Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 November 28; 13(44): 5813-5821
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Ignacio Gil-Bazo, MD, PhD, *Series Editor*

Moving forward in colorectal cancer research, what proteomics has to tell

Nerea Bitarte, Eva Bandrés, Ruth Zárate, Natalia Ramirez, Jesus Garcia-Foncillas

Nerea Bitarte, Eva Bandrés, Ruth Zárate, Natalia Ramirez, Jesus Garcia-Foncillas, Laboratory of Pharmacogenomics, Center for Medical Applied Research, Unit of Clinical Genetics, University Clinic, Department of Oncology and Radiotherapy, University Clinic, University of Navarra, Pamplona 31008, Spain
Correspondence to: Jesús García-Foncillas, MD, PhD, Laboratory of Pharmacogenomics, Center for Medical Applied Research, Department of Oncology and Radiotherapy, University Clinic, University of Navarra, Avda Pio XII 36, Pamplona 31008, Spain. jgfoncillas@unav.es
Telephone: +34-948-194700-1008 Fax: +34-948-194718
Received: July 30, 2007 Revised: September 12, 2007

Abstract

Colorectal cancer is the third most common cancer and is highly fatal. During the last several years, research has been primarily based on the study of expression profiles using microarray technology. But now, investigators are putting into practice proteomic analyses of cancer tissues and cells to identify new diagnostic or therapeutic biomarkers for this cancer. Because the proteome reflects the state of a cell, tissue or organism more accurately, much is expected from proteomics to yield better tumor markers for disease diagnosis and therapy monitoring. This review summarizes the most relevant applications of proteomics the biomarker discovery for colorectal cancer.

Key words: Proteomics; Colorectal cancer; Biomarker

Bitarte N, Bandrés E, Zárate R, Ramirez N, Garcia-Foncillas J. Moving forward in colorectal cancer research, what proteomics has to tell. *World J Gastroenterol* 2007; 13(44): 5813-5821

http://www.wjgnet.com/1007-9327/13/5813.asp

INTRODUCTION

Cancer is not a single disease, but an accumulation of genetic and epigenetic events. It is characterized by uncontrolled growth of cells that can invade and destroy normal tissues. These abnormal cells can also spread through the bloodstream or lymph system to start new tumors in other parts of the body. The disease is a great challenge to clinicians and scientists.

Recent progress in molecular biology has allowed the identification of markers usefull for patient management through the identification of genetic alterations and an understanding of chemotherapy molecular targets. Several examples in digestive oncology underline the relevance of molecular biology in clinical research[1].

Colorectal cancer is a common malignancy with an annual incidence of over 945 000 cases worldwide and an annual mortality of 492 000[2]. Surgery is the treatment of choice offering a potential cure. However, 30%-40% of patients have local regionally advanced or metastatic disease on presentation, which cannot be cured by surgery alone[3]. In addition, more than half of patients initially believed to be cured develop recurrence and die of the disease[4].

Advances in genomics and proteomics contribute to our understanding of pathways that control growth, differentiation, and death of cells. In these processes, the identification of candidate disease genes and modifier genes by integrated study of gene expression and metabolite levels is instrumental for future health care. This approach, called systems biology, can recognize early onset of disease and identify new molecular targets for novel drugs in cancer[5].

Proteomics analyzes proteins within a cell or in the corresponding tissue; the proteins of interest are identified, but their function and interactions are not determined. The research provides complete and detailed data about structure, expression, and function of genes, but failes to demonstrate how all the information implicated in the genome is used. In the "post-genomic era," proteomics might be the key to understand systems biology. During the past few years, proteomics has been utilized in many fields of science, medicine, pharmacy, industry and agriculture[6]. In most of the applications proteomics is used to determine expression profiles of proteins in cells and tissues in normal or disease states[7] that are responsible for abnormal cell proliferation. The identification of proteins that are characteristic for cancer development can potentially uncover diagnostic, or prognostic markers, or novel drug targets, and could help understand the mechanisms underlying tumor formation (Figure 1).

Currently, proteomic technology has been used in two areas of cancer research, in early diagnosis and in the treatment of patients, that also includes prediction of response. This technology, when combined with

genomic analysis, may provide more information about the molecular basis of carcinogenesis and the development of more effective anti-cancer therapies. This review focuses on the proteomic studies applied in colorectal cancer.

PROTEOMIC TECHNIQUES IN CANCER RESEARCH

Sample preparation in proteomic

Sample preparation is the most critical step in any proteomics study. This is important because it affects reproducibility as a result of the heterogeneity of proteins derived from cell populations[8]. From the time of sample collection to when proteins are processed for analysis, multiple factors come into play. Mechanical methods, such as surface scrapping and fine needle aspiration, have been used for capturing cancer cells[9]. Calcium depletion and other nonenzymatic methods, such as immunomagnetic separation, have been used to obtain pure populations of cancer cells[10]. An important advancement in sample preparation has been the development of laser capture microdissection (LCM). The LCM system permits obtaining pure populations of cancer cells from frozen, paraffin-embedded, stained, and unstained tissues for molecular analysis. The system is based on visualizing a tissue section via light microscopy and procurement of cells by activating a 7.5-30 micron diameter infrared laser beam which adheres the tissue to a plastic cap. Intact deoxyribonucleic acid, RNA, and protein are then extracted from the adhered tissue which then can be analyzed using conventional methods[11,12]. Protein expression has been compared using 2-D PAGE and differentially expressed proteins identified by mass spectrometry, permitting the discovery of a novel colorectal cancer biomarker[13,14].

Two-dimensional gel electrophoresis and tumor protein detection (2D)

Traditional proteomic studies are based on 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE) to compare protein expression patterns from different tissues or cell lines. The first dimension separates proteins by pH, isoelectric focusing, and the second dimension by molecular mass, sodium dodecyl sulfate PAGE. Although, 2-D PAGE has been available for several decades, improvements in this technology have dramatically improved sensitivity, resolution and reproducibility.

The more important application of this technique in disease proteomics is the discovery of proteins which might serve as prognostic biomarkers for survival of cancer patients. A novel application of 2-D PAGE has been in the discovery of circulating autoantibodies in cancer patients. In some cancer patients, there is evidence that a humoral immune response against tumor antigens might be elicited, and this might be used in serum assays of disease progression or in the development of anticancer vaccines.

An advantage of 2-D PAGE is that it has the capacity to resolve and investigate protein, abundance in a single sample and the possibility to directly detect changes in diseased and healthy tissue.

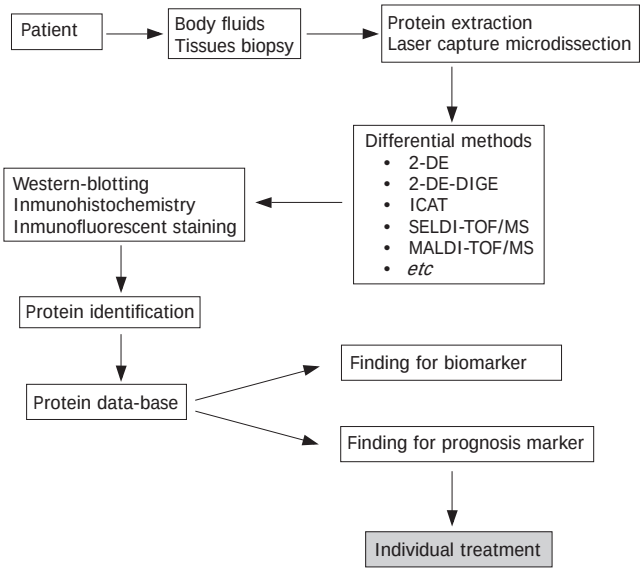

Figure 1 Proteomic differential display methods.

The major disavantage of 2-D PAGE is that it is laborious and does not resolve highly basic or proteins, smaller than 10 kDa. Because most clinical biomarkers are high large proteins 2-D PAGE is an ideal technology for the study of cancer biomarkers. Therefore, 2-D PAGE, complemented with mass spectrometry, has been used to identify protein changes associated with a variety of human cancers[12].

Two-dimensional difference gel electrophoresis (2D-DIGE)

One of the most recent technical advances in 2-DGE has been multiplexing fluorescent 2D-DIGE[15]. This method directly labels lysine groups in proteins with cyanine (Cy) dyes prior to IEF and can allow for quantitative comparisons between patients and control samples when different fluorescent labels are used for each sample.

The critical aspect of 2D-DIGE technology is the ability to label 2-3 samples with different dyes and then electorphorese all samples on the same 2-D gel. This ability reduces spot pattern variability and the number of gels in an experiment making spot matching much more simple and accurate[16]. The single positive charge of the CyDye replaces the single positive charge present in the lysine at neutral and acidic pH keeping the pI of the protein relatively unchanged. A mass of approximately 500 Da is also added by the CyDye to the labeled protein. The individual protein data from the control and diseased/treatment (Cy5 or Cy3) samples are normalized against the Cy2 dye-labeled sample, Cy5:Cy2 and Cy3:Cy2. These logarithm abundance ratios are then compared between the control and diseased/treatment samples from all the gels using statistical analysis (*t*-test and ANOVA)[17,18]. The principal disavantage of this technique is that it has a low thoughtput (three samples per gel) (Figure 2).

Antibody, protein and peptide arrays

Antibody array based measurement technologies have long provided an important tool to detect and manipulate specific biological molecules. While previous uses of

antibodies and related affinity reagents have focused on single targets, recent developments have included multiplexed use of antibodies in arrays, so that many targets can be measured in parallel, sometimes in very small sample volumes. The uses of such arrays are varied and new applications and formats continue to evolve[19].

The experimental features of microarrays have advantages for cancer research. The low sample volumes result in the consumption of small amounts of both precious clinical samples and expensive antibodies. The assays can be run efficiently in parallel, making possible studies on the large populations of samples that are necessary for marker detection and validation. In addition, these assays have good reproducibility, high sensitivity, and quantitative accuracy over large concentration ranges[20]. Antibody and protein arrays are complementary and in some aspects preferable to separation based and mass spectrometry based technologies. Reproducibility and throughput can be higher, and the identities of the considered proteins are known or can be readily characterized. Therefore, specific hypotheses regarding the nature of molecular alterations can be tested, and biologically interpreted[21]. Applications of antibody array methods to cancer research are increasing in scale and effectiveness.

Protein and peptide arrays are effective for probing the interactions of protein and peptides with other antibodies, protein or other molecules. Protein microarrays are an emerging class of nanotechnology for analysing many different proteins simultaneously. Much progress has been made for applications in basic science[22]. These approaches are likely to recapitulate at the protein level the mRNA expression profiling studies by arraying various protein probes on top of specific surfaces, and then determining interactions with specific proteins in complex samples. The most advanced format in this setting is the antibody microarray, where the proteins are specific antibodies printed on solid surfaces.

Protein arrays recently have confirmed the use for probing the abundance of specific proteins in biological samples, this phase call "reverse phase". Protein lysates from cell culture or tissue samples are spotted in microarrays on nitrocellulose membranes. A labeled antibody specific for a particular protein is incubated on a microarray, and quantification of the bound antibody reveals the amount of that protein in each sample[23,24]. Therefore, reverse phase array experiments quantify a single protein in many samples, in contrast to antibody arrays that quantify many proteins in one sample. Numerous demonstrations that this technology uses for profiling proteins in cancer have appeared.

The various methods presented here are complementary with each other and with other proteomic methods, and they may be used together for added benefit as demonstrated in a study of proteins in breast cancer cells using cytokine arrays, reverse phase arrays, and bead-based arrays in conjunction with two-dimensional gels (Figure 3).

TOF-Mass Spectrometry applications in clinical oncology

SELDI-TOF MS is a commonly used non-gel based method. The technique combines protein separation directly with presentation to the mass spectrometer. Various types

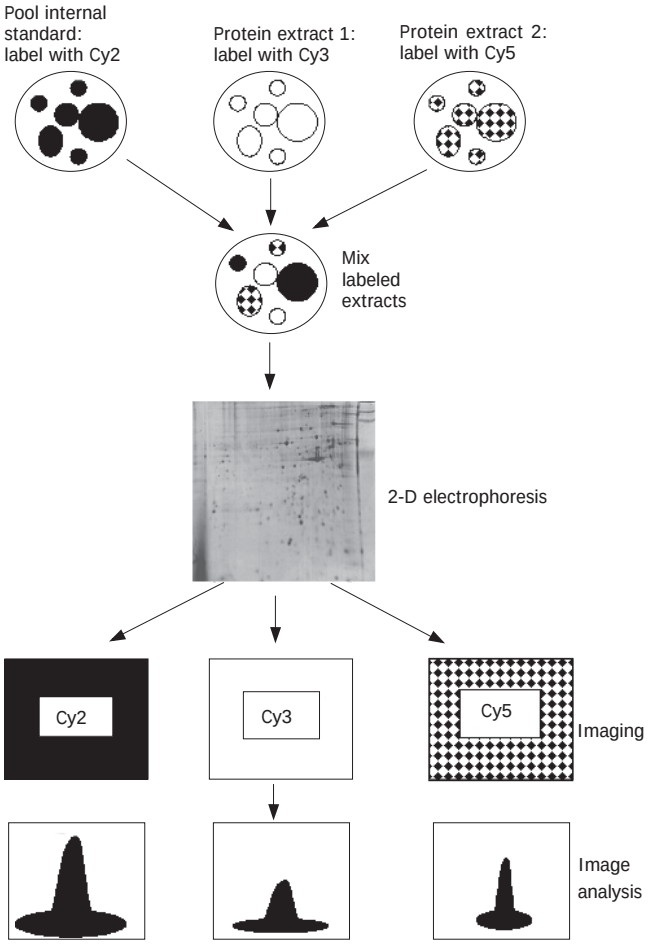

Figure 2 2D-DIGE techniques. Cy2, Cy3 and Cy5 are different fluorescent dyes.

of substrates have different affinities for different proteins, thus it is possible to increase protein representation when combining various arrays. The combination of these arrays with up-front prefractionation chromatography (e.g. anion exchange) permits the detection of up to 2000 protein species from serum[25,26]. The resulting spectral masses are analyzed using univariate and multivariate statistical instruments to provide a single marker or multimarker pattern that can classify clinical samples. Discriminator protein pinnacles are then purified and submitted to the MSbased identification process (Figure 4).

The SELDI technique was developed to profile clinical biological fluids, notably serum and/or plasma, and became important when numerous studies showed its potential in identifying unique biomarkers or complex patterns with diagnostic value, allowing its use for screening and early diagnosis in various cancers[27,28]. One major criticism of the technique relies on the overall lack of sensitivity and capability to detect tumor-specific protein traces within a large amount of nonspecific protein species[29]. However, even though still controversial in its reproducibility and ability to detect actual specific tumor signatures, SELDI has several advantages, such as easy of use, high throughput, and relatively reasonable cost, all making it a very attractive technique for working with large clinical sample.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), is a

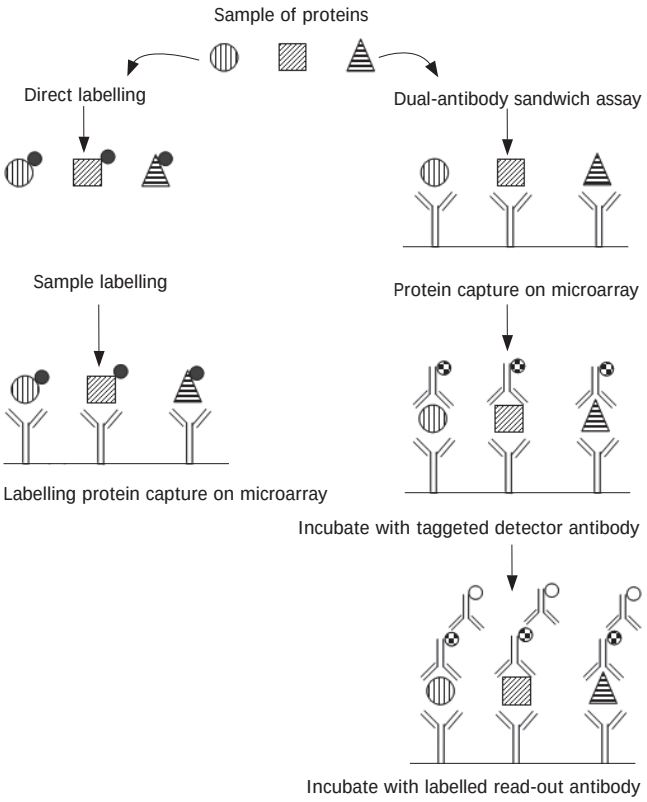

Figure 3 Representation of the two antibody microarray experimental formats. Direct labelling: single-capture antibody experiments; all proteins in a sample are labelled (black circles) thereby providing a means for detecting bound proteins following incubation on an antibody microarray. Dual-antibody (capture and read-out antibody) sandwich immunoassays: proteins captured on an antibody microarray are detected by a cocktail of tagged detection antibodies, which are matched to the spotted antibodies. The detector antibody tag is then measured by binding of a labelled (empty circles) read-out antibody.

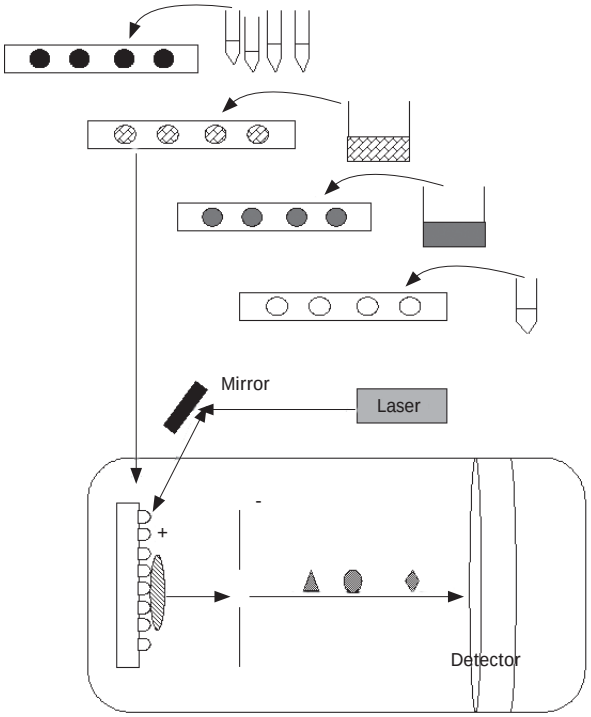

Figure 4 Principles of SELDI-TOF MS. The application of sample from to an eight-spot array with hydrophilic, hydrophobic, cationic, anionic or immobilized-metal affinity capture chromatography surface (black colour). The addition of an appropriate binding buffer (purple colour). On-chip sample purification using one or more wash buffers (grey colour). The application of energy-absorbing matrix for the absorption of laser energy (empty colour). Laser irradiation desorbs bound proteins and positively ionizes them. Owing to the electric field, they migrate in the mass analyser: (small diamond) and multiply charged proteins (oval) faster than large and single-charged ones (triangle). Thus, the proteins are separated. Time of flight (t) is proportional to protein mass per charge.

technique to analyze peptides and proteins in relatively complex samples. It has even been used for the direct analysis of tissue specimens[30]. In MALDI-TOF MS, a small quantity of specimen containing peptides and protein is dried on a target plate together with a light-absorbing matrix molecule.

Two technical advancements have improved resolution of MALDI-TOF MS to its current state. First, use of an electronic mirror (reflectron) to reflect ions substantially increases resolution, and second, delayed extraction introduced after sample vaporization and earlier than the electric potential is applied. Shorter times are optimal for small molecules, and longer times for large molecules. The standard detector for MALDI-TOF MS is a microchannel plate, which acts as an electron multiplier for ions reaching the detector. Detector replys relate to the number of ions reaching the detector and ion velocities.

MALDI-TOF MS permits a rapid determination of molecular masses and the heterogeneity of small amounts of peptides and proteins. Usually, intact molecular ions are formed and determination of polypeptide mass.

LC-MS and LC-MS-MS in comparative proteomic

Capillary-scale HPLC-MS/MS (LC-MS) is rapidly emerging as a method of choice for large scale proteomic analysis[31]. LC-MS systems can be used to identify and track the relative abundance of thousands of molecules[32]. For standard bottom-up profiling experiments, the molecules in question are peptides derived by proteolysis of intact proteins. For very complex protein samples, such as blood, the peptide mixtures are resolved by chromatographic separation prior to injection into the mass spectrometer. This generates a more informative map, that consists of both the unique elution of individual peptides. Distinct peptides of interest are induced by collision fragmentation followed by database matching for the purpose of sequence identification, while the recorded pattern of precursor ion intensities can be used to infer the relative quantities of the various proteins between samples[33].

LC-MS systems consists of different instruments to separate peptide mixtures based on physicochemical properties, separate ions on the basis of m/z ratios and registers the relative abundance of ions at discrete m/z.

In LC-MS-MS technique, precursor ions are recorded in full-scan mode, followed by selective ion isolation and fragmentation for sequence identification[33] (Figure 5).

Isotope-coded affinity tags (ICAT and iTRAQ)

This is the prototypical and the most popular method for quantitative proteome analysis based on stable isotope affinity tagging and MS[34].

The ICAT reagent is a sulphydryl-directed alkylating agent composed of iodoacetate attached to biotin through

a short oligomeric coupling arm (d0). The exchange of 8 deuterium atoms for hydrogen atoms in the coupling arm produces a heavy isotope version of the reagent (d8). Thus the reagent comprises of a cysteine reactive group, a linker containing the heavy or light isotopes (d8/d0) and a biotin affinity tag. This method involves *in vitro* derivatization of cysteine residues in protein with d0 or d8 followed by enzymatic digestion of the combined sample. All the cysteine residues thus tagged with biotin are selectively separated by avidin column and the cysteine-containing peptides are further separated followed by MS analysis[35].

The iTRAQ technique capable of multiplexing samples is primarily based on the ICAT technique and compared in detail. The iTRAQ technique uses four isobaric reagents allowing the multiplexing of four different simples in a single LC-MS-MS experiment. The multiplexing capability of iTRAQ allows a control sample to be compared with different points in time of a disease state, as well as with respect to different drug treatments. One of the major advantages of this technique is its ability to label multiple peptides per protein, which increases the confidence of identification and quantitation[35].

There are numerous differences (advantages and disadvantages) between the select proteomic technologies for protein profiling (Table 1).

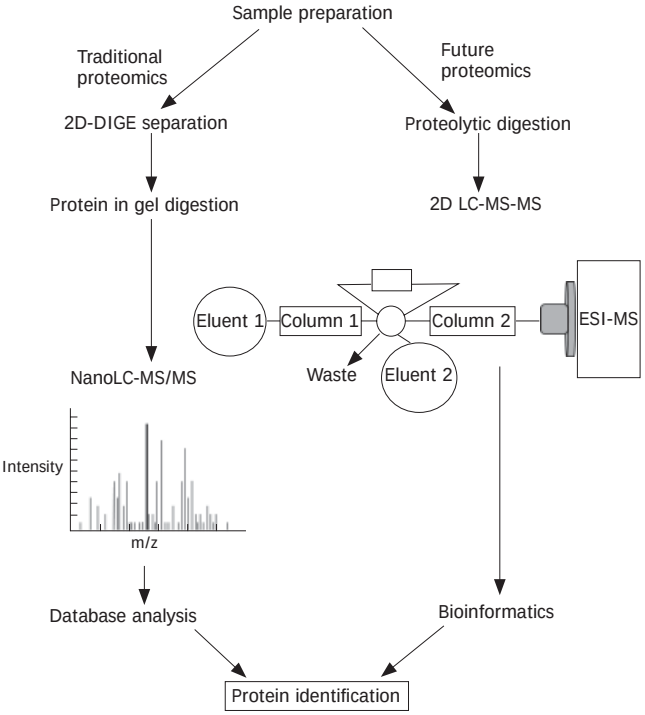

Figure 5 Different strategies for proteomic studies.

High-resolution hybrid quadrupole TOF

One of the first major advances used in any developing area of research was a high-resolution hybrid quadrupole TOF (QqTOF) MS fitted with a SELDI ion source to acquire proteomic patterns from serum. A recent study was designed to determine whether there is any diagnostic advantage provided by acquiring the proteomic patterns of serum samples using a high-resolution, high mass accuracy MS instrument. Results were analyzed on the exact same ProteinChip surface, thus eliminating all experimental variability apart from the use of two different instruments. Different combinations of bioinformatic heuristic parameters were used to generate different diagnostic models using the data acquired from the two distinct mass spectrometers[36]. These parameters included the similarity space for cluster classification, and the learning rate in training of the genetic algorithm. The diagnostic models generated from mass spectra acquired using the higher-resolution Qq-TOF MS were statistically superior[37].

Proteomic analysis software

The result of the analysis of a complex proteomic mixture by SELDI-TOF-MS is a low resolution profile of the protein or peptide species that were subsequently ionized from ProteinChip surface. It has been the development and combination of sophisticated bioinformatic algorithms for the analysis of SELDI-TOF-MS data. The intention of this bioinformatic analysis has led to the potential application of this technology as a major advancement in the diagnosis of cancer and other diseases. There are several different types of bioinformatic algorithms, such as single classification trees, neural nets, genetic algorithms, and random forest algorithms, which have been applied to enable SELDI-TOF-MS data to be investigated as a diagnostic technology. Although they function in different protocols, these algorithms share a common goal: to construct a classifier and discover peak intensities most likely to be responsible for segregating classes of samples[38]. Since its inception, SELDI-TOF-MS has been used to develop diagnostic platforms for several different cancers.

PROTEOMIC ANALYSIS IN COLORECTAL CANCER

During the past decade, genomic analyses have been introduced into cancer studies with variable success. It has become recognized that genomic techniques are insufficient to study the complex pathways of carcinogenesis; this has led to the application of proteomic techniques, which allow for the reliable analysis of complex mixtures of proteins[39].

Colorectal cancer is the third most common cancer in the world. It is well known that the adenomatous polyposis coli (APC) gene is mutated in patients with familial adenomatous polyposis (FAP) and sporadic colorectal cancer, and that mutations initiate colorectal carcinogenesis. It is now suggested that many colorectal cancers arise from preexisting adenomas. Following several steps of mutation of oncogenes and tumor suppressor genes, adenomas develop to colorectal cancers[40].

Many groups have reported the proteomic analyses of colorectal cancers. Dundas *et al* found that mortalin, also known as mitochondrial HSP70, is involved in cell cycle regulation with important roles in cellular senescence and immortalization pathways and was over-expressed in colorectal adenocarcinomas and correlated with poor survival[41]. Lane *et al* identified over-expressed multiple cytochrome P450 enzymes in human colorectal cancer tissues and metastases[42]. Cytochrome P450 proteins

Table 1 Advantages and disadvantages of proteomic technologies for protein profiling

Technique	Methods	Advantages	Disavantages
2D	Separation on a gel of the protein content of a sample in two dimensions according to mass and charge; gels are stained and spot intensities in samples are compared among different gels	High separation (thousands of proteins per gel)	Low throughput laborious (one samples per gel); poor resolution for extreme masses and extremely acidic or basic proteins; no direct protein identification; large amount of starting material compared with other techniques
2D-DIGE	Measuring three samples per gel; each of them is labelled with a different fluorescent dye, and the intensities of each gel spot for each sample are measured at a wavelength specific for the label	Direct comparison of samples on one gel: better reproductibility	Low throughput (three samples per gel)
Protein microarrays	Binding of a targeted protein in one sample to spotted probes on a 'forward' microarray; conversely, binding of specific probes to a targeted protein in spotted samples on a 'reverse' microarray; detection of bound proteins by direct labelling or by labelled secondary antibodies	High throughput in terms of number of probes per (forward) array or number of samples per (reverse) array; biomarker identity or class readily known	Synthesis of many different probes necessary; identity or class of targeted proteins must be known; limited to detection of proteins targeted by the probes
SELDI-TOF MS	Selected part of a protein mixture is bound to a specific chromatographic surface and the rest washed away	High throughput; direct application of whole sample (fast on-chip sample cleanup); small amount of starting material	Unsuitable for high molecular weight proteins; limited to detection of bound proteins; lower resolution and mass accuracy than MALDI-TOF
MALDI-TOF MS	Application of a protein mixture onto a gold plate; desorption of proteins from the plate by laser energy and measurement of the protein masses; comparison of peak intensities between multiple samples	High throughput	Need for sample fractionation of complex samples; more starting material needed for sample fractionation; unsuitable for high molecular weight proteins
LC-MS-MS	Separation of a mixture of peptides (resulting from protein digestion with trypsin) by one-, two-or three-dimensional LC and measurement of peptide masses by MS-MS	Direct identification of several hundred proteins per sample by MS-MS of peptides	Low throughput; time consuming; detection by MS–MS often not comprehensive, tus complicating comparison of different samples
ICAT	Chemical tagging of proteins on cysteine residues with a heavy or light stable isotopic; after labelling samples are mixed, proteins are digested with trypsin, and labelled peptides isolated by affinity chromatography; both samples are analysed concomitantly by LC-MS-MS	Direct identification of biomarkers by MS-MS of peptides; relative quantitation; less sample complexity than with iTRAQe; MS-MS of only differentially expressed proteins	Low throughput; tagging of only cysteine-containing peptides
iTRAQ	Chemical tagging of proteins on their amine groups with stable isotopic labels of identical mass ('isobaric'); four different labels are available for four different samples; after labelling, samples are mixed, proteins digested with trypsin and analysed concomitantly by LC-MS-MS	Direct identification of biomarkers by MS-MS of peptides; owing to isobaric labels, selection for MS-MS of the same peptide in all four samples in the same single MS run	Low throughput (four samples per run); for generating signature ion, MS-MS of all peptides in a sample is necessary; high sample complexity and limited resolution of LC (even three dimensional), confounding by co-eluting isobaric peptides

(CYPs) in the liver are known to be of major importance to the fate of anticancer agents; however, their expression and role in tumours has received little attention. CYP-mediated metabolism is generally viewed as a route to drug detoxification and increased elimination, although CYP activation of certain anticancer drugs. The presence of metabolically active CYPs in a colon metastastic deposit is likely to be important in determining the metabolic fate of chemotherapeutic agents and hence the outcome of treatment. Stulik *et al* performed proteomic differential display between the matched sets of macroscopically normal colon mucosa and colorectal cancer tissues. They report that the expression of HSP70, S100A9, S100A8, S100A11 and S100A6 was up-regulated in colorectal cancer tissues compared to normal colon mucosa, and the levels of liver fatty acid-binding protein, actin-binding protein/smooth muscle protein 22-a and cyclooxygenase 2 were down-regulated in transformed colon mucosa[43]. The S100A6 protein was the first S-100 protein specifically identified as being related to the state of cellular proliferation. The possible correlation between increased expression of some members of the S100 protein

Table 2 Proteomic analysis in human colorectal cancer tissues

Up-regulated		Down-regulated
Annexin Ⅳ		NCF2
MTA-1		PMM2
SSX5 protein		Serpin 1
Dynein heavy chain		CNRC
Cytochrome P450		Annexin Ⅴ
CPT1		APC
Keratin 10		VAV3 protein
Keratin 8		RSP 4
Keratin 19		SPARC like protein 1
Vimentin		PDI
β-actin		GN6ST
REL1		Cathepsin D
HSP60		Calreticulin
Mortalin	Cathepsin fragment	SM31
		PDA6
	Proteasome subunit a type 6	ApoA1 precursor
Cytochrome P450 enzymes		ATP synthase b chain
(in cancer tissues and metastatic tissues)	Triosephosphate isomerase 14-3-3 proteins	Albumin
		Liver fatty acid-binding protein
HSP70	GST-P	Actin-binding protein/smooth muscle protein 22-a
S100A9		
S100A8	P13693 translationally controlled tumor protein	Cyclooxygenase 2
S100A11		
S100A6		Puromycin-sensitive aminopeptidase
	Nucleoside diphosphate kinase A	
		NADH-ubiquinone oxidoreductase
Adenosyl homocysteinase	Calgranulin B; S100 A9	
Leukocyte elastase inhibitor, claude B		Succinate dehydrogenase subunit A
Macrophage capping protein		
Biliverdin reductase A		Aldehyde dehydrogenase, cytosolic, class Ⅰ
Annexin 1 fragment		
α-tubulin		
Elongation factor 1-d		Selenium-binding protein
Tropomyosin a1		Creatin kinase B chain
Tropomyosin a4 chain		Placental thrombin inhibitor
Actin fragment		Vimentin
Annexin 5		Desmin
Microtuble-associated protein RP/EB		Tubulin b 5 chain
Pyridoxal kinase		Carbonic anhydrase Ⅰ
Annexin 3		Myosin regulatory light chain 2
Annexin 4		

family and colon carcinogenesis is also supported by the finding that documents the participation of the S100A4 protein in the progression and metastasis of colorectal carcinogenesis. Alfonso *et al* reported the up-regulation of annexin Ⅳ, MTA-1 and others in colorectal cancer tissues, and the down-regulation of NCF2, PMM2 and others[44]. Several functional groups of proteins were affected, including regulators of transcription, structural proteins, and those involved in protein synthesis and folding. The MTA-1 gene encodes a protein that was identified in metastatic cells, specifically, mammary adenocarcinoma cell lines. Expression of the MTA-1 gene has been associated with the progression of several carcinomas in colon, lung, prostate, and liver. A annexin Ⅳ is a calcium-binding protein and I involved in cellular communication and signal transduction, for this reason it was up-regulated in colorectal cancer. Friedman *et al* identified adenosyl homocysteinase, leukocyte elastase inhibitor and others as up-regulated proteins, and puromycin-sensitive aminopeptidase, NADH-ubiquinone oxidoreductase and others as down-regulated proteins in colorectal cancer tissues[45].

Minowa *et al* identified truncated β-tubulins as a protein specific to polyp samples from APC gene-mutant mice by proteomic analysis of the small intestine and colon epithelia[46]. The adenomatous polyposis coli gene (APC) is mutated in patients with familial adenomatous popyposis (FAC) and sporadic colon cancer, and these mutations initiate colon carcinogenesis. Simpson *et al*[47] performed membrane proteomic analysis of the human colon carcinoma cell line LIM 1215 to search for novel tumor marker proteins expressed during various stages of cancer progression, although the data are not shown.

Given the continual rise in the number of potential biomarkers of CRC, future studies will increasingly employ genomic and proteomic technologies, which enable the measurement and analysis of numerous potential biomarkers simultaneously. These techniques are able to produce gene or protein 'profiles' associated with clinical outcome, the analysis of which may then yield novel biomarkers with prognostic and/or therapeutic potential[48] (Table 2).

At this moment, biomarkers whose sensitivity and

specificity are better than bloody stool examination have not yet been found. Since the bloody stool test is easier than examination using cancer specimens and easier to handle than sera, from a clinical aspect, the bloody stool examination is better than biomarkers[34].

In another recent study, the detection of upregulated α-defensins 1, 2 and 3 in colorectal cancer tissue were reported in two independent, but similar analyzes. In both studies, SELDI-TOF MS results in tissue correlated with serum levels that were determined using ELISA or SELDI-TOF MS. This provides an interesting approach for finding new serum markers because biomarkers identified first in tissue could prove to be more specific. Unfortunately, α-defensin levels are also increased in serum during, for example, infection[49]. α-defensin and β-defensin are major components of the epithelial mammalian innate immune system. Defensins are small cationic peptides with high activity against a variety of microbials, encoded by genes and some are regulated in response to challenge with bacterial antigens. Gastrointestinal α-defensins (HD5 and HD6) are almost exclusively expressed in and secreted from Paneth cells of the small intestine, while β-defensins (hBD-1, hBD-2, hBD-3) are secreted by virtually all gastrointestinal epithelial cells to a varying extent.

CONCLUSIONS AND FUTURE PERSPECTIVES

Rapidly developing techniques that considerably enhanced information gained from proteomes integrate proteomics with other disciplines such as cell biology, biochemistry, molecular genetics, and chemistry. This consolidation certainly demonstrates incredible power and possibilities of proteomics for further applications. It is necessary to cross the barriers of limited resolution, mass range, detection level, and other reasons for protein underrepresentation in analyzed proteomes. Once achieved, the door that allows complete identification of specific protein markers will open and the comprehension of complex networks of protein/peptide interactions involved in cancer will begin to be elucidated[6]. While the application of computational and statistical methods to proteomic profiling is relatively new, it is rapidly gaining interest. Hence, it is worthwhile suggesting fruitful avenues for moving forward. It was suggested above that simultaneous LC-MS data alignment and normalization may be beneficial for comparative profiling.

Proteomic technologies are now in place to examine simultaneously and comprehensively many protein expression differences that result from disease and treatment, with the ultimate payoff being the use of specific protein profiles for the early diagnosis of patients and for patient-tailored therapies[49].

REFERENCES

1 **Lievre A**, Laurent-Puig P. Molecular biology in clinical cancer research: the example of digestive cancers. *Rev Epidemiol Sante Publique* 2005; **53**: 267-282
2 **Weitz J**, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. *Lancet* 2005; **365**: 153-165
3 **Penland SK**, Goldberg RM. Current strategies in previously untreated advanced colorectal cancer. *Oncology* (Williston Park) 2004; **18**: 715-722, 727; discussion 727-729
4 **Midgley RS**, Kerr DJ. ABC of colorectal cancer: adjuvant therapy. *BMJ* 2000; **321**: 1208-1211
5 **Tanke HJ**. Genomics and proteomics: the potential role of oral diagnostics. *Ann N Y Acad Sci* 2007; **1098**: 330-334
6 **Drabik A**, Bierczynska-Krzysik A, Bodzon-Kulakowska A, Suder P, Kotlinska J, Silberring J. Proteomics in neurosciences. *Mass Spectrom Rev* 2007; **26**: 432-450
7 **Kuramitsu Y**, Nakamura K. Proteomic analysis of cancer tissues: shedding light on carcinogenesis and possible biomarkers. *Proteomics* 2006; **6**: 5650-5661
8 **Herbert B**. Advances in protein solubilisation for two-dimensional electrophoresis. *Electrophoresis* 1999; **20**: 660-663
9 **Simpson RJ**, Dorow DS. Cancer proteomics: from signaling networks to tumor markers. *Trends Biotechnol* 2001; **19**: S40-S48
10 **Page MJ**, Amess B, Townsend RR, Parekh R, Herath A, Brusten L, Zvelebil MJ, Stein RC, Waterfield MD, Davies SC, O'Hare MJ. Proteomic definition of normal human luminal and myoepithelial breast cells purified from reduction mammoplasties. *Proc Natl Acad Sci USA* 1999; **96**: 12589-12594
11 **Bonner RF**, Emmert-Buck M, Cole K, Pohida T, Chuaqui R, Goldstein S, Liotta LA. Laser capture microdissection: molecular analysis of tissue. *Science* 1997; **278**: 1481,1483
12 **Emmert-Buck MR**, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA. Laser capture microdissection. *Science* 1996; **274**: 998-1001
13 **Srinivas PR**, Verma M, Zhao Y, Srivastava S. Proteomics for cancer biomarker discovery. *Clin Chem* 2002; **48**: 1160-1169
14 **Ornstein DK**, Tyson DR. Proteomics for the identification of new prostate cancer biomarkers. *Urol Oncol* 2006; **24**: 231-236
15 **Unlu M**, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* 1997; **18**: 2071-2077
16 **Tannu NS**, Hemby SE. Methods for proteomics in neuroscience. *Prog Brain Res* 2006; **158**: 41-82
17 **Tonge R**, Shaw J, Middleton B, Rowlinson R, Rayner S, Young J, Pognan F, Hawkins E, Currie I, Davison M. Validation and development of fluorescence two-dimensional differential gel electrophoresis proteomics technology. *Proteomics* 2001; **1**: 377-396
18 **Alban A**, David SO, Bjorkesten L, Andersson C, Sloge E, Lewis S, Currie I. A novel experimental design for comparative two-dimensional gel analysis: two-dimensional difference gel electrophoresis incorporating a pooled internal standard. *Proteomics* 2003; **3**: 36-44
19 **Haab BB**. Applications of antibody array platforms. *Curr Opin Biotechnol* 2006; **17**: 415-421
20 **Haab BB**, Dunham MJ, Brown PO. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol* 2001; **2**: RESEARCH0004
21 **Haab BB**. Antibody arrays in cancer research. *Mol Cell Proteomics* 2005; **4**: 377-383
22 **Becker KF**, Metzger V, Hipp S, Hofler H. Clinical proteomics: new trends for protein microarrays. *Curr Med Chem* 2006; **13**: 1831-1837
23 **Petricoin EF**, Liotta LA. SELDI-TOF-based serum proteomic pattern diagnostics for early detection of cancer. *Curr Opin Biotechnol* 2004; **15**: 24-30
24 **Petricoin EF**, Liotta LA. Proteomic approaches in cancer risk and response assessment. *Trends Mol Med* 2004; **10**: 59-64
25 **Issaq HJ**, Veenstra TD, Conrads TP, Felschow D. The SELDI-TOF MS approach to proteomics: protein profiling and biomarker identification. *Biochem Biophys Res Commun* 2002; **292**: 587-592
26 **Clarke CH**, Buckley JA, Fung ET. SELDI-TOF-MS proteomics of breast cancer. *Clin Chem Lab Med* 2005; **43**: 1314-1320
27 **Zhang Z**, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, Rosenzweig JM, Cameron B, Wang YY, Meng XY, Berchuck A, Van Haaften-Day C, Hacker NF, de Bruijn HW, van der Zee AG, Jacobs IJ, Fung ET, Chan DW. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res* 2004; **64**: 5882-5890

28 **Bertucci F**, Birnbaum D, Goncalves A. Proteomics of breast cancer: principles and potential clinical applications. *Mol Cell Proteomics* 2006; **5**: 1772-1786

29 **Won Y**, Song HJ, Kang TW, Kim JJ, Han BD, Lee SW. Pattern analysis of serum proteome distinguishes renal cell carcinoma from other urologic diseases and healthy persons. *Proteomics* 2003; **3**: 2310-2316

30 **Hortin GL**. The MALDI-TOF mass spectrometric view of the plasma proteome and peptidome. *Clin Chem* 2006; **52**: 1223-1237

31 **Tyers M**, Mann M. From genomics to proteomics. *Nature* 2003; **422**: 193-197

32 **Aebersold R**, Mann M. Mass spectrometry-based proteomics. *Nature* 2003; **422**: 198-207

33 **Listgarten J**, Emili A. Statistical and computational methods for comparative proteomic profiling using liquid chromatography-tandem mass spectrometry. *Mol Cell Proteomics* 2005; **4**: 419-434

34 **Gygi SP**, Rist B, Griffin TJ, Eng J, Aebersold R. Proteome analysis of low-abundance proteins using multidimensional chromatography and isotope-coded affinity tags. *J Proteome Res* 2002; **1**: 47-54

35 **Tannu NS**, Hemby SE. Methods for proteomics in neuroscience. *Prog Brain Res* 2006; **158**: 41-82

36 **Banez LL**, Prasanna P, Sun L, Ali A, Zou Z, Adam BL, McLeod DG, Moul JW, Srivastava S. Diagnostic potential of serum proteomic patterns in prostate cancer. *J Urol* 2003; **170**: 442-446

37 **Ye B**, Cramer DW, Skates SJ, Gygi SP, Pratomo V, Fu L, Horick NK, Licklider LJ, Schorge JO, Berkowitz RS, Mok SC. Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic profiling and mass spectrometry. *Clin Cancer Res* 2003; **9**: 2904-2911

38 **Garcia-Foncillas J**, Bandres E, Zarate R, Remirez N. Proteomic analysis in cancer research: potential application in clinical use. *Clin Transl Oncol* 2006; **8**: 250-261

39 **Chuthapisith S**, Layfield R, Kerr ID, Eremin O. Principles of proteomics and its applications in cancer. *Surgeon* 2007; **5**: 14-22

40 **Kuramitsu Y**, Nakamura K. Proteomic analysis of cancer tissues: shedding light on carcinogenesis and possible biomarkers. *Proteomics* 2006; **6**: 5650-5661

41 **Dundas SR**, Lawrie LC, Rooney PH, Murray GI. Mortalin is over-expressed by colorectal adenocarcinomas and correlates with poor survival. *J Pathol* 2005; **205**: 74-81

42 **Lane CS**, Nisar S, Griffiths WJ, Fuller BJ, Davidson BR, Hewes J, Welham KJ, Patterson LH. Identification of cytochrome P450 enzymes in human colorectal metastases and the surrounding liver: a proteomic approach. *Eur J Cancer* 2004; **40**: 2127-2134

43 **Stulik J**, Koupilova K, Osterreicher J, Knizek J, Macela A, Bures J, Jandik P, Langr F, Dedic K, Jungblut PR. Protein abundance alterations in matched sets of macroscopically normal colon mucosa and colorectal carcinoma. *Electrophoresis* 1999; **20**: 3638-3646

44 **Alfonso P**, Nunez A, Madoz-Gurpide J, Lombardia L, Sanchez L, Casal JI. Proteomic expression analysis of colorectal cancer by two-dimensional differential gel electrophoresis. *Proteomics* 2005; **5**: 2602-2611

45 **Friedman DB**, Hill S, Keller JW, Merchant NB, Levy SE, Coffey RJ, Caprioli RM. Proteome analysis of human colon cancer by two-dimensional difference gel electrophoresis and mass spectrometry. *Proteomics* 2004; **4**: 793-811

46 **Minowa T**, Ohtsuka S, Sasai H, Kamada M. Proteomic analysis of the small intestine and colon epithelia of adenomatous polyposis coli gene-mutant mice by two-dimensional gel electrophoresis. *Electrophoresis* 2000; **21**: 1782-1786

47 **Simpson RJ**, Connolly LM, Eddes JS, Pereira JJ, Moritz RL, Reid GE. Proteomic analysis of the human colon carcinoma cell line (LIM 1215): development of a membrane protein database. *Electrophoresis* 2000; **21**: 1707-1732

48 **Neal CP**, Garcea G, Doucas H, Manson MM, Sutton CD, Dennison AR, Berry DP. Molecular prognostic markers in resectable colorectal liver metastases: a systematic review. *Eur J Cancer* 2006; **42**: 1728-1743

49 **Engwegen JY**, Gast MC, Schellens JH, Beijnen JH. Clinical proteomics: searching for better tumour markers with SELDI-TOF mass spectrometry. *Trends Pharmacol Sci* 2006; **27**: 251-259

S- Editor Liu Y **L- Editor** Rippe RA **E- Editor** Lu W