



Approach of Proteomics in Cancer Therapy

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Abstract:

Cancer is one of the leading causes of death all over the world. Every year millions of death occurs due to this malignant disease. Therefore early diagnosis and prevention are key factors needed to reduce the mortality and morbidity of all types of cancer. But, currently available cancer screening tools such as mammography and invasive needle or surgical evaluation for breast cancer; or chest X-ray for lung cancer, etc. are not sensitive enough for early detection of the disease. It is essential to develop non invasive techniques that differentiate between patients with and without cancer, as well as between stages of cancer. Recently, significant progress has been made in the development of new proteomics technology. The progress that has been made in this field is helpful in identifying biomarkers that can be used for early diagnosis of cancer and improving the understanding of the molecular etiological mechanism of cancer. This article focuses on the present status of proteomics in this field.

Key word:

Cancer, mammography, proteomics, biomarker.

Introduction:

Human genome contains between 30,000 to 35,000 genes. It was found that one gene can produce more than one protein, each with a different functional capability. The generation of multiple proteins from a single gene can occur as a result of alternate splicing where a single DNA template can produce several different messenger RNAs, each of which is then used to make different proteins (Venter et al., 2001). In addition, the protein may undergo modification by cellular processes after it is created (termed post-translational modification). The result is that one gene can produce as many as 1,000 different proteins. On average, however, a gene produces five to ten different proteins. Genomics is the efficient use of information on the expression, regulation and structural association of genes. It is used in genetic analysis, measurement of gene expression and determination of gene function. As genomics has proven inadequate to predict the structure and dynamic properties of all proteins, a new field of protein study termed proteomics has developed. This is the large-scale study of protein expression, structure and function. It aims to correlate the structural and functional diversity of proteins with underlying biological processes, including disease processes (Liotta et al., 2003). In this review, proteomics approaches in cancer studies have been represented and discussed.

II) Proteomics





The term “proteome” was introduced by Marc Wilkins all describe “all proteins expressed by a genome or tissue” (Wilkins et al., 1997). An alternative but similar definition of a proteome is a “set of all expressed proteins in a cell, tissue or organism at a certain point in a time” (Pennington et al., 1997). Proteomics is the study of the proteome and involves the technology used to identify and quantify the various proteins, protein-protein and protein-nucleic acid interactions within the proteome, as well as the post-translational modifications that affect protein activity (Hewick et al., 2003; Jhanker et al., 2012). Proteomics, indeed, is the link between genes, proteins and disease (Lohr and Faissner, 2004).

Proteomics is considered the next step in the study of biological systems after genomics (Tyagi et al., 2010). It is much more complicated than genomics mostly because while an organism's genome is more or less constant, the proteome differs from cell to cell and from time to time. This is because distinct genes are expressed in distinct cell types. This means that even the basic set of proteins which are produced in a cell needs to be determined. In the past this was done by mRNA analysis, but this was found not to correlate with protein content (Rogers et al., 2008). It is now known that mRNA is not always translated into protein (Dhingraa et al., 2005), and the amount of protein produced for a given amount of mRNA depends on the gene it is transcribed from and on the current physiological state of the cell. Proteomics confirms the presence of the protein and provides a direct measure of the quantity present. Proteomics has yielded a set of technologies that are significantly advancing in the field of cancer diagnostics. These technologies allow efficient means of identifying new biomarkers for the early detection of cancer and promise hope of new methods of diagnosis.

III) Techniques for proteomic analysis

Proteomic technologies with computational methods have been advanced recently over many other complementary techniques. This enables scientists to screen large numbers of proteins within clinically distinct samples that helps to discover disease biomarkers, identify and validate drug targets, design more effective drugs, assessment of drug efficacy and patient response, i.e., to interfere with almost every steps in modern drug discovery process (Ahn et al., 2008).

The identification of low abundance proteins may often be hindered by the abundant presence of other proteins. Hence, separation science, cellular proteomics and improvement in sensitivity, resolution and mass accuracy of mass spectrometry will play important roles in cancer (Roy and Shukla 2008). Much of the improvement in sensitivity for proteomic analysis has come from both new instruments and from sample fractionation to reduce the complexity of proteins. The table below summarizes the techniques used in proteomics and their uses (Akhter et al., 2010).





Technology	Uses
2-D Gel electrophoresis	Used to identify low abundance proteins in complex biological samples such as blood, urine and oral fluid.
Tandem mass spectrometry	Used to separate ions based on a sample's electronic mass, to study inborn errors of metabolism and metabolic profiles, and to identify therapeutic drugs, drugs of abuse, disease markers and toxic compounds.
Mass spectrometry MALDI-TOF (Matrix Assisted Laser Desorption Ionisation-Time Of Flight)	Deals with thermolabile, non-volatile organic compounds and those of high molecular mass. It is used in for the analysis of proteins, peptides, glycoproteins, oligosaccharides and oligonucleotides.
ICP-MS (Inductively Coupled Plasma-Mass Spectrometry)	Involves the formation of gas containing electrons, ions and neutral particles from Argon gas. Technology is used for ultrasensitive quantification of proteins and peptides down to low attomole range.
X-ray Tomography	Used to determine the location of labelled proteins or protein complexes in an intact cell. Frequently correlated with images of cells from light based microscopes.
Microarray 'chips'	These are matrix-support surfaces for binding selected proteins and allowing high-throughput screening for disease associated proteins.
Other methods: 1. Affinity chromatography 2. Yeast two hybrid techniques 3. Fluorescence Resonance Energy Transfer (FRET) 4. Surface Plasmon Resonance (SPR)	These methods are used for detection of drug-protein, hormone-protein, protein-protein, DNA-protein, carbohydrate-protein, and lipid-protein interactions.

IV) Clinical applications of proteomics

One of the most challenges in the field of cancer research is to determine malignancy as a characteristic agent in an early stage. These agents require molecular level examination of the diseases. Many studies using proteomic techniques have been performed on biomarkers to investigate potentials of early cancer diagnosis (McLeod and Evans, 2001).

Prostate cancer: The world wide incidence of prostate cancer ranks third among cancers in men. The highest incidence of prostate cancer in the world is found in American men. The Japanese and mainland Chinese populations have the lowest rates of prostate cancer (Odedina et al., 2009). Since the advent of prostate specific antigen (PSA) screening, a significant number of men have had a PSA test performed and this has led to a significant increase in the number of diagnosed cases (Jones et al., 2002). However, the PSA lacks sensitivity and therefore, evaluating multiple proteins will be essential to establishing signature proteomic patterns that distinguish cancer from non-cancer as well as identify all genetic subtypes of the cancer and their biological activity. In one study, proteomic analysis of prostate cancer patients versus healthy controls was carried out by looking for differences in protein patterns between the two groups. Using blood samples from 167 prostate cancer patients, 77 patients with benign prostate hyperplasia and 82 healthy men, protein patterns developed as a classification system had correctly classified 96 percent of the samples as either prostate cancer or non-cancer (Schiffer, 2007). A further proteomic approach is to determine whether the changes in specific phosphoproteins believed to be involved in cellular signalling events





and cancer progression in prostate cancer patients have been speculated to serve as a biomarker of early disease (Adam et al., 2002).

Pancreatic cancer: Pancreatic cancer is one of the leading causes of cancer death. In recent years, proteomics profiling techniques combined with various data analysis methods have been successfully used to gain critical insights into processes and mechanisms underlying pathologic conditions, particularly as they relate to cancer. The LOCCANDIA (Lab-On-Chip based protein profiling for CANcerDIagnosis) project is primarily concerned with validating the application of plasma protein profiling for early pancreatic cancer diagnosis by means of developing an innovative nano-technology based (lab-on-a-chip) platform integrated in a full proteomics analysis chain (Honda, 2005).

Renal Cancer: Renal cancer is the most deadly of urological malignancies. Molecular bases of this treatment-resistant neoplasm has been studied widely recently (Laird et al., 2013). The first evaluation of renal carcinoma cancer (RCC) proteome was a comparison between normal renal and cancer type in 1997 in which 2-D PAGE was applied to determine normal and tumor kidney tissues in ten patients suffering from RCC. Among 2789 separated polypeptides, 43 of them were found through gel comparison, amino acid analysis, N-terminal sequencing, and/or immune detection. Protein expression among normal and tumor kidney tissues proved four polypeptides not present in RCC. One of them was identified as ubiquinol cytochrome C reductase (UQCR) and the second was mitochondrial NADH-ubiquinone oxidoreductase complex I. In one study in 2004, heat shock protein 27 over-expression was identified as a potential biomarker by 2-D PAGE separation, mass spectrometry, and Western blotting immune detection methods. The result was also validated by immunohistochemistry on tissue sections. Base on one recent proteomic study, expression levels of profilin-1 (Pfn1), 14-3-3 zeta/delta (14-3-3), and galectin-1 (Gal-1) changed in RCC patients. In clustering analysis of changed expression proteins showed that protein expression profile for metastatic RCC in aggressive and non-aggressive RCC is different (Masui et al., 2013). Another study investigated on validates diagnostic and prognostic serum markers using proteomic profiling which several peptides were identified as having independent prognostic but not diagnostic significance on multivariable analysis (Wood et al., 2010).

Lung cancer: Lung cancers are also a leading cause of cancer death in developed countries. The diagnosis of patients with lung cancer is generally poor, with an overall five-year survival rate for patients receiving treatment of only 14% (Hoffman et al, 2000). Therefore, early diagnosis of lung cancer is necessary to improve patient survival. Plasma is a preferred specimen for the early diagnosis of lung cancer because samples are easily available by non-invasive methods. However, the currently available





plasma tumor markers such as TPA, chromogranin, CA19-9, Cyfra 21 have limited sensitivity and specificity for early diagnosis and novel plasma markers are required (Tarro et al., 2005). Alfanzo and colleagues provided in 2004 a more comprehensive understanding of the disease progression and constituted a method to complement histopathological diagnosis by carrying out 2-DE, MALDI and peptide mass fingerprinting. Another study used technique like SELDI-TOF-MS to discriminate glioblastomas from oligodendrogliomas and led to the identification of three potential biomarkers thus, direct tissue proteomics analysis is an original application of SELDI-TOF-MS technology that can expand the use of clinical proteomics as a compliment to anatomopathological diagnosis (Bouamrani et al., 2006).

Bladder Cancer: Bladder cancer incidence varies widely throughout the world. The risk of bladder cancer increases with age with over 70 percent of people diagnosed are older than 65 years (Freedman et al., 2006). Biological characteristics of urothelial carcinomas range from benign, superficial, low-grade, non-life threatening, papillary lesions, that respond well to resection and adjuvant treatment but are prone to recurrence to highly invasive malignant carcinomas with grave outcome. Several laboratories have successfully demonstrated that specific protein patterns can be detected from tumor tissue and these could discriminate adequately between diseased and healthy tissue. In the case of bladder cancer, proteomics analysis has identified several keratin proteins that are expressed in different amounts as the disease progresses from the early transitional epithelium stage to full blown squamous cell carcinoma. The measurement of keratin levels in bladder cancer biopsies can therefore be used to monitor the progression of the disease. Another protein, psoriasin, is found in the urine of bladder cancer patients and can be used as an early diagnostic marker for the disease. The study and utilization of these novel markers support the notion that proteomics, but not DNA arrays, can be used in cancer diagnosis. Urine, in common with most bodily fluids, contains proteins but no RNA (Celis and Gromov, 2003; Trevino et al., 2007).

Ovarian Cancer: Ovarian cancer represents the sixth most commonly diagnosed cancer among women in the world, and causes more deaths per year than any other cancer of the female reproductive system. It is a major focus of early biomarker discovery because it is usually diagnosed at an advanced stage with a median five-year survival rate of about 20 percent (Peltonen and McKusick, 2001). To evaluate the potential use of proteomics as a diagnostic tool, a group of researchers from the National Cancer Institute (NCI) in Bethesda, MD, collected serum from 50 ovarian cancer patients and 50 controls and used a computer algorithm to search for the protein patterns that distinguished cancer cells from non-cancer cells. They have shown that with a set of blinded serum samples, the test pattern correctly identified all 50





patients with cancer, and was able to discriminate them from 63 out of 66 patients without cancer or had benign disease. Using the same approach, two other groups reported similar results (Triche et al., 2001 and Jurisicova et al., 2008).

Breast cancer : It is the second leading cause of cancer death in American women. Despite advances in understanding the biology of this disease, early diagnosis and intervention is the most important factor affecting survival. Mammography is a significant advancement, but is inadequate in detection of non-calcified premalignant and noninvasive disease. Nipple aspirate fluid initially appeared as a promising source for the detection of potential biomarkers, as it is a direct sampling of breast epithelial cells. Application of SELDI-based technologies to serum screening in breast cancer is very promising these days. Zhang and colleagues detected in 2006 the serum proteomic pattern in breast cancer patients by SELDI-TOF-MS protein chip array techniques to screen biomarker candidates and build diagnostic models in order to evaluate their clinical significance. Another study used LCM to investigate the HER2/neu status in pure populations of breast cancer cells. Their results indicate that LCM is a powerful technique for isolating pure populations of cells from paraffin-embedded tissue sections (Seth et al., 2006).

Conclusion:

Proteomics play a vital role in scientific disciplines like; new drug targets according to the molecular profile of the cancer cell and have the potential to aid the development of cancer therapy. The proteomic approaches are a means of establishing the nature of post-translational modifications too. Despite the promise of proteomic technologies in clinical cancer research, there are limitations that need to be overcome to increase sensitivity and enhance the information capture.

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