Journal of
Proteomics & Bioinformatics

Doustjalali et al., J Proteomics Bioinform 2014, 7:3
http://dx.doi.org/10.4172/jpb.1000305

Review Article Open Access

Two Dimensional Gel Electrophoresis: An Overview of Proteomic Technique in Cancer Research

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Abstract

Proteomics can widely be used as one of the tool for protein level analysis which has largely become a necessity because the study of genes by genomics might not be adequately predict the structural dynamics of proteins. There is, however, a strong and synergistic relationship between proteomics and genomics as the two disciplines investigate the molecular organization of the cell at the complementary levels and increase the effectiveness of each other. In proteomics, the most promising technique with sufficient resolving power is two-dimensional gel electrophoresis (2DE) that provides a unique platform for the simultaneous separation of proteins in a complex mixture. However, the performance of optimization techniques in proteomics research using 2DE has its own limitations. In order to overcome these limitations, herein we have aimed for reviewing the application of 2DE proteomics in previously published well-known cancer research and in precise identification of sensitive and specific tumor markers over a period of time. To the authors' knowledge, this review represents the most promising strategies for application of the 2DE proteomic technique towards focusing on the bright future of cancer research is discussed.

Keywords: Proteomics; Cancer; 2DE; Tumor markers; Proteomics technique

Abbreviations: 2DE: Two-Dimensional Gel Electrophoresis; AGE: Advanced Glycation Endproduct; CRC: Colon and Rectal Cancer; RCC: Renal Cell Carcinomas; AML: Acute Myeloid Leukemia; TAA: Tumor-Associated Antigens; BC: Breast Cancer; AAT: α1-Antitrypsin; FDB: Fibrocystic Disease of the Breast; ACT: α1-Antichymotrypsin; NPC: Nasopharyngeal Carcinoma; CPL: Ceruloplasmin; ITIH4: Inter-α-Trypsin inhibitor Heavy Chain H4; CRT: Calreticulin; SNCG: γ-Synuclein; s-COMT: Soluble Isoform of Catechol-o-Methyltransferase; IEF: Isoelectric Focusing; pI: Isoelectric Points; SDS: Sodium Dodecyl Sulphate; MW: Molecular Weight

Introduction

Cancer refers to a group of many related ailments that is initiated within cells. Normally, cells grow and divide to attain functional maturity before eventually undergoing senescence and apoptosis. However, some cells do not undergo apoptosis but continue to divide uncontrollably and metastasize leading to formation of special tissue masses which call tumors that could be benign or malignant [1]. Advances in molecular genetics have provided a better understanding of the genesis of cancerous cells. The proliferation of normal cells is thought to be regulated by growth-promoting oncogenes and is counter balanced by growth-constraining tumor suppressor genes. The development of cancer appears to involve the activation or alteration in the expression of the oncogenes [2] or alternatively the inactivation of tumor suppressor genes [3].

Prompt diagnosis of cancer is challenging and represents one of the important ways of reducing mortality owing to cancer. The goal is to diagnose cancer when the tumor is still small enough to be completely removed surgically. Furthermore, early detection may lead to effective treatment. Unfortunately, most cancers are asymptomatic until the tumors are either too large or become malignant. Though, surgical interventions and other modes of therapeutic strategies, such as chemotherapy or radio therapy are often effective in successfully treating most of the tumor cells, but still challenging [1]. Therefore, application of novel technologies to achieve prompt and timely detection of cancers may have an important impact in clinical sciences. To achieve this goal, specific and sensitive tumor markers are required. A tumor marker is a biochemical substance present in or secreted by tumor cells itself or alarming signals produced in response to onset of cancer by the host cells.

Markers produced by cancer cells include enzymes, isoenzymes, hormones, oncofetal antigens, carbohydrate epitopes recognized by monoclonal antibodies, receptors, oncogene products and genetic changes. These markers can be used, to differentiate a tumor from normal tissue, to determine the presence of a tumor based on measurements in the blood or other body secretions, to identify the stages of cancer, to monitor the progression of cancer and effectiveness of therapy [4]. An ideal tumor marker should be both specific for a

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Received January 06, 2014; **Accepted** March 03, 2014; **Published** March 10, 2014

Citation: Doustjalali SR, Bhuiyan M, Al-Jashamy K,Linn NH, Abdul Kadir SY, et al. (2014) Two Dimensional Gel Electrophoresis: An Overview of Proteomic Technique in Cancer Research. J Proteomics Bioinform 7: 077-081. doi:10.4172/jpb.1000305

given type of cancer and sensitive enough to detect minute quantities for early diagnosis or during screening. Unfortunately, most known tumor markers are not highly sensitive and specific to use in timely detection. Therefore, this arise a need to identify more sensitive and specific indicators that can be used as potential surrogate markers to diagnose earlier and monitor cancer progress.

Two-dimensional gel electrophoresis (2DE) is one of the most promising proteomic approach developed to achieve above mentioned goal in cancer biology. However, the performance of optimization techniques in proteomics research using 2DE has its own limitations. In order to overcome these limitations, herein we have focused for reviewing the application of 2DE proteomics in previously published well-known various cancer researches and will discuss on precise identification of sensitive and specific tumor markers over a period of time.

Proteomics: A New Addition to Biological Sciences

The term 'Proteome' was first introduced in 1995, meaning the protein complement of a genome. In the cascade of regulatory events leading from the evolution of a gene to an active protein, the proteome can be seen as an end product of the genome. While the genome is comparatively static, proteome is a highly dynamic entity, as the protein content of a given cell varies with respect to changes in the surrounding environment, physiological state of the cell (e.g. position in cell cycle), stress, drug administration, health and disease. Moreover, different cell type within a multicellular organism will have different proteomes, while the genome is held relatively constant [5-7]. Proteomics is considered to be a mass-screening approach, which aims to document the overall distribution of proteins in the cell, identify and characterize individual proteins of interest, and ultimately to elucidate their relationships and functional roles. Such direct protein level analysis has become a necessity because the study of genes by genomics may not adequately predict the structural dynamics of proteins. However, there is a strong and synergistic relationship between proteomics and genomics as the two disciplines investigate the molecular organization of a cell at complementary levels (proteins and genes). Further, each discipline provides information that increases the effectiveness of the other [8].

Protein technology is inherently more complex than DNA-based technology. Not only is the basic alphabet bigger with 20 unmodified and many more modified amino acids for proteins as opposed to 4 nucleotides for DNA, but also some genes can be variously spliced, therefore making numerous different products from a single stretch of DNA. Additionally, mRNA editing is relatively common, leading to modified message and corresponding protein products. There are also many ways in which proteins are modified after they have been synthesized. It can be argued with some justification that possibly all eukaryotic proteins are post-translationally modified in some way (e.g. truncation at the N- or C-terminus, by protein splicing (rarely), or by addition of various substituents such as sugars, phosphate, sulphate, methyl, acetyl or lipid groups). There are many proteomes to make matters more complicated, while an organism has effectively a single genome (if unusual circumstances such as the genes involved in antibody production in B and T lymphocytes are set aside). Even in a unicellular organism, the expressed proteins (proteome) will be different depending on the growth conditions. Hence, as mentioned, there can be various ways in which proteins can be modified, one of the famous dogmas of biology, the one gene one enzyme hypothesis of Beadle and Tatum is no longer tenable [9].

In proteomic approach, the technique mostly used with sufficient resolving power is 2DE, which provides a platform for the simultaneous separation of proteins in a complex mixture (Figure 1). The technique, which involves separation of proteins via their differences in molecular weights and net charges, may be used to analyse a wide spectrum of protein sample from animals, plants and microorganisms [10-18].

Proteins that are separated can subsequently be identified by mass-spectrometry and N-terminal protein sequencing. Mass analysis follows the conversion of proteins or peptides to molecular ions. This is accomplished by the mass analyzers in the mass spectrometer, which resolve the molecular ions on the basis of their mass and charge in a vacuum. Information on the mass to charge ratio (m/z) obtained can be used to identify the protein by searching nucleotide and protein database. In addition, the m/z ratio can also be used to determine the type and location of protein modifications [10,11,17-24]. The N-terminal protein sequencing was first introduced by Edman [25] in 1949 and may also be used to search for complementary sequence in known protein database such as the blast search. However, not all N-termini of proteins may be sequenced as some may be blocked due to modification of their structures. Thus identification of proteins using this technique is also restricted [26]. The proteomic approach via 2DE has been used widely as one of the tool for studying cancerous tissues, serum and urine.

Proteomics Approach in Histopathology

Breast cancer

Researchers have identified advanced glycation endproduct (AGE) modified proteins from clinical breast cancer tissues using 2DE-immunoblot and mass-spectrometry [27]. They identified proteins: serotransferrin, fibrinogen gamma chain, glycerol-3-phosphate dehydrogenase, lactate dehydrogenase, annexin II, prohibitin and peroxiredoxin 6, which have established diagnostic roles in cancer detection. Other researchers have developed a general 2DE method that was suitable to yield good results with human breast cancer cell line (MCF-7) [28]. Specific protein differences in normal versus malignant human breast tissues were detected by others [29]. They detected a

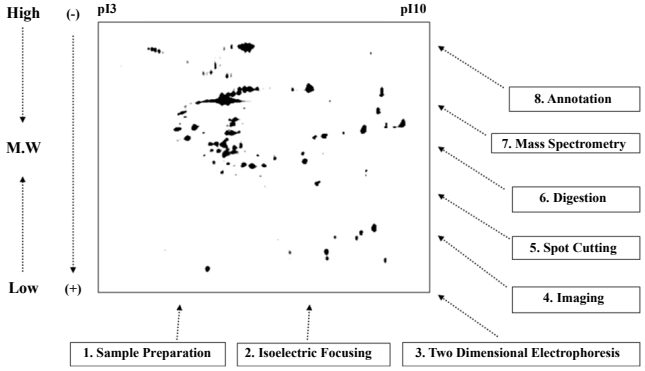

Figure 1: Flowchart of 2DE proteomic technique. Two-dimensional gel electrophoresis (2DE) is a powerful method for the analysis of complex protein mixtures from biological samples. This technique sorts proteins according to two different properties in discrete steps. First dimension electrophoresis, isoelectric focusing (IEF) will separate complex mixture of proteins according to their isoelectric points (pI). The second dimension step, sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis will further separate the focused proteins based on their molecular weights (M.W.). After image analysis the differentially expressed protein spots will be cut, digested and identified by mass spectrometry.

relationship between the expression of estrogen and progesterone receptors and certain other polypeptides. Giometti et al. extensively studied breast cancerous cell lines and developed a database of human breast epithelial cell proteins using quantitative 2DE [30]. They determined the proteins present in different types of human breast cells (milk producing and nonproducing, oestrogen receptor positive and negative, normal and malignant) and changes in abundance of the proteins in response to stimuli that trigger cell differentiation, growth, or death. By using proteomic approach Rasmussen et al. also analyzed human breast carcinoma proteins and studied proteins that bind to specific ligands [31].

Brain cancer

Proteomics approach by two-dimensional gel electrophoresis was used followed by spot picking and analysis of proteins/peptides by mass spectrometry. Ten different novel proteins/peptides were identified, which were differentially expressed in brain cancer glioblastoma multiforme [32,33]. Hanash et al. [33] subjected samples obtained from nine neuroblastoma and thirteen other cell lines to 2DE separation and studied their protein patterns. They scored more than 600 polypeptides and found one promising polypeptide, which was present in all neuroblastoma cell lines but not in the other types of cells. Other researchers studied elegantly by combining genomic and proteomic approach. They mentioned that the benefit of their 2DE approaches was the efficiency of scale and the ease with which abundant proteins or multi-copy genomic fragment could be detected, identified and quantitatively analysed [34].

Lung cancer

A total of 19 proteins were successfully identified in the lung cancer by using 2DE. Out of which twelve proteins were up-regulated, and seven proteins were down-regulated in the cancerous tissue compared with the tumor-adjacent normal tissue [35]. Variation in the expression of several proteins was correlated with different histopathological types [36]. Hirano et al. [37] also analyzed the relationship between the histopathological findings in primary lung malignancies. They thought that it was more precise to classify primary lung carcinomas based on the 2DE finding than to rely on morphology alone.

Colon cancer

Protein expression in colon and rectal cancer (CRC) and paired normal tissues was examined by 2DE to identify differentially expressed proteins. When compared with normal colorectal mucosa, protein abnormal expression of 65 spots varying more than 1.5 times were found in 2DE gels from colorectal cancer samples [38]. Ward et al. [39] isolated a number of proteins from a crude cell extract of a human colon carcinoma cell line by 2DE and blotting techniques. 2DE database of human colon carcinoma cells were established, which lists proteins from normal crypts and several colorectal cancer cell lines [40,41].

Renal cancer

The 2DE based proteomic analysis was used to outline differences in protein levels among different subtypes of renal cell carcinomas (RCC), including clear cell carcinomas, papillary lesions, chromophobe tumors and renal oncocytomas. Spot pattern was compared to the corresponding normal kidney from the same patients and distinctive, differentially expressed proteins were characterized by mass spectrometry [42]. Sarto et al. [43] established a human kidney protein map to study normal and tumor kidney tissue from proximal tubular epithelium. Ubiquinol cytochrome c reductase and mitochondrial NADH-ubiquinone oxido-reductase complex I were identified. These results indicated that mitochondrial dysfunction might play a major role in renal cell carcinoma genesis or evolution.

Ovarian cancer

In one of the study when tissues from the ovarian cancer and normal ovary were examined by 2DE, five proteins (galectin-1, cathepsin B, ubiquitin carboxy-terminal hydrolase L1, HLA class II antigen DRB1-11 and heat shock protein 27) were up-regulated and seven proteins (cellular retinol-binding protein, transthyretin, SH3 binding glutamic-rich-like protein, tubulin-specific chaperone A, DJ-1, gamma-actin and tropomyosin 4) were down-regulated [44]. Lawson et al. [45] measured and compared quantitative protein charges in metastatic and primary epithelial ovarian carcinoma by 2DE techniques.

Bone marrow

Proteomic analysis of different subtypes of acute myeloid leukemia (AML) cells was carried out using 2DE. Proteins identified were more significantly altered between the different AMLs belonged to the group of suppressor genes, metabolic enzymes, antioxidants, structural proteins and signal transduction mediators. Among them, seven identified proteins were found significantly altered in almost all the AML blast cells analyzed in relation to normal mononuclear blood cells [46,47]. For a number of years, Hanash et al. [48-54] extensively studied childhood leukaemia. 2DE allowed them to detect 11 polypeptide markers that distinguished between subtypes of acute lymphoblastic leukaemia and between acute lymphoblastic and acute myelocytic leukaemia. In children with otherwise undifferentiated leukaemia, they found cellular proteins revealing myeloid origin of the blast cells. They also found a new marker for common acute lymphoblastic leukaemia. Keim et al. [55] also studied the proliferating cell nuclear antigen expression in childhood acute leukaemia and found differences in its expression according to leukaemia subtype. They believed that these differences were not related to the initial peripheral white blood count, age, or sex, but that they reflected differences in proliferative activity between subtypes of acute leukaemia.

Proteomics Approach in Serology

López-Árias et al. [56] employed an immunoproteomic approach, combining 2DE, Western blot, and MALDI-MS to identify tumor-associated antigens (TAA) in the sera of patients diagnosed with infiltrating ductal or in situ carcinoma breast cancer. Sera obtained from the patients with stage II breast cancer (BC) and healthy volunteers were evaluated for the presence of novel TAA. They suggested that Alpha 1-antitrypsin (AAT) and auto-antibodies against alpha 1 antitrypsin may be useful serum biomarkers for early-stage BC screening and diagnosis.

Doustjalali et al. [10] analyzed unfractionated sera of newly diagnosed patients with breast carcinoma (BC), prior to treatment, and patients with fibrocystic disease of the breast (FDB) by 2DE and silver staining. The relative expression of alpha1-antichymotrypsin (ACT), clusterin, and complement factor B was significantly higher in all BC patients as compared to normal controls. However, the expression of alpha1-antitrypsin (AAT) in BC patients was apparently lower than those of the controls. Similar differential expression of ACT was detected in the FDB patients also. The differential expression of the serum proteins was apparently abrogated in a six month follow

up study on the BC patients subsequent to treatment. Doustjalali et al. [11] used also the proteomics approach to study the simultaneous expression of serum proteins in patients with nasopharyngeal carcinoma (NPC). They subjected unfractionated whole sera of newly diagnosed Malaysian Chinese patients with WHO type III NPC to 2DE and image analysis. Their data demonstrated that the serum high abundance 2DE protein profiles of NPC patients were generally similar to that of the controls, with exception of the ceruloplasmin (CPL) spots (identified by mass spectrometric analysis and MASCOT database search), showing higher expression. When follow-up 2DE studies were performed on NPC patients who responded positively to six months treatment, the difference in CPL expression was no longer significant [46].

Mohamed et al. [12] reported that expression of the 35 kDa inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) cleavage fragment was demonstrated to be significantly enhanced in sera of patients with breast carcinoma, epithelial ovarian carcinoma, and germ cell ovarian carcinoma but not in patients with nasopharyngeal carcinoma and osteosarcoma. The lectin-based electrophoretic bioanalytical method adopted in their study may be used to assess the physiological relevance of ITIH4 fragmentation and its correlation with different malignancies, their stages and progression. Rui et al. [57] used 2DE and MALDI-TOF. They reported the up-regulation of HSP27 and down-regulation of 14-3-3 proteins in the sera of BC patients.

Proteomics Approach in Urology

Significant reduced levels of CD59, kininogen-1 and a 39 kDa fragment of inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4), and enhanced excretion of a 19 kDa fragment of albumin, were detected by 2DE analysis in the urine of patients with ovarian carcinoma compared to the control subjects [58]. Rehman et al. [59] used 2DE proteomic approach for analysis of voided urine obtained after prostatic massage from patients with prostate cancer. They reported that the calgranulin B/MRP-14 may have potential as novel marker for prostate cancer. Using 2DE proteomic analysis, Iwaki et al. [60] identified calreticulin (CRT), gamma-synuclein (SNCG) and a soluble isoform of catechol-o-methyltransferase (s-COMT) as novel candidates for tumor markers in bladder cancer.

Conclusions

Due to wider and expanding network of cancerous conditions, precise identification, prompt and timely detection of cancer is only feasible by using sophisticated technologies such as proteomics. 2DE is the most promising proteomics technique with sufficient resolving power that provides a unique platform for the simultaneous separation of proteins in a complex mixture. However, the performance of optimization techniques in proteomics research using 2DE has its own limitations. In order to overcome these limitations, herein we have reviewed the application of 2DE proteomics in previously published well-known cancer research and in precise identification of sensitive and specific tumor markers over a period of time. Thus, exploring better concepts in employing proteomics using 2DE is necessary to ensure successful development of novel proteomics to improve quality of life of humankind. In conclusion, there is a great interest in the use of 2DE proteomic technique to do cancer research. Knowledge about proteomics technique is extremely beneficial in understanding the holistic approach. This review represents the most comprehensive description of two-dimensional gel electrophoresis (2DE) technique and future studies may reveal more effective strategy in cancer research.

Competing Interests

The authors declare they have no competing interests.

Authors' Contributions

Saeid R Doustjalali, Munira Bhuiyan, Karim Al-Jashamy, Negar S Sabet have been involved in drafting the manuscript. Samiah Yasmin Abdul Kadir, Nyan Htain Linn, Vinothini Appalanaidu, Hafiza Arzuman, Anitadevi Krishnan, Wai Ma Lin, Alireza Saraji, Kaliappan Gopal, Esaki Muthu Shankar, Shahhosseini Fatemeh revised the draft. All authors read and approved the final manuscript to be published.

Acknowledgements

The author would like to thank the co-authors for their valuable comments and suggestions to improve the quality of the paper.

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