ELSEVIER

European Journal of Cancer 40 (2004) 2623–2632

European Journal of Cancer

www.ejconline.com

Genomic and proteomic technologies for individualisation and improvement of cancer treatment

Julia Wulfkuhle [a,*], Virginia Espina [a], Lance Liotta [a], Emanuel Petricoin [b]

[a] *NCI/FDA Clinical Proteomics Program, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bldg. 29A/2B20, HFM 710, 8800 Rockville Pike, Bethesda, MD 20892, USA*

[b] *NCI/FDA Clinical Proteomics Program, Office of Cell and Gene Therapy, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892, USA*

Received 4 December 2003; accepted 20 May 2004
Available online 6 August 2004

Abstract

The development of microarray-based technologies for characterising tumours, both at the genomic and proteomic levels, has had a significant impact on the field of oncology. Gene expression profiling of various human tumour tissues has led to the identification of expression patterns related to disease outcome and drug resistance, as well as to the discovery of new therapeutic targets and insights into disease pathogenesis. Protein microarray technologies, such as reverse-phase protein arrays, provide the unique opportunity to profile tissues and assess the activity of signalling pathways within isolated cell populations. This technology can be used to identify patients likely to benefit from specific treatment modalities and also to monitor therapeutic response in samples obtained during and after treatment. Routine application of genomic and proteomic microarray technologies in clinical practice will require significant efforts to standardise the techniques, controls and reference standards, and analytical tools used. Extensive, independent validation using large, statistically-powered datasets will also be necessary. Inclusion of concomitant genomic and proteomic-based molecular profiling techniques into clinical trial protocols will bring us closer to the reality of patient-tailored therapy.
Published by Elsevier Ltd.

Keywords: Molecular profiling; Individual targeted therapy; Genomics; Proteomics; Microarrays; Protein microarrays; Cancer

1. Introduction

The past decade has seen a revolution in technologies surrounding the field of molecular profiling in disease research. These developments have clearly had a most significant impact in the field of oncology. The complex genetic and proteomic alterations that lead to cancer progression and result in significant clinical heterogeneity among individual tumours and patients are difficult to measure by traditional pathological classification methods based on morphology, degree of differentiation, and the presence or absence of metastases. Microarray technologies used to profile tumours at the DNA, RNA and protein levels have led to the discoveries of disease susceptibility genes, therapeutic targets and expression profiles related to disease outcomes and drug sensitivity and resistance [1–8]. Detailed molecular classification of individual patient tumours will provide a means for clinicians to select therapies suited to each patient based on their molecular profiles and promises to change the way medicine is practiced [9].

At a functional level, cancer is both a proteomic and genomic disease. At the genetic level, certain chromosomal deletions, rearrangements, and gene mutations are selected out during cancer progression because these defect(s) ultimately alter protein signalling networks and generate a survival advantage for the cancer cell [10–12]. The function of proteins is closely tied to their cellular, tissue and physiological contexts and the protein–protein interactions that drive biological outcomes can be characterised as a

* Corresponding author. Tel.: +1-301-402-0211; fax: +1-301-480-5005.
E-mail address: wulfkuhle@cber.fda.gov (J. Wulfkuhle).

0959-8049/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/j.ejca.2004.05.020

fluctuating information flow within the cell and organism through protein pathways and networks. Defective, hyperactive or dominating signal pathways drive cancer growth, survival, invasion and metastasis [13]. A comprehensive analysis of the molecular basis of cancer requires integration of the distinct, but complementary, fields of genomics and proteomics. In the future, if physicians are to individualise therapy to each patient, they will need to be provided with genomic and proteomic data regarding the diagnosis and prognosis of the disease. In this review, we will examine recent work in the genomic and proteomic profiling of cancer that is bringing medicine closer to the reality of personalised medicine and discuss what limitations still must be addressed if this goal is to be realised.

2. Applications of gene expression profiling

Evidence is emerging to support the concept that each patient's cancer might have a unique complement of pathogenic molecular derangements. Consequently, a given class of therapy might be effective for only a subset of patients who harbour tumours with susceptible molecular alterations. This provides strong justification to develop strategies for selection from a menu of treatment choices or treatment combinations that best match the individual molecular profile of a tumour [9,13–23]. One of the first steps and a major focus along the path to individualised therapeutics for cancer has been the large-scale analysis of gene expression using DNA array technology [3,24]. This powerful technology is being used to study many biological processes. The experimental and clinical goals stemming from gene expression profiling technologies range from insights into pathogenesis, cancer diagnosis and prediction of clinical outcome to identification of therapeutic targets. The technology and experimental approach used may be different depending on which goal is being sought. Typically, a DNA array consists of rows and rows of oligonucleotide strands, or complementary DNAs (cDNAs), lined up in dots on a miniature silicon chip or glass slide (Fig. 1). Today, arrays for gene expression profiling can accommodate over 30 000 oligonucleotides or cDNAs. Expression levels can be compared across many samples, normal and pathological. Pattern recognition software and clustering algorithms allow identification of groups of genes whose expression varies in similar ways or tumour tissue specimens with similar repertoires of expressed genes. These analysis tools are powerful because they can reveal hidden patterns of relationships that transcend the histological appearance of a tumour and lead to hypotheses about gene function and tumour pathology.

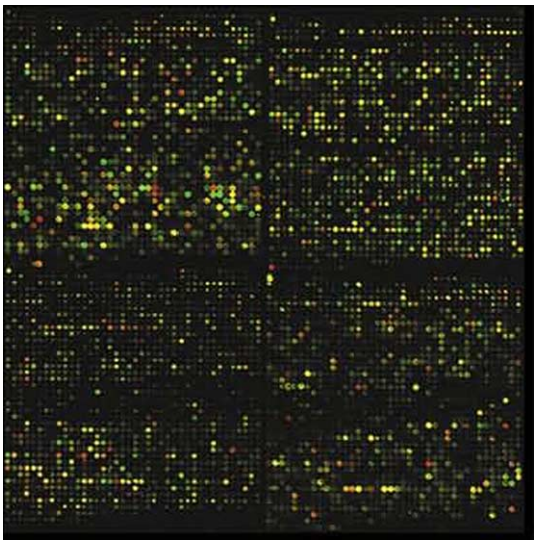

Fig. 1. Gene microarray format for transcriptional profiling. Oligonucleotide arrays consist of a series of short segments of DNA, which are complementary to the RNA transcript for the gene of interest. To use the arrays, RNA is extracted from tumour tissue, amplified, and labelled with a fluorescent or radioactive probe and hybridised with the chip. The spot intensity reflects the abundance of its matching mRNA species and hence reflects the expression level for its gene. With appropriate pattern recognition software, it is then possible to identify groups of genes whose expression varies in similar ways. Specialised cancer arrays have been developed (e.g. the lymphoid "lympho-chip" [27]).

2.1. Gene discovery, tumour classification and clinical outcome correlation

Ultimately, our ability to identify individuals at high-risk of developing cancer and to rationally design new drugs and treatment strategies to effectively treat the disease will require a much more detailed understanding of the complex interactions and disruptions that occur at the genetic and protein levels which lead to neoplastic transformation and tumour progression. Genetic and array-based gene expression profiling has been used to identify a number of genes involved in an inherited predisposition to disease, such as *BRCA1/2* and *APC*, and to elucidate the pathways these gene products operate within to lead to disease [5]. Cluster analysis of metastatic and non-metastatic medulloblastomas pinpointed the platelet-derived growth factor receptor (PDGFRα) and the Ras/metogen-activated protein kinase (MAPK) pathway for involvement in cell adhesion and motility [12]. Gene expression profiling studies in melanoma have led to the discovery of Wnt5a as a molecule that is highly correlated with metastatic potential in cutaneous melanoma [25].

Many studies have been published recently showing how DNA microarrays can be applied to the analysis and classification of cancer tissues. The pioneering

studies demonstrating that gene expression profiling could be used to predict and discern tumour classes and clinical outcomes of disease were performed in leukaemias and lymphomas [26,27]. The first report identified a set of 50 genes that was able to distinguish acute myelogenous leukaemia (AML) from acute lymphocytic leukaemia (ALL). This cluster of genes was able to distinguish between these two tumour types in an independent set of patient samples [26]. The second study used gene expression profiling to classify diffuse large-B-cell lymphomas [27] (Fig. 1). Profiling segregated the tumours into two large groups, one representing profiles similar to germinal centre B-cells and the other containing profiles reminiscent of *in vitro*-activated B-cells. Interestingly, these groupings correlated strongly with the clinical outcome of this disease, with patients harbouring the germinal centre B-cell-like lymphoma profiles having longer overall survival.

Subsequently, many other groups have successfully applied gene expression profiling to tumour classification and outcome prediction in various types of tumours [4,9,25,28–44]. In one impressive recent study, van 't Veer and colleagues used a cohort of breast tumours from young patients diagnosed with lymph node-negative disease to identify a gene expression signature predictive of poor prognosis [36]. The authors found that tumours not normally predicted to have poor outcomes based on clinical parameters could in fact display the "poor prognosis" signature, indicating that metastatic phenotypes may be programmed at early stages of tumour progression. Consensus guidelines based on clinical and histological parameters suggest that as many as 90% of node-negative, young breast cancer patients are candidates for adjuvant systemic chemotherapy. However, most of these women would not develop metastatic disease, even without treatment, and thus they potentially suffer the side-effects of therapy without any real benefit. A comparison of the efficacy of the microarray classifier with the current consensus criteria for adjuvant systemic therapy demonstrated that the "poor prognosis" profile was as effective at selecting patients at high risk for metastatic disease and also reduced the number of patients unnecessarily treated. This study provides a clear example of how gene expression profiling could potentially impact treatment decisions, and, ultimately, disease outcome.

2.2. Targeted therapeutics, drug sensitivity and resistance profiling

Beyond using genomic profiling for diagnostic and prognostic purposes, researchers also employ this technology to drug development and drug sensitivity and resistance profiling. Expression profiling has led to the identification of new therapeutic targets, and the development and approval of a number of targeted therapeutic agents for the treatment of cancer, such as trastuzumab (Herceptin^{TM}) for the treatment of ErbB2-overexpressing breast and ovarian tumours, iniatinib mesylate (Gleevec^{TM}), targeted against the Bcr-Abl fusion and c-Kit kinases for treatment of chronic myelogenous leukaemia and ALLs and gastrointestinal stromal tumours (GIST), and gefitinib (Iressa^{TM}, 2D1839) a tyrosine kinase inhibitor that targets the epidermal growth factor receptor, which is important in a number of epithelial cancers [45]. These therapeutic agents represent the beginning of a new era in rational drug design in which specific molecular targets drive the drug development process. This new era presents challenges for clinicians to readily identify those patients whose tumours have the appropriate molecular signatures to benefit from these therapies. The availability of the human genome sequence has recently permitted more large-scale analysis of the complex genetic contributions to drug sensitivity and response [46]. Great benefits will be gained by the identification and analysis of how single-nucleotide polymorphisms (SNPs) contribute to individual drug response [45,47–49]. Expression microarrays have been used to profile chemosensitivity in transformed cell lines *in vitro* [50], animal models [51,52], as well as in human tissues. Profiling studies correlating drug response to clinical outcome in oesophageal cancer following adjuvant therapy identified a battery of 52 genes correlated with prognosis and possibly, by extension, drug sensitivity or resistance [53]. A small study comparing pre- and post-treatment biopsy specimens from breast tumours identified distinguishing profiles between patients responding to systemic therapy and non-responders [32]. Another recent study of breast tumours by Chang and colleagues identified 92 genes with differential expression that correlated with therapeutic response to docetaxel [54]. While very intriguing, it should be taken into consideration that these studies were small and carried out limited independent validation, so it remains unclear whether these predictors truly reflect sensitivity to treatment or more fundamental facets of tumour biology related to disease progression.

2.3. Challenges of routine genomic profiling

The value of applying gene expression profiling to disease classification and prognosis is clear. However, there are several limitations and issues that must be addressed before these techniques can be applied routinely in the clinic. An assessment of the current DNA microarray literature found that there is little standardisation in the field with regard to the methods, analysis and controls used, and also data validation [55]. Small study sets currently limit the statistical significance of many molecular classifiers. Another issue

constantly faced in research using human tissues is small tumour size and limited availability of specimens for study. While techniques such as laser capture microdissection (LCM) and RNA amplification allow one to utilise biopsy specimens and small tumours for study, it will be important that future clinical trials incorporate tissue accrual components to allow for evaluation by molecular techniques. Finally, incorporation of genomic profiling into the realm of patient treatment will require the complete standardisation and development of straightforward techniques that can be incorporated into clinical laboratory testing.

3. Clinical applications of proteomic profiling

Molecular profiling using gene arrays has shown considerable potential for the classification of patient populations according to disease stage or survival outcome. Nevertheless, transcript profiling, by itself, provides an incomplete picture, because gene transcript levels often do not correlate with the phosphorylated or otherwise functional state of the encoded proteins. Moreover, gene transcripts provide little information about protein–protein interactions and the state of the cellular circuitry [11,56–59]. Consequently, the application of molecular profiling to select the appropriate treatment strategy should include direct proteomic pathway analysis of patient material.

Proteomic technologies such as mass spectrometry (MS), 2-dimensional electrophoresis (2-DE), bead capture and micro-ensyme-linked immunosorbent assays (ELISA) are currently successfully employed for drug discovery and biomarker identification [58,60–62]. However, despite their sophistication, these technologies have substantial limitations when they are applied to tissue and blood samples. Discovery platforms, such as 2D gels, isotope-coded affinity tagging (ICAT), multidimensional liquid chromatography–MS platforms and antibody arrays, require large cellular input samples that are orders of magnitude greater than those procured during a clinical biopsy [63–73]. Clinical specimens might contain only a few hundred cells as the starting point for analysis, rendering many proteomic tools inconsequential. The use of clinical trial material for proteomic analysis requires the development of new technologies that can utilise these small amounts of cellular material for discovery and profiling, and, ultimately, validation of potential targets in patients. A second limitation of the newer proteomic technologies is a requirement for denatured proteins. As denaturation will break protein complexes apart and destroy the three-dimensional protein conformation, these methods might not adequately probe the state of the cellular circuitry mediated by protein–protein interactions.

Most current therapeutics are directed at protein targets. Because cultured cell lines cannot fully model human disease, source material for the identification of new protein targets has shifted away from *in vitro* models to the use of actual diseased human tissue. While tissues are heterogeneous and often composed of hundreds of interacting cell populations, they provide the opportunity for the discovery of changes in the cellular proteome dependent on the cellular microenvironment. LCM has made it possible to analyse diseased cells in the tissue section itself [74], or to physically separate the desired cells directly from the surrounding contaminating cells. This technology has been applied to discover dozens of new protein targets that are either a cause or a consequence of the disease process in human tissues [75–84].

3.1. Protein microarrays

Realignment of the concepts and techniques from genomic profiling and applying them to proteomics has led to the development of protein microarrays. Protein microarrays represent an emerging technology that examines protein-protein recognition events in a global, high-throughput manner, can be used to profile the working state of cellular signal pathways in a manner not possible with gene arrays [9,85–95]. In the simplest sense, protein microarrays are immobilised protein spots [9,89,95,96]. The spots may be homogeneous or heterogeneous and may consist of a bait molecule, such as an antibody, a cell or phage lysate, a nucleic acid, drug or a recombinant protein or peptide (Fig. 2) [88,90,92,93, 95,97–101]. Protein microarrays may be used for drug discovery, biomarker identification and molecular profiling of cellular material.

The post-translational modifications of protein networks can be profiled employing protein microarrays by comparing the proportion of total (activated and non-activated) protein to the phosphorylated (activated) protein. This information, in general, reflects the state of information flow through a protein network. Monitoring the total and phosphorylated proteins over time, before and after treatment, or between disease and non-disease states may allow us to infer the activity levels of the proteins in a particular pathway in real-time [13,102]. The utility of protein microarrays lies in their ability to provide a map of known cell signalling proteins. Identification of critical nodes, or interactions, within the network is a potential starting point for drug development and/or the design of individual therapy regimens [13,102].

3.1.1. Reverse-phase protein microarrays

A new type of protein array, the reverse-phase protein array [92,103,104], has demonstrated a unique ability to

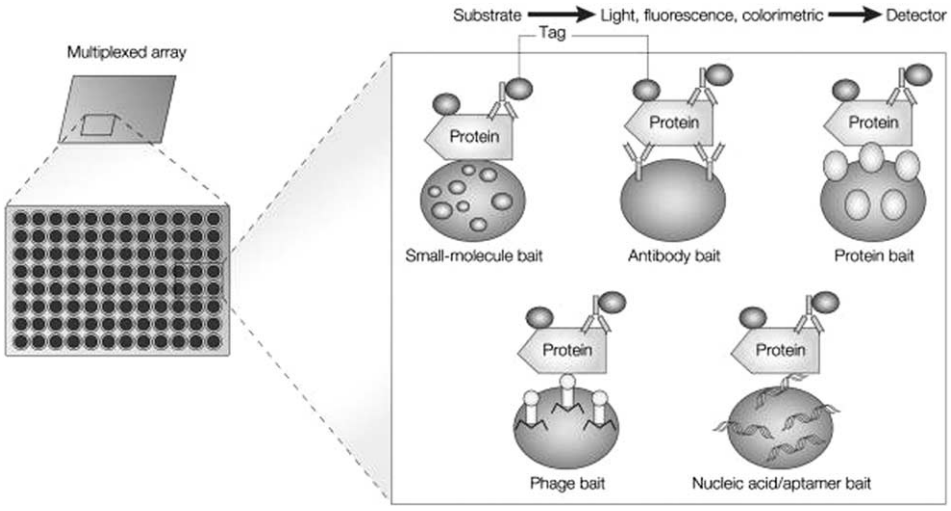

Fig. 2. Protein microarray formats. Protein microarrays consist of an array of protein samples, or protein baits, immobilised on a solid phase. The array can be queried with a mixture of labelled proteins containing analytes of interest. The analyte proteins are captured and can be detected using colorimetric, fluorescent, or chemiluminescent means.

analyse signalling pathways using small numbers of human tissue cells that were microdissected from biopsy specimens procured during clinical trials. Employing this approach, LCM-procured pure cell populations are taken from human biopsy specimens, and a protein lysate is arrayed onto nitrocellulose slides (Fig. 3). Key technological components of this method offer unique advantages over tissue arrays [105] or antibody arrays [71,72,83]. First, the reverse-phase array can use denatured lysates, so that antigen retrieval, which is a large limitation for tissue arrays, is not problematic. Protein microarrays can also consist of non-denatured lysates derived directly from LCM-procured tissue cells, so that protein–protein, protein–DNA and/or protein–RNA complexes can be detected and characterised. During printing, each patient sample is arrayed in a miniature dilution curve, providing an internal standard curve. This allows for direct quantitative measurement once antibody dilutions are determined to be in the linear range of detection. Finally, reverse-phase protein microarrays do

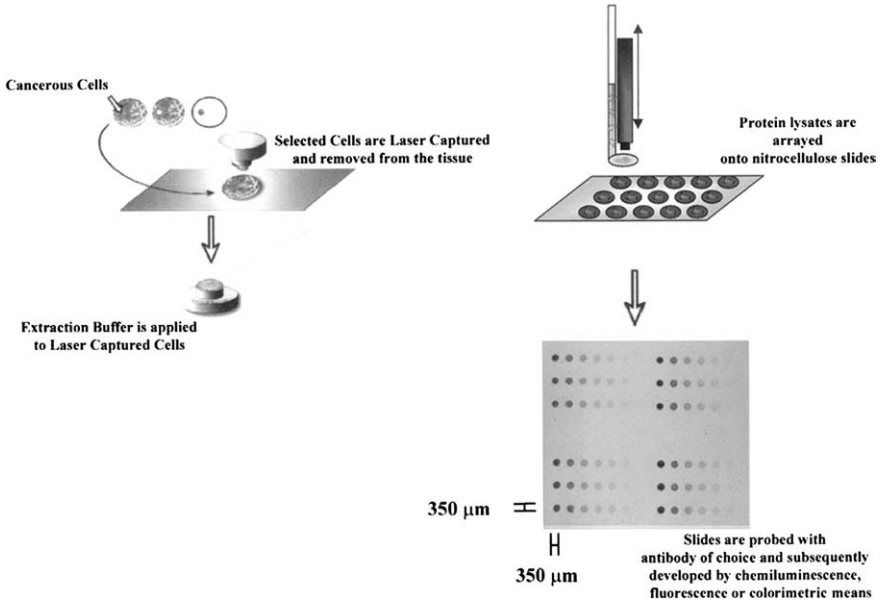

Fig. 3. Reverse-phase protein arrays. A new class of protein array is the reverse-phase array, which immobilises the cellular lysate sample to be analysed. Lysates are prepared from cultured cells or microdissected tissues and are arrayed in miniature dilution curves. The analyte molecule contained in the sample is detected by a separate labelled probe (e.g., antibody) applied to the surface of the array. This array is highly linear, very sensitive and requires no labelling of the sample proteins.

not require direct tagging of the protein as a readout for the assay, which allows a marked improvement in reproducibility, sensitivity and robustness of the assay over other techniques [106].

3.1.2. Potential of reverse-phase arrays in clinical research

The reverse-phase array platform was employed to address the basic question of whether premalignant transformation is caused by an increase in the cell growth rate through the activation of mitogenic growth pathways or by a decrease in the cell death rate through activation of apoptosis-inhibiting prosurvival signalling pathways [92]. Analysis of LCM-procured patient-matched normal epithelial, premalignant and invasive prostate carcinoma cell study sets revealed that phosphorylation and activation of Akt occurred as a critical early step in the progression of cancer. Thus, the accumulation of cells that is seen during early-stage prostate cancer (prostatic intraepithelial neoplasia) is caused by an alteration of the cellular turnover by a decrease in the death rate and not induction of the growth rate. Consequently, inhibition of Akt activity through molecular targeted therapeutics may have a profound impact on the treatment and prevention of prostate cancer progression.

Our laboratory has also utilised reverse-phase arrays for more global signal transduction profiling in large tumour sets [103,104]. In one study, we arrayed microdissected cell lysates from a number of ovarian cancers to determine if the activation state of key molecules involved in pro-survival and mitogenic signalling in ovarian cancers correlate with the tumour histotype or disease stage [104]. Profiles of the levels of phosphorylated ERK1/2 did not vary significantly with tumour histotype or disease stage and strongly suggested that pathway activation profiles in ovarian tumours may be patient-specific (Fig. 4). In another small study of prostate tissue, pathway profiling of microdissected cells from normal, stroma and prostate tumour revealed the preliminary finding that activation of protein kinase Cα PKCα is down-modulated in prostate disease. If validated, this finding could have profound effects on the rationale behind some current therapies. A randomised phase II trial of antisense oligonucleotides, ISIS 3521, directed to PKCα, examined the effects of these drugs on patients with hormone-refractory prostate cancer (HRPC) [107]. Based on our findings, knocking out the expression of PKCα for prostate cancer would not be effective. In fact, this is exactly what was seen; 31 patients with HRPC were randomised to receive the compound and no partial or complete responders were noted in this study. This illustrates the importance of proteomic technology coupled to signal pathway profiling in providing new and unexpected insights into cellular processes. Furthermore, it highlights the potential advantage of the reverse phase protein array technology for examining the status of multiple signalling events simultaneously in patients prior to initiating molecular-targeted therapies.

3.2. Current challenges of protein microarray technologies

Gene transcript profiling was catalysed by the ease and throughput of manufacturing probes with known, specific, and predictable affinity constants. In contrast, the probes (e.g., antibodies, aptamers, ligands, drugs) for protein microarrays cannot be manufactured with

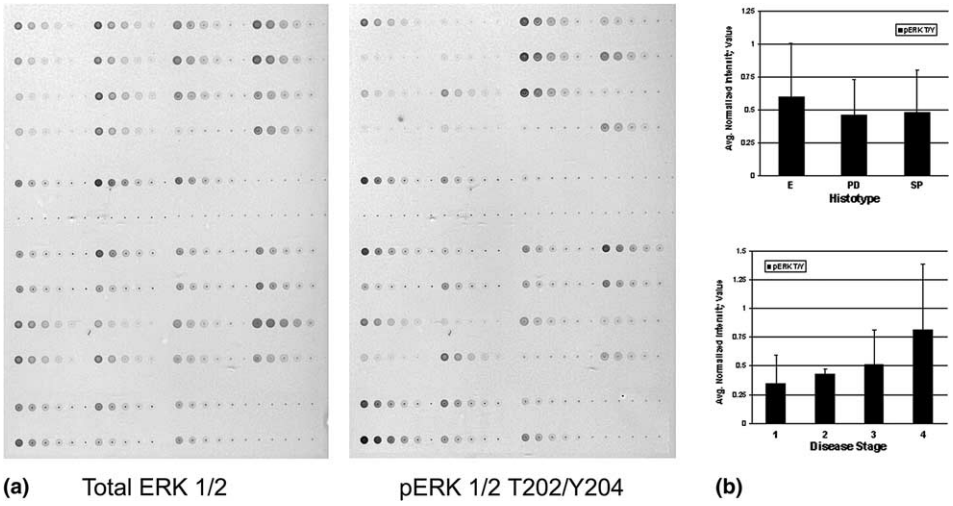

Fig. 4. (a) Reverse-phase arrays stained with ERK1/2 antibodies. Identical arrays of lysates from microdissected ovarian tumours were stained with antibodies as indicated. Each dilution curve represents an individual tumour. Left panel: image of total ERK 1/2 antibody staining. Right panel: image of phospho ERK 1/2 T202/Y204 antibody staining. (b) Comparisons of phospho ERK 1/2 levels in ovarian tumours. Normalised intensity values for phospho ERK 1/2 antibody staining were averaged among tumours of the same histotype (upper panel) or disease stage (lower panel): Error bars, standard deviation (SD) from the mean; E, endometrioid; PD, poorly differentiated; SP, serous papillary.

predictable affinity and specificity. The availability of high quality, specific antibodies or suitable protein binding ligands is the limiting factor, and starting point, for the successful utilisation of this technology [97]. In addition, prior to use on any array format, the antibody specificity must be thoroughly validated (e.g., single, appropriate-sized band on a Western blot) using a complex biological sample similar to that applied and analysed on the array. Post-translational modifications or protein–protein interactions of an individual protein will contain critical biological meaning that cannot be ascertained merely by measuring the total concentration of the analyte. Consequently, a significant challenge for protein microarrays is the need for antibodies or similar detection probes, that are specific for the modification or activation state of the target protein. Sets of high-quality, modification state-specific antibodies are now available commercially. Unfortunately, high-quality antibodies are currently available for only a small percentage of the known proteins involved in signal networks and gene regulation. A significant challenge for cooperative groups, funding agencies, and international consortia is the generation of large comprehensive libraries of fully characterised specific antibodies, ligands and probes. A major initiative of Human Proteome Organization (HUPO) is the production and qualification of antibody libraries that will be made available to the scientific community [108,109].

4. The future of individualised therapy

At present, cancer therapy has been directed at a single molecular target. In the future, we can imagine targeting an entire set of nodes all along the pathogenic signalling pathway. Such an approach could achieve, theoretically, a higher efficacy with a lower toxicity. Combinatorial therapy, an alternative approach to single-agent therapy, offers the promise of higher specificity at lower treatment doses [110–112]. It is possible that a correctly chosen series of inhibitors, acting at several points along a signalling pathway, could be used at lower concentrations than would be required for single agent therapy, and yet still result in a complete shutdown of the pathway. The advantage is realised because the inhibitors work in series at different points along the pathway. This means that output of one node in the pathway is inhibited before it reaches the next node. Consequently, a lower concentration of inhibitor is required at each successive level [113]. With this concept in mind, a redefined goal of molecular profiling is to map the cellular circuit so as to define the optimal set of interconnected drug targets. The use of combinatorial therapy for increased efficacy could also yield a decrease in unwanted toxic side-effects, as each drug can now be given at a lower treatment dose. However, this will need to be proved, and will require a higher degree of vigilance during the implementation of the regime to monitor the combined toxic effects of the drugs on normal cell populations. So, the use of clinical proteomic tools, such as whole-body protein arrays [113], becomes even more relevant to this emerging era of patient-tailored molecular medicine, and could aid in the analysis of desired drug effects on target pathways and unwanted toxic effects on the circuitry within normal cell populations.

We can envision a future in medicine that routinely employs technologies to map the state of gene expression and protein signalling pathways within biopsy samples [13,102]. Functional maps of the state of key pathways within that patient's tumour cells will become the starting point for personalised therapy. Under this scenario, therapy can be tailored to the individual tumour's molecular defect. Moreover, it should be feasible to administer combination therapy targeting multiple interdependent points along a pathogenic pathway, or targeting separate pathways. Following rebiopsy or molecular imaging, the effect of the treatment can be monitored in real-time [13,102,106]. On-going clinical trials at the National Cancer Institute have currently incorporated reverse-phase microarray technology for evaluation of the sample procurement and processing technologies, as well as a means to elucidate hypotheses concerning treatment effects on cell signalling networks. As more cell signalling information is gleaned from protein microarrays, we envision an enhanced ability to develop/identify new combinatorial therapies or targeted therapies. The ability to discern treatment effectiveness, *via* protein microarrays, at an early time point during treatment can lead to early intervention and positive patient outcomes.

Conflict of interest

None.

References

1. Petricoin III EF, Hackett JL, Lesko LJ, Puri RK, Gutman SI, Chumakov K, *et al*. Medical applications of microarray technologies: a regulatory science perspective. *Nat Genet* 2002, **32**(Suppl.), 474–479.
2. Baak JPA, Path FRC, Hermsen MAJA, Meijer G, Schmidt J, Janssen EAM. Genomics and proteomics in cancer. *Eur J Cancer* 2003, **39**, 1199–1215.
3. Holland K, Sullivan M. Cancer genomics: the chips are on the table. *New Zeal Med J* 2002, **115**, U204–U221.
4. Ciro M, Bracken AP, Helin K. Profiling cancer. *Curr Opin Cell Biol* 2003, **15**, 213–220.
5. Mariadason JM, Augenlicht LH, Arango D. Microarray analysis in the clinical management of cancer. *Hematol Oncol Clin North Am* 2003, **17**.

6. Nevins JR, Huang ES, Dressman H, Pittman J, Huang AT, West M. Towards integrated clinico-genomic models for personalized medicine: combining gene expression signatures and clinical factors in breast cancer outcomes prediction. *Hum Mol Genet* 2003, **12**, R153–R157.
7. Onyango P. Genomics and cancer. *Curr Opin Oncol* 2002, **14**, 79–85.
8. Omenn GS. Genetic advances will influence the practice of medicine: examples from cancer research and care of cancer patients. *Genet Med* 2002, **4**, 15S–20S.
9. Liotta L, Petricoin E. Molecular profiling of human cancer. *Nat Rev Genet* 2000, **1**, 48–56.
10. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000, **100**, 57–70.
11. Hunter T. Signaling-2000 and beyond. *Cell* 2000, **100**, 113–127.
12. MacDonald TJ, Brown KM, LaFleur B, Peterson K, Lawlor C, Chen Y, *et al*. Expression profiling of medulloblastoma: PDGFRa and the RAS/MAPK pathway as therapeutic targets for metastatic disease. *Nat Genet* 2001, **29**, 143–152.
13. Liotta LA, Kohn EC, Petricoin EF. Clinical proteomics personalized molecular medicine. *J Am Med Assoc* 2001, **286**(18), 2211–2214.
14. Karpati G, Li H, Nalbantoglu J. Molecular therapy ofr glioblastoma. *Curr Opin Mol Ther* 1999, **1**, 545–552.
15. Brown CK, Kirkwood JM. Targeted therapy of malignant melanoma. *Curr Oncol Rep* 2001, **3**, 344–352.
16. Frankel AE, Sievers EL, Scheinberg DA. Cell surface receptor-targeted therapy of acute myeloid leukemia: a review. *Cancer Biother Radiopharm* 2000, **15**, 459–476.
17. Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, *et al*. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. *Proc Natl Acad Sci USA* 1992, **89**, 9267–9271.
18. Cimoli G, Bagnasco L, Pescarolo MP, Avignolo C, Melchiori A, Pasa S, *et al*. Signaling proteins as innovativ targets for antineoplastic therapy: our experience with the signaling protein c-myc. *Tumori* 2001, **87**, S20–S23.
19. Gasparini G, Gion M. Molecular-targeted anticancer therapy: challenges related to study design and choice of proper endpoints. *Cancer J Sci Am* 2000, **6**, 117–131.
20. Wiestner A, Staudt LM. Towards molecular diagnosis and targeted therapy of lymphoid malignancies. *Semin Hematol* 2003, **40**, 296–307.
21. von Mehren M. Gastrointestinal stromal tumors: a paradigm for molecularly targeted therapy. *Cancer Invest* 2003, **21**(553–563).
22. Abou-Jawde R, Choueiri T, Alemany C, Mekhail T. An overview of targeted treatments in cancer. *Clin Ther* 2003, **25**, 2121–2137.
23. Gale DM. Molecular targets in cancer therapy. *Semin Oncol Nurs* 2003, **19**, 193–205.
24. Liang P, Pardee AB. Analysing differential gene expression in cancer. *Nat Rev Cancer* 2003, **3**, 869–876.
25. Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, *et al*. Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature* 2000, **406**, 536–540.
26. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, *et al*. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 1999, **286**(5439), 531–537.
27. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000, **403**, 503–511.
28. Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, *et al*. Delineation of prognostic biomarkers in prostate cancer. *Nature* 2001, **412**, 822–826.
29. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, *et al*. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001, **98**, 10869–10874.
30. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, *et al*. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003, **100**, 8418–8423.
31. Sotiriou C, Neo SY, McShane LM, Korn EL, Long PM, Jazaeri A, *et al*. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci USA* 2003, **100**, 10393–10398.
32. Sotiriou C, Powles TJ, Dowsett M, Jazaeri AA, Feldman AL, Assersohn L, *et al*. Gene expression profiles derived from fine needle aspiration correlate with response to systemic chemotherapy in breast cancer. *Breast Cancer Res* 2002, **4**, R3.
33. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, *et al*. Molecular portraits of human breast tumors. *Nature* 2000, **406**, 747–752.
34. Schwartz DR, Kardia SLR, Shedden KA, Kuick R, Michailidis G, Taylor JMG, *et al*. Gene expression in ovarian cancer reflects both morphology and biological behavior, distinguishing clear cell from other poor-prognosis ovarian carcinomas. *Cancer Research* 2002, **62**, 4722–4729.
35. Schoch C, Kohlmann A, Schnittger S, Brors B, Dugas M, Mergenthaler S, *et al*. Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. *Proc Natl Acad Sci USA* 2002, **99**, 10008–10013.
36. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AAM, Mao M, *et al*. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002, **415**, 530–536.
37. Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, *et al*. Predictionof central nervous system embryonal tumour outcome based on gene expression. *Nature* 2002, **415**, 436–442.
38. Nutt CL, Mani DR, Betensky RA, Tamayo P, Cairncross JG, Ladd C, *et al*. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res* 2003, **63**, 1602–1607.
39. Ramaswamy S, Tamayo P, Rifkin R, Mukherjee S, Yeang C-H, Angelo M, *et al*. Multiclass cancer diagnosis using tumor gene expression signatures. *Proc Natl Acad Sci USA* 2001, **98**, 15149–15154.
40. Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. *Nat Genet* 2003, **33**, 49–54.
41. Beer DG, Kardia SLR, Huang C-C, Giordano TJ, Levin AM, Misek DE, *et al*. Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med* 2002, **8**, 816–824.
42. Shipp MA, Ross KN, Tamayo P, Weng AP, Kutok JL, Aguiar RC, *et al*. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat Med* 2002, **8**, 68–74.
43. Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, *et al*. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell* 2002, **1**, 133–143.
44. Jazaeri AA, Lu K, Schmandt R, Harris CP, Rao PH, Sotiriou C, *et al*. Molecular determinants of tumor differentiation in papillary serous ovarian carcinoma. *Mol Carcinog* 2003, **36**, 53–59.
45. Workman P, Clarke PA. Innovative cancer drug targets: genomics transcriptomics and clinomics. *Expert Opin Pharmacother* 2001, **2**, 911–915.
46. Huang Y, Sadee W. Drug sensitivity and resistance genes in cancer chemotherapy: a chemogenomics approach. *Drug Disc Today* 2003, **8**, 356–363.
47. McLeod HL, Yu J. Cancer pharmacogenomics: SNPs, chips, and the individual patient. *Cancer Invest* 2003, **21**, 630–640.

48. Zhou W. Mapping genetic alterations in tumors with single nucleotide polymorphisms. *Curr Opin Oncol* 2003, **15**, 50–54.
49. Ross JS, Ginsburg GS. Integration of molecular diagnostics with therapeutics: implications for drug discovery and patient care. *Expert Rev Mol Diag* 2002, **2**, 531–541.
50. Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, *et al*. A gene expression database for the molecular pharmacology of cancer. *Nat Genet* 2000, **24**, 236–244.
51. Zembutsu H, Ohnishi Y, Tsunoda T, Furukawa Y, Katagiri T, Ueyama Y, *et al*. Genome-wide cDNA microaray screening to correlate gene expression profiles with sensitivity of 85 human-cancer xenografts to anticancer drugs. *Cancer Res* 2002, **62**, 518–527.
52. Zembutsu H, Ohnishi Y, Daigo Y, Katagiri T, Kikuchi T, Kakiuchi S, *et al*. Gene-expression profiles fo human tumor xenografts innude mice treated with the EGFR tyrosine kinase inhibitor ZD1839. *Int J Oncol* 2003, **23**(29–39).
53. Kihara C, Tsunoda T, Tanaka T, Yamana H, Furukawa Y, Ono K, *et al*. Prediction of sensitivity of esophageal tumors to adjuvant chemotherapy by cDNA microarray analysis of gene-expression profiles. *Cancer Res* 2001, **61**, 6474–6479.
54. Chang JC, Wooten EC, Tsimelzon A, Hilsenbeck SG, Gutierrez MC, Elledge R, *et al*. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *The Lancet* 2003, **362**, 362–369.
55. Ntzani EE, Ioannidis JPA. Predictive ability of DNA microarrays for cancer outcomes and correlates: an empirical assessment. *The Lancet* 2003, **362**, 1439–1444.
56. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature* 2001, **411**, 355–365.
57. Bowden ET, Barth M, Thomas D, Glazer RI, Mueller SC. An invasion-related complex of cortactin, paxillin and PKCmu associates with invadopodia at sites of extracellular matrix degradation. *Oncogene* 1999, **18**, 4440–4449.
58. Celis JE, Gromov P. Proteomics in translational cancer research: toward an integrated approach. *Cancer Cell* 2003, **3**, 9–15.
59. Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL. The large-scale organization of metabolic networks. *Nature* 2000, **407**, 651–654.
60. Chen G, Gharib TG, Huang C-C, Thomas DG, Shedden KA, Taylor JMG, *et al*. Proteomic analysis of lung adenocarcinoma: identification of a highly expressed set of proteins in tumors. *Clinical Cancer Research* 2002, **8**, 2298–2305.
61. Delehanty JB, Ligler FS. A microarray immunoassay for simultaneous detection of proteins and bacteria. *Anal Chem* 2002, **74**, 5681–5687.
62. Pandey A, Mann M. Proteomics to study genes and genomes. *Nature* 2000, **405**, 837–846.
63. Herbert BR, Sanchez JC, Bini L. Two-dimensional electrophoresis: the state of the art and future directions in proteome research. In Wilkens MR, Williams KL, Appel RD, Hochstrasser DF, eds. *Proteome research: new frontiers in functional genomics*. New York, Springer-Verlag, 1997, pp 13–34.
64. Shen Y, Tolic N, Zhao R, Pasa-Tolic L, Li L, Berger SJ, *et al*. High-throughput proteomics using high-efficiency multiple-capillary liquid chromatography with on-line high-performance ESI FTICR mass spectrometry. *Anal Chem* 2001, **73**, 3011–3021.
65. Li J, Wang C, Kelly JF, Harrison DJ, Thibault P. Rapid and sensitive separation of trace level protein digests using microfabricated devices coupled to a quadrupole-time-of-flight mass spectrometer. *Electrophoresis* 2001, **21**, 198–210.
66. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotechnol* 1999, **17**, 994–999.
67. Washburn MP, Wolters D, Yates JR. Large scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat Biotechnol* 2001, **19**, 242–247.
68. Washburn MP, Ulaszek R, Deciu C, Schieltz DM, Yates JR. Analysis of quantitative proteomic data generated via multidimensional protein identification technology. *Anal Chem* 2002, **74**, 1650–1657.
69. Krutchinsky AN, Kalkum M, Chait BT. Automatic identification of proteins with a MALDI-quadrupole ion trap mass spectrometer. *Anal Chem* 2001, **73**, 5066–5077.
70. Zhou H, Ranish JA, Watts JD, Aebersold R. Quantitative proteome analysis by solid-phase isotope tagging and mass spectrometry. *Nat Biotechnol* 2002, **20**, 512–515.
71. Zhou G, Li H, DeCamp D, Chen S, Shu H, Gong Y, *et al*. 2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers. *Mol Cell Proteomics* 2002, **1**, 117–124.
72. Sreekumar A, Nyati MK, Varambally S, Barrette TR, Ghosh D, Lawrence TS, *et al*. Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins. *Cancer Res* 2001, **61**, 7585–7593.
73. MacBeath G. Proteomics comes to the surface. *Nat Biotechnol* 2001, **19**(9), 828–829.
74. Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM. Imaging mass spectrometry: A new technology for the analysis of protein expression in mammalian tissues. *Nature Med* 2001, **7**, 493–496.
75. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, *et al*. Laser capture microdissection. *Science* 1996, **274**(5289), 998–1001.
76. Emmert-Buck MR, Gillespie JW, Paweletz CP, Ornstein DK, Basrur V, Appella E, *et al*. An approach to proteomic analysis of human tumors. *Mol Carcinog* 2000, **27**, 158–165.
77. Craven RA, Totty N, Harnden P, Selby PJ, Banks RE. Laser capture microdissection and two-dimensional polyacrylamide gel electrophoresis. Evaluation of tissue preparation and sample limitations. *Am J Pathol* 2002, **160**, 815–822.
78. Ornstein DK, Gillespie JW, Paweletz CP, Duray PH, Herring J, Vocke CD, *et al*. Proteomic analysis of laser capture microdissected human prostate cancer and *in vitro* prostate cell lines. *Electrophoresis* 2000, **21**, 2235–2242.
79. Wulfkuhle JD, Sgroi DC, Krutzsch H, McLean K, McGarvey K, Knowlton M, *et al*. Proteomics of human breast ductal carcinoma *in situ*. *Cancer Res* 2002, **62**, 6740–6749.
80. Wulfkuhle JD, McLean KC, Paweletz CP, Sgroi DC, Trock BJ, Steeg PS, *et al*. New approaches to proteomic analysis of breast cancer. *Proteomics* 2001, **1**, 1205–1215.
81. Wulfkuhle JD, Liotta LA, Petricoin III EF. Proteomic applications for the early detection of cancer. *Nat Rev Cancer* 2003, **3**, 267–275.
82. Jones MB, Krutzsch H, Shu H, Zhao Y, Liotta LA, Kohn EC, *et al*. Proteomic analysis and identification of new biomarkers and therapeutic targets for invasive ovarian cancer. *Proteomics* 2002, **2**, 76–84.
83. Knezevic V, Leethanakul C, Bichsel VE, Worth JM, Prabhu VV, Gutkind JS, *et al*. Proteomic profiling of the cancer microenvironment by antibody arrays. *Proteomics* 2001, **1**, 1271–1278.
84. Ahram M, Best CJM, Flaig MJ, Gillespie JW, Leiva IM, Chuaqui RF, *et al*. Proteomic analysis of human prostate cancer. *Mol Carcinog* 2002, **33**, 9–15.
85. Charboneau L. Utility of reverse phase protein microarrays: Applications to signaling pathways and human body arrays. *Brief Funct Genomics Proteomics* 2002, **1**, 305–315.
86. Cutler P. Protein arrays: The current state-of-the-art. *Proteomics* 2003, **3**, 3–18.
87. Ge H. UPA, a universal protein array system for quantitative detection of protein–protein, protein–DNA, protein–RNA and protein–ligand interactions. *Nucleic Acids Res* 2000, **28**, e3.
88. Lal SP, Christopherson RI, dos Remedios CG. Antibody arrays: and embryonic but rapidly growing technology. *Drug Discov Today* 2002, **7**, S143–S149.

89. MacBeath G. Protein microarrays and proteomics. *Nat Genet* 2002, **32**(Suppl.), 526–532.
90. MacBeath G, Schreiber SL. Printing proteins as microarrays for high-throughput function determination. *Science* 2000, **289**(5485), 1760–1763.
91. Miller JC, Zhou H, Kwekel J, Cavalio R, Burke J, Butler EB, *et al*. Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. *Proteomics* 2003, **3**, 56–63.
92. Paweletz CP, Charboneau L, Bichsel VE, Simone NL, Chen T, Gillespie JW, *et al*. Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene* 2001, **20**, 1981–1989.
93. Wilson DS, Nock S. Recent developments in protein microarray technology. *Angew Chem Int Ed Engl* 2003, **42**, 494–500.
94. Zhu H, Snyder M. Protein arrays and microarrays. *Curr Opin Chem Biol* 2001, **5**(40–45).
95. Zhu H, Snyder M. Protein chip technology. *Curr Opin Chem Biol* 2003, **7**, 55–63.
96. Haab BB, Dunham MJ, Brown PO. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol.* 2001;**2**:RESEARCH0004.
97. Templin MF, Stoll D, Schrenk M, Traub PC, Vohringer CF, Joos TO. Protein microarray technology. *Trends Biotechnol* 2002, **20**(160–166).
98. Humphery-Smith I, Wischerhoff E, Hashimoto R. Protein arrays for assessment of target selectivity. *Drug Discov World* 2002, **4**, 17–27.
99. Petach H, Gold L. Dimensionality is the issue: use of photoaptamers in protein microarrays. *Curr Opin Biotechnol* 2002, **13**, 309–314.
100. Schaeferling M, Schiller S, Paul H, Kruschina M, Pavlickova P, Meerkamp M, *et al*. Application of self-assembly techniques in the design of biocompatible protein microarray surfaces. *Electrophoresis* 2002, **23**, 3097–3105.
101. Weng S, Gu K, Hammond PW, Lohse P, Rise C, Wagner RW, *et al*. Generating addressable protein microarrays with PROfusion covalent mRNA-protein fusion technology. *Proteomics* 2002, **2**, 48–57.
102. Petricoin EF, Zoon KC, Kohn EC, Barrett JC, Liotta LA. Clinical proteomics: Translating benchside promise into bedside reality. *Nat Rev Drug Discov* 2002, **1**, 683–695.
103. Grubb RL, Calvert VS, Wulfkuhle JD, Paweletz CP, Linehan WM, Phillips JL, *et al*. Signal pathway profiling of prostate cancer using reverse phase protein microarrays. *Proteomics* 2003, **3**, 2142–2146.
104. Wulfkuhle JD, Aquino JA, Calvert VS, Fishman DA, Coukos G, Liotta LA, *et al*. Signal pathway profiling of ovarian cancer from human tissue specimens using reverse-phase protein microarrays. *Proteomics* 2003, **3**, 2085–2090.
105. Torhorst J, Bucher C, Kononen J, Haas P, Zuber M, Kochli OR, *et al*. Tissue microarrays for rapid linking of molecular changes to clinical endpoints. *Am J Pathol* 2001, **159**, 2249–2256.
106. Espina V, Mehta AI, Winters ME, Calvert V, Wulfkuhle J, Petricoin III EF, *et al*. Protein microarrays: molecular profiling technologies for clinical specimens. *Proteomics* 2003, **3**, 2091–2100.
107. Tolcher AW, Reyno L, Venner PM, Ernst SD, Moore M, Geary RS, *et al*. A randomized phase II and pharmacokinetic study of the antisense oligonucleotides ISIS 3521 and ISIS 5132 inpatients with hormone-refractory prostate cancer. *Clin Cancer Res* 2002, **8**, 2530–2535.
108. Tyers M, Mann M. From genomics to proteomics. *Nature* 2003, **422**, 193–197.
109. Hanash S. Disease proteomics. *Nature* 2003, **422**, 226–232.
110. Moasser MM, Basso A, Averbuch SD, Rosen N. The tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res* 2001, **61**, 7184–7188.
111. Normanno N, Campiglio M, De LA, Somenzi G, Maiello M, Ciardiello F, *et al*. Cooperative inhibitory effect of ZD1839 (Iressa) in combination with teastuzumab (Herceptin) on human breast cancer cell growth. *Ann Oncol* 2002, **13**, 65–72.
112. Zwick E, Bange J, Ullrich A. Receptor tyrosine kinases as targets for anticancer drugs. *Trends Mol Med* 2001, **8**, 17–23.
113. Petricoin EF, Liotta LA. Clinical Applications of Proteomics. *J Nutr* 2003, **133**, 2476S–2484S.