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TUMOR BIOMARKERS

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Biomarkers are biochemical or molecular parameters associated with the presence and severity of specific disease states. Biomarkers are detectable and measurable by a variety of methods including various laboratory assays and medical imaging systems like computed tomography (CT), magnetic resonance imaging (MRI), or nuclear medicine techniques such as positron emission tomography (PET). In addition to these established techniques, there are a variety of imaging methods in various stages of development that hold great promise in the imaging of biomarkers, such as molecular imaging, a method that allows detection of specific molecules in living organisms. Tumor biomarkers are substances, usually proteins that are produced by the body in response to cancer growth or by the cancer tissue itself. Some tumor biomarkers are specific, while others are seen in several cancer types. Many of the well-known biomarkers are also seen in non-cancerous conditions. Consequently, these tumor biomarkers are not diagnostic for cancer.

There are only few well-established tumor biomarkers that are being routinely used by physicians. Many other potential biomarkers are still being researched. Some biomarker tests cause great excitement when they are first discovered but, upon further investigation, prove to be no more useful than biomarkers already in use. The goal is to be able to screen for and diagnose cancer early, when it is the most treatable and before it has had a chance to grow and metastasize. So far, very few tumor biomarkers have gained wide acceptance as a general screen. Other biomarkers are either not specific enough with high false positives, leading to expensive and unnecessary follow-up testing or they are not elevated early enough in the disease process. Some people are at a higher risk for particular cancers because they have inherited a genetic mutation. While not considered tumor makers, there are tests

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that look for these mutations in order to estimate the risk of developing a particular type of cancer. BRCA1 and BRCA2 are examples of gene mutations related to an inherited risk of breast cancer and ovarian cancer.

Examples of biomarkers include genetic biomarkers (e.g., nuclear aberrations [such as micronuclei], gene amplification, and mutation), cellular biomarkers (e.g., differentiation biomarkers and measures of proliferation, such as thymidine labeling index), histologic biomarkers (e.g., premalignant lesions, such as leukoplakia and colonic polyps), and biochemical and pharmacologic biomarkers.

Tumor biomarkers are not diagnostic in themselves. A definitive diagnosis of cancer is made by looking at biopsy specimens (e.g., of tissue) under a microscope. However, tumor biomarkers provide information that can be used for:

Screening: Some biomarkers may be suited for general screening in those with a strong family history of a particular cancer. In the case of genetic biomarkers, they may be used to help predict risk in family members, e.g. PSA for Prostate cancer.

Diagnosis: In the diagnosis of cancer tumor biomarkers can be used as an aid. A patient presenting with a symptoms, tumor biomarkers may be used to help identify the source of the cancer, such as CA-125 for ovarian cancer, and to help differentiate it from other conditions.

Guiding Treatment: Some tumor biomarkers will give doctors information about what treatments their patients may respond to e.g. Her2/neu positive breast cancer and respond to Herceptin treatment.

Monitoring Treatment: Tumor biomarkers can be used to monitor the effectiveness of treatment, especially in advanced cancers. If the biomarker level drops, the treatment is working; if it stays elevated, adjustments are needed e.g. CEA in colorectal cancer.

Determining recurrence: Currently, one of the biggest uses for tumor biomarkers is to monitor for cancer recurrence. If a tumor biomarker is elevated before treatment, low after treatment, and then begins to rise over time, then it is likely that the cancer is returning. (If it remains elevated after surgery, then chances are that not all of the tumor was removed.)

A list of commonly used tumor biomarkers is given in Table 1 and tumor biomarkers being evaluated is listed in Table 2.

Genetic Alterations in Tumors as Biomarkers

The post human genome project era, and the advances in biotechnology have generated many candidate biomarkers in cancers with potential clinical value. These biomarkers can be a signature for cancer staging at the time of diagnosis and/or personalization of therapy which

Table 1 : Common Tumor biomarkers Currently in Use

Tumor Biomarkers	Tumors	Applications	Sample
AFP (Alpha-feto protein)	Liver, germ cell cancer of ovaries or testes	Diagnose, monitor treatment and determine recurrence	Blood
B2M (Beta2 microglobulin)	Multiple myeloma and lymphomas	Determine prognosis	Blood
BTA (Bladder tumor antigen)	Bladder Cancer	Diagnose and determine recurrence	Urine
CA-125 (Cancer antigen 125)	Ovarian Cancer	Diagnose, monitor treatment and determine recurrence	Blood
CEA (Carcino-embryonic antigen)	Colorectal, lung, breast, thyroid, pancreatic, liver, cervix, and bladder	Monitor treatment and determine recurrence	Blood
CA 19-9 (Cancer antigen 19-9)	Pancreatic, sometimes colorectal and bile ducts	Stage disease, monitor treatment and determine recurrence	Blood
CA 15-3 (Cancer antigen 15-3)	Breast and others including lung, ovarian	Stage disease, monitor treatment and determine recurrence	Blood
hCG (Human chorionic gonadotropin)	Testicular and trophoblastic	Help diagnose, monitor treatment and determine recurrence	Blood, Urine
Estrogen receptors and Her-2/neu	Breast cancer	Determine prognosis and guide treatment (Hormones and Herceptin)	Tissue
Monoclonal immunoglobulins	Multiple myeloma and Waldenstrom's macroglobulinemia	Help diagnose, monitor treatment, and determine recurrence	Blood, Urine
NSE (Neuron-specific enolase)	Neuroblastoma, small cell lung cancer	Monitor treatment	Blood
PSA (Prostate specific antigen)	Prostate cancer	Screening, diagnose, monitor treatment, and determine recurrence	Blood
Thyroglobulin	Thyroid cancer	Determine recurrence	Blood

Table 2 : Tumor biomarkers being evaluated

Tumor Biomarkers	Tumors	Applications	Sample
Cytokeratin 18 (CK18), bladder tumor fibronectin (BTF), Bladder tumor antigen (BTA)	Bladder cancer	Help diagnose and determine recurrence	
TA-90	Metastatic melanoma	Help diagnosis	
CA242, CAM 17.1 and Tissue polypeptide specific antigen (TPS)	Pancreatic cancer -	Help diagnosis	

could improve patient care. Assessment of genotypes at single locus may contribute to evaluation of cancer risk, diagnosis and prediction of disease progression. In case of sporadic cancers we are at a stage where there is a lot of information and research which is being translated to the clinic. There are molecular biomarkers which are clinically applied in selecting the patients for specific therapy and to monitor the prognosis. The HER2 gene is over expressed in ~30% of breast cancers, increasing the aggressiveness of the tumor. Herceptin (or trastuzumab), recombinant monoclonal antibody against HER2, is now part of the ideal management in patients who over express this receptor. This was first conclusively shown in a phase 3 clinical trial in women with metastatic breast cancer who over expressed HER2. When added to conventional chemotherapy the antibody resulted in a significantly longer time to disease progression, a higher rate of response, a longer duration of response, and improved overall survival (1). In oligodendroglioma, several studies have shown that 1p and 19q loss are 100% responsive to PCV (Procarbazine, Carbamazipine and Vincristine) therapy and have an average survival of 12 years instead of 2 years before the era of PCV. It also helps avoid patients with no loss of 1p/19q, who are not responsive to PCV, from unnecessary risk of chemotherapy. In tumor monitoring and in determining the response of hematological

malignancies, molecular typing is increasingly used. The BCR-ABL fusion protein of its translocation can be determined by PCR for monitoring CML. N-myc amplification correlates with poor prognosis in neuroblastoma patients. Amplified N-myc confers resistance to certain drugs like cisplatin used in the therapy of the disease. There is a vast body of literature on p53 that has yet to be transformed to clinical practice. For example in prostate cancer, mutations in the *p53* gene are associated with metastasis and may serve as a biomarker for progression (2).

Hereditary cancers

In the case of hereditary cancers, assessing the molecular alteration may contribute to evaluation of cancer risk and early diagnosis. The linkage of mutations in the BRCA1 and BRCA2 genes to susceptibility to both breast and ovarian cancers is now used in clinical practice. Given this risk, otherwise healthy individuals who test positive for BRCA1/2 mutations may opt to undergo prophylactic bilateral mastectomy and oophorectomy (3). The same reasoning can be used for screening for p53 mutations in detecting 'at risk individuals' in families of Li Fraumeni syndrome. Similar mutation in APC gene contribute both to hereditary and sporadic colorectal cancers, making screening for mutations in this gene a possible component of colorectal cancer risk assessment and clinical management (4). HNPCC (hereditary nonpolyposis colon cancer) is a hereditary syndrome that is caused when a person inherits a mutation in different mismatch repair genes, mutation in two of the gene MLH1 and MSH2 account for the vast majority of detectable mutations. Members of families with an error in one of the genes associated with HNPCC should strongly consider some special colon cancer screening and prevention options.

Biomarker discovery in post genomic era : Molecular profiling of cancers

Biomedical research in general is in the midst of an informational and technological revolution. Over the past decade, molecular profiling has emerged as a dynamic new discipline, capable of generating a global view of DNA alterations, mRNA and protein patterns in various cell types and disease processes by integrating the expanding genetic databases from the Human Genome Project with newly developed expression analysis technologies. It helps in explaining the relationship between genotype and phenotype in humans, which is still largely unknown. It also provide with the identification of new molecules for development of new diagnostic and therapeutic targets for clinical intervention.

The underlying cause of each patient's disease is typically unique to the individual. Molecular profiling provides advanced analysis and tools to better help the physician determine the molecular characteristics of each patient's disease so that they may better modify the

medical strategy specific to the individual. Hence molecular profiling with Genomic-based & Proteomic-based approaches and tissue arrays is being used to discover next generation biomarkers which help to obtain more global views on cancer genes. These include:

DNA-based biomarkers - Genetic changes and mutations in oncogenes, tumor suppressor genes and genes involved in maintaining the cellular integrity.

Mitochondrial DNA based biomarkers.

Pattern-based RNA expression analysis - Demonstrated in a number of tumor types.

Epigenetic biomarkers - Potential to guide treatment decisions for a number of marketed and developmental agents.

Specific proteomic patterns associated with disease.

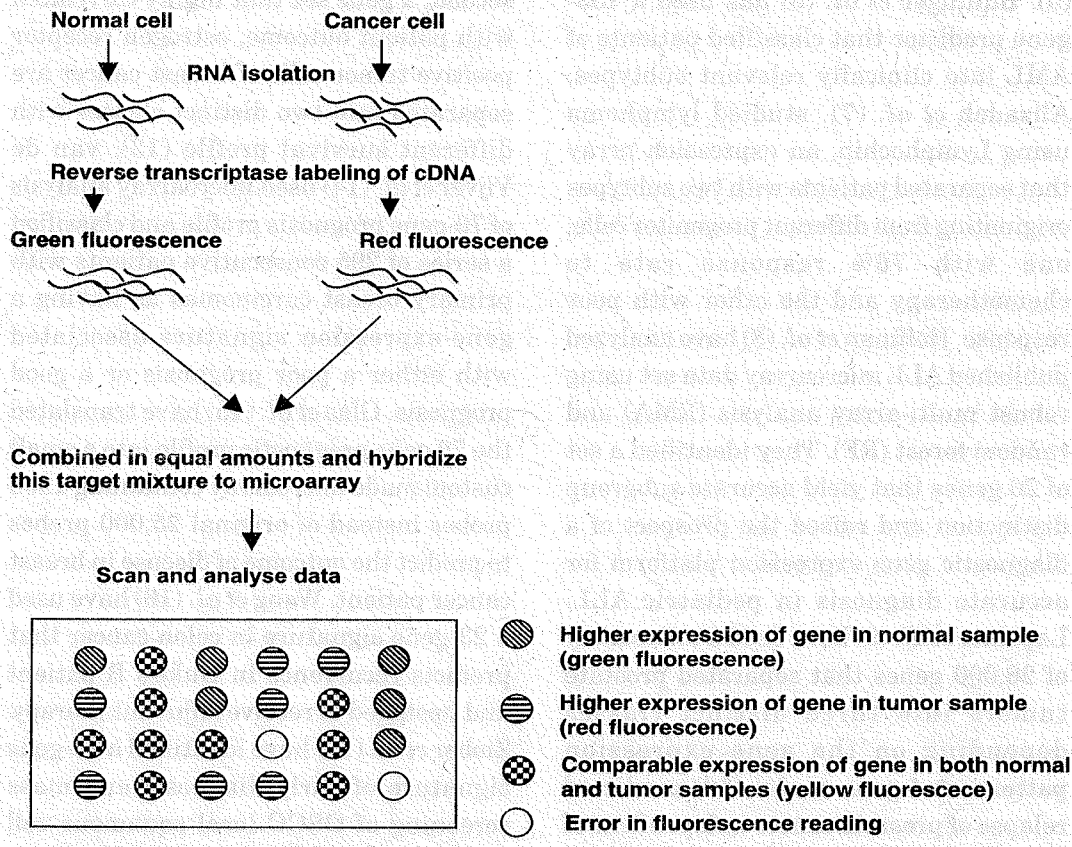
Genomic based approaches: Genomics involves the study of complex set of genes, their expression and interaction with each other using microarray system. The cancer development is dependent on many different genes, their expression and interaction with each other to create a favorable environment for disease to develop. With the advancement in the study of genomic analysis, the identification of a set of genes (rather than single genes) expressed in the tumor is possible that may provide far more specific and reliable information regarding the tumors.

DNA microarrays: These are the cornerstone of genomic analysis. They comprise a number of (often thousands) of genes spotted onto a glass slide in a precise manner. There are two types of DNA microarrays cDNA array and oligonucleotide array. cDNA array is used mostly for gene expression profiling. RNA isolated from the tumor tissues are labeled and then hybridized to the microarray (chip) which contain known DNA molecules (~100 nts) immobilized on a solid surface (Figure1). An array can accommodate ~20 thousand specific sequences on a single chip, either chosen randomly or deliberately biased to represent collection of genes typically expressed in a cell type of interest. Oligonucleotide array contain short nucleic acids upto 25 nts immobilized on a glass slide surface. They are used to screen for sequence variations (mutations) of specific genes and for genotyping where labeled DNA from an individual is tested for genetic markers like single nucleotide polymorphisms (SNPs), microsatellite markers to yield a fingerprint, which in turn may be linked to the risk of developing diseases

In cancer DNA microarray can be applied to

- Study the global gene expression pattern in tumors contributing to malignancy i.e. snapshots of genes either up- or down-regulated in tumors.

Figure1: Schematic representation of Microarray analysis



The intensity and color of each spot encode information on a specific gene from the tested samples.

- Molecular classification of neoplasms by gene expression signatures
- Discovery of new prognostic or predictive indicators and biomarkers of therapeutic response
- Identification and validation of new molecular targets for drug development
- Identification of genes conferring drug resistance
- Prediction or selection of patients most likely to benefit from, or suffer from particular side effects of drugs (pharmacogenomics)

There are reports in the literature where microarray analyses was done to classify and to predict the clinical outcome of cancers. In case of acute myeloid leukemia a 13,000-gene array was used that separated acute myeloid leukemia patients into classes, including one class

with a particularly poor clinical outcome (5). Bullinger *et al.* (6) has used a 133-gene predictor that classified patients of AML into clinically relevant subtypes. Alizadeh *et al.* (7) studied lymphoma using Lymphochip, an expression array that separated patients with two subtypes originating from different progenitor cells, one with 76% response rate to chemotherapy and the other with poor response. Hoffman *et al.* (8) have analyzed published ALL microarray data set using robust multi-array analysis (RMA) and random forest (RF). They identified a set of 26 genes that yield accurate subgroup distinction and raised the prospect of a diagnostic gene expression platform for accurate diagnosis in pediatric ALL. Lapointe *et al.* (9) have used a microarray of 26,000 genes that separated prostate tumors into three distinct classes depending on the gene expression pattern. A 4-gene model (10) predicted relapse of prostate cancer independent of stage and grade. Biopsy specimen of lung cancer was analyzed (11) using a 99-gene profile and a 42-gene profile associated with increased risk of death. A review article (12) has discussed the molecular profiling of non-small cell lung cancer, the progress made thus far in relation to its etiology, pathogenesis, molecular classification and potential biomarkers that may be of use in diagnosis, screening, and assessing the effectiveness of therapy. By molecular profiling of breast cancer using two different gene sets: first, a set of 456 cDNA clones that reflect the intrinsic properties of the tumors and, second, a gene set that highly correlated with patient outcome, estrogen receptor positive tumor cells of breast cancer are separated into two distinct classes with different survival profile (13). van de Vijver *et al.* (14) used microarray analysis of 70-gene prognosis profile and classified a series of 295 consecutive patients with primary breast carcinomas as having a gene-expression signature associated with either a poor prognosis or a good prognosis. Glas *et al.* (15) have translated the 70 gene prognostic profile into a small custom made microarray containing 1900 probes instead of original 25,000 probes to predict the outcome of disease in breast cancer patient. Wang *et al.* (16) have used a 23-gene signature in colon cancer that predicts recurrence in Duke's B patient and upstaged to receive adjuvant therapy. Ziober *et al.* (17) have identified a 25-gene signature of early diagnosis and mass screening of OSCC (oral squamous cell carcinomas). This gene signature was found to be 96% accurate on cross validation. These arrays are measuring up well when compared with conventional classification and prognostication methods

Arrays are also used to study the drug response in different malignancies. A 31 gene profile distinguishes individual with CML that achieved major cytogenetic response treated with imatinib (tyrosine kinase inhibitor) (18). A 95 gene profile was identified that predicted imatinib sensitivity in ALL (19). Gene expression

pattern in childhood ALL and response to drugs like prednisolone (33 genes), vincristine (40 genes) L-asparaginase (35 genes) or daunorubicin (20 genes) was studied by (19). Xu *et al.* (20) did pharmacogenomic profiling of PI3K/PTEN-AKT-mTOR pathway in many solid tumors and identified variable expression of all the signaling protein in this pathway and predicted sensitivity to mTOR inhibitor (rapamycin). Potti *et al.* (21) have developed gene expression signatures that predict sensitivity to individual chemotherapeutic drugs and also to multidrug regimens in malignancies of different tissue origin.

After the discovery stage has been completed using microarrays, a smaller subset of targets is spotted on a 'macroarray' which can be used more conveniently. The differences between microarray and macroarray are given in Table 3.

Table 3: Microarray Vs Macroarray

Microarray	Macroarray
Molecular portrait of the whole genome	Molecular portrait of the biological pathways
DNA/Oligo's spot size smaller than 200 microns in diameter	Spot size over 300 micron in diameter
Contain thousands of spots per array	Fewer spots are present usually hundreds or less per array
Capture a molecular portrait of the living cell or tissue	Molecular signature obtained by parallel comparison of the portrait among samples of different physiological and pathological origin
Done for discovering or for research purpose	Clinical application, for tumor classification, prognostication and therapeutic response

Proteomics based approach

Proteomics: The goal of Proteomics is to obtain a more global and integrated view of biology by studying all the proteins of a cell rather than each one individually. The growth of proteomics is a direct result of advances made in large scale nucleotide sequencing of expressed sequence tags (ESTs) and genomic DNA as without this information protein could not be identified. Proteomics is important as many type of information cannot be obtained from the study of gene alone. Protein expression and function are subject to modulation through transcription as well as through posttranscriptional and posttranslational events. More than one RNA can result from one gene through a process of differential splicing. Additionally, there

are more than 200 post translation modifications that proteins could undergo that affect function, protein-protein and nucleotide-protein interaction, stability, targeting, half-life etc., all contributing to a potentially large number of protein products from one gene. Proteins are responsible for the phenotypes of cells, and it is only through the study of proteins can protein modification be characterized and the targets of drugs identified. Depending on the type of study different approaches are applied in proteomics like

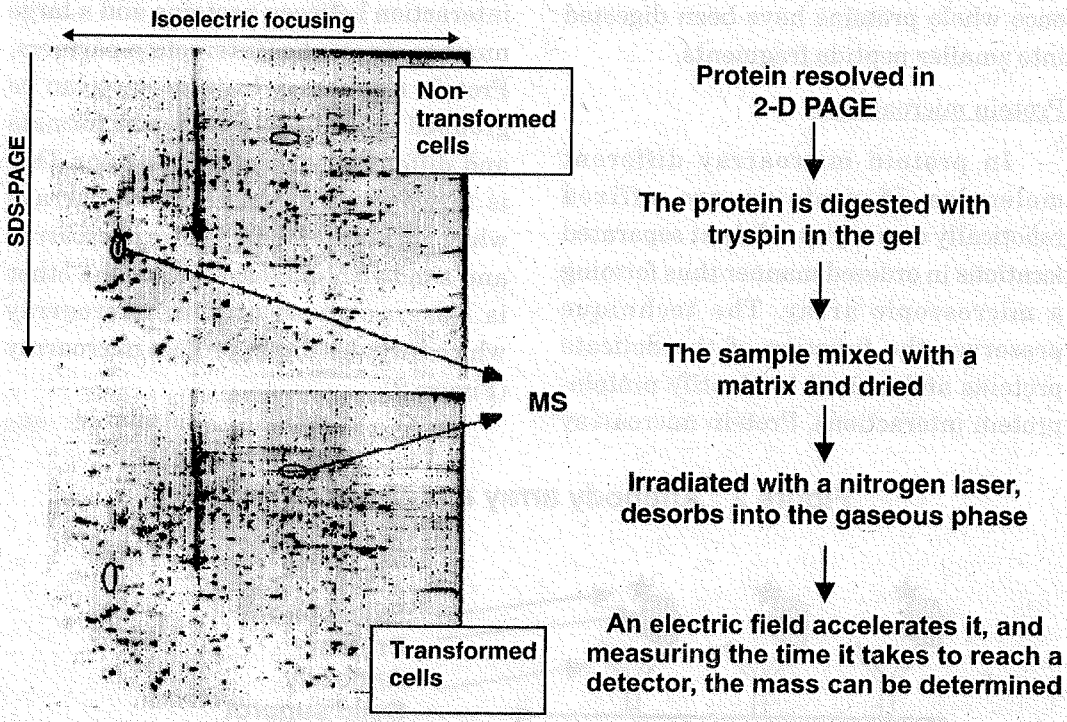
Two-dimensional Gel electrophoresis (2-DE): 2-DE is done for separation and isolation of different proteins in a sample. 2-DE is able to resolve proteins that have undergone some form of posttranslational modification and/or different forms of proteins that arise from alternative mRNA splicing or proteolytic processing. A number of improvements have been made in 2-DE over the years like introduction of immobilized pH gradients (improved the reproducibility of 2-DE), the use of fluorescent dyes (improved the sensitivity of protein detection), specialized pH gradients (able to resolve more proteins) and automation of the process of 2-DE from gel running to image analysis and spot picking.

Mass spectrometry (MS): MS is an important emerging method for the characterization of proteins and acquisition of protein structure information. MS can give sequence data from any Coomassie stained band or gel spot. The protein is digested with trypsin in the gel, peptides eluted and fractionated by reverse phase chromatography and introduced into the mass spectrometer. The mass spectrometer determines the mass of the peptides and the sequence (by collisionally induced dissociation). From the masses of the peptide fragments, sequence data is determined by comparison with known sequences or by manual interpretation. The methods for ionization of whole proteins are electrospray ionization (ESI), matrix-assisted laser desorption/ ionization (MALDI) and surface enhanced laser desorption ionization (SELDI).

ESI: Samples are analyzed by loading into an ion trap mass spectrometer using an ElectroSpray Ionization (ESI). Peptides require some form of purification after in gel digestion and manual loading of the samples in individual microcapillary tubes. It is tedious and slow.

MALDI: Most often, masses are determined by Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MALDI-TOF) (Figure 3). In this technique, the sample is mixed with a matrix, commonly cyanohydroxycinnamic acid for peptides or sinapinic acid for proteins and dried. When irradiated with a nitrogen laser, the matrix adsorbs energy, which is transferred to the peptide molecule, which desorbs into the gaseous phase. An electric field accelerates it, and by

Figure 3 : Protein separation by 2DE and protein identification by MALDI (Matrix assisted laser Desorption ionization).



measuring the time it takes to reach a detector, the mass can be determined. Sample application can be performed by a robot, the entire process including data collection and analysis can be automated and the samples can often be used directly without any purification after in-gel digestion (Qin *et al* 1997).

SELDI: SELDI overcomes many of the problems associated with sample preparation inherent with MALDI-MS. The underlying principle in SELDI is surface enhanced affinity capture through the use of specific probe surface or chips. A 2-DE analysis separation is not necessary because it can bind protein molecules on the basis of its defined chip surfaces. Chips with broad binding properties, including immobilized metal affinity capture, and with biochemically characterized surfaces such as antibodies and receptors, form core of SELDI (22). This MS technology enables both biomarker discovery and protein profiling from the sample source without preprocessing.

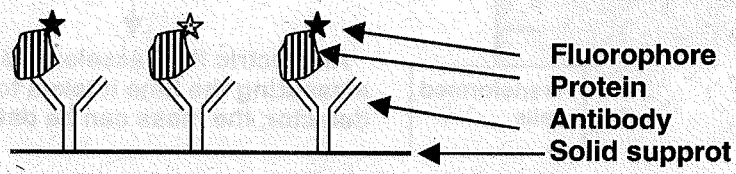
Mass analysis of proteolytic peptides is a much more popular method of protein

characterization, as cheaper instrument designs can be used for characterization. Additionally, sample preparation is easier once whole proteins have been digested into smaller peptide fragments.

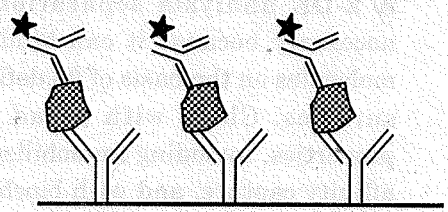
Protein microarrays:

In protein microarray different molecules of proteins are affixed robotically over a glass slide at separated locations in ordered manner thus forming a microscopic array. The technique preserves the function of the delicate proteins and is used to identify protein-protein interactions. Protein microarray technology has the potential to monitor complex intracellular protein expression and to simultaneously study the interaction between proteins and a large number of potential interaction partners. Protein microarray based assays can be grouped according to different formats and different types of applications. One is forward phase protein microarrays which include protein capture microarray and sandwich microarray and the other is Reverse phase protein microarray which includes direct protein microarray (Figure 2).

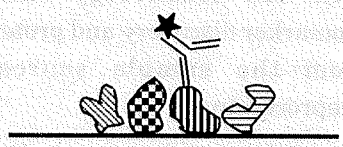
Figure 2 : Antibody array and protein array



Protein capture microarray (Forward phase protein microarray)



Sandwich microarray (Forward phase protein microarray)



Direct protein microarray (Forward phase protein microarray)

Protein expression proteomics:

The quantitative study of protein expression between samples that differ by some variable is known as expression proteomics. In this approach, protein expression of the entire proteome or of subproteomes between samples can be compared. Information from this approach can identify novel proteins in signal transduction or identify disease-specific proteins.

Structural proteomics:

Structural proteomics attempts to identify all the proteins within a protein complex or organelle, determine where they are located, and characterize all protein-protein interactions. An example of structural Proteomics was the recent analysis of the nuclear pore complex. Isolation of specific subcellular organelles or protein complexes by purification can greatly simplify the Proteomic analysis. This information will help piece together the overall architecture of cells and explain how expression of certain proteins gives a cell its unique characteristics.

Functional proteomics:

"Functional proteomics" is a broad term for many specific, directed proteomics approaches. This could include the isolation of protein complexes or the use of protein ligands to isolate specific types of proteins. This approach allows a selected group of proteins to be studied and characterized and can provide important information about protein signaling, disease mechanisms or protein-drug interactions.

Application of proteomics in cancer

New proteomic technology allows us to gain an overview of thousands of proteins simultaneously as a proteomic pattern, analyze the individual protein signaling pathways being utilized by neoplastic cells, characterize the neoplasm biologically, and select specific targeted treatment modalities, known as "personalized" molecular medicine.

Several studies have already shown that protein fingerprints can reproducibly distinguish between normal and tumor samples e.g. Breast cancer (23, 24) and bladder cancer (25). Moreover, protein profiling facilitates the differentiation between samples (including cytological material) obtained from histologically different types of tumor like non-small-cell lung cancer (26), leukemias (27). Refining diagnosis at the molecular level would obviously greatly improve the classification of tumors, ultimately ameliorating our ability to predict clinical outcome and to identify individuals at a higher risk of disease recurrence.

The potential utility of chip-based proteomics in the early detection of cancer has been demonstrated by (28 a,b), who have identified a serum proteomic pattern that accurately distinguishes individuals with ovarian and prostates cancer from control subjects. Valerio *et al.* (29) have applied proteomic analysis of serum samples to the differential diagnosis of tumors from tumor-like diseases, such as pancreatic carcinoma and chronic pancreatitis. Sera from individuals with

melanoma and healthy volunteers have been analyzed by MALDI-MS (Matrix Assisted Laser Desorption Ionization-Mass Spectrometer) This (30) showed the presence and abundance of proteins with molecular weights of 2500–3500 Da, which were completely absent in control subjects, indicating the potential of protein profiling not only in early diagnosis but also in disease prognosis. de Noo *et al.* (31) have used MALDI-TOF to assess the feasibility of this approach for the detection of breast cancer. Preoperative serum samples were obtained from 78 breast cancer patients and 29 controls. High sensitivity (100%) and specificity (97%) were shown for the detection of breast cancer and indicated the potential usefulness of serum protein profile in breast cancer detection. Miguet *et al.* (32) have used SELDI-TOF-MS technology to discover and identify potential biomarkers for accurate diagnosis of the different forms of chronic mature B-cell lymphomas. New biomarkers were observed in 3 mass ranges (m/z = 13000, m/z = 9000, m/z = <2000). These markers were observed in 38% of the patient's sera but in none of the control sera.

Besides providing new insights into cancer pathogenesis, proteomics could have an unprecedented impact on some vital areas of cancer patient management, such as

- Early detection of disease, by using proteomic patterns of body fluid samples (33, 34)
- Cancer diagnosis and/or prognosis, based on proteomic signatures of tumor samples as a complement to histopathological evaluation (26, 35, 25.)
- The development of new disease and/or patient-specific therapeutic strategies after the identification of differential display between normal tissue and tumor tissue (36,37) and
- A rational modulation of therapy according to changes in protein profiles associated with drug resistance (38, 39, 40).

Tissue microarrays

Compared with the high-throughput techniques of genomics and proteomics, most tissue based molecular analyses are slow, cumbersome and require extensive manual interaction. Using conventional molecular pathology technique only single sample can be process, by cutting thin 5μm section and analyzing the specimen by immunostaining or in-situ hybridization, and it is cumbersome and slow. To overcome these limitations of conventional technique and to enable genome scale molecular pathology studies, Tissue microarray (TMA) technology was developed. TMAs facilitate the analysis of molecular alterations in thousands of tissue specimens in a massively parallel fashion. Construction of TMAs is achieved by acquiring cylindrical core specimen from up to 1000 fixed and paraffin embedded tissue specimens and arraying them at high density into a recipient TMA block.

The composite array block is sliced into sections that are placed on a glass slide. The slide now contains hundreds of tumor samples. Immunohistochemical staining or *in situ* hybridization can be used to query the array for specific molecules such as insulin-like growth factor binding protein (e.g. IGFBP1), apoptosis related proteins (e.g. BCL2), heat-shock proteins (e.g. HSPs) and a transcription factors (e.g.GATA2).

Studies are done using tissue array method to look for the increased expression of various proteins in cancer tissues. Singer *et al.* (41) have used tissue arrays to investigate the expression pattern of transglutaminase-2 in 57 invasive breast cancer biopsies and 62 ovarian cancers. Transglutaminase-2 has a role in cell growth and is also known to be associated with cell adhesion, metastasis and extracellular matrix modulation. Increased protein expression was seen in 84% of breast tumors and 58% of ovarian tumors. Ortiz-Rev *et al.* (42) have investigated the frequency and pattern of expression of CD10 and renal cell carcinoma (RCC) marker in 40 clear cell renal cell carcinomas using two tissue arrays prepared from paraffin blocks. They suggested that CD10 and RCC were often expressed by clear cell renal cell carcinomas and they may be useful markers to suggest the renal origin of carcinomas.

Conclusions

Recent technological advances in genomics and proteomics could revolutionize the way of doing research in medicine by providing researchers with a formidable high-throughput laboratory tool to study gene and protein expression profiles and functions in health and disease. New diagnostics and therapeutics biomarkers will also be discovered using methods that provide global views of cellular function. The greatest potential in the care of cancer patients could lie in the personalization of diagnosis and treatment which could be possible using molecular profiling that provides the global gene/protein information. Despite the promise demonstrated with molecular profiling, several barriers must be overcome prior to routine diagnostic implementation for patient intervention. One barrier is the cost of microarray technology for determining the molecular profile of the tumor. This technology is expensive and requires special handling procedures. Efforts are underway to reduce the problems associated with molecular profiling in order to bring this technology from bench to bedside. Small number of genes selected from a larger expression data set can be tested for clinical relevance. In case of B-cell lymphoma clinically relevant outcomes have been predicted using biomarkers of a set of 6-gene reverse transcription (RT)-PCR that can be easily standardized in a clinical laboratory (43). And biotechnology companies are trying to commercialize the array chips for different cancers.

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