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Proteomics in translational cancer research: Toward an integrated approach

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Proteomics provides powerful tools for the study of clinically relevant samples in the context of translational cancer research. Here we briefly review applications of gel-based proteomics for the study of bladder and lung cancer using fresh tissue biopsies. In general, these studies have emphasized the potential of the technology for biomarker discovery, as well as for addressing the issue of cancer heterogeneity.

Introduction

The sequencing of the human and other important genomes is only the beginning of the quest to understand the functionality of cells, tissues, and organs, both in health and disease. Together with advances in bioinformatics, this development has paved the way to the revolution in biology and medicine that we are experiencing today. We are rapidly moving from the study of single molecules to the analysis of complex biological systems, and the current explosion of emerging technologies within proteomics and functional genomics promises to elicit major advances in medicine in the near future.

Cancer, being a complex disease that affects a significant fraction of the population, is foreseen as a prime target for the new technologies, as tools for the high throughput analysis of genes and proteins might expedite the applications of basic research findings into daily clinical practice through translational research. In particular, proteomic technologies are expected to play a key role in the study and treatment of cancer, as they provide invaluable resources to define and characterize regulatory and functional networks, to investigate the precise molecular defect in diseased tissues and biological fluids, and for developing specific reagents to precisely pinpoint a particular disease or stage of a disease. For drug discovery, proteomics assist with powerful tools for identifying new clinically relevant drug targets, and provide functional insight for drug development.

Proteomics encompasses many platform technologies for protein separation and identification, for determining their biomolecular interactions, function, and regulation, and for annotating, storing, and distributing protein information (Figure 1) (Celis et al., 1998; Aebersold and Goodlett, 2001; Chakravarti et al., 2002; Figeys, 2002; Gavin et al., 2002; Liu et al., 2002; Mylvaganam et al., 2002; Panisko et al., 2002; Yanagida, 2002, and references therein). The proteome is much more complex and dynamic than the genome, and the task of deciphering it even in a single cell type is daunting, as there may be many thousands of proteins, including splice variants, posttranslational modifications, and cleavage products, present in a cell at any given time. Moreover, the dynamic range of protein expression expands over several orders of magnitude, a fact that limits their characterization and analysis.

Today, the application of novel technologies from proteomics to the study of cancer is slowly shifting to the analysis of clinically relevant samples such as biopsy specimens (Celis et al., 1996a, 1999a, 1999b, 2002; Alaiya et al., 2000, 2002; Lawrie et al., 2001; Wulfkuhle et al., 2001; Ahram et al., 2002; Chen et al., 2002a, 2002b; Gharib et al., 2002; Jones et al., 2002; Meehan et al., 2002) and fluids (Petricoin et al., 2002a, 2002b), as the ultimate aim of translational research is to bring basic discoveries closer to the bedside (Celis, 2002; Petricoin et al., 2002c). The implementation of discovery-driven translational research will not only require coordination of basic research activities, facilities, and infrastructures, but also the creation of an integrated and multidisciplinary environment with the participation of a dedicated team of clinicians, oncologists, pathologists, and epidemiologists, as well as industrial partners. Issues related to sample collection, handling, and storage, number of patients, availability of normal controls, tissue banks, quality of the clinical information, follow-up studies, and ethical considerations are critical, and must be carefully considered. As we redefine the manner in which we approach cancer, changes also need to be made in the way cancer research is funded.

Translational cancer research has only recently gathered momentum among the proteomic research community, and as a result there are just a few long-term programs that have been initiated using tissue biopsies and biological fluids. A recent example of what has been achieved so far using fluids is illustrated by the work of Petricoin, Liotta, and coworkers, who have used a combination of mass spectra generated by surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) and artificial intelligence-based informatic algorithms to search for protein patterns or features in serum that detect ovarian and prostate cancer (Petricoin et al., 2002a, 2002b). These studies, which have been recently reviewed by Daly and Ozols (2002) in this journal, require further validation using a larger sample number, but have clearly illustrated the clinical potential of the chip technology in the diagnosis of some cancers.

Analysis of tissue biopsies is by far more complicated than the analysis of fluids, due to the heterogeneous nature of the samples. Today, two-dimensional polyacrylamide gel electrophoresis (2D PAGE) (O'Farrell, 1975), often referred as gel-based proteomics, multidimensional chromatography, and protein biochips, in combination with mass spectrometry (McDonald and Yates, 2002; Yip and Lomas, 2002; Wu and McCoss, 2002, and references therein), are among the proteomic tools that are available for biomarker and drug target discovery. Considerable work is currently underway to explore applications of non-gel-based proteomics in various areas of biology, as this technology has much to offer. What follows below is a short description of the gel-based technology as applied to whole fresh tissue specimens in the context of trans-

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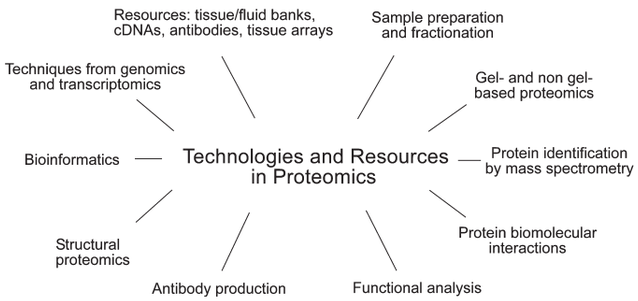

Figure 1. Technologies and resources in proteomics

Proteomics encompasses many platform technologies for protein separation and identification, for determining their biomolecular interactions, structure, function, and regulation, and for annotating, storing, and distributing protein information. In the context of translational cancer research, proteomics requires well-established tissue banks as well as tools from genomics and transcriptomics.

lational research. We also provide selected examples of what has been accomplished in bladder and lung cancer, two of the most comprehensively studied malignancies using 2D gels in combination with other technologies.

Gel-based proteomics: A platform technology for the analysis of tissue biopsies

2D PAGE separates proteins based on their molecular masses and isoelectric points (pIs), and is the method of choice to analyze complex tissue samples that are often composed of different cell types (Celis et al., 1996a). For many years, the 2D PAGE technology relied on the use of carrier ampholytes to establish the pH gradient, but this technique suffers from lack of reproducibility due to variations in the batches of ampholytes used to generate the pH gradients. Lately, however, with the introduction of immobilized pH gradients (IPGs) (Görg et al., 2000, and references therein), which are an integral part of the polyacrylamide matrix, it has been possible to obtain more reproducible focusing patterns. IPGs avoid some of the problems associated with carrier ampholytes, such as cationic drift, allow a higher loading capacity for micropreparative runs, and provide increased resolution when narrow pH gradients are used (approximately 0.05 pH/cm; zoom gels). Critical issues in gel-based proteomics relate mainly to sample preparation and standardization of the gel running conditions (Celis and Gromov, 1999).

For 2D PAGE-based protein profiling, one usually chooses a condition of interest and lets the cells or tissue reveal the global protein response, as all detected proteins can be analyzed quantitatively with respect to each other. Figure 2 shows 2D gel isoelectrofocusing (IEF) autoradiograms of proteins from fresh biopsies obtained from normal urothelium (transitional epithelium) and an invasive transitional cell carcinoma (TCC, Gr. III, T2-4) metabolically labeled with [^{35}S]-methionine. Interpretation of the expression patterns of tissues demands prior knowledge of specific protein markers for the individual cell types contributing to the overall picture, a fact that requires considerable investment in time at the onset of the project (Celis et al., 1996a). Alternatively, selected groups of cells can be recovered from hematoxylin or immunostained tissue sections using microdissection procedures (Emmert-Buck et al., 1996). The number of cells required for gel analysis, however, is in the order of 50,000 cells.

Protein profiles can be scanned and quantitated to search for changes in the levels of preexisting proteins, induction of new products, and groups of coregulated polypeptides, and interesting targets or molecular signatures can be identified using mass spectrometry (Aebersold and Goodlett, 2001, and references therein) and immunoblotting (Celis et al., 1995), provided that specific antibodies are available. The latter will reveal posttranslational modified variants as well as spliced variants that may be difficult to identify using a combination of 2D PAGE and mass spectrometry. Antibodies are invaluable to validate protein expression data (levels as well as cell type involved) using immunohistochemistry on frozen or paraffin sections (Figure 2).

Protein expression data gathered by 2D PAGE in combination with other technologies can be stored in comprehensive databases that document protein expression in normal and tumor tissues, as exemplified by the TCC (http://proteomics.cancer.dk) (Figure 3) and lung proteomic databases (Oh et al., 2001). The TCC database is available online and is updated regularly. By clicking on a spot, one can readily retrieve information on any protein, known or unknown. Files for known proteins have links to other databases available online. As these databases achieve a critical mass of data, they will become valuable resources of information for expediting the identification of signaling pathways and components that are affected in various types of cancers. In addition, they are expected to facilitate the identification and validation of targets with a proven role in the disease.

As we stand today, one could expect to visualize a maximum of about 4,000 to 5,000 cellular and secreted proteins with the gel-based technology using [^{35}S]-methionine labeled whole tissue samples. This fraction is still short of the total number of polypeptides that may be present in a given cell type, but still sufficient to start biomarker discovery. Missing polypeptides are either not resolved by the pH gradient (too basic or too acidic), or do not enter the gel due to solubilization problems (membrane proteins) or molecular weight size, or simply because they are not detected due to limitations in the sensitivity of the current procedures, which are based mainly on isotope incorporation (autoradiography, fluorography) and staining with silver nitrate or fluorescent dyes (Patton, 2002, and references therein). Some of these limitations can be partly overcome by applying extensive prefractionation procedures to enrich for the desired protein, protein fraction, or subcellular component.

Applications of gel-based proteomics to the study of cancer

Even though there are only a few large cancer proteomic projects that are well underway at the moment, such as the bladder and lung programs (see below), there is evidence in the literature indicating that the gel-based technology can be applied to the analysis of a variety of tumor types that include breast (Franzen et al., 1996; Wulfkuhle et al., 2001), colon (Stulik et al., 2001; Lawrie et al., 2001; J.E.C., unpublished data), prostate (Ahram et al., 2002; Meehan et al., 2002; Alaiya et al., 2000), ovary (Jones et al., 2002; Alaiya et al., 2002), and kidney (J.E.C. and H. von der Maasse, unpublished observations). Moreover, there have been several reports on leukemia and hematological malignancies (Melhem et al., 1997). Applications to bladder and lung cancer, two of the most comprehensively studied malignancies using the gel-based technology in the context of translational research, are briefly reviewed below.

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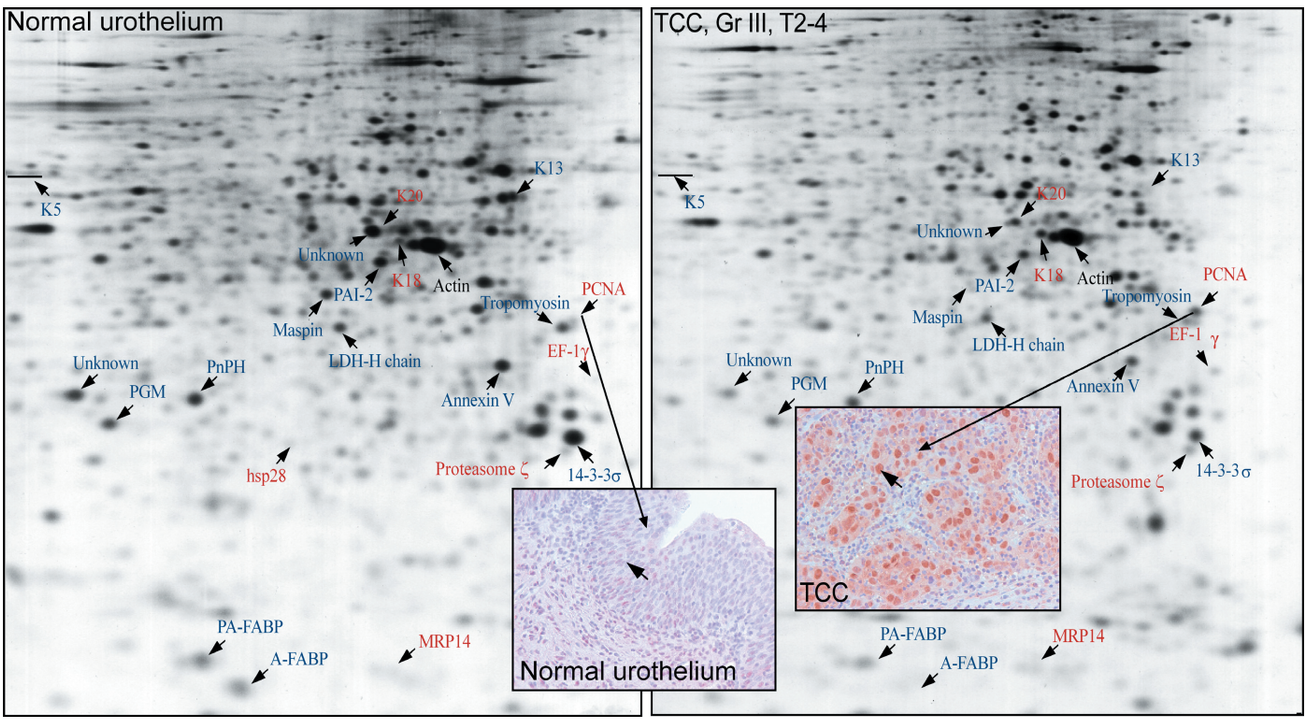

Figure 2. Highly deregulated proteins in invasive TCCs

[^{35}S]-methionine-labeled proteins synthesized by normal urothelium and an invasive TCC (Grade III, T2-4) were separated by 2D PAGE (IEF) and visualized by autoradiography. Proteins indicated with red are upregulated in the tumor, while those indicated in green are downregulated. The position of actin is indicated for reference.

Bladder cancer

Bladder cancer comprises a broad spectrum of tumors with various histological types that include TCCs, adenocarcinomas, and squamous cell carcinomas (SCCs) (Friedell et al., 1983). TCCs are by far the more prevalent tumors and represent nearly 95% of all bladder cancers in the Western Hemisphere. SCCs, on the other hand, encompass a small percentage (2%–3%) of all bladder lesions diagnosed in Europe and America, but are very frequent (80%) in areas of Africa and the Middle East, where *Schistosoma haematobium*, a parasite that induces bladder SCCs in humans, is prevalent (El-Bolkainy, 1983).

For several years, our group has collaborated with clinicians to explore the possibility of using proteome expression profiles of bladder tumors as fingerprints to objectively subclassify histopathological types, and as a staring point for searching for protein markers that may form the basis for diagnosis, prognosis, and treatment. These studies have been complemented with cDNA array analysis (Celis et al., 2000), and by genome-wide studies of gene copy numbers, transcripts, and protein levels in pairs of noninvasive and invasive TCCs (Ørntoft et al., 2002).

More than 1000 fresh specimens that include tumors, normal biopsies, and cystectomies have been systematically analyzed so far using 2D PAGE, and we have established protein expression databases of TCCs and SCCs, as well as other cell types that are available through the Internet (http://proteomics.cancer.dk) (Figure 3). So far, these studies have revealed several protein markers for TCC progression (Celis et al., 1996a) and a marker, psoriasin, that is produced specifically by SCCs and that is externalized to the urine (Celis et al., 1996b). In addition, this work has led to the development of novel strategies for the identification of tumor heterogeneity among low-grade papillary TCCs (Celis et al., 2002) as well as early metaplastic lesions in SCC-bearing patients (Celis et al., 1999a; see below).

Proteomic strategies to identify tumor heterogeneity among low-grade papillary TCCs

Papillary TCCs are typically superficial at first presentation and are often multifocal. These tumors exhibit a high frequency of recurrence (>60%), and 10%–15% of them will progress to life-threatening malignancies over a long period of time (Cheng et al., 2000). Presently, it is not possible to assess with certainty the biological behavior of these tumors based on clinical or morphological criteria alone, and as a result it is important to identify tumor subtypes that will develop recurrences and/or progress to invasive disease. Moreover, it is vital to distinguish those lesions that have no significant effect on the life expectancy of the patient, as all tumor-bearing patients are diagnosed with cancer, a fact that has practical and economic implications as well as a profound psychological effect on the patient.

The strategy for identifying cancer heterogeneity among low-grade papillary TCCs encompasses a blind and systematic study of the proteome expression profiles of fresh biopsy specimens from both normal and tumor origin (Celis et al., 2002). Large numbers of samples are required to define a baseline of normal and abnormal protein expression (Celis et al., 1996a, 2002). First, one identifies major proteins that are differentially

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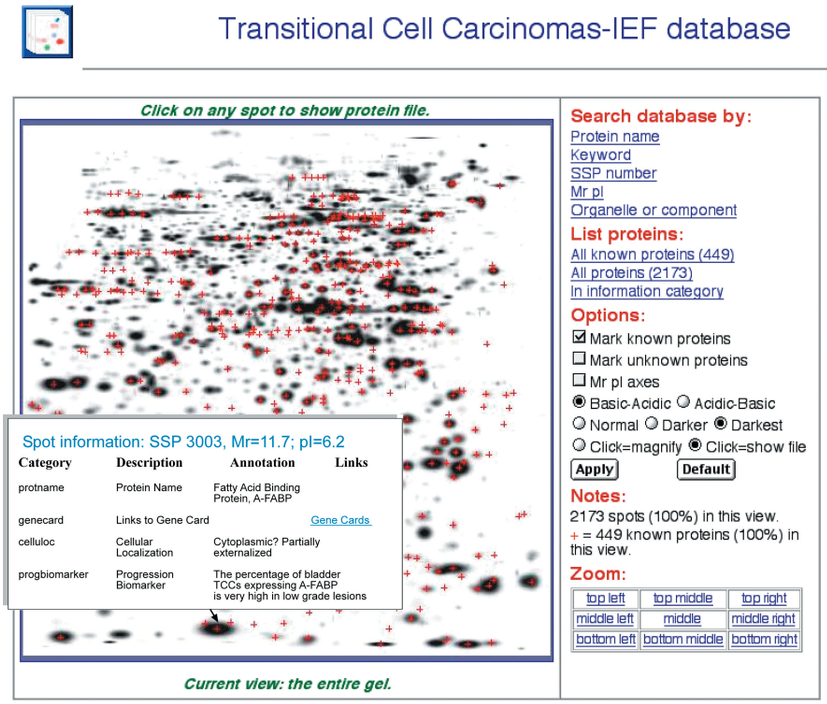

Figure 3. Master synthetic image of human bladder TCC proteins separated by IEF 2D PAGE as depicted online (http://proteomics.cancer.dk)

Proteins labeled with a cross correspond to known proteins. By clicking on any spot, it is possible to open a file that contains protein information available in the database as well as links to other related websites. Only part of the file for A-FABP is shown.

expressed in invasive lesions (Gr III, T2-4) as compared to normal urothelium (Figure 2, only a few highly deregulated proteins are indicated), and thereafter uses specific antibodies against these proteins to immunostain cryostat sections of low-grade papillary tumors diagnosed as having the same grade of atypia and stage (Gr I, Ta) by the pathologist. The rate-limiting step in the procedure lies in the preparation of specific antibodies that will work both in immunoblotting as well as in immunofluorescence. So far, these studies have identified several types of cancer heterogeneity (Celis et al., 2002) that affect either the basal proliferative compartment (Figures 4Aa and 4Ab), the umbrella cells (Figure 4Ac), or the suprabasal layers (not shown). Control staining of normal urothelium with these antibodies is shown in Figures 4Ad–4Af. Analysis of the clinical data gathered through a period of five years indicated that lesions displaying phenotypic alterations in the basal, proliferative compartment (Figures 4Aa and 4Ab) showed the highest number of recurrences. Even though a long-term prospective study involving a larger sample size with regular follow-up is required to assess the biological potential of these lesions, the data clearly illustrate the potential of combining proteomics with immunohistochemistry to reveal cancer heterogeneity and to correlate it with outcome.

Identification of early metaplastic lesions in SCC-bearing patients

An important task in cancer control and prevention is the detection of the disease at an early stage to allow rapid intervention and treatment. SCCs of the bladder are highly malignant and have a bad prognosis, and the success of treatment relies heavily on early detection. Thus, the identification of early precancerous lesions as well as markers in the urine may prove to be instrumental in the treatment of the disease.

To search for precancerous lesions, we have pursued a systematic analysis of the proteome expression profiles of fresh tumors as well as urothelial tissue from patients that have undergone removal of the bladder due to invasive disease (cystectomy) (Celis et al., 1999a). Since bladder cancer is a field disease, that is, a large part of the urothelium is at risk of developing the disease, we surmised that cystectomy specimens might exhibit a spectrum of abnormalities ranging from early metaplastic stages to invasive disease. First, we identify proteins that are differentially expressed by pure SCCs and normal urothelium, and thereafter we use antibodies against these putative markers to stain serial cryostat sections (immunowalking) obtained from bladder cystectomies of SCC-bearing patients. Indeed, these studies have revealed several types of metaplastic lesions that can be differentiated based on the staining with antibodies against keratin 19 (expressed by normal urothelium, Figure 4Bg), 14 (expressed by pure SCCs, Figure 4Be), and other markers identified by the gel analysis (Celis et al., 1999a). Figures 4Ba–4Bc depict a series of 3 cryostat sections from a region close to the tumor showing transition from "normal" urothelium to a keratin 19 negative metaplastic type lesion that is located at the right side of the section (Figure 4Ba). This particular lesion closely resembles the tumor phenotype, as it stains with keratin 14 antibodies (Figure 4Bb; compare with Figure 4Be), and the basal cells do not express keratin 13 (Figure 4Bc), a protein not expressed by the tumor (Figure 4Bf). It is possible that this particular tumor arose from the expansion of the basal cell compartment of this type of lesion. As additional antibodies become available against specific markers, we will be able to accurately define the phenotype of precancerous lesions. Specific membrane markers, or secreted proteins derived from these lesions (Celis et al., 1996b), may provide with potential biomarkers for early diagnosis using urine samples.

Lung cancer

Lung cancer is the most common cause of cancer death in women and men and is a major public health concern in the Western world. Hanash and colleagues have analyzed over 1000 lung cancer-related samples using 2D PAGE in combination with mass spectrometry, and have constructed the lung proteomic database (Oh et al., 2001) that integrates protein and gene expression data at the RNA level. The aim of their studies is to identify biomarkers for the early detection of cancer, for developing novel classification of tumors, and for revealing novel targets for therapeutic intervention (Hanash et al., 2001; Chen et al., 2002a; Gharib et al., 2002).

The parallel transcriptomic and proteomic analysis of lung tumors has permitted this group to compare mRNA and protein

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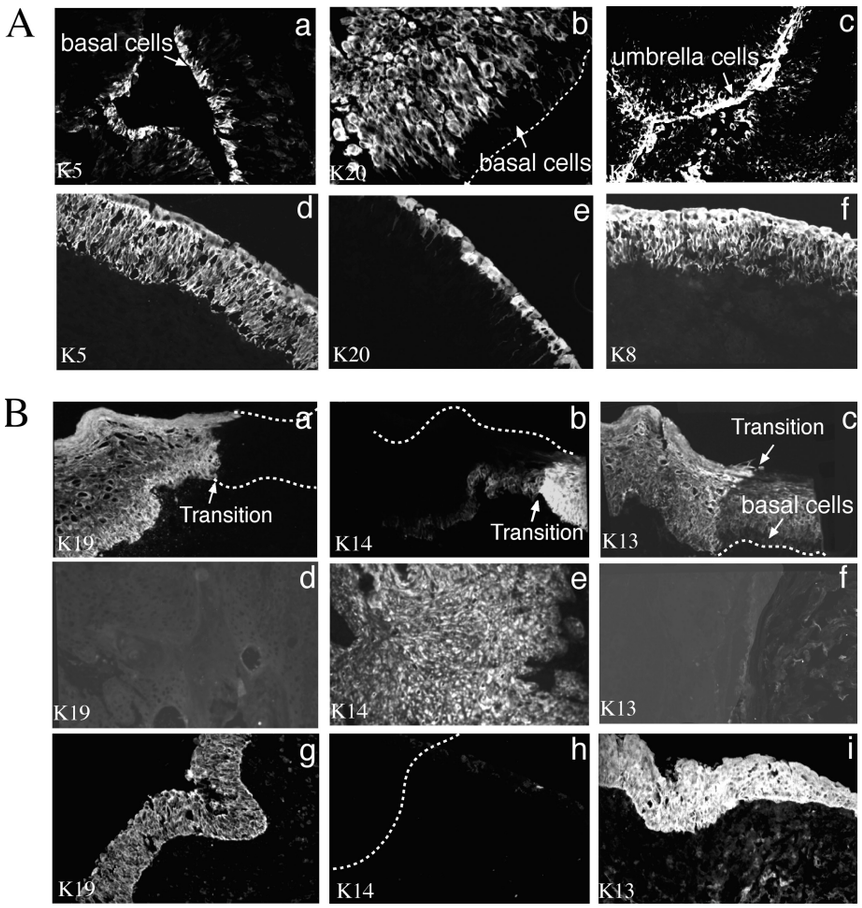

Figure 4. Tumor and tissue heterogeneity in bladder cancer

A: Cancer heterogeneity among low-grade papillary TCC (Grl, Ta).
a–c show cancer heterogeneity as revealed by staining cryostat tumor sections with antibodies against keratins 5, 20, and 8, respectively. **d–f** show control staining of normal urothelium with these antibodies. From Celis et al. (2002); reproduced by permission from *Molecular Cell Proteomics*.
B: Identification of metaplastic lesions in SCC cystectomy 884-1. Serial cryostat sections were reacted with antibodies against proteins differentially expressed by normal urothelium (**g–i**) and the corresponding SCC (**d–f**). **a–c** show 3 serial sections reacted with antibodies against keratins 19, 14, and 13, respectively (immunowalking). White arrows indicate reference points for comparison. From Celis et al. (1999a); reproduced by permission from *Cancer Research*.

levels in the same tumors (Chen et al., 2002b). The integrated intensities of 165 protein spots representing protein products of 98 genes were analyzed in 76 lung adenocarcinomas and 9 unaffected lung tissues using 2D PAGE. For the same 85 samples, mRNA levels were determined using oligonucleotide microarrays. Only 21 out of the 98 genes analyzed (21.4%) showed a statistically significant correlation between protein and mRNA levels ($r > 0.2445$; $p < 0.05$). The mRNA/protein correlation coefficients also varied between isoforms of the same protein, suggesting that isoform-specific mechanisms may be involved in regulating protein abundance. Recently, the group identified a battery of genes (Beer et al., 2002) and proteins, which included specific cytokeratin isoforms that are predictive of survival in lung cancer (Gharib et al., 2002).

Hanash and colleagues have also searched for circulating tumor antigens by screening for autoantibodies in lung cancer patients using Western blotting (Brichory et al., 2001a). Briefly, 2D gel blots of the lung adenocarcinoma cell line A549 have been incubated with sera from patients with lung adenocarcinomas, other cancers, and noncancer controls. These studies have revealed autoantibodies against glycosylated annexins I and II in 60% of the patients with lung adenocarcinomas and 33% of patients with SCCs. Similarly, studies involving sera from 64 newly diagnosed patients with lung cancer, 99 patients with other types of cancer, and 71 noncancer controls revealed a tumor antigen, protein gene product 9.5 (PGP 9.5, a neurospecific protein), that induces a humoral response in lung cancer (Brichory et al., 2001b).

In an effort to speed up the analysis of serum for autoantibodies to lungs bearing antigens, Madoz-Gurpide et al. (2001) have developed a novel approach that combines liquid phase protein separations with microarray technologies. Whole cell or tissue lysates are fractionated by isoelectric focusing and reverse phase chromatography into hundreds of fractions, which are then arrayed onto nitrocellulose-coated slides and incubated with the sera. Such biochips require low sample volume and provide a rapid procedure to molecularly profile the antibody response to tumor antigens in cancer.

Perspectives

Today it is becoming increasingly clear that translational cancer research must make use of the entire armamentarium of technology and resources available within proteomics, genomics, and functional genomics (Figure 1). No single laboratory or research institution has the critical mass or the resources to tackle such a complex biological problem, and as a result, it is essential to join forces in order to make an impact on the disease. With this goal in mind, the Danish Cancer Society recently supported the establishment of the Danish Centre for Translational Research in Breast Cancer (DCTB), a network that brings together basic scientists working in various areas of cancer research (cell cycle, metastasis, apoptosis, signaling pathways), with clinicians, oncologists, surgeons, pathologists, and epidemiologists, in an integrated environment for the study of one of the most common malignancies and causes of cancer death in women worldwide. Briefly, the strategy is based on the analysis of fresh tissue biopsies (normal and cancer) obtained from the same patient using an array of state-of-the-art technologies from genomics, transcriptomics, proteomics, and functional genomics. Small tissue samples dissected from the same tumor or normal tissue are distributed to members of the Centre who apply various experimental paradigms and share their data

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via databases that will be linked with clinical information to generate a prognostic index and molecular profile for each patient. The aim of the program is to elucidate the biochemical pathways affected, to develop noninvasive diagnostics able to detect cancer in the very early stages, to develop and/or improve methodologies to determine the various stages of breast cancer and to predict outcome, and to apply molecular classification to identify the best responders to therapy. Biomarkers of interest might be tested using the Danish Breast Cancer Group (DCBG) tumor repository bank, which contains tissue samples from about 10,000 breast cancer patients with a full clinical follow-up. These studies will be complemented by the analysis of serum samples as well as nipple fluid aspirate. Altogether, these capabilities are expected to accelerate drug development and improve clinical trial methodologies. The immediate outcome of these investigations will manifest as enhanced patient survival and improved quality of life.

Acknowledgments

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References

Aebersold, R., and Goodlett, D.R. (2001). Mass spectrometry in proteomics. Chem. Rev. *101*, 269–295.

Ahram, M., Best, C.J., Flaig, M.J., Gillespie, J.W., Leiva, I.M., Chuaqui, R.F., Zhou, G., Shu, H., Duray, P.H., Linehan, W.M., et al. (2002). Proteomic analysis of human prostate cancer. Mol. Carcinog. *33*, 9–15.

Alaiya, A., Roblick, U., Egevad, L., Carlsson, A., Franzen, B., Volz, D., Huwendiek, S., Linder, S., and Auer, G. (2000). Polypeptide expression in prostate hyperplasia and prostate adenocarcinoma. Anal. Cell. Pathol. *21*, 1–9.

Alaiya, A.A., Franzen, B., Hagman, A., Dysvik, B., Roblick, U.J., Becker, S., Moberger, B., Auer, G., and Linder, S. (2002). Molecular classification of borderline ovarian tumors using hierarchical cluster analysis of protein expression profiles. Int. J. Cancer *98*, 895–899.

Beer, D.G., Kardia, S.L., Huang, C.C., Giordano, T.J., Levin, A.M., Misek, D.E., Lin, L., Chen, G., Gharib, T.G., Thomas, D.G., et al. (2002). Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nat. Med. *8*, 816–824.

Brichory, F.M., Misek, D.E., Yim, A.M., Krause, M.C., Giordano, T.J., Beer, D.G., and Hanash, S.M. (2001a). An immune response manifested by the common occurrence of annexins I and II autoantibodies and high circulating levels of IL-6 in lung cancer. Proc. Natl. Acad. Sci. USA *98*, 9824–9829.

Brichory, F., Beer, D., Le Naour, F., Giordano, T., and Hanash, S. (2001b). Proteomics-based identification of protein gene product 9.5 as a tumor antigen that induces a humoral immune response in lung cancer. Cancer Res. *61*, 7908–7912.

Celis, J.E. (2002). A new start in Madrid: Symposium on basic and translational cancer research. EMBO Rep. *3*, 718–723.

Celis, J.E., and Gromov, P.S. (1999). 2D protein electrophoresis: can it be perfected? Curr. Opin. Biotechnol. *10*, 16–21.

Celis, J.E., Rasmussen, H.H., Gromov, P., Olsen, E., Madsen, P., Leffers, H., Honore, B., Dejgaard, K., Vorum, H., Kristensen, D.B., et al. (1995). The human keratinocyte two-dimensional gel protein database (update 1995): mapping components of signal transduction pathways. Electrophoresis *16*, 2177–2240.

Celis, J.E., Ostergaard, M., Basse, B., Celis, A., Lauridsen, J.B., Ratz, G.P., Andersen, I., Hein, B., Wolf, H., Orntoft, T.F., and Rasmussen, H.H. (1996a). Loss of adipocyte-type fatty acid binding protein and other protein biomarkers is associated with progression of human bladder transitional cell carcinomas. Cancer Res. *56*, 4782–4790.

Celis, J.E., Rasmussen, H.H., Vorum, H., Madsen, P., Honore, B., Wolf, H., and Orntoft, T.F. (1996b). Bladder squamous cell carcinomas express psoriasin and externalize it to the urine. J. Urol. *155*, 2105–2112.

Celis, J.E., Ostergaard, M., Jensen, N.A., Gromova, I., Rasmussen, H.H., and Gromov, P. (1998). Human and mouse proteomic databases: novel resources in the protein universe. FEBS Lett. *430*, 64–72.

Celis, J.E., Celis, P., Ostergaard, M., Basse, B., Lauridsen, J.B., Ratz, G., Rasmussen, H.H., Orntoft, T.F., Hein, B., Wolf, H., and Celis, A. (1999a). Proteomics and immunohistochemistry define some of the steps involved in the squamous differentiation of the bladder transitional epithelium: a novel strategy for identifying metaplastic lesions. Cancer Res. *59*, 3003–3009.

Celis, A., Rasmussen, H.H., Celis, P., Basse, B., Lauridsen, J.B., Ratz, G., Hein, B., Ostergaard, M., Wolf, H., Orntoft, T., and Celis, J.E. (1999b). Short-term culturing of low-grade superficial bladder transitional cell carcinomas leads to changes in the expression levels of several proteins involved in key cellular activities. Electrophoresis *20*, 355–361.

Celis, J.E., Kruhoffer, M., Gromova, I., Frederiksen, C., Ostergaard, M., Thykjaer, T., Gromov, P., Yu, J., Palsdottir, H., Magnusson, N., and Orntoft, T.F. (2000). Gene expression profiling: monitoring transcription and translation products using DNA microarrays and proteomics. FEBS Lett. *480*, 2–16.

Celis, J.E., Celis, P., Palsdottir, H., Ostergaard, M., Gromov, P., Primdahl, H., Orntoft, T.F., Wolf, H., Celis, A., and Gromova, I. (2002). Proteomic strategies to reveal tumor heterogeneity among urothelial papillomas. Mol. Cell Proteomics *1*, 269–279.

Chakravarti, D.N., Chakravarti, B., and Moutsatsos, I. (2002). Informatic tools for proteomic profiling. Biotechniques (Suppl.) *4–10*, 12–15.

Chen, G., Gharib, T.G., Huang, C.C., Thomas, D.G., Shedden, K.A., Taylor, J.M., Kardia, S.L., Misek, D.E., Giordano, T.J., Iannettoni, M.D., et al. (2002a). Proteomic analysis of lung adenocarcinoma: identification of a highly expressed set of proteins in tumors. Clin. Cancer Res. *8*, 2298–2305.

Chen, G., Gharib, T.G., Huang, C.C., Taylor, J.M., Misek, D.E., Kardia, S.L., Giordano, T.J., Iannettoni, M.D., Orringer, M.B., Hanash, S.M., and Beer, D.G. (2002b). Discordant protein and mRNA expression in lung adenocarcinomas. Mol. Cell Proteomics *1*, 304–313.

Cheng, L., Neumann, R.M., Nehra, A., Spotts, B.E., Weaver, A.L., and Bostwick, D.G. (2000). Cancer heterogeneity and its biologic implications in the grading of urothelial carcinoma. Cancer *88*, 1663–1670.

Daly, M.B., and Ozols, R.F. (2002). The search for predictive patterns in ovarian cancer: Proteomics meets bioinformatics. Cancer Cell *1*, 111–112.

El-Bolkainy, M.N. (1983). Schistosomasis and bladder cancer. In The Pathology of Bladder Cancer, Bryan, G.T., and Cohen, S. M., eds. (Boca Raton, FL: CRC Press, Inc.), vol. I, pp. 57–90.

Emmert-Buck, M.R., Bonner, R.F., Smith, P.D., Chuaqui, R.F., Zhuang, Z., Goldstein, S.R., Weiss, R.A., and Liotta, L.A. (1996). Laser capture microdissection. Science *274*, 998–1001.

Figeys, D. (2002). Functional proteomics: mapping protein-protein interactions and pathways. Curr. Opin. Mol. Ther. *4*, 210–215.

Friedell, G.H., Nagy, G.K., and Cohen, S.M. (1983). Pathology of human bladder cancer and related lesions. In The Pathology of Bladder Cancer, Bryan, G.T., and Cohen, S.M., eds. (Boca Raton, FL: CRC Press, Inc.), vol. I, pp. 11–42.

Franzen, B., Linder, S., Uryu, K., Alaiya, A.A., Hirano, T., Kato, H., and Auer, G. (1996). Expression of tropomyosin isoforms in benign and malignant human breast lesions. Br. J. Cancer *73*, 909–913.

Gavin, A.C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J.M., Michon, A.M., Cruciat, C.M., et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature *415*, 141–147.

Gharib, T.G., Chen, G., Wang, H., Huang, C.C., Prescott, M.S., Shedden, K., Misek, D.E., Thomas, D.G., Giordano, T.J., Taylor, J.M., et al. (2002). Proteomic analysis of cytokeratin isoforms uncovers association with survival in lung adenocarcinoma. Neoplasia. *4*, 440–448.

Görg, A., Obermaier, C., Boguth, G., Harder, A., Scheibe, B., Wildgruber, R., and Weiss, W. (2000). The current state of two-dimensional electrophoresis

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with immobilized pH gradients. Electrophoresis *21*, 1037–1053.

Hanash, S.M., Bricory, F., and Beer, D. (2001). A proteomic approach to the identification of lung cancer markers. Dis. Markers *17*, 295–300.

Jones, M.B., Krutzsch, H., Shu, H., Zhao, Y., Liotta, L.A., Kohn, E.C., and Petricoin, E.F., 3rd. (2002). Proteomic analysis and identification of new biomarkers and therapeutic targets for invasive ovarian cancer. Proteomics *2*, 76–84.

Lawrie, L.C., Curran, S., McLeod, H.L., Fothergill, J.E., and Murray, G.I. (2001). Application of laser capture microdissection and proteomics in colon cancer. Mol. Pathol. *54*, 253–258.

Liu, H., Lin, D., and Yates, J.R., 3rd. (2002). Multidimensional separations for protein/peptide analysis in the post-genomic era. Biotechniques *32*, 898–902.

Madoz-Gurpide, J., Wang, H., Misek, D.E., Brichory, F., and Hanash, S.M. (2001). Protein based microarrays: a tool for probing the proteome of cancer cells and tissues. Proteomics *1*, 1279–1287.

McDonald, W.H., and Yates, J.R., 3rd. (2002). Shotgun proteomics and biomarker discovery. Dis. Markers *18*, 99–105.

Meehan, K.L., Holland, J.W., and Dawkins, H.J. (2002). Proteomic analysis of normal and malignant prostate tissue to identify novel proteins lost in cancer. Prostate *50*, 54–63.

Melhem, R., Hailat, N., Kuick, R., and Hanash, S.M. (1997). Quantitative analysis of Op18 phosphorylation in childhood acute leukemia. Leukemia *11*, 1690–1695.

Mylvaganam, S.E., Prabhakaran, M., Tudor, S., Moezzi, S., and Ramnarayan, K. (2002). Structural proteomics: methods in deriving protein structural information and issues in data management. Biotechniques (*Suppl*), 42–46.

Oh, J.M., Brichory, F., Puravs, E., Kuick, R., Wood, C., Rouillard, J.M., Tra, J., Kardia, S., Beer, D., and Hanash, S. (2001). A database of protein expression in lung cancer. Proteomics *1*, 1303–1319.

O'Farrell, P.H. (1975). High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. *250*, 4007–4021.

Ørntoft, T.F., Thykjaer, T., Waldman, F.M., Wolf, H., and Celis, J.E. (2002). Genome-wide study of gene copy numbers, transcripts, and protein levels in pairs of non-invasive and invasive human transitional cell carcinomas. Mol. Cell Proteomics *1*, 37–45.

Panisko, E.A., Conrads, T.P., Goshe, M.B., and Veenstra, T.D. (2002). The postgenomic age: characterization of proteomes. Exp. Hematol. *30*, 97–107.

Patton, W.F. (2002). Detection technology in proteome analysis. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. *771*, 3–31.

Petricoin, E.F., Ardekani, A.M., Hitt, B.A., Levine, P.J., Fusaro, V.A., Steinberg, S.M., Mills, G.B., Simone, C., Fishman, D.A., Kohn, E.C., and Liotta, L.A. (2002a). Use of proteomic patterns in serum to identify ovarian cancer. Lancet *359*, 572–577.

Petricoin, E.F., 3rd, Ornstein, D.K., Paweletz, C.P., Ardekani, A., Hackett, P.S., Hitt, B.A., Velassco, A., Trucco, C., Wiegand, L., Wood, K., et al. (2002b). Serum proteomic patterns for detection of prostate cancer. J. Natl. Cancer Inst. *94*, 1576–1578.

Petricoin, E.F., Zoon, K.C., Kohn, E.C., Barrett, J.C., and Liotta, L.A. (2002c). Clinical proteomics: translating benchside promise into bedside reality. Nat. Rev. Drug Discov. *1*, 683–695.

Stulik, J., Hernychova, L., Porkertova, S., Knizek, J., Macela, A., Bures, J., Jandik, P., Langridge, J.I., and Jungblut, P.R. (2001). Proteome study of colorectal carcinogenesis. Electrophoresis *22*, 3019–3025.

Wu, C.C., and McCoss, M.J. (2002). Shotgun proteomics: tools for the analysis of complex biological systems. Curr. Opin. Mol. Ther. *4*, 242–250.

Wulfkuhle, J.D., McLean, K.C., Paweletz, C.P., Sgroi, D.C., Trock, B.J., Steeg, P.S., and Petricoin, E.F., 3rd. (2001). New approaches to proteomic analysis of breast cancer. Proteomics *1*, 1205–1215.

Yanagida, M. (2002). Functional proteomics; current achievements. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. *771*, 89–106.

Yip, T.T., and Lomas, L. (2002). SELDI ProteinChip array in oncoproteomic research. Tech. Cancer Res. Treatment *1*, 273–274.