Molecular pathology

Proteomic applications (II)

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INTRODUCTION

The majority of human diseases as defined through clinical pathology should be regarded as multifactorial, and their complexity must be understood at the molecular level. The dramatic advances in genomic sequencing and mRNA-based analysis of gene expression have generated important descriptive information. However, it is becoming apparent that purely gene-based expression analysis is not sufficient for the dissection of a disease phenotype at the molecular level.

The proteomic approach differs from all physiologically or biochemically driven approaches by making no hypothesis on the pathophysiological nature of the changes to be detected. This approach compares two-dimensional electrophoresis patterns of proteins from one group of samples with the corresponding patterns in a different sample and is thereby able to detect yet unknown and completely unsuspected quantitative and qualitative biochemical changes in a given tissue. The ultimate objectives of proteomics go beyond the simple cataloging of the proteins that cells express in health and disease states. The eventual goal is to elucidate the organization and dynamics of the metabolic, signaling and regulatory networks through which the life of the cell is transacted. Moreover, proteomics seeks to understand how these networks become dysfunctional in disease, and to predict how their function can be manipulated through interventions such as drugs and genetic manipulations.

The potential of 2-D electrophoresis in clinical investigation was recognized with the first publications of the combination of IEF with SDS-PAGE in 1974 and 1975. However, the maximum effect of proteomics-based approaches on biomedical research has not yet been achieved, partially because of the lack of awareness in the research community about the technological advances that have made such an approach feasible on a large or small scale, and partially because of the naturally occurring lead-in time after any technological advancement.

There is a great deal of interest in the application of proteomics to the study of individual diseases, and research in these areas is continually expanding (Table 1).

Table 1. Proteomic applications.

- Cancer
- Cardiovascular diseases
- Neurological diseases
- Other diseases
- Toxicology – drug development
- Microbiology – infectious diseases

There are now numerous examples of proteomics-based clinical studies and the number of projects is increasing monthly. Consequently, there is a need for basic studies regarding the correlation between the histopathological picture and quality of the 2-D electrophoretic pattern. This is essential in order to increase the possibilities of identifying subtle alterations. Identifying disease markers, proteins that appear or disappear during the course of a disease, does not necessarily require that all expressed proteins in a clinical sample be identified, although the more complete the proteome, the more complete will be any set of markers.

CANCER

Carcinogenesis is a multistep process and a tumor cell results from several independent genetic or epigenetic damage events occurring sequentially within a single cell. Genetic alterations lead to changes of the polypeptide expression level, which can be analyzed in detail by 2-D PAGE. Studies of global protein expression in human tumors have led to the identification of various polypeptide markers which are potentially useful as diagnostic tools (Table 2). Many changes in gene expression recorded between benign and malignant human tumors are due to post-translational modifications not detected by analyses of mRNA. Proteome analyses have also yielded information about tumor heterogeneity and the degree of relatedness between primary tumors and their metastases. In addition, proteomics has the potential to unravel basic tumor biological questions regarding mechanisms involved in the pathogenesis of cancer.

Genetic markers, detected cytogenetically or by mutation detection, are now entering clinical practice, but some changes likely to be important in carcinogenesis, diagnosis and prognosis, such as abnormal expression of proto-oncogenes, may not be associated with a detectable genetic lesion. Precise and accurate knowledge of the repertoire of proteins associated with human cancers will likely provide insights into the fundamental mechanisms of tumor development, tumor progression, and/or provide new drug targets, vaccine antigens, or markers for early detection.

Table 2. Cancer and proteimics.

- Proteins associated with tumor development
- Proteins associated with tumor progression. Metastatic process
- Provide new drug targets. Study of drug resistance
- Vaccine antigens
- Markers for early detection
- Create artificial learning models

Bladder cancer

Celis *et al.* have reported the results of the analysis of squamous bladder tumors by 2-D electrophoresis (1). The approach first made use of proteomic technology to identify and reveal proteins that were differentially expressed in bladder cancer and normal urothelium. Thereafter, specific antibodies against the differentially expressed proteins were used to immunostain cryostat sections of biopsies. Tumors with a low degree of differentiation showed decreases in the expression of some cytokeratins and of psoriasin (S100-A7), galectin 7 and stratifin (2). All bladder cancer studied externalized psoriasin to the urine, supporting the contention that this protein, alone or in combination with other polypeptides, may be a useful marker for the early detection of these lesions (3).

Kidney cancer

Studies on phenotypical expression of human kidney tissue and on post-translational modifications in renal cell carcinoma have not yet provided a marker for early diagnosis. Sarto *et al.* reported the comparison of protein between normal kidney and renal cell carcinoma (4). Four proteins were found to be expressed only in normal kidney tissue but absent in renal cell carcinoma. Two were identified as ubiquinol cytochrome *c* reductase and as mitochondrial nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase complex I. Interestingly, both of these genetic loci are on chromosome 19, at positions 19p12 and 19p13.3, respectively, which could indicate a genetic lesion. The absence of these two mitochondrial proteins suggests that mitochondrial dysfunction may play a major role in renal cell carcinoma genesis or evolution. Two further abnormalities in renal cell carcinoma have also been described, namely increased expression of manganese superoxide dismutase and loss of plasma glutathione peroxidase.

Breast cancer

Breast cancer is a heterogeneous disease at the molecular level, raising the possibility of a future functional classification based on mechanisms rather than morphology (5). These molecular phenotypes will also confer predictive value on the potential of the tumor to invade, metastasize and respond to or resist new therapeutic strategies. Due to early diagnosis by screening programs, the percentage of patients who do not have spreading of breast cancer to regional lymph nodes at the time of diagnosis has gradually increased. This has led to an increased need for other prognostic markers. Some biological markers are available, including estrogen/progesterone receptors, nuclear DNA content, and proliferative activity, but additional markers are required. The work by Franzen *et al.* has documented protein expression patterns in clinical breast tumors of different histopathological types and grades (6). Invasive carcinomas showed high expression of some members of stress protein (HSP90, pHSP60 and calreticulin) and down-regulation of 14-3-3σ chaperone (7).

Lung cancer

Since the lung is a common site for metastases from tumors growing at other sides in the body, there is a need for markers that can distinguish primary lung carcinomas from distant metastases and malignant mesotheliomas. A pair of polypeptide markers specific for lung adenocarcinoma was described using 2-D PAGE. These 35 kDa markers, TA01/TA02, are expressed in about 90% of all primary lung adenocarcinomas (8, 9). Recently, the amino acid sequence of TA02 has been determined, and it has been reported that TA02 is identical to the aspartic protease napsin A. Bergman *et al.* detected an increase expression of cathepsin D, an aspartic lysosomal endoproteinase, in lung adenocarcinoma, and nuclear ribonucleoprotein C1/C2 in small-cell lung carcinoma (10).

Using 2-D electrophoresis, our group has recently identified that semenogelins are expressed and secreted into the medium by cultured small-cell lung cancer cells (Fig. 1). Other lung cancer cell types did not express these proteins, suggesting that these proteins may be useful markers for detecting small-cell lung cancer (SCLC) (11).

Using proteomic techniques Hirano *et al.* analyzed the relationship between the histopathological findings in 45 primary lung malignancies and the expression of a number of polypeptides (12). Sixteen polypeptides were judged to be associated with histopathological features. These polypeptides seem to be valuable as differentiation markers. High expression of β-tubulin, heat shock proteins 73 and 90, lamin B, and proliferating cell nuclear antigen (PCNA) were observed in small cell lung

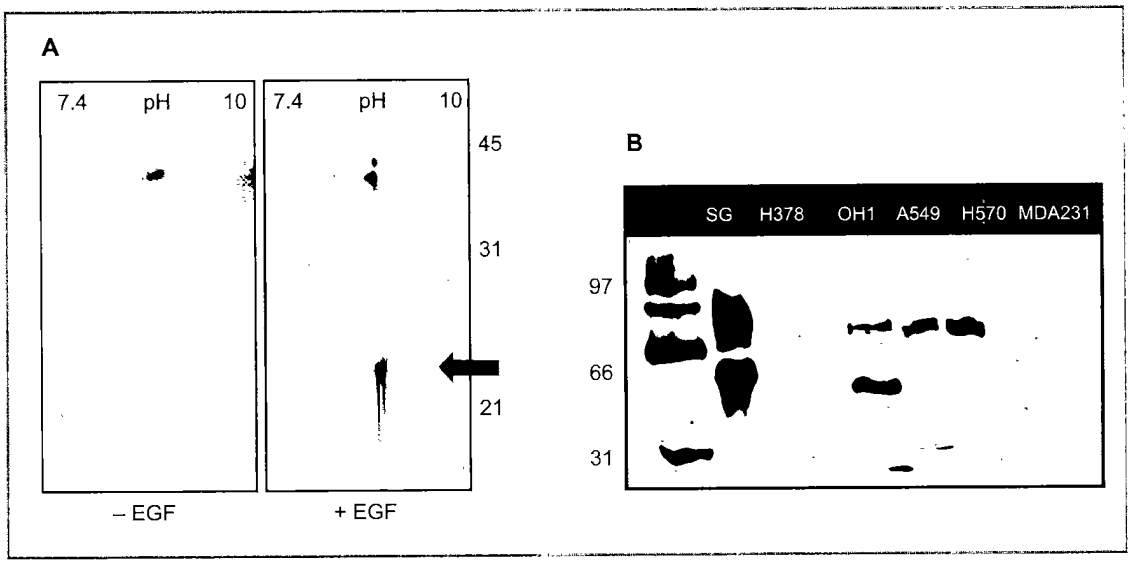

Figure 1. (A) Induction of semenogelin I and II association with adhesion complexes by epidermal growth factor (EGF) in small-cell lung cancer cells. Matched portions of the 2-D PAGE are presented, demonstrating the presence of two protein spots, with Mr 25,000-28,000, isoelectric point = 9 (black arrow). (B) Specific expression of semenogelin proteins in lung cancer cell lines, detected by Western blot.

carcinomas. One polypeptide of unknown identity (35 kDa, pI 5.5) was significantly overexpressed in primary lung adenocarcinomas compared with SCLC, squamous cell lung carcinomas, metastatic lung adenocarcinomas from colon and rectum, and normal tissue (8).

Colorectal cancer

Recently Stulik *et al.* have started a study directed towards the analysis of polypeptide changes associated with malignant transformation of colon mucosa (13). The levels of liver fatty acid-binding protein, actin-binding protein/smooth muscle protein 22-α and cyclooxygenase 2 were down-regulated in colorectal carcinoma compared to normal colon mucosa. Conversely, the expression of a novel variant of heat shock protein 70 and several members of the S100 protein family of calcium-binding proteins (two isoforms of S100A9, S100A8, S100A11 and S100A6) were up-regulated in transformed colon mucosa. The researchers detected a protein with a molecular mass of 13 kDa and a pI value of 5.6, whose expression was restricted to tumor tissue only. They found overexpression of this protein in cases of polyps with a moderate degree of dysplasia and also in polyps of low-degree dysplasia from patients suffering from either colorectal carcinoma or long-term ulcerative colitis. This peptide was successfully sequenced and further verified by MALDI-MS, and corresponded to calgranulin B (14).

Hepatocellular carcinoma

Studies using 2-D electrophoresis, performed by Zeindl-Eberhart *et al.*, detected several protein variants in *N*-methyl-*N*-nitrosourea-induced rat hepatomas (15). They identified one of these peptides by internal amino acid microsequencing as hepatoma-derived aldose reductase-like protein (35 kDa/pI 7.4). Immunohistochemistry using an FR-1 antibody direct against hepatoma-derived aldose reductase-like protein revealed that this protein is already strongly expressed in preneoplastic and in early neoplastic stages of chemically induced hepatocarcinogenesis, but not in normal surrounding liver tissue (16). In human hepatocarcinomas a protein was identified as a homolog to rat hepatoma-derived aldose reductase-like protein. This human aldose reductase-like protein appears to be selectively expressed in human tissue.

Prostate cancer

Prostate cancer is biologically heterogeneous with unpredictable aggressive behavior. Despite progress in cancer genetics, the molecular events underlying the development and progression of prostate cancer are incompletely understood. 2-D electrophoresis studies showed significant increases in the level of expression of PCNA, calreticulin, HSP90, HSP60, oncoprotein 18, elongation factor 2, glutathione-S-transferase-α, superoxide dismutase and triosephosphate isomerase (17). In addition, decreased levels of tropomyosin-1 and -2 and cytokeratin 18 were observed in prostate carcinomas compared to benign prostatic hyperplasias.

Ovarian cancer

Epithelial ovarian cancers are characterized by a broad spectrum of biological behavior ranging from tumors with excellent prognosis to tumors that progress rapidly and have a very poor prognosis. Work from the group of Alaiya has lead to proteomic definitions of benign, borderline, and malignant tumors (18, 19). The patterns of expression were similar to those observed in prostate cancer, suggesting a high degree of similarity in the protein expression patterns among different tumor types of epithelial origin. The combined score of nine polypeptides was found to discriminate between malignant and benign ovarian tumors, whereas borderline tumors were classified as intermediate or benign. The intratumoral variation in protein expression was low (low degree of heterogeneity). In contrast, large differences were observed when the protein profiles of different tumors were compared (20).

Ovarian carcinoma has an increased expression of retinoic acid-binding protein, carbohydrate-binding proteins, lipoproteins, and proteins protective against oxidative processes in the cell. In malignant ovary tissue, galectin-1 was up-regulated (10).

Esophageal cancer

Normal esophageal squamous epithelium and corresponding tumor cells were procured by laser-capture microdissection and studied by 2-D electrophoresis by Emmert-Buck *et al.* (21). Seventeen proteins showed

tumor-specific alterations, including 10 that were uniquely present in the tumors and seven that were observed only in the normal epithelium. Two of the altered proteins were characterized by mass spectrometry and immunoblot analysis and were identified as cytokeratin 1 (overexpressed) and annexin I (underexpressed).

A previous study on esophageal carcinoma was performed by Isoda *et al.* in 1990 (22). They studied and compared the patterns of proteins of surgically resected esophageal carcinomas and normal mucosa by 2-D PAGE with silver staining. Four spots were observed in all of the esophageal carcinomas that were not present in any of the normal mucosa. The molecular weights and isoelectric points were 46,000 and 5.3; 46,000 and 5.2; 36,000 and 4.7; and 33,000 and 5.1, respectively. One spot was observed in all of the normal mucosa but not in any of the esophageal carcinomas. Its molecular weight and isoelectric points were 27,000 and 5.3, respectively.

Recently, the protein expression patterns of normal, metaplastic and malignant esophageal tissues were analyzed by 2-D PAGE to identify changes associated with Barrett's metaplasia and transformation to esophageal adenocarcinoma (23). In normal esophagus, heat-shock protein 27 (Hsp27), a small heat-shock protein that is protective against cytotoxic stresses was abundant. However, Hsp27 expression was markedly lower in Barrett's metaplasia and esophageal adenocarcinomas. This finding was confirmed by immunohistochemical analysis. These results demonstrate abundant constitutive expression of the stress-response protein Hsp27 in the normal esophagus, and suggest that low-level expression in Barrett's metaplasia may be one factor, which may influence susceptibility to esophageal adenocarcinoma development.

Astrocytoma

In 1986 Narayan *et al.* used 2-D gel electrophoresis with silver staining to study protein patterns in various malignant human brain tumors obtained at surgery. These samples included 20 high-grade astrocytomas (anaplastic astrocytomas and glioblastomas), one low-grade astrocytoma, six juvenile astrocytomas, four ependymomas, and five medulloblastomas. Each type of tumor was found to have a characteristic protein profile that set it apart from the other tumors studied (24). Recently, Luider *et al.* applied the 2-D PAGE technique to glioma samples in an attempt to discriminate the glioma subtypes (25). It was found that the presence of glial fibrillary acidic protein fragments distinguishes oligodendroglioma from astrocytoma. A cluster of protein spots with a molecular mass of 36 kDa and two spots with slightly larger molecular mass were found in the six samples of astrocytoma, but not seen in the oligodendroglioma specimens. These results were confirmed by immunohistochemistry in tissue sections of the tumors.

Metastatic process

To study the complex process of metastasis and its possible negative regulation by specific gene products, the expression of specific proteins between the highly metastatic and nonmetastatic HEp-3 cells was investigated by 2-D PAGE (26). The increased cellular expression of four distinct proteins directly correlated with the loss of the metastatic phenotype. Two of the four proteins were associated with isolated cellular membranes (36 kDa, pI 5.7; 22kDa, pI 5.6), one protein fractionated with the cytoplasm (65 kDa, pI 6.2), and one protein was enriched in the nuclei fraction (32 kDa, pI 5.8). In breast cancer, the expression of tropomyosin-1 was found to be 1.7-fold higher in primary tumors with metastatic spread to axillary lymph nodes compared to primary tumors with no evidence of metastasis (6). These data indicate that 2-D electrophoresis can be used to identify specific proteins in subcellular compartments that are candidates for negative or positively regulators of the metastatic or invasion process.

Other fields in cancer research

The pattern of cellular protein expression and phosphorylation after an apoptotic stimulus can be studied using proteomic techniques. In cultured endothelial cells induced to undergo apoptosis using a combination of a cytokine and protein synthesis inhibitors such as cycloheximide, 2-D electrophoresis revealed specific proteolysis of distinct proteins, some at an early stage of apoptosis and some at a later stage (27). Apoptosis-associated processes in prostate epithelial cells include solubilization of the rigid intermediate filament network by specific proteolysis, as well as increased levels of endo-

plasmic reticulum proteins with chaperone functions (28). In this study apoptotic cells showed significant reduction in the levels of the intermediate filament proteins, keratins-18, -19, vimentin and the associated 14-3-3 adapter proteins. At the same time, molecular chaperones such as glucose-regulated protein 94, calreticulin, calnexin, and protein disulfide isomerase exhibit marked accumulation in these apoptotic cells. The mitochondrial (29), the Fas (30) and p53 (31) pathways of cellular apoptosis has also been studied recently using proteomic methods.

Although chemotherapeutic drugs are effective in the cure or palliation of some human cancers, both intrinsic and acquired drug resistances remain the major problem in the treatment of many types of cancer. In particular, human malignant melanoma is known for its high therapeutic resistance. Recently, Sinha *et al.* studied the phenotypical differences between melanoma cell lines and their chemoresistant counterparts using proteomic technology (32). They found four proteins overexpressed in all chemoresistant melanoma cell lines. The significance of these findings is now being verified using transfection experiments.

An interesting potential application of proteome data is to use 2-D electrophoresis data sets to create artificial learning models (17). The artificial learning approach has potential to improve tumor diagnosis and cancer treatment prediction. In this sense, Alaiya *et al.* established a learning model to artificially classify ovarian tumors using multivariate analysis of a set of 170 polypeptides from 2-D electrophoresis gels (19). This approach has also been applied in lung cancer (33).

CARDIOVASCULAR DISEASES

Cardiovascular diseases are characterized by a host of changes to the cellular processes that affect the contractility of the heart. The identification of modified or altered proteins in the disease state will provide new diagnostic markers of myocyte injury. In comparison to other organs the heart is a relatively homogeneous organ, and is therefore well suited for a proteomic investigation by 2-D electrophoresis (34). M.J. Dunn's group at Harefield Hospital, UK, has been developing one of the major clinical applications of proteomics in the study of heart disease (35). The preliminary biomedical relevant results of these investigations are proteins whose amounts are either increased or decreased. These proteins can now be investigated by other methods, such as immunohistochemistry.

Dilated cardiomyopathy

A large number of qualitative and quantitative changes in cardiac proteins have been described in biochemical or pathological studies, or proposed on the basis of mRNA studies in a combination of animal model and human disease samples. However, recently 2-D electrophoresis methods have been applied for the identification of protein alterations specific for dilated cardiomyopathy. The group of Corbett *et al.* found a total of 88 proteins that displayed decreased abundance in dilated cardiomyopathy *versus* ischemic heart disease, while five proteins had elevated levels in the dilated cardiomyopathy group (36). It has been suggested that increased proteolysis may at least in part be responsible for these findings. The most prominent changes occurred in the contractile protein myosin light chain 2 and in the group of proteins identified as desmin. Significant changes were also observed in a variety of metabolic enzymes, and several proteins implicated in the stress response.

In a recent animal model study of heart failure, 69 proteins were significantly altered, of which 42 were decreased and 27 were elevated. Of these proteins, 20 were identified. Ten of these were associated with mitochondria and energy production, and the cytoskeletal protein desmin was detected in reduced quantities (37). These results indicate that the development of heart failure involves alterations in mitochondrial energy production, the cytoskeleton and calcium activation.

Hypertensive heart disease

Hypertrophy of cardiac myocytes is a primary response of the heart to overload, and is an independent predictor of heart failure and death. Distinct cellular phenotypes are associated with hypertrophy resulting from different causes. Two groups have focused on the study of hypertensive heart disease using proteomic methods (38, 39). In the study of Arnott *et al.* (39), 2-D electrophoretic patterns were compared from cultured normal rat car-

diac myocytes and myocytes with phenylephrine-induced hypertrophy. Eleven proteins displayed quantitative changes in expression in cardiac hypertrophy, with three showing a decrease and eight an increase. Five of these proteins were found to be isoforms of the myosin light chain, at least one form of which is known to be associated with cardiac hypertrophy. The others represented proteins not previously known to be associated with the condition (39). In the study of Pleissner *et al.*, however, 20 protein patterns from the left ventricle of early hypertensive and normotensive rats were compared and only one myocardial spot (MW: 41.3; pI: 6.3) was decreased by more than twofold in hypertension (38).

NEUROLOGICAL DISEASES

Proteomic approaches appear particularly suitable for a molecular dissection of disease phenotypes in the central nervous system, which is largely inaccessible to meaningful mRNA expression-based analysis of primary human material, since post mortem delays in primary human brain tissue affects mRNAs more readily than proteins. Changes in the protein composition of cerebrospinal fluid may be indicative of altered central nervous system protein expression pattern with a causative or diagnostic disease link (40-42). Insights obtained from the study of cerebrospinal fluid proteins may be further extended through a subsequent proteomic analysis of specific brain areas implicated in the particular pathology (43, 44). In order to investigate, a more detailed analysis of individual cell types such as glial cells *versus* neurons, may be necessary. This may be achieved through laser capture microdissection.

Neurodegenerative disorders

Alzheimer's disease is the most common single cause of dementia. The characteristic neuropathological brain lesions are senile plaques and cytoplasmic neurofibrillary tangles. Comparative 2-D PAGE was used to show differences from normal individuals and brains of patients with Alzheimer's disease. Five protein spots were increased in the Alzheimer's group, 28 were decreased, and nine were uniquely expressed. The study did not determine the protein identities but provides a useful reference for future studies (45, 46).

In other recent study Edgar *et al.* collected *post mortem* hippocampal tissue from the brains of schizophrenic, Alzheimer's disease and control patients (47). In comparison with the control hippocampal proteome, eight proteins in the schizophrenic were found to be decreased and eight increased in concentration, whereas, in the Alzheimer's disease, 35 proteins were decreased and 73 were increased in concentration. One protein, which was decreased in concentration in both diseases, was characterized as diazepam binding inhibitor.

Other neurological diseases

Prion diseases have been studied by proteomic approaches. Neuropathological diagnosis requires a brain biopsy or necropsy sample, although many sporadic cases have a typical clinical picture. However, with the emergence of a new variant of the disease associated with the bovine spongiform encephalopathy epidemic, the search for diagnostic and screening tests that can be used before death has intensified. In Germany, cerebrospinal fluid samples from 58 definite (neuropathologically verified), 46 probable, and 34 possible Creutzfeldt-Jakob disease (CJD) cases, and from 44 patients without CJD were analyzed by 2-D electrophoresis (48). The protein p130/131 was detected in 81% of definite, 80% of probable, 68% of possible CJD cases, and in none of the other 44 cases. 2-D electrophoresis for p130/131 was a specific test for the diagnosis of CJD. Detection of p130/131 could be used as a criterion for the diagnosis of probable CJD in addition to the currently accepted clinical and ECG criteria. The value of these proteins as markers for CJD in patients with dementia has since been confirmed in several studies (49, 50).

A proteomic approach has also been used to investigate demyelination in a mouse model (51). The expression of proteins was examined in the optic nerves of transgenic mice with a c-myc-induced degenerative myelin disorder. However, since many myelin-specific proteins are highly basic, they cannot readily be analyzed by standard isoelectric focusing 2-D electrophoresis that affords separation primarily in the pI range of 4-8. An alternative method, nonequilibrium pH gradient electrophoresis (NEPHGE)-2-D electrophoresis has been optimized for the analysis of myelin proteins with basic pIs by Yama-

guchi and Pfeiffer (52). Proteomic technology has also been applied to the study of muscle diseases, such as muscle denervation (53) and dystrophic skeletal muscle (54).

OTHER DISEASES

Brief overviews of other biomedical areas are given below to illustrate the potential of the proteomic approach. Although not detailed here, proteomics offer great potential in unraveling complex biological problems such as the nature of particular molecular complexes or pathways in disease pathogenesis. In this way, the pathogenesis of the aging process (54), autoimmune diseases such as rheumatoid arthritis or Sjogren's syndrome (55, 56), interstitial lung diseases (57), or cystic fibrosis (58) are being studied with proteomic techniques.

TOXICOLOGY: DRUG DEVELOPMENT

Recent progress in genomics and proteomics technologies has created the unique opportunity to make a significant impact on the pharmaceutical drug development processes. The perception that cells and whole organisms express specific inducible responses to stimuli such as drug treatment implies that unique expression patterns, molecular fingerprints, indicative of a drug's efficacy and potential toxicity, are accessible.

Toxicology is likely to prove one of the most important applications of proteomics. 2-D electrophoresis is a highly sensitive means of screening for toxicity and probing toxic mechanisms. Most drugs exert their effects on proteins. By comparing proteins expressed following treatment with a given drug with those present under untreated conditions, it is possible to identify changes in biochemical pathways via observed alterations in sets of proteins that may be related to the drug's efficacy or toxicity. Based on the assumption that changes in gene and protein expression precede manifestations at the morphological level, these proteins can be used as efficacy or toxicity markers in high throughput screening assays to test large sets of lead compounds.

In one ongoing study, a group in the United Kingdom recently reported an ongoing 2-D electrophoresis study of glomerular nephrotoxicity in the rat following exposure to puramycin aminonucleoside (59). By monitoring the proteins in urine, the study has permitted a more detailed understanding of the nature and progression of the proteinuria associated with glomerular nephrotoxicity than has previously been possible.

Proteomics played a key role in the discovery of novel molecular mechanisms involved in cyclosporin A nephrotoxicity (60, 61). An analysis of cyclosporin A toxicity revealed a novel toxic mechanism involving the calcium-binding protein calbindin-D. In cyclosporin A-treated human kidney-transplant recipients with renal vascular or tubular toxicity, a marked decrease in renal calbindin-D 28 kDa protein level was found in most of the kidney biopsy sections. Calbindin is a marker for cyclosporin A nephrotoxicity. The discovery of calbindin-D 28 kDa being involved in cyclosporin A toxicity has evolved from the application of 2-D electrophoresis, proving that proteomics can provide essential information in mechanistic toxicology.

INFECTIOUS DISEASES

The techniques of proteomics have been used for many years to investigate protein synthesis and gene expression in bacteria. Proteomics provides qualitative data on the proteins encoded by the bacterial genomes together with quantitative data on the response of protein synthesis under defined environmental conditions (62). The main aim of most studies has been the search for new diagnostic markers, candidate antigens for vaccines, and determinants of virulence.

The applications of proteomics to medical microbiology are diverse (63, 64). Early studies used 2-D electrophoresis to analyze cellular protein synthesis on a global scale in a nonselective manner and in many cases the identities and functions of the proteins were unknown. This is illustrated by the use of 2-D electrophoresis as a tool to investigate the molecular epidemiology and taxonomy of microorganisms. For the investigation of pathogenic determinants and antibiotic resistance mechanisms, the identification of the proteins involved and studied by 2-D electrophoresis is clearly a crucial requirement.

Mycobacterium tuberculosis is probably the most studied microorganism by 2-D electrophoresis. Several research groups have characterized the mycobacterial

proteomes, particularly the secreted proteins (65, 66). Several of the antigens identified by these studies have now been incorporated into trial vaccines. Recently, the serological proteome analysis in conjunction with tandem mass spectrometry has identified and sequenced a novel protein, Mtb81, which may be useful for the diagnosis of tuberculosis (67).

The immune response to infection with *Helicobacter pylori* has been studied and 20 proteins have been identified which were reactive with the serum of infected patients. All the proteins were identified as *H. pylori* proteins (68). A follow-up study investigated the possibility of using one of these proteins as an antigen for generating an *H. pylori* vaccine, due to its consistently high reactivity.

Microbial drug resistance is a significant concern and the utility of protein profiling via proteomics has much to contribute to the understanding of the various microbial physiological states under various normal conditions and following the development of drug resistance. For example, protein expression profiling of erythromycin-resistant *Streptococcus pneumoniae* has demonstrated a unique phenotype in these resistant bacteria in which glyceraldehyde-3-phosphate dehydrogenase is more abundantly expressed and post-translationally modified than in erythromycin-susceptible forms (69).

CONCLUSION

The technologies of 2-D PAGE and mass spectometry are the driving force in contemporary proteomics and are likely to remain so in the immediate future, although new approaches including antibody-based techniques and protein chips may challenge their preeminence.

The development of proteomic techniques is a new method of studying pathological processes at the molecular level. These techniques are already leading to improvements in the understanding of many processes. It will be possible in the near future to combine genomic and proteomic information to obtain a more comprehensive picture of many pathological conditions.

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