

Review

The use of proteomics for the assessment of clinical samples in research

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Abstract

Proteomics, the analysis of expressed proteins, has been an important developing area of research for the past two decades [Anderson, NG, Anderson, NL. Twenty years of two-dimensional electrophoresis: past, present and future. *Electrophoresis* 1996; 17:443–53]. Advances in technology have led to a rapid increase in applications to a wide range of samples; from initial experiments using cell lines, more complex tissues and biological fluids are now being assessed to establish changes in protein expression. A primary aim of clinical proteomics is the identification of biomarkers for diagnosis and therapeutic intervention of disease, by comparing the proteomic profiles of control and disease, and differing physiological states. This expansion into clinical samples has not been without difficulties owing to the complexity and dynamic range in plasma and human tissues including tissue biopsies. The most widely used techniques for analysis of clinical samples are surface-enhanced laser desorption/ionisation mass spectrometry (SELDI-MS) and 2-dimensional gel electrophoresis (2-DE) coupled to matrix-assisted laser desorption ionisation [Person, MD, Monks, TJ, Lau, SS. An integrated approach to identifying chemically induced posttranslational modifications using comparative MALDI-MS and targeted HPLC-ESI-MS/MS. *Chem. Res. Toxicol.*, 2003; 16:598–608]-mass spectroscopy (MALDI-MS). This review aims to summarise the findings of studies that have used proteomic research methods to analyse samples from clinical studies and to assess the impact that proteomic techniques have had in assessing clinical samples.

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Introduction

Proteomic technology

Since the completion of the human genome in 2002 and the recognition that this cannot provide all the answers to the aetiology of disease, attention has turned to assessing changes in the expressed proteins of a given genome. As a consequence, proteomics has been a rapidly growing area of research that can examine not only the presence or absence of particular proteins, but also post-synthetic changes [1]. However, to assess the proteome of any biological sample requires specialised technology and expertise. The most common method of assessment of a given sample proteome combines 2-D polyacrylamide gel electrophoresis (2-DE) and mass spectrometry (MS), although similar analyses are possible using chromatography-MS or tandem MS (MS-MS). A schematic illustration of the most common proteomic methods which are discussed within this review is shown in Fig. 1. These technologies have advantages and disadvantages and it is beyond the scope of this review to assess all proteomic technologies in detail. Few of these technologies are available to clinical laboratories for identification of novel markers; however, 2-DE technologies are widely available within a research setting, and a number of proteomic facilities are now accessible and provide a service to many clinical laboratories. Therefore, this review will focus on the application of 2-DE technologies.

2-DE comprises iso-electric focussing in the 1st dimension, where proteins are separated based on charge and polyacrylamide gel electrophoresis [3] in the 2nd dimension where proteins are separated based on size/mass. The combination of these techniques results in proteins resolved on a gel as spots when stained using silver, fluorescent dyes or Coomassie blue [4]. 2-DE is not a new technique, and indeed a great number of studies have used this technique to assess proteins of interest [5,6]. The application of 2-DE in assessment of clinical samples is comprehensively reviewed by Young and Tracy [7]. The authors review studies involving human-based samples that used 2-DE analysis in the 3 years prior to publication.

Due mainly to cost, many laboratories have moved into the proteomics field by investigation of 2-DE and matrix-assisted laser desorption ionisation [2]-MS; however, proteomic technologies encompass many more techniques. A number of studies have investigated changes in protein abundance by MS-MS or by stable isotope tagging [8,9]. Many of these studies have been conducted in yeast or simple cell systems, with very few taking the analyses forward into eukaryote systems or even into clinical

samples. HPLC-MS allows the separation of a number of biological fluids such as plasma or urine, but requires the digestion of proteins, preventing the identification of native proteins as biomarkers for disease.

Surface-enhanced laser desorption/ionisation mass spectrometry (SELDI-MS) has been used to identify key proteins in a number of cancers and allows high sample throughput. However, there are limitations involving sample resolution as analysis is most adept for proteins of molecular mass lower than 20 kDa. Hence many higher molecular mass proteins are poorly resolved and may be eliminated from the study. SELDI-MS also involves the use of chip technology, in which proteins are selected by chemical properties or by biochemical properties. Although many different chip arrays may be used on one sample, the choice of chip array can require some prior knowledge of the changes that may take place between control and test or disease subjects, in order to select the correct chip array to yield proteins of interest. For example, chip arrays can be specific for hydrophobic anionic, cationic or hydrophilic proteins, and they can also use antibody–antigen interactions, receptor–ligand interactions or DNA–protein interactions to bait specific proteins. Hence, prior knowledge may be required to identify if an intervention or disease was expected to increase expression of hydrophobic proteins, or anionic proteins, or even one specific target protein. Despite these limitations, the use of chip arrays allows minimal sample preparation and this technology can yield important results [10,11].

Protein resolution

One of the most important challenges in analysis of proteins by 2-DE is how well proteins are resolved and how many proteins are visible. For example, in plasma, the low-abundance proteins, such as cytokines, are often important in proteomic studies, and most often they are frequently masked by high-abundance proteins, such as albumin. This is demonstrated very well in an overview of 2-DE electrophoresis by Garfin [12]. This review describes a number of methods to increase relative amounts of low-abundance proteins in a sample, including pre-fractionation and chromatographic methods. There are also a number of ways to enhance the separation and visualisation of proteins without the need for extensive sample manipulation or fractionation. The greatest resolution of proteins can be seen using large format gels (30 × 30 cm); however, the difficulty in handling this size of gel makes their routine use impractical. Smaller format gels (15 × 10 cm) are more practical to use but can result in some overcrowding of proteins within certain regions of the gel. Narrow range

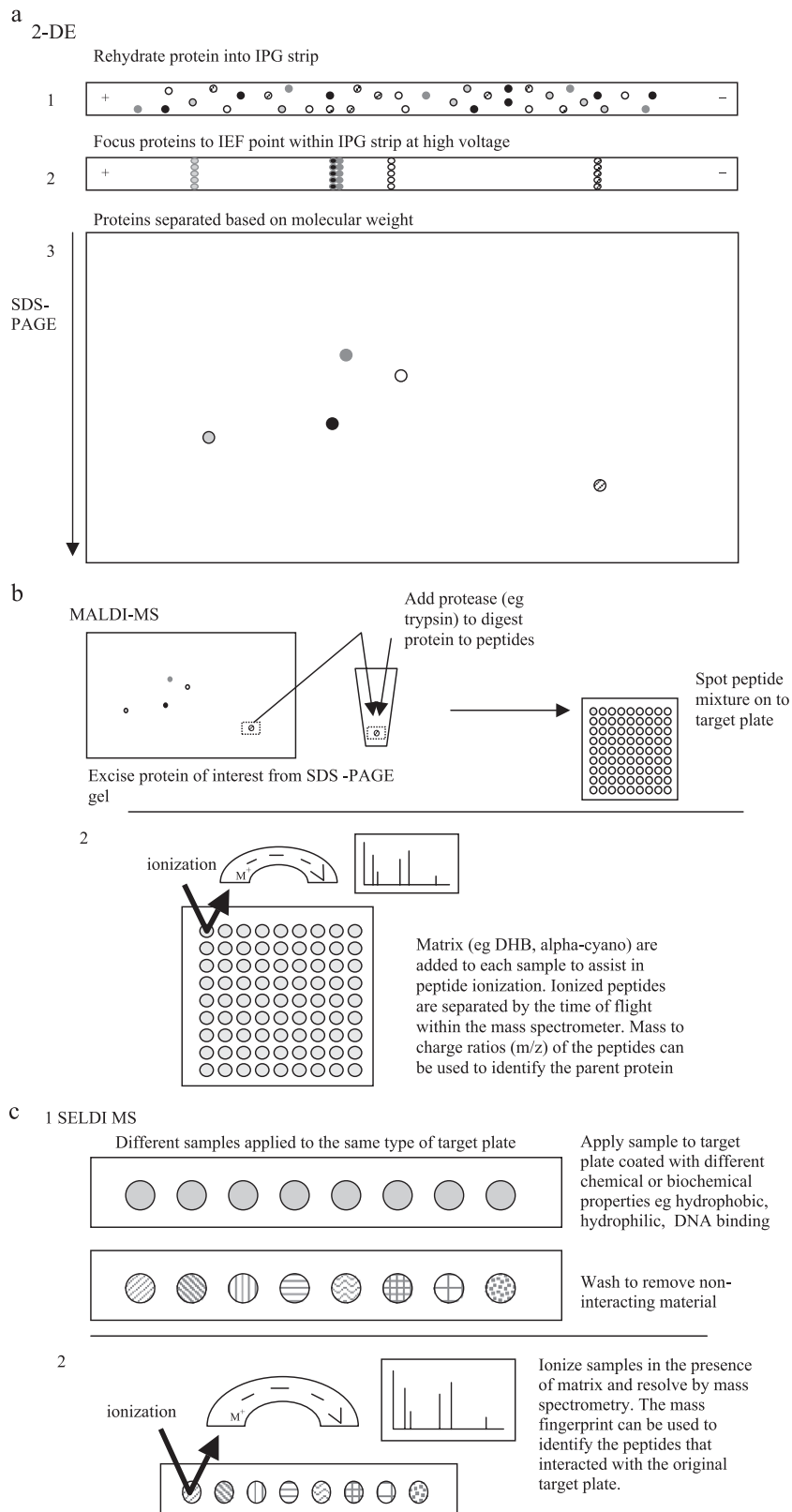


Fig. 1. (a) Schematic diagram of 2-dimensional electrophoresis (2-DE). (b) Schematic diagram of matrix-assisted laser desorption mass spectrometry (MALDI-MS) [2]. (c) Schematic diagram of surface enhanced laser desorption/ionisation mass spectrometry (SELDI-MS).

strips can allow separation of proteins within 1 pH unit (pH 5.5–6.5 for example) and afford benefits such as greater resolution and identification of low-abundance proteins that are not visible on wider range strips (spanning a pH range 3–10) [13–15]. Wider-range strips offer the advantage of assessing the proteins visible within a sample, from acidic to basic pH and can be used to give an overview of the proteome before selecting proteins in a narrower range for further investigation (Fig. 2).

Sample choice and preparation

Perhaps the largest body of research using clinical samples in the proteomics field to date has been undertaken using plasma samples, due to the ease of accessibility of plasma. Other biological fluids such as cerebrospinal fluid or synovial fluid may share some proteins present in plasma, but collection procedures are more invasive and require specialist collection. In addition to the major plasma proteins such as albumin, lipoproteins and immunoglobulins, plasma may contain numerous tissue proteins that have been released as markers. For example, proteins that normally function in heart muscle and are restricted to the tissue during normal health may be released into plasma as a result of cell death or damage. These include cardiac troponins or myoglobin that are released after myocardial infarction [16]. Analysis of plasma using proteomics has been restricted by the presence of albumin at high concentrations, undoubtedly masking the changes to other low-abundance proteins. There is a limit to the total amount of protein that can be loaded and therefore only femtogram quantities of low abundance proteins may be loaded in the presence of microgram quantities of albumin. The dynamic range of proteins within plasma spans 9 orders of magnitude; in contrast cellular proteins have a range of 5 or 6 orders of magnitude [17]. Proteins at the low-abundance end do not fall into the range of sensitivity for protein detection with standard staining techniques, as albumin and immunoglobulins constitute 60–90% of all plasma proteins. The removal of albumin has become commonplace or even routine, and allows increased detection sensitivity of low-abundance proteins [18]. Selective depletion can greatly enhance the visualisation of proteins with molecular parameters (pI , mass) similar to the

proteins removed. Techniques for the removal of albumin often involve the dye-binding properties of the protein and more specific antibody-based procedures [19]. It should be considered, however, that depletion of a plasma or serum sample for albumin or immunoglobulins may also exclude or deplete other proteins to which notable changes have taken place [20]. A number of techniques used to remove albumin, such as cibacron blue chromatography, are also known to bind and remove other proteins due to the nature of the protein binding [21]. Cibacron blue mimics nicotinamide adenine dinucleotide and thus will bind any protein with a dinucleotide fold [22]. Despite these limitations, a number of studies have successfully assessed plasma samples for altered protein expression in disease using proteomic techniques resulting in the identification of potentially useful markers of disease.

Protein identification

Analysis of the protein patterns produced by 2-DE is a complex process, and when clinical samples are involved, the number of variations between samples can be overwhelming. Proteins can only be assessed with the aid of gel matching by image analysis software. However, this process requires a high degree of input by the operator to verify that protein matches that have been identified by the software are correct. Very often, image software may match the wrong protein in a diseased sample set to one in a control sample set. Correct protein matching is essential to identify change in protein expression in diseased samples. Voss and Haberl [23] highlighted this mismatching in two 2-DE gels run and processed in parallel. The study identified distorted spot patterns within the gels and poor reducibility in staining intensities. They concluded that correct protein matching requires additional manual matching and as a result of this limited numbers of proteins within samples sets can be assessed reliably. However, studies that have specifically studied the effectiveness of software packages have highlighted how effective they can be in spot detection and quantification. When comparing PDQuest software with progenesis, Rosengren et al. [24] observed that the quality of the gel critically influenced the spot detection and protein matching. When a previous study that compared Z3 and Melanie

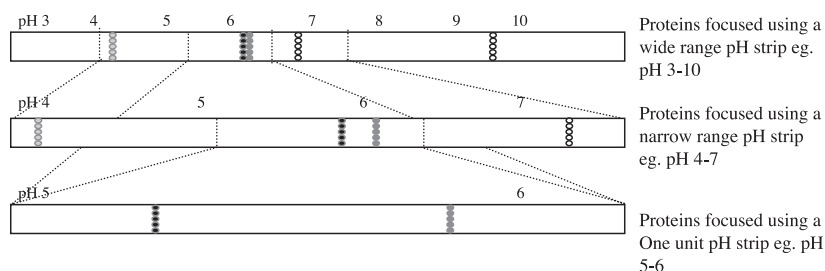


Fig. 2. A schematic of isoelectric focussing using narrow- and wide-range IPG strips.

software packages was included [25] and all four software packages were compared, all software packages matched over 85% of proteins.

Post-translational modifications

The analysis of post-translational modifications represents one of the most challenging aspects of clinical proteomic research. Phosphorylation is required for activation and/or functionality of as many as one in three proteins [26], and is catalysed by protein kinases. The most common sites of phosphorylation in eukaryotic proteins are tyrosine, serine and threonine and there are potentially tens of thousands of phosphorylation sites, of which fewer than 2000 are known [27]. The most well studied phosphorylation events are those involving phosphorylation of tyrosine residues; however, phosphorylation of serine and threonine is more frequent. The lack of research in this area has partly been due to a lack of suitable antibodies [28], a limitation that is becoming less of a problem. Investigation of phosphorylation events involved in signalling pathways will undoubtedly allow a greater understanding of the mechanisms and networks controlled by phosphorylation; however, analysis is also often difficult due to the low abundance of phosphorylated proteins [29] and the speed of removal of phosphate groups. Nevertheless, Gronborg et al. [28] identified novel phosphoproteins involved in intracellular signalling following immunoprecipitation and blotting of phosphoserine and phosphothreonine proteins using specific antibodies. Yamagata et al. [30] identified 5% of proteins visible on a 2-DE gel in rat skin fibroblast culture as phosphorylated. Soskic et al. [31] also successfully identified phosphorylated proteins using anti-phosphoserine and anti-phosphotyrosine antibodies following 2-DE. Although these studies involved cell lines, they highlight possible directions for proteomic research in 2-DE protein mapping and blotting.

Reactive oxygen or nitrogen can cause a number of chemical modifications to proteins, some of which are irreversible. Nitration and/or oxidation of proteins can modify their function or action and proteomic methods are increasingly being used to identify proteins that have been modified in this way [32]. Oxidation of proteins has been shown to cause formation of carbonyl groups, which react with 2,4-dinitrophenylhydrazine (DNPH). By reacting proteins with DNPH following 2-DE, oxidised proteins can be identified using anti-DNP antibodies [33,34]. The different types of post-translational modification are shown in Table 1, with examples studies that have identified each modification and the method of detection.

Plasma and blood samples

The accessibility of blood samples and the routine drawing of blood for other analyses make the use of plasma and blood ideal candidates for the identification of biomarkers for clinical studies. One of the first studies to

Table 1
Post-translational modifications and methods of detection

Modification	Methods of detection	Reference
Reactive oxygen species		
Protein carbonyl	Western blotting	[35]
Specific amino acid modifications	Mass spectral or HPLC techniques	[36]
Reactive nitrogen species		
Nitration	Western blotting Mass spectral techniques	[37]
Glycosylation		
Addition of carbohydrates either singly or in oligomers proteins	Gel shift Mass spectral techniques	[38]
Phosphorylation		
Addition of phosphate to either serine, threonine or tyrosine	Western blotting Gel shift Mass spectral techniques Radiochemical detection	[39] [30] [29]
Acylation	Mass spectral techniques [2]	[40]
Sulfation	Mass spectral techniques and gel manipulation software	[41]
Vitamin C		
Glyoxal formation	Western blotting	[42]
Selenium modifications		
Selenocysteine	Selenocysteine insertion	[43]

suggest that 2-DE could be used to distinguish protein spot patterns between disease states and control was by Tissot et al. [44]. Plasma and serum were compared between blood donor controls and pathological samples from patients with diseases including liver cirrhosis, acute hepatic failure, renal failure, and immunohemolytic anaemia. Despite the complexity of the protein profiles, differences were detected between disease and control samples, characterised by presence or absence of protein spots, or by reduction or enlargement of protein spots. The study concluded that identification of plasma protein alterations in specific disease groups can allow the diagnosis of a disease solely based on the protein map modifications.

Protein markers of rheumatoid arthritis in blood samples

In order to demonstrate the validity of the technique for this analysis, Doherty et al. [45] identified 19 plasma proteins of interest prior to analysis for comparison between patients with rheumatoid arthritis and controls. The study assessed these predefined proteins by 2-DE and found five of them were significantly elevated in diseased patients, and seven were significantly decreased. This type of analysis, where a virtual protein map is used to assess a number of predefined proteins of interest is unusual in clinical sample analyses. Instead, studies use 2-DE coupled with image analysis software to give an overview of the total plasma proteome, and identify altered proteins for further analysis.

Protein markers of cancer in blood samples

Proteomic approaches are likely to offer rapid developments in the field of tumour marker discovery. Whilst

several studies have focussed on analysis of the tumour itself [46,47], Lehrer et al. [48] used SELDI-MS to screen plasma samples from 11 men with prostate cancer and 12 men with benign prostatic hyperplasia. The study identified three protein peaks between 15 and 17.5 kDa mass range, which were present in serum of cancer subjects and which were not present in control serum. These results highlight the possible use of proteomic plasma screening for diagnosis and monitoring of such diseases as prostate cancer. Plasma screening has also been successful in identifying biomarkers for ovarian cancer. Rai et al. [49] used protein chip analysis to identify a panel of seven protein biomarkers that were unique to plasma samples from ovarian cancer sufferers. Cancer antigen 125 (CA125) is currently used as a tool for population based screening for early detection and diagnosis of ovarian cancer. Of the seven markers identified in this study, four were shown to improve the sensitivity of this existing population-based screening method if used in combination with CA125. Mitsaides et al. [25] used 2-DE to investigate the signalling profile of tumour cells in plasma of patients with Waldenstrom's macroglobulinemia (WM) and multiple myeloma (MM). Analysis of plasma samples showed considerable overlap in B-cell proteomic profiles between the two conditions; however, distinct differences were also observed, indicating a role for proteomic analysis in investigation of B-cell malignancies.

Protein markers of Alzheimer's disease in blood samples

The analysis of post-translational modification of proteins in plasma is in the early stages of development. Nikov et al. [37] assessed nitrated proteins by in gel labelling and affinity chromatography coupled to MS, and successfully presented a method to identify specific protein targets of nitration and intact modified proteins in plasma or serum. Choi et al. [51] assessed only oxidised proteins in plasma from patients with Alzheimer's disease. Seven principal oxidised protein spots were observed and identified by MALDI-MS. The proteins were oxidised isoforms of fibrinogen γ -chain precursor protein and α -1-antitrypsin precursor, which have both previously been implicated in the pathology of the disease [52]. In addition, Mattila and Frey [53] investigated changes in blood cells in patients with Alzheimer's disease by 2-DE. Although no qualitative protein changes were seen, a number of significant quantitative changes were observed. Platelets, lymphocytes and red blood cell membranes (RBCM) were isolated from five patients with Alzheimer's disease and a total of 114 spots were identified as displaying a significant quantitative change across the three cell types. The most interesting of these was actin, which showed a marked reduction in intensity in Alzheimer's disease platelets and lymphocytes.

Protein markers of disease in blood cells

Imam-Sghiouar et al. [54] successfully identified phosphorylated proteins in B-lymphoblasts of patients suffering

from Scott syndrome. Scott syndrome is a very rare inherited disorder affecting the membrane of red cells, platelets and lymphocytes. This study found phosphorylated fascin, an actin-binding protein, in control lymphoblasts but not in lymphoblasts of Scott patients, suggesting a role for actin remodelling in the mechanism for this disorder.

Cerebrospinal fluid

2-DE of cerebrospinal fluid (CSF) is as complex to optimise and resolve as 2-DE of plasma, since the most abundant proteins present in CSF are albumin and immunoglobulins. In order to assess changes to lower abundance proteins, it is necessary to deplete these proteins. However, Terry and Desiderio [55] have demonstrated that studies involving the CSF proteome can attain a high level of reproducibility. The reproducibility of the CSF proteome was assessed by comparing seven CSF samples to one another and to a designated reference CSF sample. The number of proteins detected were assessed in a pooled CSF sample run 10 times, and showed very little variation (CV = 6%).

Davidsson et al. [56] found the use of narrow range IPG strips improved resolution and identification of proteins of interest in CSF. This study identified six key proteins that had altered expression in Alzheimer's disease, including apolipoprotein A1 (Apo A1) and apolipoprotein E (Apo E). Following depletion and use of narrow range strips, Puchades et al. [14] also used CSF samples to identify proteins with altered expression in Alzheimer's disease compared to controls. These proteins were identified again as Apo A1 and Apo E. This study also highlighted the involvement of a number of glycosylated proteins with altered expression in Alzheimer's CSF that were previously unknown to play a role in the disease. Apo E is a constituent of lipoproteins involved in the transport and metabolism of lipids. It is produced by the liver and in the brain where Apo E-lipid complexes are involved in neuronal regeneration. [57]. Apo E has three known isoforms, E2, E3 and E4, with E4 being linked to Alzheimer's disease [58]. Hesse et al. [59] demonstrated that this isoform could be identified by 2-DE coupled to MALDI-MS in CSF of Alzheimer's patients. Following 2-DE separation, the known change of Cys to Arg in position 112 of the E4 isoform was identified by MALDI-MS. This peptide was not detected in CSF from healthy controls.

Jiang et al. [60] also used 2-DE and MALDI-MS in the analysis of CSF from schizophrenic patients. This study identified significant decreases in apolipoprotein A-IV in schizophrenia compared to controls. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a hereditary condition causing stroke and dementia. Unlu et al. [61] used 2-DE to assess CSF samples from sufferers. Following electrophoresis, MS analysis identified a protein unique to this disease as complement factor B, which may suggest an alternative complement pathway in CADASIL.

Tissue samples

Separation and analysis of proteins from tissue samples and tumour biopsies has proved very successful in identifying novel markers. Jungblut et al. [62] successfully identified two peptides from calgranulin B, which were elevated in colorectal tumour samples and pre-neoplastic polyps. Calgranulin B is involved in calcium binding and is also expressed during an inflammatory response. Although this study highlighted some initial difficulty in mapping these proteins to online maps, a number of studies have successfully identified proteins of interest in tumour samples by 2-DE. Chen et al. [63] identified nine proteins by 2-DE/MS that were overexpressed in lung adenocarcinoma. A sample set of 93 tumours were assessed by 2-DE and overexpressed candidate proteins were identified by MALDI-MS. Altered expression in the identified proteins ranged from 1.4-fold to 10.6-fold increases when compared to uninvolved lung tissue. Zhukov et al. [64] used SELDI-MS to identify protein peak profiles that were unique to malignant lung tumours and pre-malignant epithelium. This study highlighted three protein peaks in the 17–23 kDa mass range, which were increased compared to normal cells, and one protein signature that was not present in control cells. This study gave similar results to a study discussed earlier by Lehrer et al. [48] when plasma samples were used to assess markers of prostatic neoplasms.

2-DE has also been successful in identifying biomarkers of bladder cancer in tumour biopsies. Bladder cancer encompasses a number of tumours including squamous cell carcinomas [13], transitional cell carcinomas (TCC), adenocarcinomas and small cell carcinomas [65]. Proteomic profiles of bladder tumour biopsies from SCC have produced comprehensive proteomic databases. Comparisons of SCC

and normal biopsies have identified several protein biomarkers expressed in tumour samples including a number of isoforms of keratin [66] and psoriasin [67]. Conversely, Celis et al. [68] identified several isoforms of keratin that were not expressed in SCC, when compared to normal urothelium. In a separate study, these authors also identified four proteins that were lost at various stages of tumour progression, including glutathione s-transferase and keratin 13 [66].

Proteomic analysis of Alzheimer's brain tissue has identified a number of protein species that are important in this disease pathology. In particular, oxidatively modified proteins have been identified to play a key role [69,70]. Korolainen et al. [71] used DNPH to identify protein carbonyls in cytosolic fractions from post mortem Alzheimer's brain tissue. This study used the same technique and methodological principle as Mattila and Frey [72] in plasma samples. Korolainen et al. [71] identified over 100 oxidised proteins by 2-DE, with six proteins having reduced protein carbonyl content and one protein shown to have increased protein carbonyl content in Alzheimer's disease tissue. At the time of publication, the proteins of interest had not been identified by MS, but used 2-DE technology alone to identify protein modifications between disease and control tissue and concluded that not only are proteins oxidised in Alzheimer's disease, but that the balance of protein oxidation and degradation is altered. Oxidatively modified proteins in Alzheimer's disease were also the focus of two large studies by Castegna et al. [73,74]. Both studies used 2-DE coupled with Western blotting to identify protein carbonylation; however, MALDI-MS was then used to identify key protein species. Specific oxidation targets within Alzheimer's disease were identified as creatine kinase BB, glutamine synthase and ubiquitin carboxyl-terminal hydrolase L-1 with these three proteins showing

Table 2
Summary of specimen type, disease and method of detection for the studies reviewed

Clinical specimen	Disease	Method of detection	Reference	
Plasma	Liver cirrhosis	2-DE/MALDI-MS	[44]	
	Rheumatoid arthritis	2-DE/MALDI-MS	[45]	
	Prostate cancer	SELDI-MS	[48]	
	Ovarian cancer	SELDI-MS	[49]	
	Waldenstrom's macroglobulinemia and multiple myeloma	2-DE/MALDI-MS	[50]	
	Alzheimer's disease	MALDI-MS	[51]	
Blood cells; red cells, platelets and lymphocytes	Alzheimer's disease	2-DE/MALDI-MS	[53]	
	Scott syndrome	2-DE/MALDI-MS	[54]	
	CSF	Alzheimer's disease	2-DE/MALDI-MS	[14,56,59]
		Schizophrenia	2-DE/MALDI-MS	[60]
Tissue samples	CADASIL	2-DE/MALDI-MS	[61]	
	Colorectal cancer	2-DE/MALDI-MS	[62]	
	Lung adenocarcinoma	2-DE/MALDI-MS	[63]	
	Lung cancer	SELDI-MS	[64]	
	Bladder cancer	2-DE/MALDI-MS	[66–68]	
	Alzheimer's disease	2-DE /antibody technology	[71,73,74]	
Urine	Cardiomyopathy	2-DE/MALDI-MS	[62,76]	
	Kidney disease	Capillary electrophoresis/MS	[78]	
	Bladder cancer	SELDI-MS	[79]	
	Bladder cancer	2-DE/MALDI-MS	[80]	

significant increased oxidation levels when compared to control brain extracts. In the second study by these authors, further protein oxidation targets were identified as dihydropyrimidinase-related protein 2, α -enolase and heat shock cognate 71, in Alzheimer's disease inferior parietal lobe.

Analysis of tissue samples by proteomics has also proved an effective method in investigation of cardiomyopathy. The heart is a relatively simple organ in proteomic terms, when compared to others such as the liver, as it is mainly made up of muscle cells. This allows greater success in separation of proteins by 2-DE and comparison of proteins between gels has been very successful [63,75]. 2-DE analyses of dilated cardiomyopathy samples identified 12 significantly different protein species when compared to controls, including ATP synthase, cytochrome *c* oxidase and haemoglobin [62]. Differences in heat shock protein 27 protein expression in cardiomyopathy have also been observed [76].

Saliva and urine

Studies assessing the proteins present in saliva from patients with rheumatoid arthritis have also highlighted the difficulty in obtaining well-resolved gels when using mixed cell populations, where an apparent change in marker level may relate only to the proportion of a particular cell type in a given sample. Initial analysis by 1-DE produced poor results, with bands of proteins indistinguishable from others. The use of 2-DE did improve resolution and successfully produced a number of proteins altered in the disease state, but proteins in these samples still appeared as ion-charged trains rather than isolated spots [77].

Wittke et al. [78] recently used capillary electrophoresis linked to MS to identify a number of polypeptides present in urine taken from patients with kidney disease that were not present in control urine. The study identified 247 polypeptides that made up a control polypeptide profile, and found 27 additional polypeptides present in diseased samples, as well as 13 polypeptides that were absent. However, although this study did identify mass fingerprints for the polypeptides, it did not speculate as to the identity of the proteins. Vlahou et al. [79] used SELDI-MS to assess urine from patients with TCC of the bladder. This study identified five novel potential TCC biomarkers that were preferentially expressed, termed Urinary Bladder Cancer markers 1–5. Ostergaard et al. [80] identified a marker of bladder cancer, psoriasin, in urine samples from patients with SCC which had previously been identified in tumour tissue. Celis et al. [81] recently highlighted the importance of proteomic methods in the early detection of bladder cancer. The studies reviewed herein are summarised in Table 2.

Conclusion

The rapidly expanding and diversifying field of proteomics has been utilised by workers in clinical fields with

success over the past decade. A wide range of samples, from plasma to post mortem brain tissue, has been assessed. As with the use of proteomics in other nonclinical settings, the analysis is limited by various factors that affect reproducibility, resolution and clarity of separation. However, even at this early stage in the application of proteomics to clinical sample investigation, positive results have been achieved and identification of disease markers has been successful.

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