can face to a great number of biological processes including physio-pathological phenomena such as cancer.

Comparative genomic hybridization (CGH), DNA sequencing, single nucleotide polymorphism (SNP) analysis have been extensively used as high-throughput tools for inspecting the hereditary and somatic components that lead to cancer at the genome level (fig. 1). While, DNA micro-array analysis, PCR- and non-PCR-based gene-expression assays are analytical tools that have also been used to know those changes in RNA abundance associated with cancer at the transcriptome level. And lately, tools and strategies (see below) to know how proteins are modified and how their levels change in tumours are being used to get a picture of cancer at the proteome level. All together, in conjunction of other coming approaches such as metabolomics, are giving us a more comprehensive and holistic view of cancer at the molecular level.

Proteomics: general background

In recent years, there has been an enormous growth in the use of genome information in science. This genome information has greatly expanded the insight into the genetic basis of cancer. The completion of the human genome sequencing project revealed that

*Supported by an unrestricted educational grant from AstraZeneca.

Correspondence: J.P. Albar.
Proteomics Facility.
Centro Nacional de Biotecnología-CSIC.
Universidad Autónoma de Madrid.
Campus de Cantoblanco.
C/ Darwin, 3.
28049 Madrid. Spain.
E-mail: jpalbar@cnb.uam.es

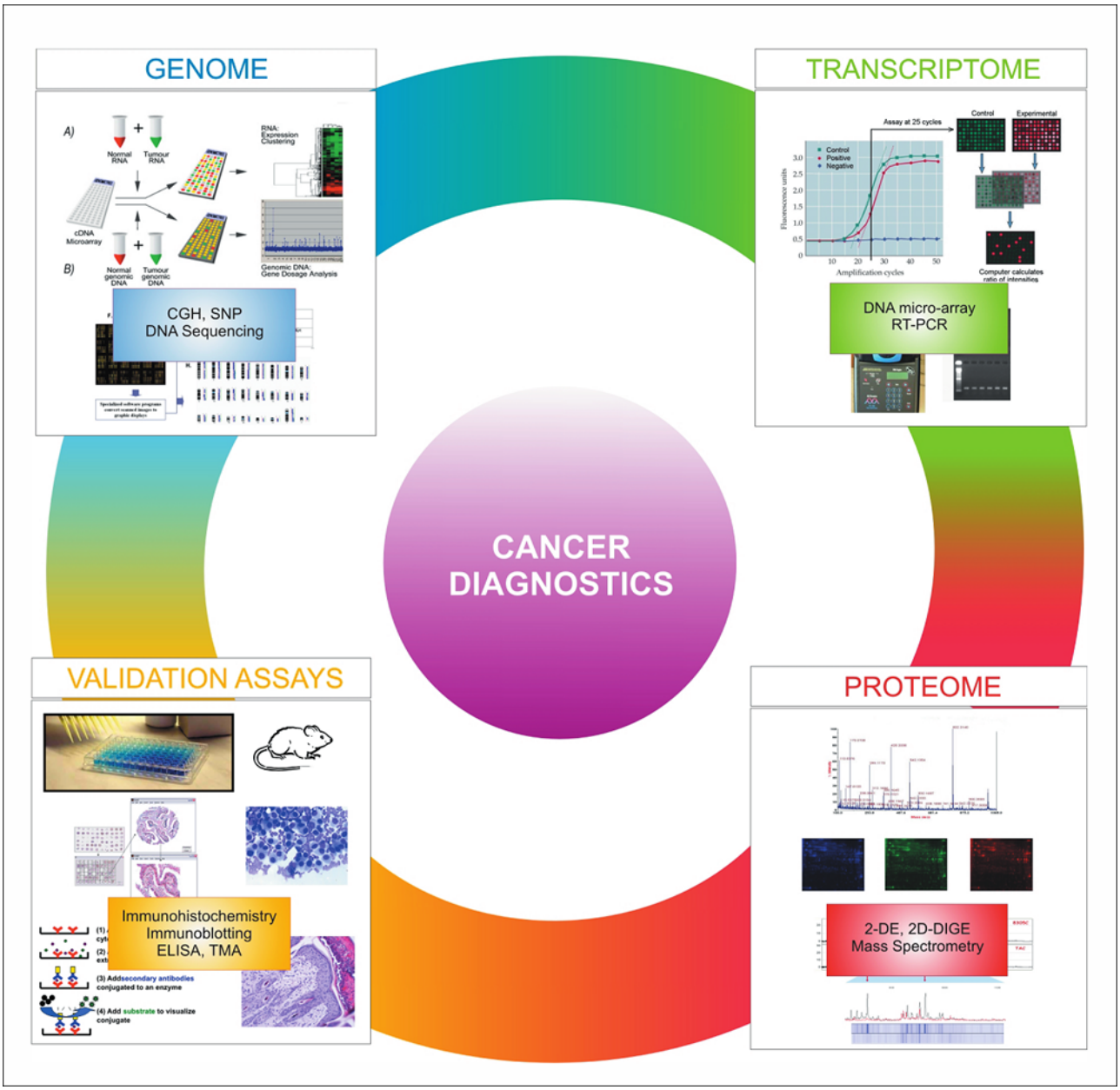

Fig. 1. ***The biological organization of the «-omes».*** **The classical view of biological organization is to consider the flow of information from the genome to the transcriptome, to the proteome and then the metabolome. However, each tier of organization depends on the other, so a perturbation in one network can affect another. Furthermore, the environment has a crucial impact on not only expression and concentrations of transcripts, proteins and metabolites, but also on the genome by selecting for adaptive changes in subpopulations of cells within a tumour.**

gene analysis alone does not tell the «whole story» and the focus of research is rapidly moving beyond transcriptomics, gene lists, and functional genomics to proteomics. This means to the task of identifying the structure, function, and interactions of those proteins produced by individual genes and their roles in specific disease processes such as the case of cancer.

The proteome encompasses all proteins that result from the genome of cells, a tissue or an organism. It is not a static parameter, as is the genome, but a dynamic collection of proteins that reflects both the intrinsic genetic programme of the cell and the impact of its immediate microenvironment[1,2]. Compared with the genome, the proteome provides a more realistic view of a biological status and is, therefore, expected to be more useful than gene analysis for evaluating, for example, disease presence, progression and response to treatment. Thus, proteomics can bridge the gap between the genome sequence and cellular behaviour.

Proteomics, also referred as functional genomics, is commonly defined as the analysis of the protein composition of a cell. Most cellular functions depend on the presence or absence of different proteins and peptides, their interactions and their modifications. Proteome analyses are not limited to the description of a current state protein composition of a cell, but are often used to identify and characterize qualitative or quantitative changes in cellular protein patterns and protein interactions that can be triggered by external or internal signals[3,4].

In current practice, proteomics encompasses four principal applications: *a)* protein mining; *b)* differential protein expression profiling; *c)* protein network mapping; and *d)* protein modification mapping. *Protein mining*: the purpose of mining proteomes is to identify as many of the components of the proteome as possible to catalogue the proteome directly, rather than to infer its composition from gene expression[5]. *Differential protein expression profile:* this involves the identification of proteins in a particular sample as a function of a particular state of the organism, or as a function of exposure to a drug, chemical or physical stimulus. The main task in protein expression profiling is to measure the expression of a set of proteins in two serial samples, and then to compare them[6]. *Protein network mapping:* this is a proteomic approach to determining how proteins interact with each other in living systems (protein–protein interactions), and therefore, it determines the function of protein[7]. *Protein modifications mapping:* this involves identifying how and where proteins are modified. The proteomic approaches are a mean of establishing the nature of post-translational modifications[8].

Most of proteomics studies done in cancer field and referred in this review are in fact differential protein expression profile studies.

Cancer proteomics

The analysis of molecular pathogenesis of cancer by analysing global protein expression in tumors, tumor cells or extracellular fluids from cancer patients[9] has been referred as «cancer proteomics». A major justification for measuring proteins instead of gene transcripts is that mRNA levels do not necessarily correlate with corresponding protein abundance[10-14].

A number of different gene products can arise from a single gene due to alternative splicing and additional complexity is provided by protein post-translational modifications, including phosphorylations, acetylations, and glycosylations, or protein cleavages[15]. These modifications are not detectable at the mRNA level but play significant roles in protein stability, localization, interactions and functions. Therefore, proteins represent a more relevant and accessible therapeutic targets than nucleic acids do.

Detection of cancer biomarkers

Although proteomic research has many biomedical fields of application, from cardiovascular[16,17] and neuromuscular diseases[18,19] to solid organ transplantation[20] and infertility[21], the most promising area is the discovery and identification of new biomarkers for cancer diagnostics[22]. The early detection of cancer is crucial for its ultimate control and prevention. Although advances in conventional diagnostic strategies, such as mammography and prostate-specific antigen (PSA) testing, have provided some improvement in the detection of disease, they still do not reach the sensitivity and specificity that are needed to reliably detect early-stage disease. In many cases, cancer is not diagnosed and treated until cancer cells have already invaded surrounding tissues and metastasized throughout the body. More than 60% of patients with breast, lung, colon and ovarian cancer have hidden or overt metastatic colonies at presentation and most conventional therapeutics are limited in their success once a tumour has spread beyond the tissue of origin[23]. Therefore it is expected that the discovery of novel biomarkers will be able to reduce the lag time between the beginning of cancer disease and its discovery. This is of particular importance in view of current evidence that the most important factor in the management of cancers is clinical stage at diagnosis[24].

A biological marker («biomarker») is defined as «a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention»[9].

A major obstacle for *tumoral markers* detection is the degree of tissue heterogeneity, a phenomenon that can affect the result obtained from comparative proteomics experiments and this may significantly affect the interpretation of the data. Immunohistochemistry is the standard method for marker determinations in routine pathological tissue diagnostics. But proteomic strategies are attracting increasing interest to be used for the identification of tissue markers and for providing data in multivariate analysis. A key technique for quantitative and comparative proteomics analysis of tumor biopsies and solid tissue is the laser capture microdissection (LCM) that permits the dissection of cancerous tissue and selection of subpopulation of cells, e.g. tumor cells from a field of normal appearing cells. Using LCM, researchers can obtain more accurate representation of cells (tumor and normal appearing cells) and make more accurate comparisons of protein expression in normal and diseased specimens[25].

Other of the biggest ongoing challenges in the field of proteomics is the detection of *serum markers* to be used for early disease detection and to follow treatment effects versus disease progression. This task has

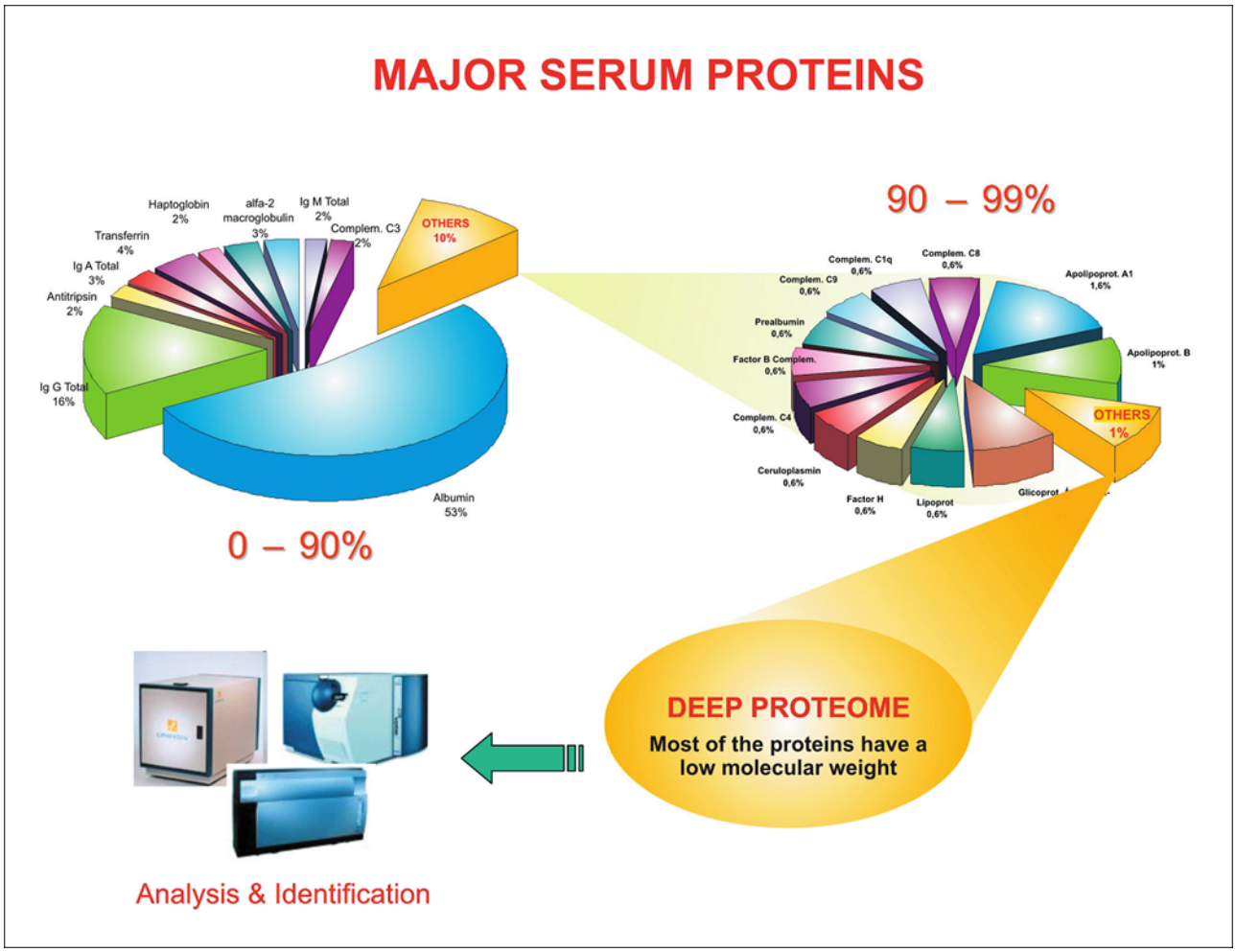

Fig. 2. ***Dynamic range of serum proteins detection.*** **The abundance of serum proteins spans eight to ten orders of magnitude (from albumin to the low-abundance interleukins) but these MS-based methods can, in most cases, detect proteins over only two to four orders of magnitude. This is why low-abundance proteins pose a challenge, especially without prior pre-fractionation or immunodepletion steps.**

to deal with several handicaps: *1)* the large dynamic range of protein concentrations ranging from ~50 mg/ml for albumin to 1–5 pg/ml for interleukins, that means 10 logs of difference[26], *2)* Twenty proteins represent 99% of the total serum protein content (fig. 2), and *3)* multiple protein isoforms are shown on 2D gels, suggesting numerous post-translational modifications such as glycosylation and phosphorylation. It is, therefore, necessary to subject samples to pre-fractionation or immunodepletion steps to remove the most abundant proteins.

PROTEOMIC APPROACHES USED IN CANCER BIOMARKER DISCOVERY

Proteomics Platforms Involving 2-DE: 2D-DIGE

Traditionally, protein extracts from cell lysates are somehow enriched and subjected to proteomic analyses by using two-dimensional gel electrophoresis (2-DE) in conjunction with mass spectrometry (MS) techniques[27]. The two-dimensional gel approach separates proteins according to two independent physicochemical parameters, charge and molecular weight. After successful separation and appropriate spots visualization, proteins of interest are extracted from gel, digested and mass spectrometry analyzed (fig. 3). Peptide mass analysis are carried out either by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or by tandem MS (MS/MS). Protein identification is finally done by correlation of the MS generated data with database sequence information[28].

Fluorescent two-dimensional difference in-gel electrophoresis (2D-DIGE) is a fairly recent improvement of the 2-DE technology[29]. Prior to electrophoresis, the proteins in different cell extracts are covalently labeled using different fluorescent dyes, e.g. cyanine (Cy2, Cy3, or Cy5) dyes (fig. 4). Typically, the test

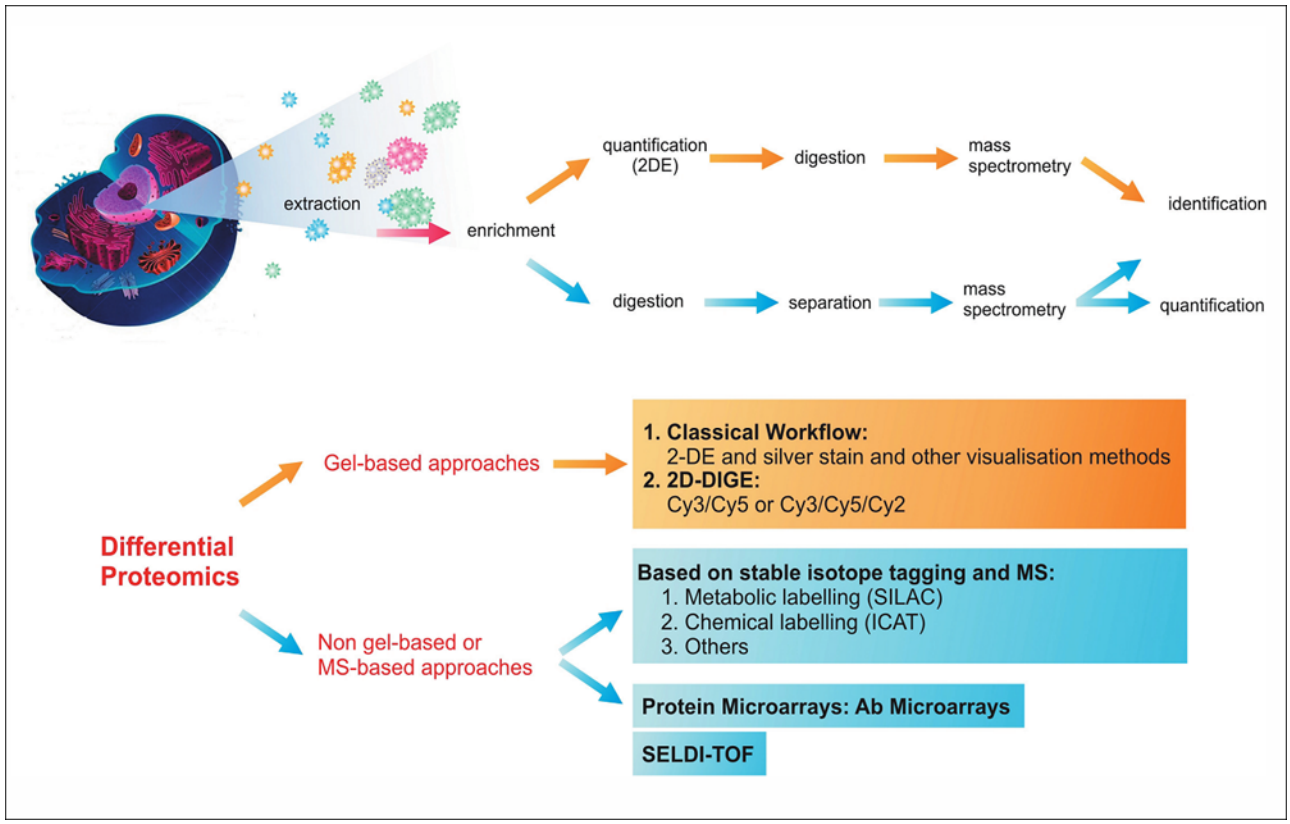

Fig. 3. ***Quantitative protein analysis from the cell to the identified protein.*** **In the upper classical workflow, 2DE is used to separate and to quantify proteins: The selected proteins are isolated and identified by mass spectrometry. In the lower workflow, LC-MS/MS or SELDI-TOF MS are used to analyze protein mixtures and accurate quantification to differential protein expression. These techniques are compatible with protein fractionation or separation methods, such as subcellular fractionation, protein complex isolation and electrophoresis and chromatography.**

sample is labeled with Cy3 and the reference sample with Cy5 or «viceversa». Equal concentrations of the two differentially-labeled protein samples are then mixed and co-separated during the same 2-DE process. The 2D-DIGE gel is then scanned at two emission wavelengths (for Cy3 and Cy5) using a fluorescence imager. A comparison of the two images generated allows the quantification of each spot. This approach eliminates or drastically reduces gel-to-gel variability associated with standard 2-DE and improves the accuracy of quantitative protein profiling showing a linear dynamic range of 4-5 orders of magnitude, by contrast with the approximately 1-2 order range of classical 2-DE staining procedures[30-32].

A large number of 2-DE based approaches have been developed in preclinical models of cancer, including cell lines, but relatively few data have been generated from clinical samples and generally involving a limited number of patients. These approaches has been applied for the identification of potential biomarkers associated to breast ductal carcinomas[25], in tissue and biological fluids such as serum[33] or nipple aspirate fluids (NAF)[34] (table 1). Ten differentially expressed proteins were validated in independent ductal carcinoma specimens and confirmed by standard immunohistochemical analysis using a limited independent tumor cohort. Many of the proteins identified were previously unconnected with breast cancer. Proteomic analysis of these specimens revealed differential expression patterns distinct from previous nucleic acid-based studies and identified new facets of the earliest stage of breast cancer progression[25]. On the other hand, sera from breast cancer and control patients have been employed to investigate protein expression alterations[33]. This work revealed proapolipoprotein A-I, transferrin, and hemoglobin as up-regulated and apolipoprotein A-I, apolipoprotein C-III, and haptoglobin alpha2 as down-regulated in patients. The serum level of transferrin correlated well with the 2D-DIGE results. However, the serum levels of apolipoprotein A-I and haptoglobin could not be detected with the clinical routine diagnostic tests. Thus, 2D DIGE can distinguish between isoforms of proteins, where the overall immunochemical quantification does fail due to a lack of isoform-specific antibodies.

In colorectal cancer tissues in comparison to adjacent normal mucosa, 34 spots were detected with statistical significance and changes in protein expression

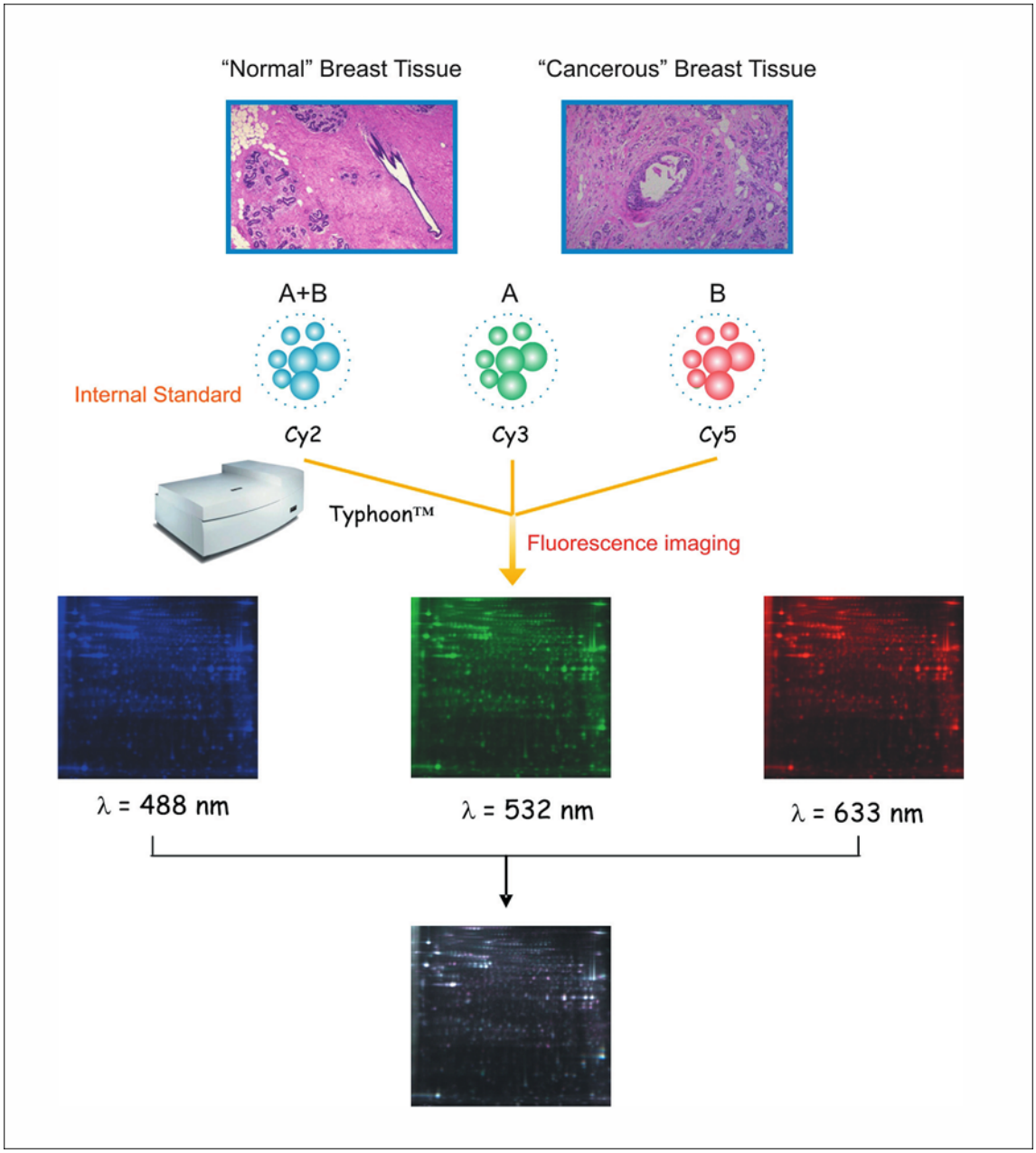

Fig. 4. 2D-DIGE approach.

levels revealed a significantly enhanced glycolytic pathway (Warburg effect), a decreased gluconeogenesis, a suppressed glucuronic acid pathway, and an impaired tricarboxylic acid cycle. These changes reveal an underlying mechanism of colorectal tumorigenesis in which the roles of impaired tricarboxylic acid cycle and the Warburg effect may be critical[55].

Proteomics platforms involving LC-based technology: ICAT, iTRAQ, SILAC, MudPIT

Other alternative proteomic approaches currently used in the field of differential protein expression profile in cancer are based on the so called «peptide-centric» approaches. Protein extracts from cell lysates are first digested and the whole peptide mixtures produced are separated, identified and quantified by two-dimensional liquid chromatography tandem mass spectrometry (2D-LC-MS/MS) (fig. 3).

Quantitative proteomics using stable isotope-label techniques

Alternative or complementary MS based approaches have been developed for differential protein expression measurements and are currently been improved.

TABLE 1. **Summary of potential biomarkers identified in cancer studies by A) Proteomics Platforms Involving 2-DE: 2D-DIGE; B) Proteomics Platforms Involving LC-based Technology: ICAT, iTRAQ, SILAC, MudPIT and Protein Microarrays**

Proteomic technologies	Cancer type	REFS
A) Gel-based approaches		
2-DE/MALDI-TOF	Breast (Tissue)	25
2D-DIGE/MALDI-TOF	Breast (Serum)	33
	Colorectal (Tissue)	35
(LCM)-ICAT/2D-LC-MS/MS	Breast (NAF)	37
	Hepatocellular (Tissue)	38
	Ovarian (Cells)	39
iTRAQ/2D-LC-MS/MS	Endometrial (Tissue)	40
	Lung (Cells)	41
SILAC/LC-MS/MS	Pancreatic (Secretome)	43
B) LC-based approaches		
MudPIT	Pancreatic (Secretome)	44
	Breast (Tissue)	45
Protein microarrays	Colorectal (Cell)	48
	Linfoma (Cell)	49

They are based on the differential labeling of perturbed and non-perturbed protein extracts with different stable isotopes ($^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{1}H/^{2}H$). In this way, the same peptide from two different samples will show the same chemical behaviour, with a difference in mass detectable by MS techniques. Peptide peak intensities can be used for relative quantification of these peptides.

– The most commonly used method for protein quantification is based on the use of Isotope-Coded Affinity Tag *(ICAT)* reagents[50,56]. ICAT profiles the relative amounts of cysteine-containing peptides derived from tryptic digests of paired protein extracts. This method has been applied[57] for quantification and identification of differentially expressed tumor-specific proteins in NAF from the tumor-bearing and contralateral disease-free breasts from unilateral early-stage breast cancer patients. In this study, alpha 2HS-glycoprotein was down-regulated, while lipophilin B, beta-globin, hemopexin, and vitamin D-binding protein precursor were overexpressed. *Western blot* analysis confirmed the overexpression of vitamin D-binding protein in tumor-bearing breasts. Thus, proteomic screening techniques using ICAT and NAF may be used to find markers for diagnosis of breast cancer (table 1).

Laser capture microdissection (LCM) and ICAT technology have been applied to investigate the qualitative and quantitative proteomes of hepatocellular carcinoma (HCC). A total of 644 proteins were qualitative identified, and 261 proteins were unambiguously quantitated. Thus, LCM coupled with 2D-LC-MS/MS and cICAT labeling technology can achieve accurate qualitative and quantitative clinical proteomic analysis including looking for tumor-interrelated proteins and finding potential biomarkers and drug targets[58].

Recently, the proteomes of cisplatin-sensitive and -resistant ovarian cancer cells were compared, and protein expression was correlated with mRNA expression profiles[59]. In resistant cells, several proteins appeared to be overexpressed at least 5-fold including cell recognition molecule CASPR3, S100 protein family members, junction adhesion molecule Claudin 4, and CDC42-binding protein kinase beta. While, in sensitive cells, proteins such hepatocyte growth factor inhibitor 1B and programmed cell death 6-interacting protein were overexpressed at least 5-fold. The direction of changes in expression levels between proteins and mRNAs were not always in the same direction, possibly reflecting posttranscriptional control of protein expression. In this study, proteins whose expression profiles correlate with cisplatin resistance in ovarian cancer cells were identified. Several proteins may be involved in modulating response to cisplatin and have potential as markers of treatment response or treatment targets (table 1).

– New LC-based quantitative method, such as *iTRAQ* (isobaric Tags for Relative and Absolute Quantitation) employs a more global strategy involving the labeling of all primary amines. iTRAQ™ is a recently developed protein quantitation technique that utilizes four isobaric amine specific tags. In single MS mode the differentially labeled versions of a peptide are indistinguishable. However, in tandem MS mode (in which peptides are isolated and fragmented) each tag generates a unique reporter ion. Protein quantitation is then achieved by comparing the intensities of the four reporter ions in the MS/MS spectra.

DeSouza et al[40] in a recent study combines these two LC-based strategies, iTRAQ and ICAT, and identified a total of nine differentially expressed proteins involved in endometrial cancer (EC). Chaperonin 10, pyruvate kinase M1/M2 isozyme, calgizzarin, hetero-

geneous nuclear ribonucleoprotein D0, macrophage migratory inhibitory factor, and polymeric immunoglobulin receptor precursor were overexpressed, while alpha-1-antitrypsin precursor, creatine kinase B, and transgelin were down-regulated markers. The use of cICAT led to identification of a higher proportion of lower-abundance signaling proteins; conversely, iTRAQ resulted in a higher percentage of the more abundant ribosomal proteins and transcription factors. A panel of these plus other markers may confer sufficient selectivity for diagnosing and screening of EC (table 1).

Differential protein expression profiling by iTRAQ-2DLC-MS/MS of lung cancer cells undergoing epithelial-mesenchymal transition shows up-regulation of proteins involved in the ontrol of cell migration, adhesion and invasion revealing a migratory/invasive phenotype[41].

– Another approach for relative quantitation of changes in protein expression involves a stable isotope labeling strategy termed *SILAC* (stable isotope labeling by amino acids in cell culture). Labeled, essential amino acids are added to amino acid deficient cell culture media and are therefore incorporated into all proteins as they are synthesized. No chemical labeling or affinity purification steps are performed, and the method is compatible with virtually all cell culture conditions, including primary cells[42]. This metabolic labeling has been applied for biomarker discovery in the secretome of pancreatic cancer-derived cells and non-neoplastic pancreatic ductal cells[45]. One hundred and forty five differentially secreted proteins (> 1.5-fold change) have been identified, several of which were previously reported as either up-regulated (e.g. cathepsin D, macrophage colony stimulation factor, and fibronectin receptor) or down-regulated (e.g. profilin 1 and IGFBP-7) proteins in pancreatic cancer, validating this approach (table 1).

– A final LC-based approach is Multidimensional Protein Identification Technology *(MudPIT)*. Two steps of HPLC (cation exchange and reverse phase) are coupled to tandem mass spectrometry (MS/MS) and database-searching algorithms allowing to rapidly analyze complex mixtures. This approach has been applied for an efficient and rapid identification of proteins released by pancreatic cancer cells, including molecules previously undescribed in this type of cancer. A series of proteoglycans, including versican, perlecan, syndecan 1 and 4 has been reported, challenging the common view that fibroblasts of tumor stroma are the sole source of these molecules[44]. In addition, this technique has also been used for identification of more than 50 enzyme activities in human breast tumors, nearly a third of which represent previously uncharacterized proteins[45] (table 1).

Protein microarrays

The general concept of a protein microarray is to selectively capture the molecule of interest from a complex sample to probe for the presence and/or abundance of that particular protein. The most popular protein arrays are constructed with antibodies *(antibody microarray)* or cytokines where the bait proteins are specific antibodies printed on solid surfaces[46]. This novel proteomic technique provides a powerful technology for analyzing the expression of hundreds of proteins simultaneously. A high-precision robot is used to print hundreds of monoclonal antibodies at a high density on a glass slide in a format that is compatible with existing hardware and software tools for DNA microarrays[47]. Protein extracts from 2 different samples are differentially labeled with fluorescent dyes. The labeled extracts are then simultaneously hybridized with the antibody array. A fluorescence-based detection procedure, analogous to that used in gene expression profiling, is exploited, in which the immobilized antibodies are used to capture fluorescently labeled antigen[25].

Although still in its infancy, this technique has been exploited in cancer research studies[48,49]. In 2001, Sreekumar et al[48] (table 1) successfully utilized antibody microarrays to monitor alterations of protein levels in LoVo colon cancer cells treated with ionizing radiation. More recently, protein microarray has also been used in proteomic analysis of mantle-cell lymphoma (MCL). A microarray containing a panel of 512 antibodies allowed the identification of 13 overexpressed proteins (2-folds). The elevated expression of some of these polypeptides was confirmed by immunoblotting and immunohistochemistry, whereas elevated expression of others could not be confirmed, illustrating the importance of confirmatory studies[49].

PROTEOMIC PROFILING TECHNOLOGY IN CANCER DIAGNOSIS

Plasma represents the golden specimen for clinical studies. Serum and plasma offer particularly promising resources for biomarker discovery because collection of these samples is minimally invasive and the blood is thought to contains the majority of protein constituents found in the body[26,50]. «Proteomic profiling» or «serum proteomic profiling» is based on the comparison of proteomic signatures of different samples[51]. The «proteomic profiling» approach for discovering new biomarkers is based on two premises: *a)* the low-molecular-weight serum proteome contains an enormous wealth of biomarker information, which has not yet been explored, and *b)* a pattern of multiple biomarkers may contain a higher level of discriminatory information than a single biomarker

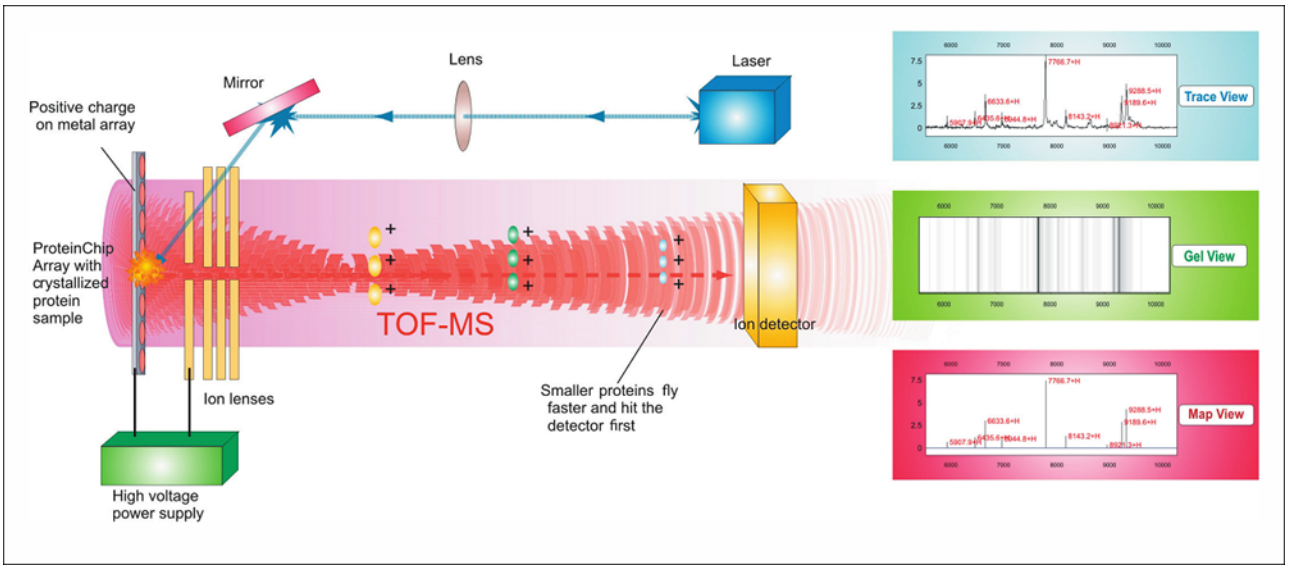

Fig. 5. ***A schematic diagram of the PBS-II TOF-MS:*** **ProteinChip Biomarker System-II (Ciphergen, CA, USA). TOF-MS: Time-of-flight mass spectrometry. After sample preparation the ProteinChip array is analyzed by laser desorption ionization TOF-MS and the smaller proteins fly faster and hit the detector first. Upper right panel shows a portion of the protein profile in spectrum view. Middle right panel is the same profile shown as pseudogel view and the last right panel is the same profile shown as map view.**

across large heterogeneous patient populations[52]. This approach applies very well for dissecting a complex multistage disease such as cancer.

Serum proteomic profiling: ProteinChip/ SELDI-TOF Platform

Currently, the prevalent approach to proteomic pattern diagnostics appears to be based on SELDI-TOF profiling[53]. The Surface-Enhanced Laser Desorption Ionization (SELDI) mass spectrometry technology, arising from the MALDI technique, combines retention chromatography with TOF-MS detection. In particular, the first dimension of protein separation uses special chemical surfaces (the ProteinChip® Array, Ciphergen Biosystems, Fremont, CA), which include anion exchange, cation exchange, normal phase, reversed phase, and immobilized metal affinity chromatography (IMAC) or biochemical surfaces (inmobilized antibody, receptor, DNA, enzyme, etc). The various types of chip surfaces have different affinity to different subsets of proteins. These proteome fractions bound to the chips are analysed by MS on the same chip, resulting in a «pattern» or «fingerprint» of proteins characterized by mass-to-charge ratio (m/z) (fig. 5). Differential expression may be determined from these protein profiles by comparing peak intensity. The resulting spectral masses are analyzed using univariate and multivariate statistical tools to yield single marker or multimarker pattern that can classify clinical samples.

SELDI-TOF MS applications and objections

ProteinChip/SELDI-TOF platform has been applied for clinical biological fluids profiling, notably serum/plasma, and gain fame when numerous studies showed promising potential in identifying unique biomarkers or complex patterns with diagnostic value, allowing to envisage its use as discovering/screening/early diagnosis tool in several types of cancer[56-61]. In particular, the impressive results in terms of specificity and sensitivity, reported by Diamandis et al[57] Petricoin et al[59] and Adam et al[62] in prostate and ovarian cancer raised enormous interest, but also some criticism. Diamandis summarised some open questions related to the application of SELDI-TOF technology for biomarker discovery as follows[52]: *a)* it is a qualitative technique where the relationships between peak height and molecular abundance are not linear, *b)* there are differences between distinguishing peaks identified by different investigators using the same technique, for the same disease; *c)* data from different laboratories are not readily reproducible, thus complicating any validation or comparison; *d)* different investigators use different optimal sample preparations; *e)* validated serum cancer markers (e.g., PSA and CA125) that could serve as internal controls are not identified by this technology; *f)* the adsorption matrices used favour extraction of high-abundance proteins/peptides at the expense of low-abundance proteins/peptides; *g)* the technique measures peptides present in high abundance in serum that is unlikely to originate from cancer tissue; they are more likely

TABLE 2A. **Summary of potential biomarkers in ovarian cancer identified by SELDI-TOF MS**

Sample type	Biomarkers (kDa)	Biomarkers ID	REFS
Ovarian cancer			
Serum	11.700	Haptoglobin-α fragment	67
Serum	28.043	ApoA-I	50
	12.828	Truncated form of transthyretin	
	3.272	Fragment of inter α trysin inhibitor heavy chain H4	
Serum	12.900	Fragment transthyretin	57, 65
	13.900	Transthyretin	
	15.900	Hb-β chain	
	28.000	ApoA-I	
	79.000	Transferrin	
Serum	15.100	Hb-α chain	68
	15.800	Hb-β chain	
Plasma	9.251	Hpt fragment	66
	54.000	Ig heavy chain	
	79.000	Transferrin	
Plasma	11.520	N-terminal arginine-truncated form SAA-1	69
	11.681	SAA-1	

ID: identify; Apo: apolipoprotein; Hb: hemoglobin; Ig: immunoglobulin; SAA: serum amyloid α.

to represent a specific cancer epiphenomena; and *h)* the relationships between distinguishing molecules and cancer biology is not known[52].

Petricoin and Liotta[25] addressed these points, underlining that instrument reproducibility and quality control have already been introduced and that standard operating procedures for sample collection, handling and shipping have been developed. Furthermore, the results of Semmes et al[63] demonstrated that «between-laboratory» reproducibility of SELDI-TOF-MS serum profiling approaches and «within-laboratory» reproducibility as determined by measuring discrete m/z peaks over time and across laboratories. Although there is even controversial in its reproducibility and ability to detect actual specific tumour signatures, SELDI has several advantages, such as ease of use, high throughput, and relatively affordable cost, making it a very attractive technique for working with large sample cohorts in a clinical setting.

SELDI-TOF MS in cancer profiling

The usefulness of the SELDI-TOF MS approach for protein profiling has been demonstrated by the huge number of published applications in the last years. In special, this technology has been applied extensively in cancer research. Prostate Specific Antigen (PSA), Cancer Antigen 125 (CA 125), Carcino Embryonic Antigen (CEA) are conventional serological antigens for testing prostate cancer, ovarian cancer, and colorectal cancer and the corresponding conventional assays detect and quantify these individual proteins, while SELDI-TOF MS uses protein patterns or «signatures» to discriminate a disease state.

Contribution of this approach to cancer biomarker discovery are summarized on table 2 and detailed below.

Ovarian cancer

The first SELDI-TOF MS study in cancer research was applied for the detection of ovarian cancer markers in serum samples by the group of Petricoin[64]. Paired samples from 50 healthy women and 50 women in different ovarian cancer stages were used in the experiment. A proteomic pattern was identified that discriminated cancer from non-cancer cases. Kozak et al[65] reported several panels of selected peaks (fragment Transthyretin, Transthyretin, Hb-β chain, ApoA-I, Transferrin) which had been observed in previous studies[58,66]. In addition, Ye et al[67] were able to identify haptoglobin á subunit as discriminating marker. This protein fragment was found to be overexpressed in 91 ovarian cancer patients when was compared with serum proteomic patterns from 91 healthy women. Zhang et al[50] identified three serum biomarkers: apolipoprotein A1 (down-regulated); a truncated form of transthyretin (down-regulated) and a cleavage fragment of inter-α-trypsin inhibitor heavy chain H4 (up-regulated) in ovarian cancer patients.

Up to fourteen new potential biomarkers associated to ovarian cancer have been reported in different studies by using this technology (table 2A).

Prostate cancer

Prostate Specific Antigen is produced by normal prostate cells in small amounts, but the higher the PSA is in serum, the higher the correlation is toward the existence of prostate cancer. The PSA screening test often generates a significant number of false positives that lead to invasive and unnecessary biopsy procedures. Thus, the application of SELDI-TOF MS

TABLE 2B. **Summary of potential biomarkers in head and neck cancer identified by SELDI-TOF MS**

Sample type	Biomarkers (kDa)	Biomarkers ID	REFS
Head and neck cancer			
Serum	10.068	Metallopanstimulin-1	97
Tissue	35.893	Annexin V	98
Tissue	3.442	HNP-1 or α-Defensin-1	99
	3.371	HNP-2 or α-Defensin-2	
	3.486	HNP-3 or α-Defensin-3	
	3.700	C-term fragment of Hb-β chain (aa 113-146)	
	9.973	Acyl-CoA-binding protein	
	11.043	Cystatin A	
	11.312	Histone H4	
	15.150	Hemoglobin α	
	15.885	Hemoglobin β	
	11.744	Calgizzarin or S100A11	
	27.805	Stratifin	

ID: identify; HNP: Hhuman neutrophil peptide; aa: aminoacid; term: terminal.

in prostate cancer has aimed at improving the currently available PSA screening test.

An innovative protein biochip immunoassay has been used to quantify and compare serum PSMA (prostate-specific membrane antigen) levels in healthy men and patients with either benign or malignant prostate disease. PSMA was captured from serum by anti-PSMA antibody bound to ProteinChip arrays, then captured PSMA was analyzed by SELDI-TOF. Initial results suggest that serum PSMA may be a more effective biomarker than prostate-specific antigen for differentiating benign versus malignant prostate disease (Xiao et al[71,72]). On the other hand, Maliki et al[73] in 2005 have demonstrated that an isoform of ApoA-II is specifically overexpressed in prostate disease. The ability of ApoA-II to detect disease in patients with normal prostate-specific antigen suggests potential utility of the marker in identifying indolent disease.

Le et al[74] reported in 2005 the identification of serum amyloid A as a biomarker to distinguish prostate cancer patients with bone lesions (table 2D).

Colorectal cancer

Carcinoembryonic antigen (CEA) is another classical biomarker that is increased in patients with colorectal, breast, lung, or pancreatic cancer. As a screening test, it can be overexpressed by many other factors than cancer; smoking for instance raises CEA levels. However, tracking post-surgery CEA levels for colon cancer is an effective way of determining the usefulness of postoperative therapy.

The combination of SELDI-TOF mass spectrometry with appropriate bioinformatics tools has been useful for finding new biomarkers and establishing patterns with high sensitivity and specificity for the detection and diagnosis of colorectal cancer[76,77]. Recently,

TABLE 2C. **Summary of potential biomarkers in colorectal cancer identified by SELDI-TOF MS**

Sample type	Biomarkers (kDa)	Biomarkers ID	REFS
Colorectal cancer			
Cell line	12.000	Prothymosin α	75
Serum	31.000	N-terminal fragment of albumin	78
	6.600	ApoC-I	
	28.000	ApoA-I	
Serum	6.640	ApoC-I	79
	6.433	truncated form of ApoC-I	
	8.940	Complement C3a-des-Arg	
	39.900	α-1-antitrysin	
	50.700	$[Transferrin]^{2+}$	
Tissue, Serum	79.100	Transferrin	
	3.443	HNP-1 or α-Defensin-1	80, 81
	3.372	HNP-2 or α-Defensin-2	
	3.486	HNP-3 or α-Defensin-3	
Tissue	11.740	Calgizzarin or S100A11	82
Tissue	10.840	Heat shock protein 10	83

ID: identify; Apo: apolipoprotein; HNP: human neutrophil peptide; Arg: arginine.

TABLE 2D. **Summary of potential biomarkers in prostate, bladder, breast, liver, renal, pancreatic, endometrial, follicular and lymphoma cancer identified by SELDI-TOF MS**

Sample type	Biomarkers (kDa)	Biomarkers ID	REFS
Prostate cancer			
Serum	8.946	ApoA-II isoform	73
Serum	11.488	SAA-1 and isoforms	74
	11.573		
	11.573		
Bladder cancer			
Tissue	10.834	Calgranulin A or S100A8	84
Urine	3.380	Defensin-α2	102
	3.450	Defensin-α1	
Breast cancer			
NAF	8.000	$[Hb\text{-}\beta\ chain]^{2+}$	90
	15.940	Hb-β chain	
	31.770	Dimer of Hb-β chain	
Serum	8.100	C-term truncated form anaphylatoxin C3a	91
	8.900	Des-Arg anaphylatoxin C3a	
Serum	7.790	High molecular weight Kininogen	92
	9.285	ApoA-II isoform	
Liver cancer			
Serum	8.900	C-terminal fragment of vitronectin precursor	94
Renal cancer			
Serum	9.200	Haptoglobin 1-α	95
	11.144	SAA-1-RSFF	
	11.432	SAA-1-RS	
	11.518	SAA-1-R	
	11.683	SAA-1	
Pancreatic cancer			
Pancreatic juice	16.572	HIP/PAP-I	96
Endometrial cancer			
Tissue	10.843	Chaperonin 10	101
Tissue	10.834	Calgranulin A	102
Tissue	9.600	Protein EC1	103
	11.300	Protein EC2	
Follicular lymphoma			
Tissue	32.500	Cyclin D3	107
Lymphoblastoid lymphoma			
Cell line	4.972	Thymosin-β	108

ID: identify; Apo: apolipoprotein; Hb: hemoglobin; HIP/PAP-I: hepatocarcinoma–intestine–pancreas/pancreatitis-associated protein I; SAA: serum amyloid α. Arg: arginine; S: serine; F: phenylalanine; aa: aminoacid; term: terminal and EC: endometrial carcinoma.

Engwegen et al[78] identified two biomarkers, ApoC-I and ApoA-I, able to distinguish colorectal cancer patients and healthy controls. The detection of upregulated α-defensins-1, 2 and -3 (HNP1-3) in colorectal cancer tissues and sera were reported in two independent but similar analyses[80,81]. In these studies, SELDI-TOF MS results in tissue correlated with serum levels that were determined using ELISA or SELDI-TOF. The direct analysis of microdissected tissue for the discovery of tumor-specific markers followed by the specific detection of these markers in serum by antibody-based methods proved to be a successful strategy in this study. Unfortunately, α-defensins levels are also increased in serum during, for example, infection. Up to 15 new potential biomarkers have been reported (table 2C). Most of these markers are well known serum proteins that, somehow, now appear in relation to colorectal cancer in the referred works.

Breast cancer

SELDI-TOF MS has been applied for the biomarker identification in breast cancer serum[85], plasma[86-87],

tissue[88], nipple aspirate fluid (NAF)[89,90] and ductal lavage fluid (DLF)[89]. Li et al[85] identified three serum proteins by protein expression profiling on serum samples of ductal carcinoma: two (8.1 kDa and 8.9 kDa) up-regulated and one (4.3 kDa) down-regulated in patients. Lately, this group[91] confirmed only two markers of those previously identified: C-terminal-truncated form of C3a(desArg) (8.1 kDa) and complement component C3a(desArg) (8.9 kDa). These biomarkers are able to discriminate breast cancer samples from control patients (table 2D).

Some studies have investigated the effect of chemotherapy-induced in serum samples. Heike et al[92] identified two serum proteins (apolipoprotein A-II and high molecular weight kininogen) related to adverse effects induced by docetaxel infusion from protein expression profiles of serum using SELDI ProteinChip system. Although promising, the potential of SELDI-TOF MS for this application has not yet been established. These results suggest that protein expression profiles determined by SELDI TOF-MS represent useful data for the identification of treatment-responsive proteins.

Other cancers

Moreover, other studies have demonstrated the feasibility of using mass spectrometric proteomic pattern analysis for the diagnosis of several categories of tumours, including lung[93], liver[94], renal[95], pancreatic[96,61], head and neck[97,99] (table 2B), nasopharyngeal[100], endometrial[101-105], bladder[84,104-106], follicular lymphoma[107], and lymphoblastoid lymphoma[108] (table 2D).

CONCLUSIONS

Clinical medicine is undergoing a revolution driven by an increasing understanding of the human genome, the human proteome, the access to high-throughput techniques and the advances in molecular biotechnology. This revolution should transform clinical practice from population-based risk assessment and empirical treatment to a predictive, individualized model based on the molecular classification of disease and risk of disease, as well as targeted therapy. In special, the field of proteomics has yielded a set of technologies and analytical techniques that are significantly advancing the field of cancer diagnostics. These technologies allow for efficient means of identifying new biomarkers for the early detection of cancer and promise a hope of new serological screening methods for diagnosis. However, such markers still require substantial further validation before being introduced into the clinical area. The confirmation and validation of these targets identified should be performed using alternative methods such as *Western blotting* or immunohistochemistry and this may be combined with a complementary RNA-based transcriptomic screening approach. Once the identification and significant expression change of a target protein has been confirmed, then further detailed studies can ensue.

In addition, the analysis of the functional pathways in which the potential biomarkers have been implicated may reveal further insights into the mechanism of action, cross-talk between cellular pathways, and may also identify novel therapeutic targets. The confirmation of the clinical importance of any potential target using samples from cancer patients requires carefully designed experiments with adequate sample numbers with relatively homogeneous characteristics.

Future improvement in instrumentation sensitivity, labeling chemistries and chromatography procedures is clearly needed to enable routine quantification of proteins/peptides by mass spectrometry and thus, identification of potential biomarkers. Nevertheless, the complementary information obtained through different methods should potentially provide a better portrait of the biological system under investigation.

References

1. López JA, Bernat A, Albar JP. Protein Expression Profiling Analysis in Hematopoietic Sttem Cells: Phenotipic of Mesenchymal Sttem Cells. In: Sánchez J-C, Corthals GL., Hochstrasser DF, eds. Biomedical Applications of Proteomic. Wiley-VCH; 2004. p. 155-70.
2. Hanash S. Integrated global profiling of cancer. Nat Rev Cancer. 2004; 8:638-44.
3. Kolch W, Mischak H, Chalmers MJ, et al.Clinical proteomics: a question of technology. Rapid Commun. Mass Spectrom. 2004;18:2565-6.
4. Kolch W, Mischak H, Pitt AR. The molecular make-up of a tumour: proteomics in cancer research. Clin. Sci. (Lond.). 2005; 108:369-83.
5. Gygi SP, Corthals GL, Zhang Y, et al. Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. Proc Natl Acad Sci. 2000;97:9390-5.
6. Wilkins MR, Gasteiger E, Bairoch A, et al. Protein identification and analysis tools in the ExPASy server. Methods Mol Biol. 1999; 112:531-52.
7. Yates JR. Mass spectrometry: from genomics to proteomics. Trends Genet. 2000;16:5-8.
8. Gatlin CL, Eng JK, Cross ST, et al. Automated identification of amino acid sequence variations in proteins by HPLC/microspray tandem mass spectrometry. Anal Chem. 2000;72:757-63.
9. Alaiya A, Al-Mohanna M, Linder S. Clinical cancer proteomics: promises and pitfalls. Proteome Res. 2005;4:1213-22.
10. Anderson L, Seilhamer J. A comparison of selected mRNA and protein abundances in human liver. Electrophoresis. 1997;18:533-7.
11. Chen G, Gharib TG, Wang H, et al. Protein profiles associated with survival in lung adenocarcinoma. Proc Natl Acad Sci USA. 2003;100:13537-42.
12. Ginestier C, Charafe-Jauffret E, Bertucci F, et al. Distinct and complementary information provided by use of tissue and DNA microarrays in the study of breast tumor markers. Am J Pathol. 2002;161: 1223-33.
13. Alaiya A, Roblick U, Egevad L, et al. Polypeptide expression in prostate hyper-plasia and prostate adenocarcinoma. Anal Cell Pathol. 2000;21:1-9.
14. Nishizuka S, Charboneau L, Young L, et al. Proteomic profiling of the NCI-60 cancer cell lines using new highdensity reverse-phase lysate microarrays. Proc Natl Acad Sci USA. 2003;100:14229-34.
15. Izzotti A, Bagnasco M, Cartiglia C, et al. Proteomic analysis as related to transcrip-

tome data in the lung of chromium(VI)-treated rats. Int J Oncol. 2004;24:1515-22.
16. Zerkowski HR, Grussenmeyer T, Matt P, et al. Proteomics strategies in cardiovascular research. J Proteome Res. 2004;3: 200–8.
17. Donners MM, Verluyten MJ, Bouwman FG. Proteomic analysis of differential protein expression in human atherosclerotic plaque progression. J Pathol. 2005;206: 39–45.
18. Pierson J, Norris JL, Aerni HR, et al. Molecular profiling of experimental Parkinson's disease. Direct analysis of peptides and proteins on brain tissue sections by MALDI mass spectrometry. J Proteome Res. 2004;3:289–95.
19. Castano EM, Roher AE, Esh CL, et al. Comparative proteomics of cerebrospinal fluid in neuropathologically-confirmed Alzheimer's disease and non-demented elderly subjects. Neurol Res. 2006;2:155-63.
20. Borozdenkova S, Westbrook JA, Patel V, et al. Use of proteomics to discover novel markers of cardiac allograft rejection. J Proteome Res. 2004;3:282–8.
21. Bohring C, Krause W. Immune infertility: towards a better understanding of sperm (auto-) immunity. The value of proteomic analysis. Human Reprod. 2003;18:915–24.
22. Clarke W, Zhang Z, Chan DW. The application of clinical proteomics to cancer and other diseases. Clin Chem Lab Med. 2003;41:1562–70.
23. Wulfkuhle JD, Liotta LA, Petricoin EF. Proteomic applications for the early detection of cancer. Nat Rev Cancer. 2003;4: 267-75.
24. Muddiman DC, Cliby W, Bergen R. Cancer biomarkers. Clin Lab News. 2003;7: 12.
25. Wulfkuhle, JD, Sgroi, DC, Krutzsch, H, et al. Proteomics of human breast ductal carcinoma *in situ*. Cancer Res. 2002;62: 6740–9.
26. Anderson NL, Polanski M, Pieper R, et al. The human plasma proteome: a nonredundant list developed by combination of four separate sources. Mol Cell Proteomics. 2004;3(4):311-26.
27. O'Farrell P. High resolution two dimensional electrophoresis of proteins. J Biol Chem. 1975;250:4007–21.
28. Mann M, Hojrup P, Roepstorff P. Use of mass spectrometric molecular weight information to identify proteins in sequence databases. Biol Mass Spectrom. 1993;22: 338–45.
29. Unlu M, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. Electrophoresis. 1997;18:2071-7.
30. Monteoliva L, Albar JP. Differential proteomics: an overview of gel and non-gel based approaches. Brief Funct Genomic Proteomic. 2004;3:220-39.
31. Zhou G, Li H, De Camp D, et al. 2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers. Mol Cell Proteomics. 2002;1:117-24.
32. Somiari RI, Somiari S, Russell S, et al. Proteomics of breast carcinoma. J Chromatogr B. 2005;815:215-25.
33. Huang HL, Stasyk T, Morandell S, et al. Biomarker discovery in breast cancer serum using 2-D differential gel electrophoresis/ MALDI-TOF/TOF and data validation by routine clinical assays. Electrophoresis. 2006;27(8):1641-50.
34. Alexander H, Stegner AL, Wagner-Mann C, et al. Proteomic Analysis to Identify Breast Cancer Biomarkers in Nipple Aspirate Fluid. Clin Cancer Res. 2004;10:7500-10.
35. Bi X, Lin Q, Foo TW, et al. Proteomic analysis of colorectal cancer reveals alterations in metabolic pathways: mechanism of tumorigenesis. Mol Cell Proteomics. 2006;5(6):1119-30. 36.
36. Gygi SP, Rist B, Gerber SA, et al. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol. 1999;17:994-9.
37. Pawlik T, Hawke D, Liu Y, et al. Proteomic analysis of nipple aspirate fluid from women with early-stage breast cancer using isotope-coded affinity tags and tandem mass spectrometry reveals differential expression of vitamin D binding protein. BMC Cancer. 2006;6:68:1471-2407.
38. Li C, Hong Y, Tan YX, et al. Accurate qualitative and quantitative proteomic analysis of clinical hepatocellular carcinoma using laser capture microdissection coupled with isotope-coded affinity tag and two-dimensional liquid chromatography mass spectrometry. Mol Cell Proteomics. 2004;3(4):399-409.
39. Stewart JJ, White JT, Yan X, et al. Proteins associated with Cisplatin resistance in ovarian cancer cells identified by quantitative proteomic technology and integrated with mRNA expression levels. Mol Cell Proteomics. 2006;5(3):433-43.
40. DeSouza, L, Diehl, MJ, Rodriguez J, et al. Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with multidimensional liquid chromatography and tandem mass spectrometry. J Proteome Res. 2005;4:377–86.
41. Keshamouni VG, Michailidis G, Grasso CS, et al. Differential protein expression profiling by iTRAQ-2DLC-MS/MS of lung cancer cells undergoing epithelial-mesenchymal transition reveals a migratory/invasive phenotype. J Proteome Res. 2006; 5(5):1143-54.
42. Ong SE, Blagoev B, Kratchmarova I, et al. Stable isotope labelling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics. 2002;1(5):376-86.
43. Gronborg M, Kristiansen TZ, Iwahori A, et al. Biomarker discovery from pancreatic cancer secretome using a differential proteomic approach. Mol Cell Proteomics. 2006;1:157-71.
44. Mauri P, Scarpa A, Nascimbeni AC, et al. Identification of proteins released by pancreatic cancer cells by multidimensional protein identification technology: a strategy for identification of novel cancer markers. FASEB J. 2005;19(9):1125-7.
45. Jessani N, Niessen S, Wei BQ, et al. A streamlined platform for high-content functional proteomics of primary human specimens. Nat Methods. 2005;2:691-7.
46. Haab BB. Antibody Arrays in Cancer Research. Mol Cell Proteomics. 2005;4:377-83.
47. Kusnezow W, Jacob A, Walijew A, et al. Antibody microarrays: an evaluation of production parameters. Proteomics. 2003; 3:254-64.
48. Sreekumar A, Nyati MK, Varambally S, et al. Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins. Cancer Res. 2001; 61:7585-93.
49. Ghobrial IM, McCormick DJ, Kaufmann SH, et al. Proteomic analysis of mantle-cell lymphoma by protein microarray. Blood. 2005;105:3722-30.
50. Zhang Z, Bast RC Yu Y, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer Res. 2004;64:5882-90.
51. Plebani M. Proteomics: the next revolution in laboratory medicine? Clin Chim Acta. 2005;357(2):115-22.
52. Diamandis EP. Point: proteomic patterns in biological fluids: do they represent the future of cancer diagnostics? Clin Chem. 2003;49:1272–8.
53. Hutchens TW, Yip TT. New desorption strategies for the mass spectrometric analysis of macromolecules. Rapid. Commun. Mass Spectrom. 1993;7:576-80.
54. Issaq HJ, Veenstra TD Conrads TP, et al. The SELDI-TOF MS approach to proteomics: protein profiling and biomarker identification. Biochem Biophys Res Commun. 2002;292:587-92.
55. Clarke CH, Buckley JA and Fung, ET. SELDI-TOF-MS proteomics of breast cancer. Clin Chem Lab Med. 2005;43:1314-20.
56. Diamandis EP. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool. Mol Cell Proteomics. 2004;3: 367–78.
57. Diamandis EP. Analysis of serum proteomic patterns for early cancer diagnosis: drawing attention to potential problems. J. Natl Cancer Inst. 2004;96:353-6.
58. Kozak KR, Amneus MW, Pusey SM, et al. Identification of biomarkers for ovarian cancer using strong anion-exchange ProteinChips: Potential use in diagnosis and prognosis. Proc Natl Acad Sci USA. 2003; 100:12343-8.
59. Petricoin III EF, Omstein DK, Paweletz CP, et al. Serum proteomic patterns for detection of prostate cancer. J. Natl Cancer Inst. 2002;94:1576–8.
60. Won Y, Song HJ, Kang TW, et al. Pattern analysis of serum proteome distinguishes renal cell carcinoma from other urologic diseases and healthy persons. Proteomics. 2005;5:2510-6.
61. Koopmann J, Zhang Z, White N, et al. Serum diagnosis of pancreatic adenocarcinoma using surface-enhanced laser desorption and ionization mass spectrometry. Clin Cancer Res. 2004;10:860-8.
62. Adam BL, Qu Y, Davies JW, et al. Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. Cancer Res. 2002;62:3609–14.
63. Semmes OJ, Feng Z, Adam BL, et al. Evaluation of serum protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry for the detection of prostate cancer: I. Assessment of platform reproducibility. Clin Chem. 2005;51(1):102-12.
64. Petricoin III EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet. 2002;359: 572–77.
65. Kozak KR, Su F, Whitelegge JP, et al. Characterization of serum biomarkers for detection of early stage ovarian cancer. Proteomics. 2005;5:4589–96.
66. Rai AJ, Zhang Z, Rosenzweig J, et al. Proteomic approaches to tumor marker discovery. Arch Pathol Lab Med. 2002;126 (12):1518-26.
67. Ye B, Cramer DW, Skates SJ, et al. Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic

profiling and mass spectrometry. Clin Cancer Res. 2003;9(8):2904-11.
68. Woong-Shick A, Sung-Pil P, Su-Mi B, et al. Identification of hemoglobin-α and -β subunits as potential serum biomarkers for the diagnosis and prognosis of ovarian cancer. Cancer Sci. 2005;96:197-201.
69. Moshkovskii SA, Serebryakova MV, Kuteykin-Teplyakov KB, et al. Ovarian cancer marker of 11.7 kDa detected by proteomics is a serum amyloid A1. Proteomics. 2005;5:3790-7.
70. Wright Jr, Cazares, LH, Leung SM, et al. ProteinChip surface enhanced laser desertion/ionization (SELDI) mass spectrometry: a novel protein biochip technology for detection of prostate cancer biomarkers in complex protein mixtures. Prostate Cancer Prostatic Dis.1999;2:264-76.
71. Xiao Z, Jiang X, Beckett ML, et al. Generation of a Baculovirus Recombinant Prostate-Specific Membrane Antigen and Its Use in the Development of a Novel Protein Biochip Quantitative Immunoassay. Protein Expression and Purification. 2000;19:12-21.
72. Xiao Z, Adam BL, Cazares LH, et al. Quantitation of Serum Prostate-specific Membrane Antigen by a Novel Protein Biochip Immunoassay Discriminates Benign from Malignant Prostate Disease. Cancer Research. 2001;61:6029-33,
73. Maliki G, Ward MD, Gupta SK, et al. Serum Levels of an Isoform of Apolipoprotein A-II as a Potential Marker for Prostate Cancer. Clinical Cancer Research. 2005;11:1073-85.
74. Le L, Chi K, Tyldesley S, et al. Identification of Serum Amyloid A as a Biomarker to Distinguish Prostate Cancer Patients with Bone Lesions. Clinical Chemistry. 2005;51(4):695-707.
75. Shiwa M, Nishimura Y, Wakatabe R, et al. Rapid discovery and identification of a tissuespecific tumor biomarker from 39 human cancer cell lines using the SELDI ProteinChip platform. Biochem Biophys Res. Commun. 2003;309:18-25.
76. Chen YD, Zheng S, Yu JK, et al. Artificial neural networks analysis of surface enhancedlaser desorption/ionization mass spectra of serum protein pattern distinguishes colorectal cancer from healthy population. Clin Cancer Res. 2004;10: 8580-5.
77. Yu JK, Chen YD, Zheng S. An integrated approach to the detection of colorectal cancer utilizing proteomics and bioinformatics. World J Gastroenterol. 2004;10: 3127-31.
78. Engwegen JY, Helgason HH, Cats A, et al. Identification of serum proteins discriminating colorectal cancer patients and healthy controls using surface enhanced laser desorption ionisation-time of flight mass spectrometry (SELDI-TOF MS). World J Gastroenterol. 2006;12:1536-44.
79. Ward DG, Suggett N, Cheng Y, et al. Identification of serum biomarkers for colon cancer by proteomic analysis. Br J Cancer. 2006;94(12):1898-905.
80. Melle C, Ernst G, Schimmel B, et al. Discovery and identification of α-defensins as low abundant tumor-derived serum markers in colorectal cancer. Gastroenterology. 2005;129:66-75.
81. Albrethsen J, Bogebo R, Gammeltoft S, et al. Upregulated expression of human neutrophil peptides 1, 2 and 3 (HNP 1-3) in colon cancer serum and tumours: a biomarker study. BMC Cancer. 2005;5(8): 1-10.
82. Melle C, Ernst G, Schimmel B, et al. Different expression of calgizzarin (S100A11) in normal colonic epithelium, adenoma and colorectal carcinoma. Int J Oncol. 2006;28(1):195-200.
83. Melle C, Bogumil R, Ernst G, et al. Detection and identification of heat shock protein 10 as a biomarker in colorectal cancer by protein profiling. Proteomics. 2006;6(8):2600-8.
84. Tolson JP, Flad T, Gnau V, et al. Differential detection of S100A8 in transitional cell carcinoma of the bladder by pair wise tissue proteomic and immunohistochemical analysis. Proteomics. 2006; 6(2):697-708.
85. Li J, Zhang Z, Rosenzweig J, et al. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. Clin Chem. 2002; 48:1296-304.
86. Pusztai L, Gregory BW, Baggerly KA, et al. Pharmacoproteomic analysis of prechemotherapy and postchemotherapy plasma samples from patients receiving neoadjuvant chemotherapy for breast carcinoma. Cancer. 2004;100:1814-22.
87. Caputo E, Lombardi ML, Luongo V, et al. Peptide profiling in epithelial tumor plasma by the emerging proteomic techniques. J. Chromatogr. B Analyt Technol Biomed Life Sci. 2005;819:59-66.
88. Traub F, Feist H, Kreipe HH, Pich A. SELDI-MS-based expression profiling of ductal invasive and lobular invasive human breast carcinomas. Pathol Res Pract. 2005;201:763-70.
89. Li J, et al. Identification of biomarkers for breast cancer in nipple aspiration and ductal lavage fluid. Clin Cancer Res. 2005; 11:8512-20.
90. Sauter ER, Shan S, Hewett JE, et al. Proteomic analysis of nipple aspirate fluid using SELDI-TOF-MS. Int J Cancer. 2005; 114:791-6.
91. Li J, Orlandi R, White CN, et al. Independent validation of candidate breast cancer serum biomarkers identified by mass spectrometry. Clin Chem. 2005;51:2229-35.
92. Heike Y, Hosokawa M, Osumi S, et al. Identification of serum proteins related to adverse effects induced by docetaxel infusion from protein expression profiles of serum using SELDI ProteinChip system. Anticancer Res. 2005;25:1197-203.
93. Xiao X, Liu, D, Tang, Y, et al. Development of proteomic patterns for detecting lung cancer. Dis Markers. 2003;19:55-9.
94. Paradis V, Degos F, Dargere D, et al. Identification of a new marker of hepatocellular carcinoma by serum protein profiling of patients with chronic liver diseases. Hepatology. 2005;41:40-7.
95. Tolson J, Bogumil R, Brunst E, et al. Serum protein profiling by SELDI mass spectrometry: detection of multiple variants of serum amyloid a in renal cancer patients. Lab Invest. 2004;84:845-56.
96. Rosty C, Ueki T, Argani P, et al. Identification of hepatocarcinoma-intestine-pancreas/ pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. Cancer Res. 2002;62:8-1875.
97. Wadsworth JT, Somers KD, Cazares LH, et al. Identification of patients with head and neck cancer using serum protein profiles. Arch. Otolaryngol. Head Neck Surg. 2004;130:98-104.
98. Melle C, Ernst G, Schimmel B, et al. Biomarker discovery and identification in laser microdissected head and neck squamous cell carcinoma with ProteinChipw technology, two-dimensional gel electrophoresis, tandem mass spectrometry, and immunohistochemistry. Mol. Cell. Proteomics. 2005;2:443-52.
99. Roesch-Ely M, Nees M, Karsai S, et al. Proteomic analysis reveals successive aberrations in protein expression from healthy mucosa to invasive head and neck cancer. Oncogene. 2006; Jul 3.
100. Cho WC, Yip TT, Yip C, et al. Identification of serum amyloid A protein as a potentially useful biomarker to monitor relapse of nasopharyngeal cancer by serum proteomic profiling. Clin Cancer Res. 2004;10:43-52.
101. Yang EC, Guo J, Diehl G, et al. Protein expression profiling of endometrial malignancies reveals a new tumormarker: chaperonin 10. J Proteome Res. 2004;3: 656-45.
102. Guo J, Yang EC, Desouza L, et al. A strategy for high-resolution protein identification in surface-enhanced laser desorption/ ionization mass spectrometry: calgranulin A and chaperonin 10 as protein markers for endometrial carcinoma. Proteomics. 2005;5:1953-66.
103. Yoshizaki T, Enomoto T, Nakashima R, et al. Altered protein expression in endometrial carcinogenesis. Cancer Lett. 2005; 226(2):101-6.
104. Vlahou, A, Schellhammer, PF, Mendrinos, S, et al. Development of a novel proteomic approach for the detection of transitional cell carcinoma of the bladder in urine. Am J Pathol. 2001;158:1491-502.
105. Langbein S, Lehmann J, Harder A, et al. Protein profiling of bladder cancer using the 2D-PAGE and SELDI-TOF-MS technique. Technol Cancer Res Treat. 2006;5 (1):67-72.
106. Krieg RC, Gaisa NT, Paweletz CP, et al. Proteomic analysis of human bladder tissue using SELDI approach following microdissection techniques. Methods Mol Biol. 2005;295:255-67.
107. Lin Z, Jenson SD, Lim MS, et al. Application of SELDI-TOF mass spectrometry for the identification of differentially expressed proteins in transformed follicular lymphoma. Mod Pathol. 2004;17:670-8.
108. Diamond DL, Zhang Y, Gaiger A, et al. Carter D. Use of ProteinChipe array surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDITOF-MS) to identify thymosin b-4, a differentially secreted protein from lymphoblastoid cell lines. J Am Soc Mass Spectrom. 2003;14:760-5.