CLINICAL PROTEOMICS: TRANSLATING BENCHSIDE PROMISE INTO BEDSIDE REALITY

Emanuel F. Petricoin, Kathryn C. Zoon‡, Elise C. Kohn§, J. Carl Barrett§ and Lance A. Liotta‖*

The ultimate goal of proteomics is to characterize the information flow through protein networks. This information can be a cause, or a consequence, of disease processes. Clinical proteomics is an exciting new subdiscipline of proteomics that involves the application of proteomic technologies at the bedside, and cancer, in particular, is a model disease for studying such applications. Here, we describe proteomic technologies that are being developed to detect cancer earlier, to discover the next generation of targets and imaging biomarkers, and finally to tailor the therapy to the patient.

**FDA–NCI Clinical Proteomics Program, Division of Therapeutic Proteins, Center for Biologic Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892, USA.*
‡Office of the Director, Center for Biologic Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892, USA.
§Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.
‖FDA–NCI Clinical Proteomics Program, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.
Correspondence to E.F.P.
e-mail: petricoin@cber.fda.gov
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Molecular medicine is moving beyond genomics to proteomics. Although the aim of proteomics is often viewed as the creation of a 'master list' of all proteins and their possible post-translational modifications, the effort expended in preparing such a list might not be rewarded by immediate new levels of functional insight, because the function of proteins is closely tied to their cellular, tissue and physiological context. So, the ultimate goal of proteomics should be to characterize the information flow within the cell and the organism through protein pathways and networks[1–6].

The information flow is mediated by protein–protein interactions (FIG. 1) — proteins 'deliver' packets of information by modifying a protein-binding partner; for example, by phosphorylation, cleavage or alteration of its conformation. The goal is to be able to access and visualize entire interconnecting circuits of proteins — both inside and outside a cell — as they coalesce after a stimulus and then disperse when the stimulus ceases. Completion of such a detailed 'protein-wiring diagram', even for only a subset of the key physiological processes, could have a profound effect on functional biology, the understanding of disease mechanisms and the rational design of targeted therapeutics.

This review describes early-stage technology designed to study the state of proteins that act as key sensors, gates or amplifiers. Profiling the level of activation of protein circuit elements under the influence of a disease process can highlight disease-related circuit derangements that could be the drug targets of the future.

Cancer as an example

Cancer, although often classified as a genetic disease, is, in a functional sense, a proteomic disease — genetic mutations can modify protein signalling pathways and thereby create a survival advantage for the cell because they force it to ignore negative inhibitory signals, or perpetually send it false positive signals. The pathogenic signalling pathways are not confined to the cancer cell, but extend to the tumour–host interface[7] (FIG. 2), and recognition that cancer is a product of the proteomic tissue microenvironment has important implications. First, it shifts the emphasis away from therapeutic targets being individual molecules — it might make more sense to target all of the deranged signalling pathways, both inside and outside the cancer cell. Second, the tumour–host communication system might involve enzymatic events and sharing of growth factors, so the microenvironment of the tumour–host interaction could be a source for biomarkers that could ultimately be shed into the serum proteome. In this article, we use

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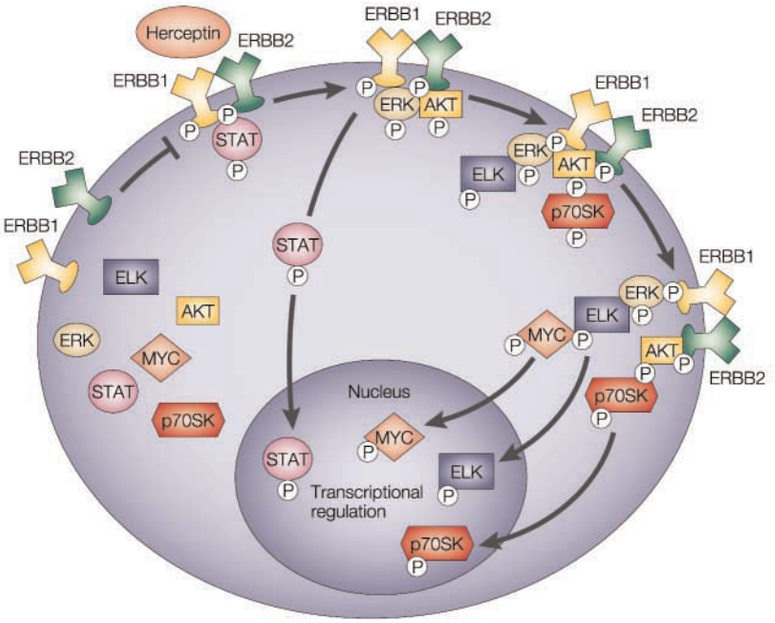

Figure 1 | **Example of a protein signalling pathway.** Protein signalling pathways and networks consist of protein complexes that assemble in response to a stimulus. Information transfer occurs through post-translational modification (for example, phosphorylation) of protein-binding partners. Example components of the ERBB1/2 pathway are shown. This pathway is a target for treatment with Herceptin, an antibody that recognizes ERBB2. AKT, v-akt murine thymoma viral oncogene homologue; ELK, member of the ETS family of transcription factors; ERBB, avian erythroblastic leukaemia viral oncogene homologue; ERK, extracellular-signal-regulated kinase; MYC, avian v-myc myelocytomatosis viral oncogene homologue; p70SK, 70 kDa ribosomal protein S6 kinase; STAT, signal transducer and activator of transcription.

cancer as an example to describe proteomic technologies that are being developed to diagnose disease earlier, to discover new therapeutic targets and biomarkers, and to facilitate individualized therapy.

Application of proteomics to early diagnosis

Unfortunately, in many cases, cancer is diagnosed and treated too late, when the cancer cells have already invaded and METASTASIZED throughout the body. More than 60% of patients with breast, lung, colon and ovarian cancer already have hidden or overt metastatic colonies. At this stage, therapeutic modalities are limited in their success. Detecting cancers at their earliest stages, even in the premalignant state, means that current or future treatment modalities might have a higher likelihood of a true cure.

Ovarian cancer is a prime example of this clinical dilemma. More than two-thirds of cases of ovarian cancer are detected at an advanced stage, when the ovarian cancer cells have spread away from the ovary surface and have disseminated throughout the PERITONEAL CAVITY[8]. Although the disease at this stage is advanced, it rarely produces specific or diagnostic symptoms. Consequently, ovarian cancer is usually treated when it is at an advanced stage[9]. The resulting five-year survival rate is 35–40% for late-stage patients who receive the best possible surgical and chemotherapeutic intervention. By contrast, if ovarian cancer is detected when it is still confined to the ovary (stage I), conventional therapy produces a high rate (95%) of five-year survival[9–13]. So, early detection of ovarian cancer, by itself, could have a profound effect on the successful treatment of this disease. Unfortunately, early-stage ovarian cancer lacks a specific symptom and a specific biomarker, and accurate and reliable diagnostic, non-invasive modalities. Proteomics offers a new approach to the discovery of early cancer biomarkers, and because of the great clinical need, a principal focus of marker discovery has been ovarian cancer.

An effective, clinically useful biomarker should be measurable in a readily accessible body fluid, such as serum, urine or saliva. The field of clinical proteomics is especially well suited to discovering such biomarkers, as serum is a protein-rich information reservoir that contains the traces of what has been encountered by the blood during its constant perfusion and percolation throughout the body. However, until now, the search for cancer-related biomarkers for early disease detection has been a 'one-at-a-time' approach, which has looked for overexpressed proteins in blood that are shed into the circulation as a consequence of the disease process[14–18]. Unfortunately, this method is laborious and time consuming, as there are potentially thousands of intact and cleaved proteins in the human serum proteome. Finding a single disease-related protein is like searching for a needle in a haystack, requiring the separation and identification of these entities individually.

Recently, serum-based proteomic pattern analysis, a new method in diagnostics and disease detection, has been described[19]. The diagnostic end point for the detection of ovarian cancer was a pattern that comprised many individual proteins, none of which could independently differentiate diseased from healthy individuals. These patterns reflect the blood proteome without knowledge of what the proteins are. The blood proteome is changing constantly as a consequence of perfusion of the diseased organ. These disease-related differences in protein levels could be the result of proteins being overexpressed and/or abnormally shed and added to the serum proteome, clipped or modified as a consequence of the disease process, or subtracted from the proteome owing to abnormal activation of the proteolytic degradation pathway. Quaternary effects due to disease-related protein–protein interactions and protein-complex formation can also modify and subtly change the serum proteome.

Using this method, one microlitre of raw, unfractionated serum from patients is analysed by surface-enhanced laser-desorption ionization time-of-flight spectrometry (SELDI-TOF) to give a proteomic signature of the serum (FIG. 3). The experimenter applies unfractionated serum directly to the surface of a treated metal bar. A subset of the proteins in the serum binds to the surface of the bar, and the unbound proteins are washed away. The adherent proteins are treated with acid (so that they become ionized by the laser energy) and are then dried down onto the bar surface. The bar containing the individual, captured serum protein samples as a row of spots (FIG. 3) is then inserted into a vacuum chamber, and a laser beam is fired at each spot.

METASTASIS
The movement or spreading of cancer cells from one organ or tissue to another. Cancer cells usually spread through the bloodstream or lymph system.

PERITONEAL CAVITY
The peritoneum is the thin membrane that lines the abdominal cavity.

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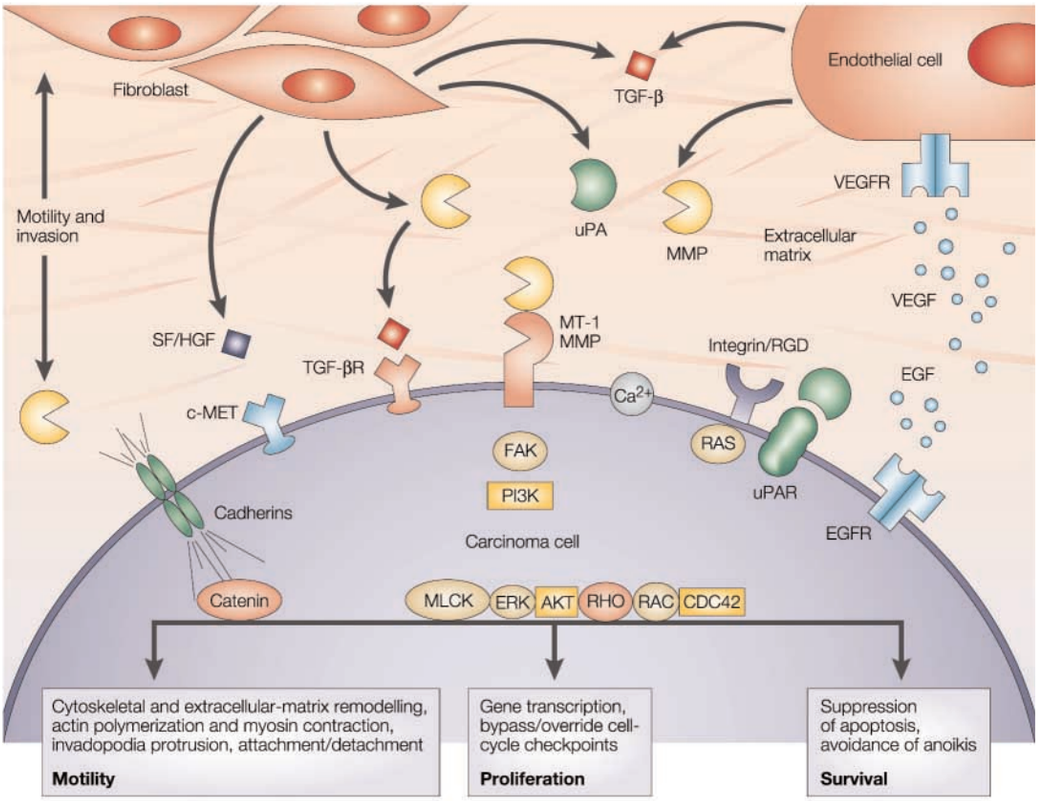

Figure 2 | **Tumour–host interaction.** Cancer is a disease of the tissue microenvironment. Interactions between the cancer cell and the host (cellular and extracellular matrix) promote tumour-cell growth, invasion, angiogenesis and survival. Examples include the exchange of growth factors, degradative enzymes and motility-stimulating molecules. AKT, v-akt murine thymoma viral oncogene homologue; CDC42, cell-division cycle 42; c-MET, MET proto-oncogene; EGF, epidermal growth factor; EGFR, EGF receptor; ERK, extracellular-signal-regulated kinase; FAK, focal adhesion kinase; MLCK, myosin light-chain kinase; MMP, matrix metalloproteinase; MT-1, metallothionein 1; PI3K, phosphatidylinositol 3-kinase; RAC, a member of the RAS superfamily of small G proteins; RGD, Arg-Gly-Asp motif; RHO, a member of the RAS superfamily of small G proteins; SF/HGF, scatter factor/hepatocyte growth factor; TGF-β, transforming growth factor-β; TGF-βR, TGF-β receptor; uPA, urokinase plasminogen activator; uPAR, uPA receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

The laser energy desorbs the ionized proteins, and the launched proteins fly down the vacuum tube towards an oppositely charged electrode. Each ion that strikes the electrode registers as a component of the data spectrum that emerges from the analysis. The mass-to-charge ratio (m/z) of each ion can be estimated by the time it takes for the launched ion to reach the electrode — small ions travel faster. So, the spectrum provides a 'time of flight' (TOF) bar code of ions ordered by size. This serum proteomic bar code consists of thousands of protein ion signatures, which require highly ordered data-mining operations for analysis.

Many bioinformatics data-mining systems are being developed, but most fall into two main types of approach. The first approach involves supervised systems that require a corpus of knowledge or data for which the outcome or classification is known ahead of time to TRAIN on. Examples of such approaches are linear-regression models, nonlinear feedforward neural networks (NLFN) and genetic algorithms (GAs)[20–27]. The second type of approach involves unsupervised systems that cluster or group records without previous knowledge of outcome or classification. Example approaches are *k*-means nearest-neighbour analysis, Euclidean distance-based nonlinear methods, fuzzy-pattern matching methods and self-organizing mapping (SOM)[28–30]. The problem, however, is the same for either type of system: finding optimal feature sets or, in this instance, proteins, in a large, unbounded information archive that is unknown at this time. A typical SELDI-TOF proteomic profile will have up to 15,500 data points representing m/z values between 500 and 20,000. Artificial intelligence (AI)-based systems that learn, adapt and gain experience over time are uniquely suited to proteomic data analysis because of the huge dimensionality of the proteome itself. The application of these AI systems to analysing mass-spectral data derived from the serum proteome has given rise to a new analytical paradigm: proteomic pattern diagnostics (BOX 1).

As each new patient is validated through pathological diagnosis using retrospective or prospective study sets, the input data from the patient can be added to an ever-expanding training set. The AI tool learns, adapts and gains experience through constant vigilant retraining.

TRAINING
A process in which a computer-driven system is provided data from a training set in which the outcome is known and is unblinded.

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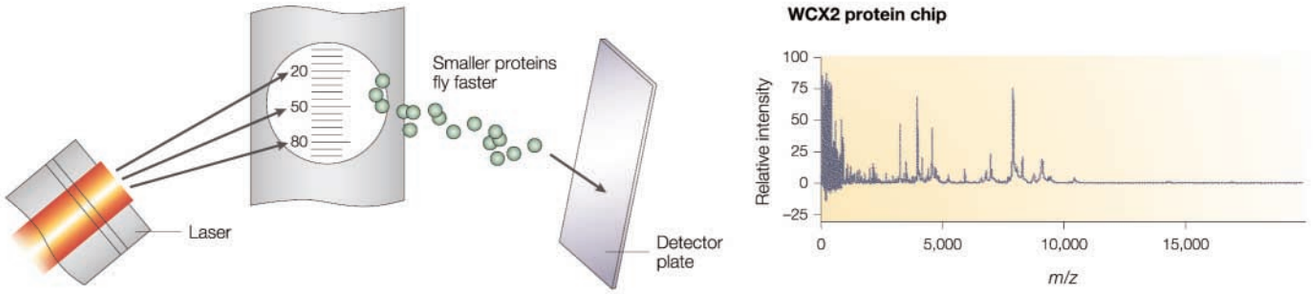

Figure 3 | **SELDI mass spectrometry.** Surface-enhanced laser-desorption ionization (SELDI) is a class of mass spectrometry that is useful for high-throughput proteomic fingerprinting of serum. Using a robotic sample dispenser, one microlitre of serum is applied to the surface of a protein-binding chip. A subset of the proteins in the sample bind to the surface of the chip. The bound proteins are treated with a MALDI (matrix-assisted laser-desorption ionization) matrix, and are then washed and dried. The chip, which contains several patient samples, is inserted into a vacuum chamber, where it is irradiated with a laser. The laser desorbs the adherent proteins, causing them to be launched as ions. The 'time of flight' (TOF) of the ion before detection by an electrode is a measure of the mass-to-charge ratio (*m/z*) of the ion. The ion spectrum can be analysed by computer-assisted tools to classify a subset of the spectrum by characteristic patterns of relative intensity. WCX2, weak cation-exchange surface.

In fact, it is possible to generate not just one, but multiple combinations of proteomic patterns from a single mass-spectral training set, each pattern combination re-adjusting as the models improve in the adaptive mode. This is exactly what has been observed with the expanding set of sera from ovarian cancer patients, which has given rise to multiple combinations of proteomic patterns that are more than 98% sensitive and specific. The initial and reported discriminatory pattern had a sensitivity of 100% and a specificity of 95% for ovarian cancer at all stages[19]. One of the newer discriminatory patterns, which has key discriminatory values at *m/z* ratios of 554, 601, 834, 5,134 and 16,292, was shown to be 100% sensitive and specific in a blinded set of 52 healthy individuals and 92 cancer patients (with stage I, II or III ovarian cancer), including 15 patients with stage I ovarian cancer. These new spectra are posted on the web site of the Clinical Proteomics Program Databank.

Disease-tailored therapeutic targets

Most current therapeutics are directed at protein targets. The source material for the identification of new protein targets is shifting away from tissue-culture cells to the discovery of proteins that change in actual diseased human tissue. The cellular proteome is constantly fluctuating depending on the cellular microenvironment. Consequently, proteomic changes in cell lines might not fully model human disease. Moreover, tissues are heterogeneous; they are composed of hundreds of interacting cell populations. New technology has now made it possible to analyse diseased cells in the tissue section itself[31], or to physically separate the desired cells directly from the surrounding contaminating cells. Laser-capture microdissection (LCM) is a technology that allows the researcher to procure pure cell populations from heterogeneous tissue sections under direct microscopic visualization[32]. 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 the actual tissue[32–39] (BOX 2).

Whole portfolios of drug targets, imaging markers and early-detection biomarkers will arise from hypothesis-generating, discovery-based proteomic platforms. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) has traditionally been the gold-standard discovery-based tool for proteomics[40–41]. Unfortunately, even with the advent of 'zoom gels', which use ultra-narrow pI GRADIENTS, only a small percentage of the entire proteome can be visualized by 2D-PAGE. Now, newer technologies that can access much more of the lower-abundance region of the proteome are being developed. Multiplexed, in-line liquid-chromatographic separation systems coupled directly to mass spectrometry (LC–LC–LC–MS/MS) to analyse affinity-tagged cellular lysates and protein mixtures are being developed[43–49], and could someday replace gel-based systems, such as 2D-PAGE. Nevertheless, 2D-PAGE remains a standard and reliable separation technology, especially for the larger-molecular-mass region of the proteome (a region that cannot be adequately resolved and analysed by even the more advanced liquid separation systems and mass spectrometers). As it is a key and complementary proteomic technology, 2D-PAGE methodology is now being adapted to higher-throughput and higher-sensitivity applications and modifications. One such adaptation uses the same CY3/CY5 dual-dye labelling strategy that has been used so successfully for complementary DNA and oligonucleotide arrays[50]. In this format, LCM-based cellular lysates from patient-matched-control and tumour epithelium are differentially labelled, each with a different fluorophore, and the lysates mixed together after labelling and run together on one gel (FIG. 4). As both lysates are run concomitantly on the same gel, a direct comparison between the two samples can be carried out more easily and in a reproducible manner. Furthermore, as fluorescence has a large, quantitative dynamic range compared with colorimetric base-staining systems, small but important changes in the relative abundances of each protein isoform can be determined. This system has distinct advantages over established methodology,

2D-PAGE
A method for separating proteins by mass and charge.

pI GRADIENTS
Isoelectric gradients are formed by subjecting a defined set of small molecules with specific net charges to an electric current, which allows the separation of proteins within the gradient, even if the proteins only differ in charge by one-thousandth of a pH unit.

CY3/CY5
Cy3 and Cy5 are water-soluble cyanine dyes that can be used as fluorescent labels for proteins and modified oligonucleotides.

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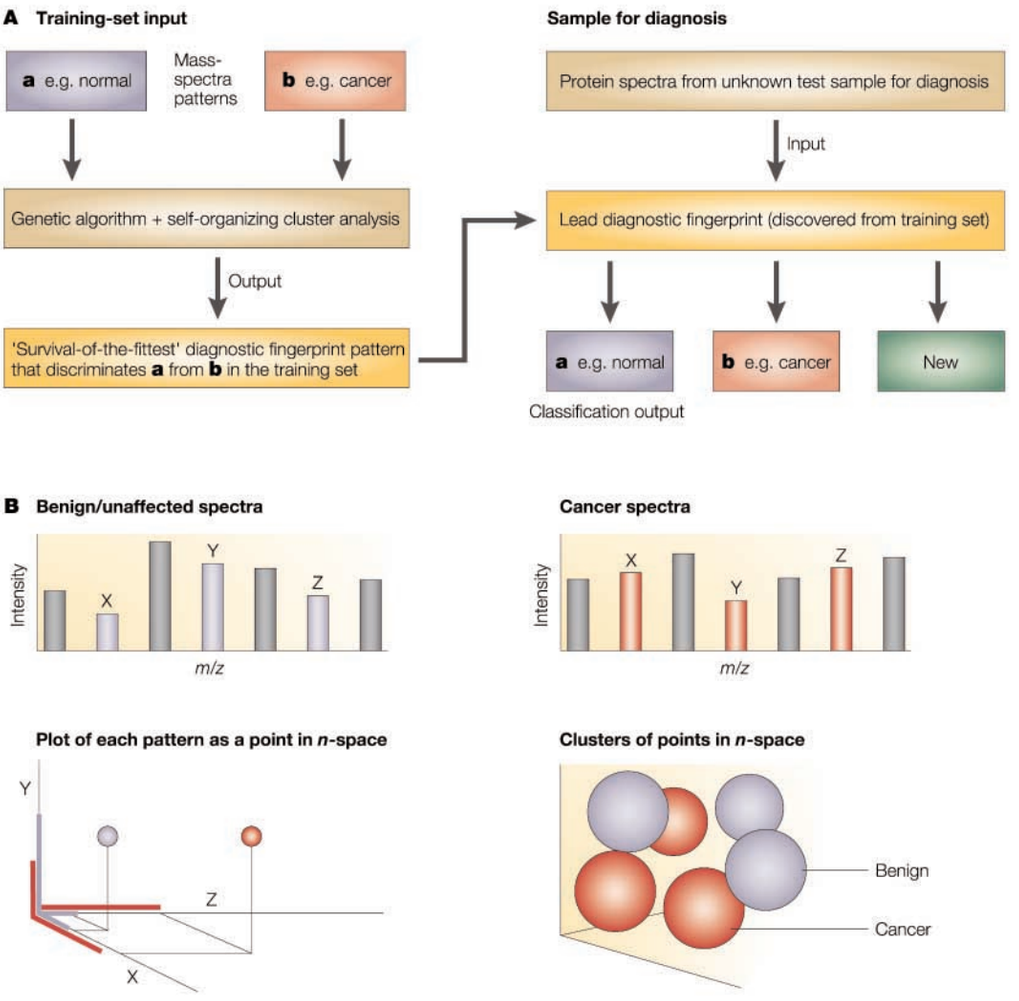
Box 1 | Proteomic pattern diagnostics

Proteomic pattern analysis begins with artificial intelligence (AI)-based computer searching of mass-spectrometry data to find the 'most-fit' combination of proteins through the use of a training set and a blinded test set (panel A). The training sets comprise serum from individuals who are healthy or have active disease at the time of serum collection. The AI engine first uses a genetic algorithm to search though the 15,500 data points by parsing the data into 'data packets' that contain 5–20 *m*/*z* (protein ion mass-to-charge ratio) values. The engine then searches through combinations of protein signatures within the training set until it finds the best combination of 5–20 proteins with combined relative abundances that are different in the disease cohort relative to the healthy population. Much of the mass spectrum is background noise, so identifying true protein ion signatures requires a system that can rapidly and iteratively search through the decision space. The parsing of data into packages of 5–20 values creates $15{,}500 \times 10^5$–$15{,}500 \times 10^{20}$ combinations, or about 1.5×10^9–1.5×10^{24} patterns. If each of these combinations was explored one at a time, it would take a computer performing 1×10^9 operations per second more than 47 million years to find the optimal discriminatory pattern. Genetic algorithms can find near-optimal solutions to these massive sets in only a few days through iterative searching, REMATING and recombination of the data packets, and applying 'SELECTIVE PRESSURE'. The systems use a fitness test, such as an unsupervised self-organizing mapping (SOM)-based adaptive clustering program.

Clusters are formed in fifth-to-twentieth-dimensional space by the vector plots of the Euclidean distance values obtained by the combined relative peak intensities that are selected at the *m*/*z* values chosen by the genetic algorithm (panel B). Once an optimal combination pattern has been found, incoming (blinded) data are analysed rapidly by the software, simply by plotting in the fifth-to-twentieth-dimensional vector space the combined relative amplitudes of the subset of the key discriminatory proteins and finding whether they fall into the clusters formed by the training set.

If the blinded spectral plot falls within an existing cluster that contains only cancer patients, then the sample is classified as cancerous; if it falls into an existing cluster that contains only healthy patients, it is classified as normal. If the *n*-dimensional vector plot falls outside any cluster, it forms its own new cluster, and the model adapts on the basis of the unblinded classification.



REMATING
A computer-driven data-analysis process that is performed by combining solutions to select and retain the best elements of each solution, and discard those elements that do not solve the problem.

SELECTIVE PRESSURE
The ability of a computer-driven data-analysis process to restrict the solution output to those that pass a fixed criteria — in this case, the ability to distinguish normal from cancerous samples.

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which requires two separate 2D-gel runs and complicated alignment and warping procedures to minimimize between-run variability.

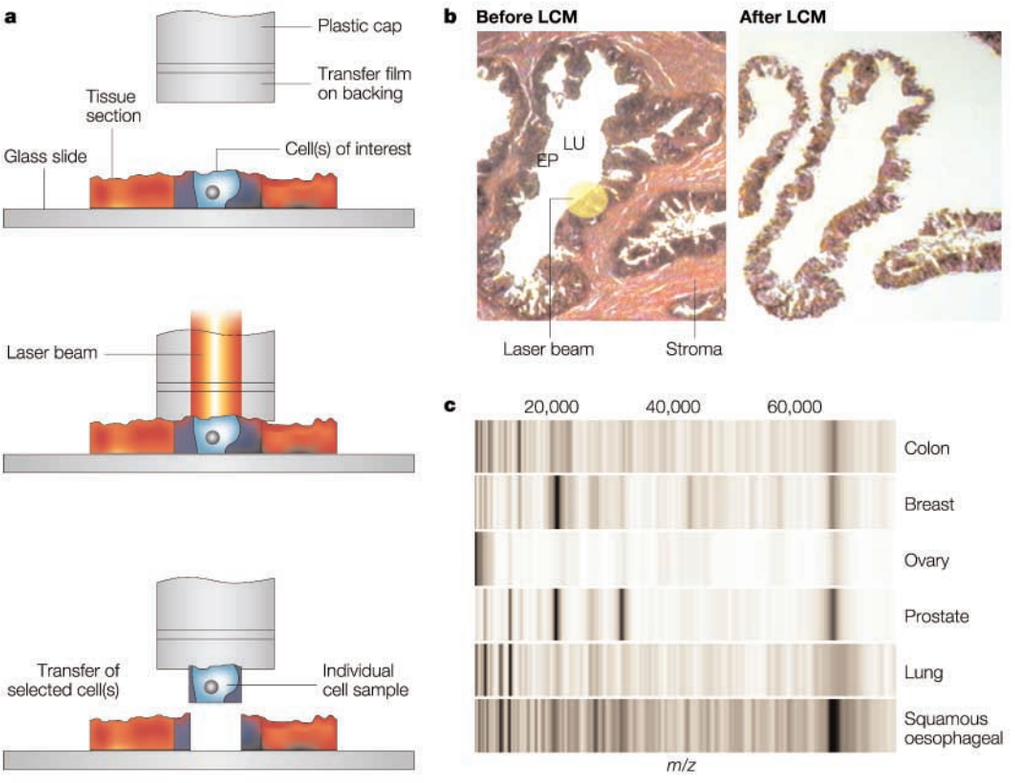
Despite their sophistication, the new proteomic technologies have substantial limitations when they are applied to tissue and blood samples. Discovery platforms, such as 2D gels, isotope-coded affinity tagging (ICAT) multidimensional LC–MS platforms and antibody arrays, require large cellular input samples that are orders of magnitude greater than those procured during a clinical biopsy[43–53]. Clinical specimens might contain only a few hundred cells as the starting point for analysis. The use of clinical-trial material for proteomic analysis requires the development of new technologies that can use these small amounts of cellular material as a starting point for discovery and profiling, and which can then be used for validation of potential targets in patients. A second limitation of the newer 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. Consequently, new microproteomic

Box 2 | **Laser-capture microdissection**

Laser-capture microdissection (LCM) is a technology for procuring pure cell populations from a stained tissue section under direct microscopic visualization. Tissues contain heterogeneous cellular populations (for example, epithelium, cancer cells, fibroblasts, endothelium and immune cells). The diseased cellular population of interest usually comprises only a small percentage of the tissue volume. LCM directly procures the subpopulation of cells selected for study, while leaving behind all of the contaminating cells.

A stained section of the heterogeneous tissue is mounted on a glass microscope slide and viewed under high magnification (panel a). The experimenter selects the individual cell(s) to be studied using a joystick. The chosen cells are lifted out of the tissue by the action of a laser pulse. The infrared laser, mounted in the optical axis of the microscope, locally expands a thermoplastic polymer to reach down and capture the cell beneath the laser pulse. When the film is lifted from the tissue section, only the pure cells for study are excised from the heterogeneous cellular population (panel b). The DNA, RNA and proteins of the captured cells remain intact and unperturbed. Using LCM, one to several thousand tissue cells can be captured in less than five minutes.

Using appropriate buffers, the cellular constituents are solubilized and subjected to microanalysis methods. Proteins from all compartments of the cell can be readily procured. Protein conformation and enzymatic activity is retained if the tissues are frozen or fixed in ethanol before sectioning. The extracted proteins can be analysed by any method that has sufficient sensitivity. An example is shown for surface-enhanced laser-desorption ionization (SELDI) fingerprinting of microdissected cancer cells[83] (panel c). Each histological type of cancer has a characteristic ion spectrum. The character of the spectra might reflect cancer-specific differences in proteomic composition. EP, epithelium; LU, lumen.



ICAT
A method in which proteins are labelled using isotope-coded affinity tags, which allows them to be systematically identified and quantified.

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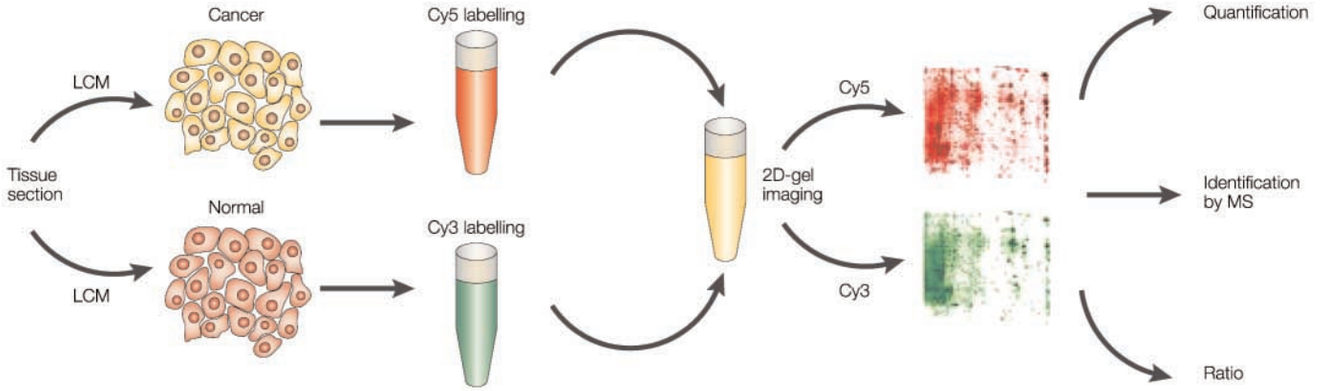

Figure 4 | **Two-colour 2D-gel methodology.** Protein lysates are prepared from laser-capture microdissected (LCM) cellular populations derived from two pathological states (for example, carcinoma and normal epithelium). Each lysate is labelled with a fluorescent dye of a different colour (Cy3, green; Cy5, red). The labelled samples are mixed and run together on a two-dimensional (2D) gel. The gel is illuminated at two wavelengths, each being specific for a particular dye. The resulting relative intensity of the spots can then be correlated with the disease state, each state serving as a control for the other. The spots representing proteins that are augmented or reduced can be cut out of the gel, further purified by chromatography and identified by sequencing using mass spectrometry (MS).

technologies need to be developed, so that clinical scientists can gain access to the information content of the cellular-circuit networks that could be targeted for therapeutic intervention.

Protein microarrays represent the first new technology that can actually profile the state of a signalling-pathway target even after the cell has lysed[54,55] (FIG. 5). A new type of protein array, the reverse-phase protein array, has shown a unique ability to analyse signalling pathways using small numbers of human tissue cells microdissected from biopsy specimens procured during clinical trials[55] (FIG. 6A). Using this approach, LCM-procured, pure cell populations are taken from human biopsy specimens, and a protein lysate is arrayed onto nitrocellulose slides. Key technological components of this method offer unique advantages over tissue arrays[56] or antibody arrays[38,50,51]. First, the reverse-phase array can use denatured lysates, so that antigen retrieval, which is a large limitation for tissue arrays, is not problematic. Second, the arrays can 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 characterized. Third, each patient sample is arrayed in a miniature dilution curve, providing an internal standard curve. Direct quantitative measurements can be ascertained,

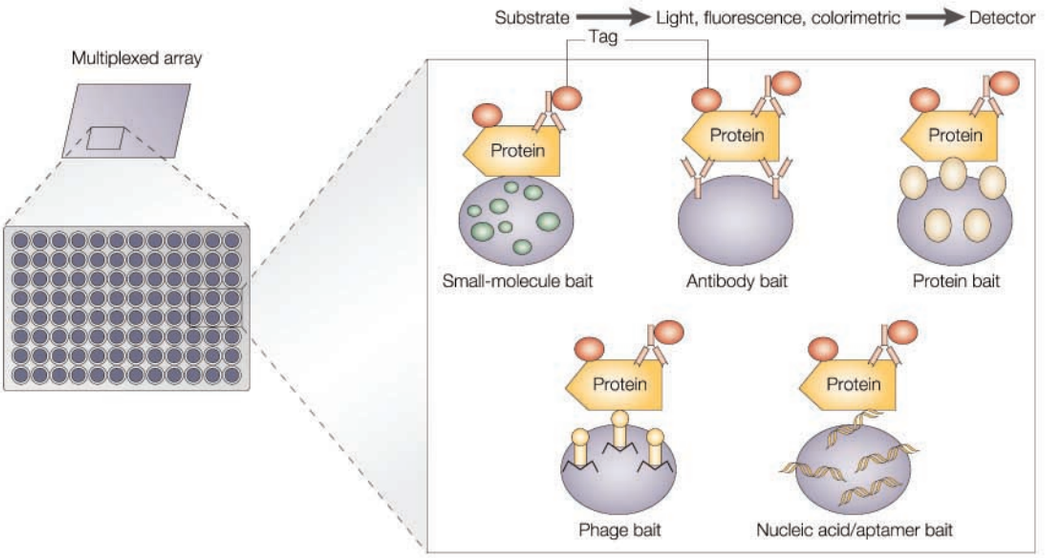

Figure 5 | **Protein microarray.** Protein microarrays consist of an array of protein samples, or protein baits, immobilized on a solid phase. The array is queried with a mixture of labelled proteins containing analytes of interest. The analyte proteins are captured and can be detected using fluorescence, colorimetric or chemiluminescence means.

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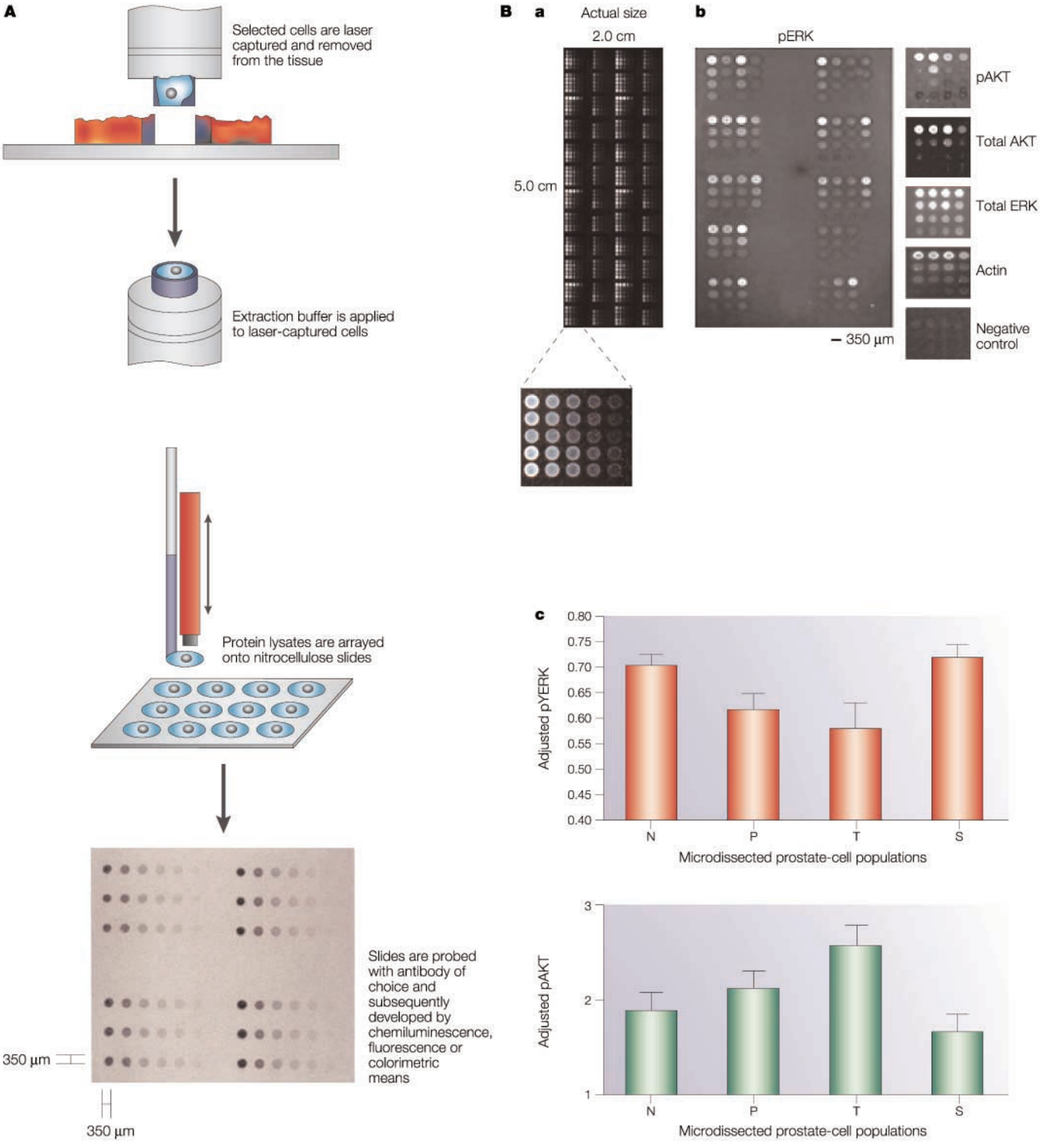

Figure 6 | **Reverse-phase arrays. A** | A new class of protein array is the reverse-phase array, which immobilizes the cellular lysate sample to be analysed. Lysates are prepared from cultured cells or microdissected tissues and arrayed in miniature dilution curves. The analyte molecule that is contained in the sample is then detected by a separate labelled probe (for example, an antibody), which is applied to the surface of the array. This array is highly linear and sensitive, and requires no labelling of the sample proteins. **B** | Signal-pathway profiling using reverse-phase arrays. **a** | Arrays comprising miniature dilution curves of hundreds of patient specimens can be placed on one array. **b** | Laser-capture microdissection (LCM)-procured, patient-matched normal, premalignant, invasive cancer and stromal cells from prostate cancer patients are analysed for ERK and AKT signalling using phosphospecific antibody reactivity. Normalization to the total cognate protein allows detailed molecular analysis. **c** | Adjusted levels of phosphorylated ERK (pERK) and AKT (pAKT) reveal an increasing activation of AKT and a concomitant decrease in the activation of ERK as the cancer cell progresses. AKT, v-akt murine thymoma viral oncogene homologue; ERK, extracellular-signal-regulated kinase; N, normal; P, pre-malignant; S, stroma; T, tumour.

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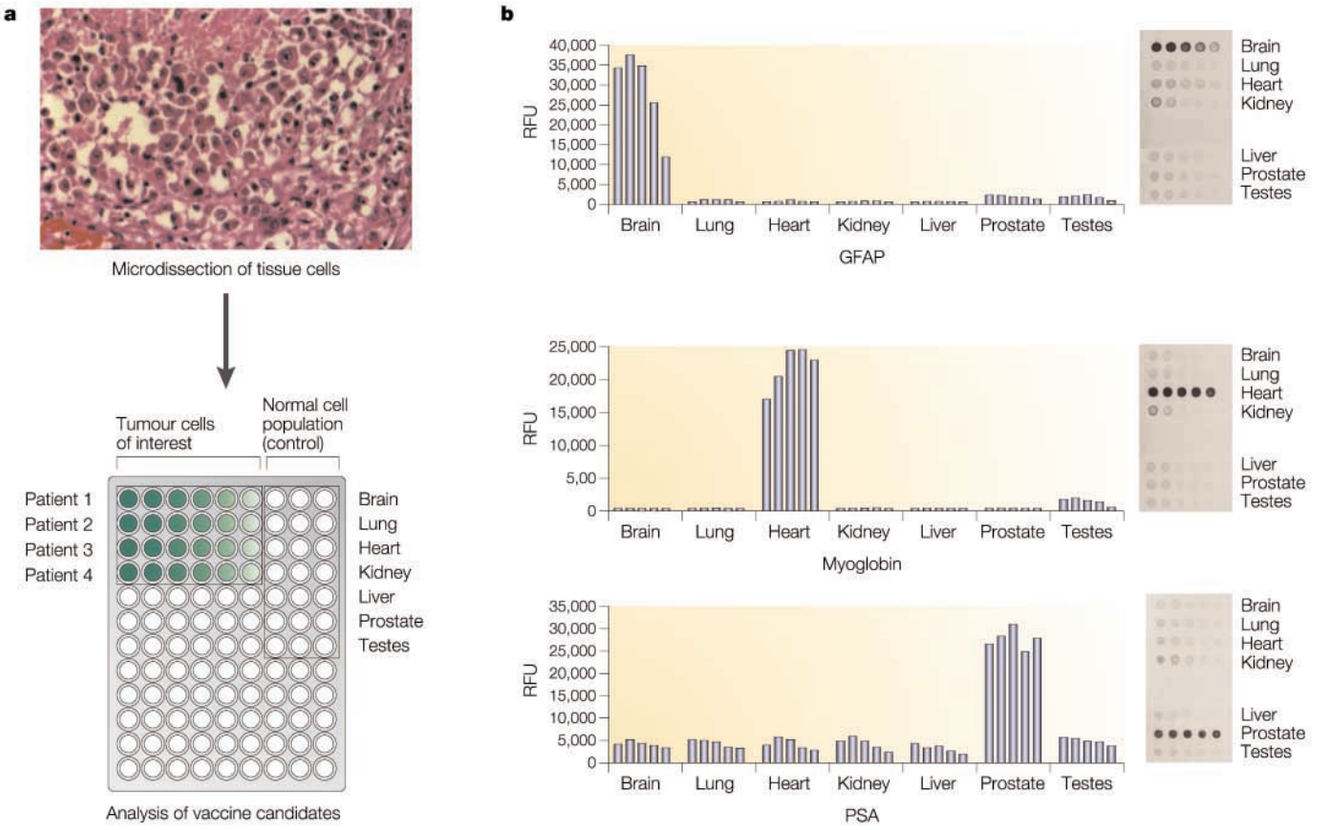

Figure 7 | **Whole-body array. a** | An extension of reverse-phase arrays is designed to profile protein samples that are derived from pure, microdissected human-organ-specific cells. Each array can directly compare cancer-cell lysates with lysates derived from all the main vital body organs. **b** | Specificity of body arrays. Organ-cell proteomic discrimination is shown using analytes that are specific for each organ type. GFAP, glial fibrillary acidic protein; PSA, prostate-specific antigen, RFU, relative fluorescent units.

as at any given point in the dilution curve, the measurement is within the linear dynamic range of the antibody–analyte interaction. Fourth, reverse-protein microarrays do 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.

Reverse-phase arrays can now be used to study key 'nodes' in the cellular circuitry and to profile the functional state of protein pathways and signalling events within the cells that are contained in biopsy samples. Recently, this platform was used to address the basic, but previously unanswered, question of whether premalignant transformation is caused by an increase in the cell growth rate through the activation of mitogenic growth pathways (for example, phosphorylation of extracellular-signal-regulated protein kinases (ERKs)), or whether early cancer is driven by a decrease in the cell death rate through the activation of apoptosis-inhibiting, pro-survival signalling pathways (for example, phosphorylation of AKT (also known as protein kinase B; PKB))[55]. Reverse-phase analysis of study sets of LCM-procured, patient-matched, normal epithelial cells, premalignant cells and invasive prostate-carcinoma cells showed that phosphorylation and activation of AKT occurred as a crucial early step in the progression of cancer[55] (FIG. 6B). So, the increase in the build-up of cells that is seen during early-stage prostate cancer (prostatic intra-epithelial neoplasia) is caused by an alteration of the cellular turnover due to a decrease in the death rate, not by induction of the growth rate. Consequently, inhibition of AKT activity through molecular-targeted therapeutics could have a profound impact in treating and preventing the progression of prostate cancer.

Moreover, the arrays can now be manufactured in a sectored-array format, in which dozens of analytes can be queried simultaneously on one slide, thereby increasing the throughput and ease of data analysis. We are now attempting to use this technology at the National Cancer Institute to record the phosphorylation status of hundreds of nodes in the cellular circuitry of cancer cells both before and after therapy, to normalize each of these outcomes against the total self-protein (for example, phospho-ERK/total ERK, phospho-aurora 2/total aurora 2) and to analyse the data through clustering analysis. This will yield a true picture of the coordination of signalling events as they change and flux in response to targeted therapy. Reverse-phase technology is applicable to the identification and characterization of targets that could be candidates for T-cell-mediated

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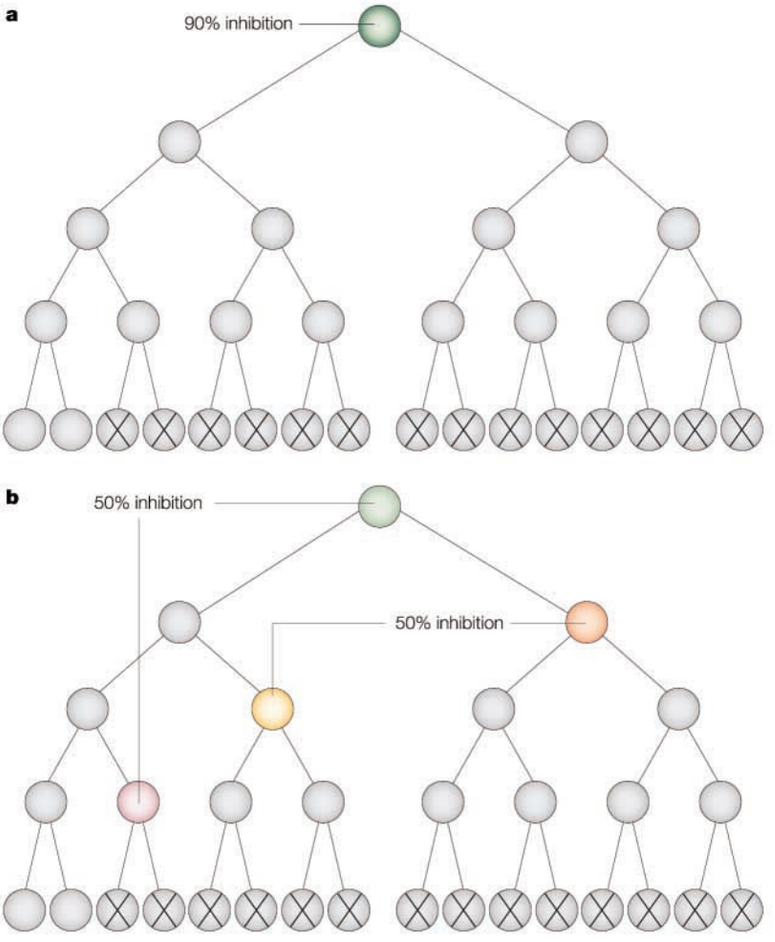

Figure 8 | **Combinatorial therapy.** A generic signalling cascade is depicted. **a** | To effectively shut off 90% of the deranged signalling emanating from the activated node, a high dose of a single drug (filled green circle) is required to shut down that one node and the pathway. **b** | By contrast, targeting with a combination of drugs, each shutting off unique but interconnected nodes (coloured circles) within the pathway that flows from the single derangement can achieve the same efficacy with a lower dose of each drug.

vaccines. At present, if an investigator feels that he/she has discovered a protein that is overexpressed in a cancer cell and warrants evaluation as a potential vaccine candidate, it is necessary for the expression status of that protein to be measured in all other normal cell types, so that immune-mediated toxicities are reduced or eliminated altogether. We are now developing microdissection-based vital-organ and whole-body arrays using the reverse-phase format to curate proteins that are specifically overexpressed in premalignant and cancer cells (FIG. 7). This array incorporates LCM-procured normal cell populations derived from patient-matched, normal-organ specimens. An organ-profiling array can be developed that represents the protein composition of cells from the brain, heart, lung, liver, kidney, testes, ovaries, prostate, uterus and so on, while containing cells from candidate cancer-biopsy sets. Specificity can be demonstrated through the use of antibodies against organ-specific antigens, such as glial fibrillary acidic protein (GFAP), myoglobin and prostate-specific antigen (PSA) for brain, heart and prostate parenchymal cells, respectively (FIG. 7). The ideal vaccine candidate should have high abundance in the cancer cells, but low abundance in the cells of vital organs. Organ arrays offer a high-throughput means to screen and assign priority to vaccine candidates. Probes for ideal vaccine targets, when applied to the array, should preferentially recognize the immobilized cancer cell proteins, but should not bind to the array sectors derived from the normal organs. Protein-array formats could also be applied to monitor and assess the efficacy of gene-therapy-based applications in which modification of stem cells or cancer cells is attempted[57,58]. However, important limitations to protein-array-based technologies are the constraints that are imposed by the fidelity, specificity and affinity of the antibodies or bait molecules that are used. So, broad-based clinical-proteomics efforts need to use these profiling tools concomitantly with discovery-based approaches, such as 2D-PAGE and expression profiling by means of mass spectrometry.

Personalized medicine

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 derangements. So, there is a strong justification to develop a strategy that could select from a menu of treatment choices, or treatment combinations, those that best match the individual molecular profile of a tumour[59–67]. Molecular profiling using gene arrays has shown considerable potential for the classification of patient populations according to disease stage or survival outcome[23,24,68]. Nevertheless, transcript profiling, by itself, might provide an incomplete picture, because gene-transcript level might bear no relationship to the phosphorylated or otherwise functional state of the encoded protein. Moreover, gene transcripts provide little information about protein–protein interactions and the state of the cellular circuitry. So, the application of molecular profiling to select the appropriate treatment strategy should include direct proteomic-pathway analysis of the biopsy material.

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 (FIG. 8). Such an approach could, theoretically, achieve a higher efficacy with a lower toxicity.

Protein kinases are the key molecules that make up these nodes in the cellular circuitry, and their aberrant function is often at the centre of many diseases, including cancer[69–74]. The new focus of narrowly focused molecular-targeted therapeutics addresses this concept. STI-571 (Gleevec, imatinib mesylate) is a key example. Treatment with Gleevec targets the dominant activity of the ABL protein kinase by binding to and blocking its ATP-binding domain. Although this pathogenic proteomic circuit has a genetic underpinning — in this case, a chromosomal translocation — it is the deranged proteomic function that dictates the biological

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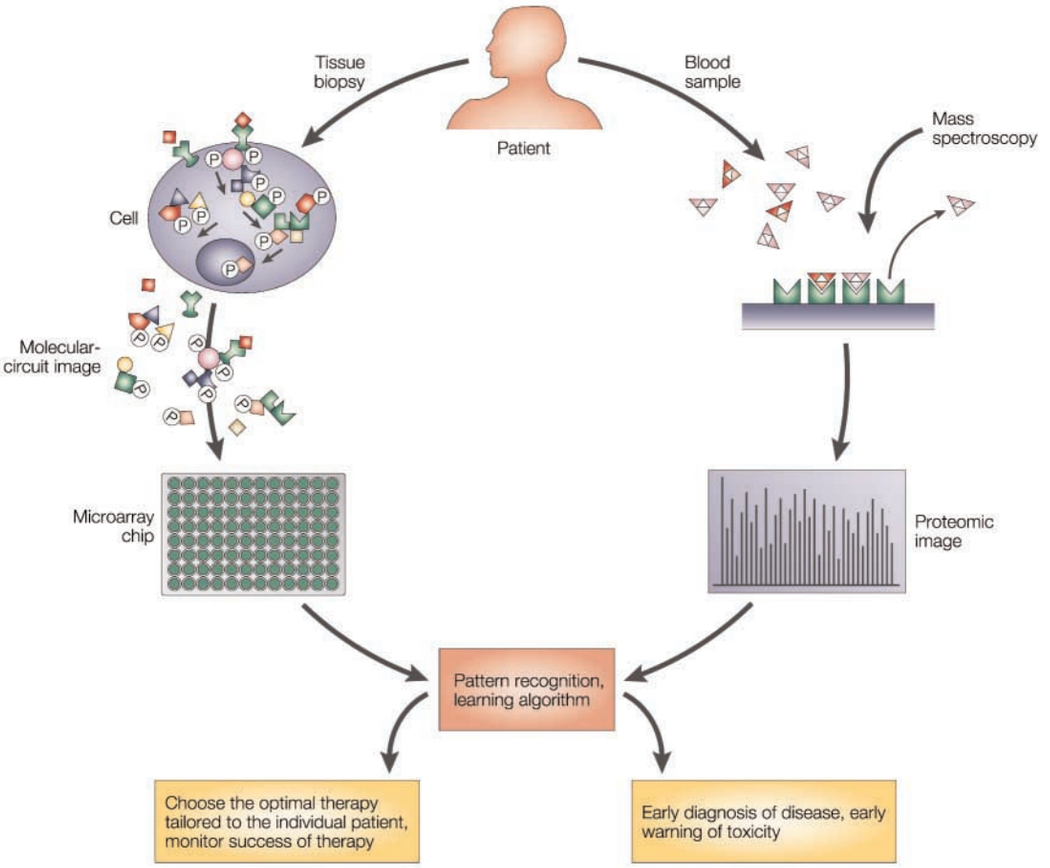

Figure 9 | **Proteomic technology applied to cancer-patient management.** Proteomic pattern analysis of serum has the potential to detect early-stage disease, toxicity or recurrence. Once the disease has been diagnosed and biopsied, protein microarrays coupled with laser-capture microdissection (LCM) offer a means to profile the individual signal pathways that are deranged in the tumour cells of the patient. In this manner, the combinatorial therapy can be tailored to, and monitored in, the individual patient.

outcome[75,76]. More than half of the estimated 1,000 kinases in the human proteome have yet to be identified, and their central roles in cellular signalling have not been defined. Drug discovery efforts that centre on the development of small-molecular-mass compounds and protein drugs that can specifically block kinases are an intense focus of the biotechnology and pharmaceutical industries owing to their key roles as 'gatekeepers' of the cellular circuitry[77]. However, other classes of molecules might also be useful targets, especially for T-cell vaccine-based therapy. At present, four molecules that block kinase activity are being investigated in Phase III trials, and as many as 30 kinase inhibitors are being evaluated in Phase I/II trials[78,79].

Proteomic signalling pathways consist of an amplification cascade of enzymatic events. The stages of the pathway can be ordered from upstream to downstream events (FIGS 1 and 8). The conventional pharmacological approach has been to select a single upstream target as the drug target. To completely shut down the entire pathway, it is necessary to treat the upstream target at a drug concentration that blocks the target with a high degree of efficiency (>85%). At this high concentration, the drug might be in the dose range that produces unwanted toxic side effects.

Combinatorial therapy, an alternative approach to single-agent therapy, offers the promise of higher specificity at lower treatment doses[80–82]. A correctly chosen series of inhibitors that act at several points along the signalling pathway can be used at low concentrations, yet the result can be a complete shutdown of the pathway. The advantage is realized 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. 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 implementation of the regime to monitor the combined toxic effects of the drugs on normal cell populations.

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So, the use of clinical-proteomic tools, such as whole-body protein arrays, becomes even more relevant to this emerging era of patient-tailored molecular medicine, and — *a priori* — could aid in the analysis of desired drug effects on target pathways and unwanted toxic effects on the circuitry within normal cell populations. Furthermore, serum proteomic pattern analysis can be used to monitor patterns associated with occult drug-induced toxicity. Proteomic pattern analysis can also be used in the lead-optimization and preclinical phases of drug development, in which serum proteomic patterns that are associated with known drug-induced toxicities can be matched against the experimental therapeutic and predictive correlates to guide and select which compounds should be taken forward or shelved.

The future of clinical proteomics

Clinical proteomics can have important direct 'bedside' applications. We can foresee a future in which the physician will use these different proteomic analyses at many points of disease management. The paradigm shift will directly affect clinical practice owing to its effects on all of the following crucial elements of patient care and management (FIG. 9): early detection of the disease using proteomic patterns of body-fluid samples; diagnosis based on proteomic signatures as a complement to histopathology; individualized selection of therapeutic combinations that best target the entire disease-specific protein network of a patient; real-time assessment of therapeutic efficacy and toxicity; and rational redirection of therapy on the basis of changes in the diseased protein network that are associated with drug resistance.

1. Liotta, L. & Petricoin, E. Molecular profiling of human cancer. *Nature Rev. Genet.* **1**, 48–56 (2000).
A description of new genomic and proteomic technologies that are allowing the reclassification of human cancer on the basis of molecular portraits instead of histology alone.
2. Ideker, T. *et al.* Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* **292**, 929–934 (2001).
3. Schwikowski, B., Uetz, P. & Fields, S. A network of protein–protein interactions in yeast. *Nature Biotechnol.* **18**, 1257–1261 (2000).
4. Legrain, P., Jestin, J. L. & Schachter, V. From the analysis of protein complexes to proteome-wide linkage maps. *Curr. Opin. Biotechnol.* **4**, 402–407 (2000).
5. Blume-Jensen, P. & Hunter,T. Oncogenic kinase signaling. *Nature* **411**, 355–365 (2001).
6. Pawson,T. Protein modules and signaling networks. *Nature* **373**, 573–580 (1995).
7. Liotta, L. A. & Kohn, E. C. The microenvironment of the tumour–host interface. *Nature* **411**, 375–379 (2001).
8. Ozols, R. F., Rubin, S. C., Thomas, G. M. & Robboy, S. J. in *Principles and Practice of Gynecologic Oncology* (eds Hoskins, W. J., Perez, C. A. & Young, R. C.) 981–1058 (Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, 2000).
9. Menon, U. & Jacobs, I. in *Principles and Practice of Gynecologic Oncology* (eds Hoskins, W. J., Perez, C. A. &, Young, R. C.) 165–182 (Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, 2000).
10. Bast, R. C. *et al.* A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N. Engl. J. Med.* **309**, 883–887 (1983).
11. Menon, U. & Jacobs, I. J. Recent developments in ovarian cancer screening. *Curr. Opin. Obstet. Gynecol.* **12**, 39–42 (2000).
12. Jacobs, I. J. *et al.* Screening for ovarian cancer: a pilot randomized controlled trial. *Lancet* **353**, 1207–1210 (1999).
13. Cohen, L. S., Escobar P. F., Scharm, C., Glimco, B. & Fishman, D. A. Three-dimensional power Doppler ultrasound improves the diagnostic accuracy for ovarian cancer prediction. *Gynecol. Oncol.* **82**, 40–48 (2001).
14. Adam, B. L., Vlahou, A., Semmes, O. J. & Wright, G. L. Jr. Proteomic approaches to biomarker discovery in prostate and bladder cancers. *Proteomics* **1**, 1264–1270 (2001).
15. Carter, D. *et al.* Purification and characterization of the mammaglobin/lipophilin B complex, a promising diagnostic marker for breast cancer. *Biochemistry* **41**, 6714–6722 (2002).
16. Rosty, C. *et al.* Identification of hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein I as a biomarker for pancreatic ductal adenocarcinoma by protein biochip technology. *Cancer Res.* **62**, 1868–1875 (2002).
17. Xiao, Z. *et al.* Quantitation of serum prostate-specific membrane antigen by a novel protein biochip immunoassay discriminates benign from malignant prostate disease. *Cancer Res.* **61**, 6029–6033 (2001).
18. Kim, J. H. *et al.* Osteopontin as a potential diagnostic biomarker for ovarian cancer. *JAMA* **287**, 1671–1679 (2002).
19. Petricoin, E. F. *et al.* Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* **359**, 572–577 (2002).
This paper describes the invention of a new process and diagnostic paradigm that is based on serum proteomic pattern analysis instead of a discovery effort based on a 'one-biomarker-at-a-time' approach.
20. Ball, G. *et al.* An integrated approach utilizing artificial neural networks and SELDI mass spectrometry for the classification of human tumours and rapid identification of potential biomarkers. *Bioinformatics* **18**, 395–404 (2002).
21. Ting, K. L., Lee, R. C., Chang, C. L. & Guarino, A. M. The relationship between the mass spectra of drugs and their biological activity — an application of artificial intelligence to chemistry. *Comput. Biol. Med.* **4**, 301–332 (1975).
22. Nicholson, J. K., Connelly, J., Lindon, J. C. & Holmes, E. Metabonomics: a platform for studying drug toxicity and gene function. *Nature Rev. Drug Discov.* **1**, 153–161 (2002).
23. Alizadeh, A. A. *et al.* Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* **403**, 503–511 (2000).
24. Golub, T. R. *et al.* Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* **286**, 531–537 (1999).
25. Lindahl, D., Palmer, J. & Edenbrandt, L. Myocardial SPET: artificial neural networks describe extent and severity of perfusion defects. *Clin. Physiol.* **19**, 497–503 (1999).
26. Lapuerta, P. *et al.* Neural network assessment of perioperative cardiac risk in vascular surgery patients. *Med. Decis. Making* **18**, 70–75 (1998).
27. Holland, J. H. (ed.) *Adaptation in Natural and Artificial Systems: an Introductory Analysis with Applications to Biology, Control, and Artificial Intelligence* 3rd edn (MIT Press, Cambridge, Massachusetts, 1994).
28. Kohonen, T. Self-organizing formation of topologically correct feature maps. *Biol. Cybernetics* **43**, 59–69 (1982).
29. Kohonen, T. The self-organizing map. *Proc. IEEE* **78**, 1464–1480 (1990).
30. Tou, J. T. & Gonzalez, R. (eds) in *Pattern Recognition Principles* (eds Tou, J. T. & Gonzalez, R.) 75–109 (Addison Weley, Reading, Massachusetts, 1974).
31. Stoeckli, M., Chaurand, P., Hallahan, D. E. & Caprioli, R. M. Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues. *Nature Med.* **7**, 493–496 (2001).
32. Emmert-Buck, M. R. *et al.* Laser capture microdissection. *Science* **274**, 998–1001 (1996).
33. Emmert-Buck, M. R. *et al.* An approach to proteomic analysis of human tumors. *Mol. Carcinog.* **27**, 158–165 (2000).
34. Craven, R. A., Totty, N., Harnden, P., Selby, P. J. & Banks, R. E. Laser capture microdissection and two-dimensional polyacrylamide gel electrophoresis: evaluation of tissue preparation and sample limitations. *Am. J. Pathol.* **160**, 815–822 (2002).
35. Ornstein, D. K. *et al.* Proteomic analysis of laser capture microdissected human prostate cancer and *in vitro* prostate cell lines. *Electrophoresis* **21**, 2235–2242 (2000).
36. Wulfkuhle, J. D. *et al.* New approaches to proteomic analysis of breast cancer. *Proteomics* **1**, 1205–1215 (2001).
37. Jones, M. B. *et al.* Proteomic analysis and identification of new biomarkers and therapeutic targets for invasive ovarian cancer. *Proteomics* **2**, 76–84 (2002).
38. Knezevic, V. *et al.* Proteomic profiling of the cancer microenvironment by antibody arrays. *Proteomics* **1**, 1271–1278 (2001).
39. Ahram, M. *et al.* Proteomic analysis of human prostate cancer. *Mol. Carcinog.* **33**, 9–15 (2002).
40. Gorg, A. *et al.* The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* **21**, 1037–1053 (2000).
41. Hanash, S. M. Biomedical applications of two-dimensional electrophoresis using immobilized pH gradients: current status. *Electrophoresis* **21**, 1202–1209 (2000).
42. Herbert, B. R., Sanchez, J.-C. & Bini, L. in *Proteome Reseach: New Frontiers in Functional Genomics* Ch. 2 (eds Wilkens, M. R., Williams, K. L., Appel, R. D. & Hochstrasser, D. F.) 13–30 (Springer–Verlag, New York, 1997).
43. Shen,Y. *et al.* High-throughput proteomics using high-efficiency multiple-capillary liquid chromatography with on-line high-performance ESI FTICR mass spectrometry. *Anal. Chem.* **73**, 3011–3021 (2001).
The design and application of an automated multiple-capillary liquid chromatography (LC) system for high-throughput proteome analysis using a high-magnetic-field Fourier transform ion cyclotron resonance (FTICR) mass spectrometer for a combined resolving power of > 6 x 10^7 components. This set provided the characterization of 1,000 proteins from a single capillary LC–FTICR analysis.
44. Li, J., Wang, C., Kelly, J. F., Harrison, D. J. & Thibault, P. Rapid and sensitive separation of trace level protein digests using microfabricated devices coupled to a quadrupole-time-of-flight mass spectrometer. *Electrophoresis* **21**, 198–210 (2000).
45. Gygi, S. P. *et al.* Quantitative analysis of complex protein mixtures using isotope coded affinity tags. *Nature Biotechnol.* **17**, 994–999 (1999).
46. Washburn, M. P., Wolters, D. & Yates, J. R. Large scale analysis of the yeast proteome by multidimensional protein identification technology. *Nature Biotechnol.* **19**, 242–247 (2001).
A method for rapid and large-scale proteome analysis by multidimensional LC coupled with tandem mass spectrometry, termed multidimensional protein identification technology (MudPIT). This approach was applied to yeast proteomic analysis, and a total of 1,484 proteins were detected and identified, including the identification of 131 proteins that are membrane localized — a highly problematic class of proteins due to their intrinsic hydrophobic nature.
47. Krutchinsky, A. N., Kalkum, M. & Chait, B. T. Automatic identification of proteins with a MALDI-quadrupole ion trap mass spectrometer. *Anal. Chem.* **73**, 5066–5077 (2001).
48. Washburn, M. P., Ulaszek, R., Deciu, C., Schieltz, D. M. & Yates, J. R. Analysis of quantitative proteomic data generated via multidimensional protein identification technology. *Anal. Chem.* **74**, 1650–1657 (2002).
49. Zhou, H., Ranish, J. A., Watts, J. D. & Aebersold, R. Quantitative proteome analysis by solid-phase isotope tagging and mass spectrometry. *Nature Biotechnol.* **20**, 512–515 (2002).
A method for directly tagging complex mixtures of peptides and proteins using a solid-phase capture and release process with the captured peptides analysed by microcapillary LC and tandem mass spectrometry (microLC–MS/MS).

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50. Zhou, G. *et al.* 2D differential in-gel electrophoresis for the identification of human esophageal squamous cell cancer-specific protein markers *Mol. Cell. Proteomics* **1**, 117–123 (2002).
51. Sreekumar, A. *et al.* Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins. *Cancer Res.* **61**, 7585–7593 (2001).
This paper describes an antibody-microarray-based platform consisting of 146 distinct antibodies to monitor alterations of protein levels induced by exposure of colon carcinoma cell lines to ionizing radiation.
52. MacBeath, G. Proteomics comes to the surface. *Nature Biotechnol.* **19**, 828–829 (2001).
53. Walter, G., Bussow, K., Lueking, A. & Glokler, J. High-throughput protein arrays: prospects for molecular diagnostics. *Trends Mol. Med.* **8**, 250–253 (2002).
54. Kuruvilla, F. G., Shamji, A. F., Sternson, S. M., Hergenrother, P. J. & Schreiber, S. L. Dissecting glucose signalling with diversity-oriented synthesis and small-molecule microarrays. *Nature* **416**, 653–657 (2002).
55. Paweletz, C. P. *et al.* Reverse-phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene* **20**, 1981–1989 (2001).
The invention of a new type of protein array in which extremely small amounts of clinical material can be immobilized and used to study signalling pathway activation.
56. Torhorst, J. *et al.* Tissue microarrays for rapid linking of molecular changes to clinical endpoints. *Am. J. Pathol.* **159**, 2249–2256 (2001).
57. Vile, R. G., Russell, S. J. & Lemoine, N. R. Cancer gene therapy: hard lessons and new courses. *Gene Ther.* **7**, 2–8 (2000).
58. Wiebe, L. I. & Knaus, E. E. Enzyme-targeted, nucleoside-based radiopharmaceuticals for scintigraphic monitoring of gene transfer and expression. *Curr. Pharm. Des.* **7**, 1893–1906 (2001).
59. Liotta, L. A., Kohn, E. C. & Petricoin, E. F. Clinical proteomics: personalized molecular medicine. *JAMA* **286**, 2211–2214 (2001).
60. Liotta, L. & Petricoin, E. Molecular profiling of human cancer. *Nature Rev. Genet.* **1**, 48–56 (2000).
61. Karpati, G., Li, H. & Nalbantoglu, J. Molecular therapy for glioblastoma. *Curr. Opin. Mol. Ther.* **1**, 545–552 (1999).
62. Brown, C. K. & Kirkwood, J. M. Targeted therapy for malignant melanoma. *Curr. Oncol. Rep.* **3**, 344–352 (2001).
63. Frankel, A. E., Sievers, E. L. & Scheinberg, D. A. Cell surface receptor-targeted therapy of acute myeloid leukemia: a review. *Cancer Biother. Radiopharm.* **15**, 459–476 (2000).
64. Cheng, J. D., Rieger, P. T., von Mehren, M., Adams, G. P. & Weiner, L. M. Recent advances in immunotherapy and monoclonal antibody treatment of cancer. *Semin. Oncol. Nurs.* **16** (Suppl. 1), 2–12 (2000).
65. Gasparini, G. & Gion, M. Molecular-targeted anticancer therapy: challenges related to study design and choice of proper endpoints. *Cancer J. Sci. Am.* **6**, 117–131 (2000).
66. Cimoli, G. *et al.* Signaling proteins as innovative targets for antineoplastic therapy: our experience with the signaling protein c-myc. *Tumori.* **87**, S20–S23 (2001).
67. Kolonin, M., Pasqualini, R. & Arap, W. Molecular addresses in blood vessels as targets for therapy. *Curr. Opin. Chem. Biol.* **5**, 308–313 (2001).
68. Rosenwald, A. *et al.* The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N. Engl. J. Med.* **346**, 1937–1947 (2002).
This paper describes the use of hierarchical clustering analysis of gene-expression profiles of biopsy samples from 240 diffuse large-B-cell lymphoma patients to build a molecular predictor of survival after chemotherapy.
69. Ponder, B. A. Cancer genetics. *Nature* **411**, 337–341 (2001).
70. Evan, G. I. & Vousden, K. H. Proliferation, cell cycle and apoptosis in cancer. *Nature* **411**, 342–348 (2001).
71. Kaptain, S., Tan, L. K. & Chen, B. HER2/NEU and breast cancer. *Diagn. Mol. Pathol.* **10**, 139–152 (2001).
72. Leyland-Jones, B. Trastuzumab: hopes and realities. *Lancet Oncol.* **3**, 137–144 (2002).
73. Sebolt-Leopold, J. S. Development of anticancer drugs targeting the MAP kinase pathway. *Oncogene* **19**, 6594–6599 (2000).
74. Santen, R. J. *et al.* The role of mitogen-activated protein (MAP) kinase in breast cancer. *J. Steroid Biochem. Mol. Biol.* **80**, 239–256 (2002).
75. Thiesing, J. T., Ohno-Jones, S., Kolibaba, K. S. & Druker, B. J. Efficacy of STI571, an ABL tyrosine kinase inhibitor, in conjunction with other antileukemic agents against BCR–ABL-positive cells. *Blood* **96**, 3195–3199 (2000).
These experiments show that combinations of Gleevec with interferon-α, hydroxyurea, daunorubicin and cytosine arabinoside have increased antiproliferative effects compared with STI571 alone, suggesting that clinical trials to test these combinations might be worthwhile.
76. Druker, B. J. *et al.* Efficacy and safety of a specific inhibitor of the BCR–ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* **344**, 1031–1037 (2001).
77. Vlahos, C. J. & Stancato, L. F. in *Platelets and Megakaryoctyes: Methods and Protocols* (eds Gibbons, J. M. & Mahaut-Smith, M. P.) (Humana, Totowa, New Jersey, 2002).
78. Traxler, P. *et al.* Tyrosine kinase inhibitors: from rational design to clinical trials. *Med. Res. Rev.* **21**, 499–512 (2001).
79. Zwick, E., Bange, J. & Ullrich, A. Receptor tyrosine kinases as targets for anticancer drugs. *Trends Mol. Med.* **8**, 17–23 (2002).
80. Normanno, N. *et al.* Cooperative inhibitory effect of ZD1839 (Iressa) in combination with trastuzumab (Herceptin) on human breast cancer cell growth. *Ann. Oncol.* **13**, 65–72 (2002).
81. Moasser, M. M., Basso, A., Averbuch, S. D. & Rosen, N. The tyrosine kinase inhibitor ZD1839 ('Iressa') inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res.* **61**, 7184–7188 (2001).
An analysis of the ability of ZD1839 (Iressa) to inhibit the phosphorylation of EGFR, HER2, HER3 and AKT and growth inhibition in a series of human breast cancer cell lines. These studies indicate that HER2-overexpressing tumours might be especially susceptible to Iressa.
82. Cuello, M. *et al.* Down-regulation of the ERBB-2 receptor by trastuzumab (Herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress ERBB-2. *Cancer Res.* **15**, 4892–4900 (2001).
83. Paweletz, C. P. *et al.* Rapid protein display profiling of cancer progression directly from human tissue using a protein biochip. *Drug Dev. Res.* **49**, 34–42 (2000).

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