PROTEOMIC APPLICATIONS FOR THE EARLY DETECTION OF CANCER

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The ability of physicians to effectively treat and cure cancer is directly dependent on their ability to detect cancers at their earliest stages. Proteomic analyses of early-stage cancers have provided new insights into the changes that occur in the early phases of tumorigenesis and represent a new resource of candidate biomarkers for early-stage disease. Studies that profile proteomic patterns in body fluids also present new opportunities for the development of novel, highly sensitive diagnostic tools for the early detection of cancer.

EARLY DETECTION

PROSTATE-SPECIFIC ANTIGEN
The serum level of this protein increases in some men who have prostate cancer or certain benign prostate conditions.

GENOMIC TECHNOLOGIES
Techniques for gene-expression analysis, including oligonucleotide arrays for determining relative levels of expression for thousands of genes between different samples (e.g. normal and tumour) that can lead to the identification of tumour-specific markers.

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The early detection of cancer is crucial for its ultimate control and prevention. Although advances in conventional diagnostic strategies, such as mammography and PROSTATE-SPECIFIC ANTIGEN (PSA) testing, have provided some improvement in the detection of disease, they still do not reach the sensitivity and specificity that are needed to reliably detect early-stage disease.

In many cases, cancer is not diagnosed and treated until cancer cells have already invaded surrounding tissues and metastasized throughout the body. More than 60% of patients with breast, lung, colon and ovarian cancer have hidden or overt metastatic colonies at presentation and most conventional therapeutics are limited in their success once a tumour has spread beyond the tissue of origin. Detecting cancers when they are at their earliest stages, even in the premalignant state, means that current or future treatment strategies will have a higher probability of truly curing the disease. So, how can early-stage cancers be detected?

Biomarkers

Biomarkers are important tools for cancer detection and monitoring. They serve as hallmarks for the physiological status of a cell at a given time and change during the disease process. Gene mutations, alterations in gene transcription and translation, and alterations in their protein products can all potentially serve as specific biomarkers for disease[1,2]. The discovery, decades ago, that free DNA was present in the serum of cancer patients began a process that has resulted in today's serum tests — for oncogene mutations, microsatellite instability and hypermethylation of promoter regions — for the detection of cancer[2] (see review by Peter Laird on page 253 in this issue). However, non-tumour cells also shed DNA into serum, so cancer-specific changes can be almost impossible to detect above the tremendous background of wild-type DNA. Their detection requires a lack of degradation, as well as amplification of this rare event.

Advances in GENOMIC TECHNOLOGIES have made it possible to rapidly screen for global and specific changes in gene expression that occur only in cancer cells[3]. In addition to requiring appropriately processed tumour tissues for analysis, a significant caveat to gene-expression analysis is that many changes in gene expression might not be reflected at the level of protein expression or function. This is an important issue to consider as most licensed tests that are available for disease detection are protein-based assays. The enzyme-linked, immunosorbent assay (ELISA) system represents the most reliable, sensitive and widely available protein-based testing platform for the detection and monitoring of cancer. These tests are robust, linear and accurate, and have reasonable throughput. Use of an ELISA system to test for the presence of disease requires a single, meticulously validated protein biomarker of disease, as well as an extremely well-characterized, high-affinity antibody that can detect the protein of interest. An effective, clinically useful biomarker should be measurable in a readily accessible body fluid, such as serum, urine or saliva. Until recently, the

Table 1 | Comparison of proteomics technologies and their contributions to biomarker discovery and early detection

	ELISA	2D-PAGE	Multidimensional protein identification technology (MudPIT)	Proteomic pattern diagnostics	Protein microarrays
Sensitivity					
	Highest	Overall low, particularly for less-abundant proteins; sensitivity limited by detection method; LCM can improve specificity via enrichment of selected cell populations	High	Medium sensitivity with diminishing yield at higher molecular weights; will improve with new MS instrumentation	Medium/high
Direct identification of markers					
	N/A	Yes	Yes	No, newer MS technologies might make this possible	Possible when coupled with MS technologies
Use					
	Detection of single, specific well-characterized analyte in body fluid or tissue; gold standard of clinical assays	Means for discovery and identification of biomarkers, not a direct means of early detection in itself	Detection and identification of potential biomarkers	Diagnostic pattern analysis in body fluids and tissues; potential biomarker identification	Multiparametric analysis of many analytes simultaneously
Throughput					
	Moderate	Low	Very low	Highest	High
Advantages/drawbacks					
	Very robust; well-established use in clinical assays; requires well-characterized antibody for detection and extensive validation; not amenable to direct discovery (strictly measurement based)	All IDs require validation and testing before clinical use; tried and true methodology, reproducible and more quantitative combined with fluorescent dyes	Significantly higher sensitivity than 2D-PAGE (much larger coverage of the proteome for biomarker discovery)	Protein IDs not necessary for diagnostic pattern analysis; reproducibility issues need to be addressed; need for validation; coupling to adaptive informatics tools might revolutionize the field of clinical chemistry	Format is flexible: can be used to assay for multiple analytes in a single specimen or a single analyte in a large number of specimens; requires prior knowledge of analyte being measured; limited by antibody sensitivity and specificity; requires use of an amplified tag detection system

2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; ID, identification; LCM, laser capture microdissection; MS, mass spectrometry.

search for cancer-related biomarkers for early disease detection has been a one-at-a-time approach to look for proteins that are overexpressed as a consequence of the disease process, and are shed into body fluids[4–8]. Unfortunately, this approach is laborious and time-consuming, as each candidate biomarker(s) must be identified from among the thousands of intact and cleaved proteins in the human serum proteome — antibodies would then need to be developed to validate and check the protein marker for specificity and sensitivity. However, the emerging field of clinical proteomics is especially well suited to the discovery and implementation of these biomarkers, as body fluids are an acellular, protein-rich information reservoir that contains traces of what the blood has encountered during its circulation through the body.

So, how are conventional and novel proteomics methods and technologies being used to discover new biomarkers for early-stage disease, and how are they being used to develop entirely new diagnostic models for disease detection?

ELISA
(Enzyme-linked, immunosorbent assay). A sensitive antibody-based method for the detection of an antigen such as a protein.

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

MASS SPECTROMETRY
A field that, in its biological applications, uses sophisticated analytical devices to determine the precise molecular weights (mass) of proteins and nucleic acids, as well as the amino-acid sequence of protein molecules.

Biomarker discovery

Two-dimensional electrophoresis. For a number of years, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) followed by protein identification using MASS SPECTROMETRY has been the primary technique for biomarker discovery in conventional proteomic analyses[9,10]. This technique is uniquely suited for direct comparisons of protein expression and has been used to identify proteins that are differentially expressed between normal and tumour tissues in various cancers, such as liver, bladder, lung, oesophageal, prostate and breast[11–19].

Despite its utility, there are several inherent disadvantages to 2D-PAGE. It requires a large amount of protein as starting material, and the technique cannot be reliably used to detect and identify low-abundance proteins (TABLE 1). Also, normal and tumour tissues are a heterogeneous mix of various cell types, all of which contribute to the proteomic profile of whole tissues on 2D gels. This represents a significant obstacle to the search for biomarkers in early-stage cancers, because these lesions

LASER CAPTURE MICRODISSECTION
A technology that is used for the rapid procurement of a microscopic and pure cellular subpopulation away from its complex tissue milieu, under direct microscopic visualization.

Summary

- Biomarkers are the foundation of cancer detection and monitoring. Most of today's licensed tests for disease detection are protein-based assays.
- Low-throughput proteomics approaches, such as 2D-PAGE (two-dimensional polyacrylamide gel electrophoresis) coupled with mass spectrometry for protein identification, have proven useful for cancer biomarker discovery, particularly when laser capture microdissection (LCM) is used to isolate cell populations of interest for analysis.
- Technologies such as multidimensional separation systems directly coupled to mass spectrometry analysis represent improvements in sensitivity and throughput when compared with traditional 2D-PAGE analysis for biomarker discovery.
- Mass-spectrometry-driven proteomic analysis is a key development for the rapid detection of cancer-specific biomarkers and proteomic patterns of tissue and body fluids.
- Proteomic pattern diagnostics combines proteomic pattern profiling of tissue and body fluids by mass spectrometry with sophisticated bioinformatics tools to identify patterns within the complex proteomic profile that discriminate between normal, benign or disease states.
- Proteomic pattern diagnostics has been successfully applied to the problems of early detection for a number of different types of cancer.
- A number of feasibility, reproducibility and standardization issues need to be addressed before proteomic pattern diagnostics can be incorporated into routine clinical practice.
- Mass spectrometry might become the preferred detection platform and clinical analyser for routine clinical and medical diagnostics.

are often small, and contamination from surrounding stromal tissue that is present in the specimen can confound the detection of tumour-specific markers.

The invention of LASER CAPTURE MICRODISSECTION (LCM) greatly improved the specificity of 2D-PAGE for biomarker discovery, as it provided a means of rapidly procuring pure cell populations from the surrounding heterogeneous tissue and also markedly enriched the proteomes of interest[20–24]. This technology has facilitated the search for early-stage disease markers in a number of tissue types[25–28]. A comparison of microdissected epithelial cells from two low-malignant potential (LMP) ovarian tumours and three invasive cancers revealed ten proteins that were more highly expressed in the LMP tumour cells and thirteen proteins — among them, RHOGDI, glyoxalase-1 and the 52-kDa FK506BP — that were more highly expressed in the invasive ovarian cancer cells[25]. In addition to identifying proteins that increase in expression, 2D-PAGE analysis can also reveal proteins that are lost during tumour progression. For example, the loss of the Ca^{2+}-dependent phospholipid-binding protein, annexin-1, has been correlated with early phases of prostate and oesophageal tumorigenesis[27]. A recent study focused on the identification of potential biomarkers in the early breast cancer lesion, ductal carcinoma *in situ* (DCIS)[28]. Four cases of patient-matched, normal ductal epithelial cells and DCIS cells were microdissected and their proteomic profiles were compared by 2D-PAGE. Differentially expressed spots from 2D-gels, for each case, were selected and sequenced by mass spectrometry. The differential expression patterns for a subset of the identified proteins were validated by immunohistochemistry with a small, independent cohort of patient-matched normal/DCIS specimens (FIG. 1). Among the proteins identified and validated were HSP27, a molecular chaperone protein that has been documented to be overexpressed in early breast cancer lesions[29], and the actin crosslinking protein transgelin, which was expressed at a higher level in normal ductal epithelial cells than in DCIS cells (FIG. 1). An analysis of transgelin gene expression in breast tissue showed that transgelin RNA levels are also lower in invasive tumours compared with normal tissue, indicating that the downregulation of protein expression might be controlled at the transcriptional level[30]. Also, the identification of differentially expressed proteins by independent methods increases their potential as candidate biomarkers and enhances their possible biological significance.

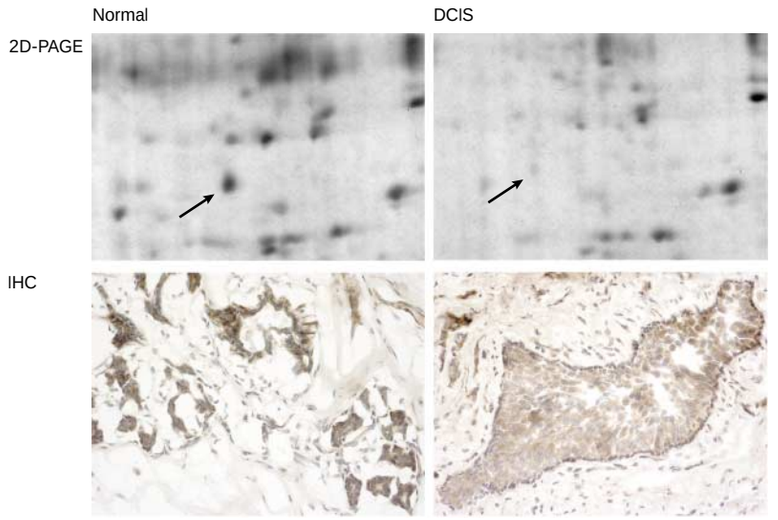
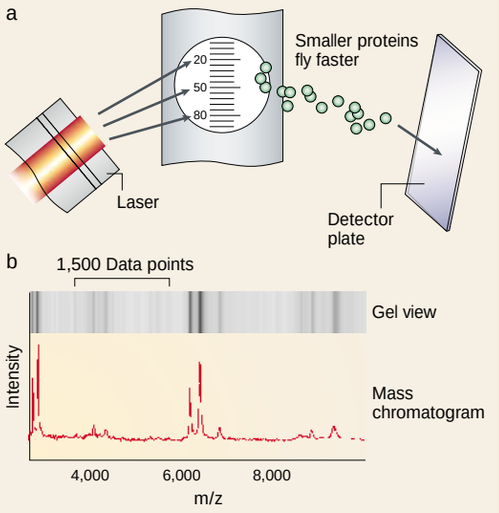


Figure 1 | **Identification and validation of differential expression of transgelin between normal and ductal carcinoma *in situ* (DCIS) epithelial cells.** Top panel, cropped images from two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of microdissected normal and DCIS breast epithelial cells, showing the decreased expression of transgelin (arrows) between normal and DCIS tissue. Lower panel, immunohistochemistry (IHC) staining of transgelin in patient-matched normal and DCIS tissue confirms the expression trend observed in 2D-PAGE analysis.

Box 1 | SELDI-TOF mass spectrometry

Using a robotic sample dispenser/processor to increase reproducibility, accuracy and speed for sample handling and delivery, one microlitre of raw, unfractionated serum is applied to the surface of a protein-binding chip. Depending on the type of chromatographic matrix used (that is, weak cation, strong anion or immobilized metal affinity), a subset of the proteins in the sample bind to the surface of the chip (Panel **a**). This interaction is specific as the chromatographic binding is based on the inherent amino-acid sequence of any given protein, as well as on the pH, detergent and salt concentration in the binding reaction buffer. Decreasing the amount of time allowed for incubation also allows the researcher to minimize non-specific binding, as the high-affinity interactions occur more quickly than low-affinity binding.

The chip is rinsed to remove unbound proteins, and the bound proteins are treated with a MATRIX COMPOUND, washed and dried (Panel **a**). The chip, containing many patient samples, is inserted into a vacuum chamber, where it is irradiated with a laser. The laser desorbs the adherent proteins, which causes them to be launched as protonated and charged ions. The time-of-flight (TOF) of the ion, before it is detected by an electrode, is a measure of the mass to charge (m/z) value of the ion. The ion spectra can be analysed by computer-assisted tools to classify a subset of the spectra by their characteristic patterns of relative intensity. Using this method, one microlitre of raw unfractionated serum from a patient is analysed by SELDI-TOF to create a proteomic signature of the serum (Panel **b**). This serum proteomic bar-code is comprised of potentially tens of thousands of protein ion signatures, which then require high-order data-mining operations for analysis. A typical low-resolution SELDI-TOF proteomic profile will have up to 15,500 data points that comprise the recordings of data between 500 and 20,000 m/z, with higher-resolution mass spectrometry instruments generating as much as 400,000 data points for 500 to 12,000 m/z.



Recent advances have led to the development of variations of the traditional 2D-gel approach, and the application of these has resulted in the identification of potential new biomarkers for early detection of disease. Differential in-gel electrophoresis (DIGE) provides a methodology that improves the reproducibility, sensitivity and quantitative aspects of 2D-gel analyses[31,32]. Cellular protein extracts are differentially labelled with fluorescent dyes, then are mixed and run on a single 2D-gel. The gel is scanned to generate a map for each labelled protein pool and the two images can be compared for differences in fluorescence intensities between labels for a given spot. This technique was recently used to identify differentially expressed proteins in oesophageal squamous-cell cancers and normal oesophageal tissue[32]. Other studies have used 2D-gels and western blotting to screen sera from cancer patients for proteins that could serve as biomarkers or immunotherapy targets using auto-antibodies against tumour-cell proteins[33–35]. Autoantibodies can be particularly useful for studying cell-surface antigens on cancer cells and could become a powerful tool for screening large numbers of antigens by protein microarray[36]. An analysis of sera from breast cancer patients identified the molecule RS/DJ-1 — a protein that regulates RNA–protein interactions — as a potential circulating biomarker for breast cancer[33]. In lung cancer patients, the protein PGP9.5 has been found to be a circulating tumour biomarker with potential clinical use in screening and diagnosis[35].

2D-PAGE and related technologies have proven to be a very reliable tool for discovery-based proteomics approaches. However, despite the availability of reagents for focusing proteins over very narrow pH ranges, only a small percentage of the proteome can be visualized by 2D-PAGE. Newer technologies such as IMAGING MASS SPECTROMETRY and multiple tandem, in-line liquid chromatography separation directly coupled to mass spectrometry analysis — otherwise known as multidimensional protein identification technology (MudPIT) — have allowed scientists to detect lower abundance proteins in the proteome[37–45] (TABLE 1). These multiplexed technologies — used to analyse tagged cellular lysates, complex protein mixtures and obtain proteomic profiles directly from intact tissue — might someday replace traditional 2D-PAGE; however, they also have drawbacks as they require a large amount of protein to begin with, which precludes their routine use with specimens such as clinical biopsies. Also, these technologies require significant time and effort on the part of the investigator, which makes them unsuitable for use in clinical testing in which throughput and cost are the final arbiters of routine use. Although these technologies have provided and will continue to provide excellent candidate molecules for early-detection tests for the presence of disease, these potential biomarkers must survive rigorous testing and high-affinity, specific antibodies must be developed

MATRIX COMPOUND
A chemical compound (organic acid) that is used to absorb laser energy and transfer this to biomolecules that are present in the sample, causing them to become protonated and ionized.

IMAGING MASS SPECTROMETRY
An application of a scanning type of mass spectrometry that allows for direct mapping of protein expression profiles that are present in tissue sections or individual cells.

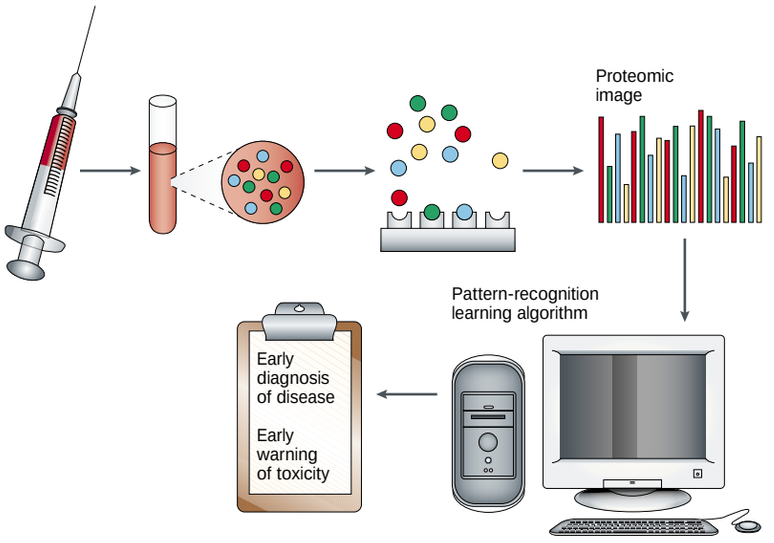


Figure 2 | **Schematic of proteomic pattern diagnostics.** A serum sample is taken from a patient, and the proteins are bound to a chip. Mass spectrometry is performed to achieve a proteomic image that can then be 'read' using bioinformatics tools. The readout could result in the early detection of cancer.

before these goals come to fruition. These issues underscore the need for higher throughput and high-sensitivity tests for the early detection of cancer.

High-throughput biomarker identification

Proteomic pattern diagnostics. Surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) mass spectrometry technology is potentially an important tool for the rapid identification of cancer-specific biomarkers and proteomic patterns in the proteomes of both tissues and body fluids (BOX 1). SELDI is a type of mass spectrometry that is useful in high-throughput proteomic fingerprinting of cell lysates and body fluids that uses on-chip protein fractionation coupled to time-of-flight separation. Within minutes, sub-proteomes of a complex milieu such as serum can be visualized as a proteomic fingerprint or 'bar-code' (FIG. 2). SELDI technology has significant advantages over other proteomic technologies in that the amounts of input material required for analysis are miniscule compared with more traditional 2D-PAGE approaches (TABLE 1). SELDI analysis is also very high throughput — data can be generated in minutes or hours for large study sets, as opposed to days for 2D-PAGE analyses. A number of studies have used SELDI technology to identify single disease-related biomarkers for several types of cancer. For example, a modified, quantitative SELDI approach has been used to show that the levels of serum prostate-specific membrane antigen are significantly higher in patients with prostate cancer than in those with benign disease[7]. Potential biomarkers for breast cancer have been identified in analyses of nipple aspirate fluid[46,47]. An early study — in which cellular fingerprints of LCM-procured cells were combined with SELDI-TOF spectral analysis — showed the diagnostic potential of a combination of peaks and patterns of distinct mass spectral features as the spectral signature could discriminate normal from preneoplastic tissues and from cancer[48]. In prostate tissue, differential expression and the relative pattern of two specific protein identities were observed during the progression of normal prostatic epithelium to intraepithelial neoplasia and invasive cancer in a patient-matched tissue set. Others have used regression analysis to identify a combination of SELDI spectral peaks that was able to discriminate normal and benign prostate signatures from signatures for diseased tissue in a small cohort of prostate tumours[49]. However, a caveat to the SELDI-TOF technology and these studies is that substantial upfront fractionation of protein mixtures and downstream purification methods are required to obtain absolute protein identification (TABLE 1).

Body fluids such as serum and urine have proven to be a rich source of biomarkers for the early detection of cancer. The blood proteome changes constantly as a consequence of the perfusion of the diseased organ adding, subtracting or modifying the circulating proteome. These disease-related differences might 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 removed from the proteome due to abnormal activation of the proteolytic degradation pathway. Effects due to disease-related protein–protein interactions and protein-complex formation can also modify and subtly change the serum proteome. As these fluids bathe or circulate through tissues, they pick up proteins that are produced by the tumour and the tumour–host microenvironment[50,51]. In fact, because the proteome is a fluctuating account of the circuitous cause and effect of the host and its response to disease, it is the ultimate record of systems biology. So, the unique tumour–host microenvironment initiates amplification cascades that are specific to the disease process, and the signatures for the presence of cancers — even at their earliest stages — might be composed of untold combinations of slight, but significant, changes in protein levels[50]. Therefore, using a combination of markers would be expected to be more effective than looking at single biomarkers[52].

The approach of proteomic pattern diagnostics combines the proteomic pattern profiling of serum by SELDI-TOF with sophisticated bioinformatics tools using the serum proteomic patterns themselves as the diagnostic medium[51] (BOX 2; TABLE 1). With this approach, the underlying identity of the individual components of the pattern is not necessary for its use as a potential diagnostic for disease. This approach is being evaluated at present for applications in early cancer detection.

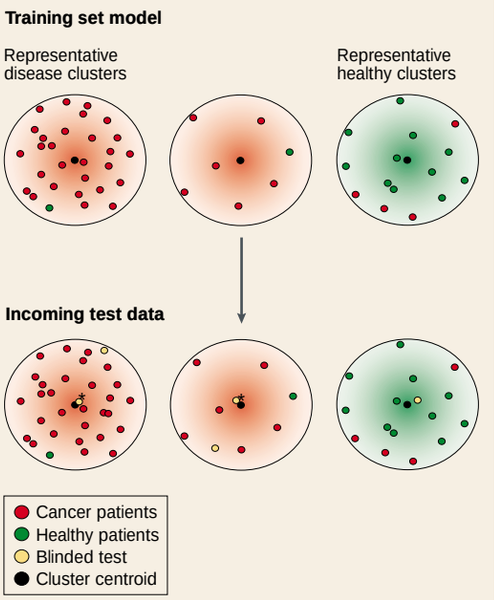
Use of proteomic pattern diagnostics to detect cancer. The first report describing the development and use of pattern recognition algorithms coupled to high-throughput mass spectrometry for proteomic pattern diagnostics applied the approach to ovarian cancer

Box 2 | Bioinformatics tools for proteomic pattern diagnostics

Many new types of bioinformatics data-mining systems are being developed, but most fall into two main types of approach. Supervised systems require knowledge or data in which the outcome or classification is known ahead of time, so that the system can be trained to recognize and distinguish outcomes[72–79]. Unsupervised systems cluster or group records without previous knowledge of outcome or classification[80–82].

The problem, however, is the same for either system: finding optimal feature sets — or, in this instance, proteins — in a large unbounded information archive that is unknown at this time. Artificial-intelligence-based bioinformatic systems that are vigilant — that is, gain experience and can identify a new and previously unseen event — are an extremely powerful tool that can be used to analyse these large complex data streams. During training of some types of these systems, clusters are formed that comprise specific n-dimensional points that represent known patients and that are based on the combined normalized intensity values from the mass spectral data streams from each of those patients (see figure). Some clusters (red = disease phenotype; green = normal phenotype) are populated by many patients that have a specific phenotype (left clusters), or can be populated with fewer patients (middle clusters). Additionally, although the algorithm hunts for homogeneity, clusters might be selected that contain both the healthy and the disease phenotype (as shown). As proteomic patterns from new patients are analysed and compared against the model that was developed during training, they are classified as healthy or diseased based on the clusters that they fall into. Importantly, however, a scoring value is obtained based on two important variables: the distance any patient value is to the theoretical centroid of any given cluster — that is, how much this particular patient 'looks' like the healthy or disease patients used in training within that particular cluster and the percent homogeneity and population density of the cluster itself. For example, two incoming patients (in yellow with asterisk) might lie identically close to the theoretical centroid of two different clusters, and might both be classified as diseased; however, the patient on the left cluster belongs to a cluster that has many more disease patients than the middle cluster, therefore it would receive a proportionately higher score based on the homogeneity and the population size. The patient on the left 'looks' more likely to have cancer than the patient in the middle. These types of informatic algorithms have the special ability to learn, adapt and gain experience over time so are uniquely suited for proteomic data analysis because of the huge dimensionality of the proteome itself. Application of these artificial intelligence (AI) systems to mass spectral data derived from the serum proteome has given rise to a new analytical model: proteomic pattern diagnostics[53]. As each new patient is validated through pathological diagnosis using retrospective or prospective study sets, its input can be added to an ever-expanding training set. The AI tool learns, adapts and gains experience through constant vigilant retraining — meaning that it can start to recognize a unique and new phenotype even though the system had not been trained or seen it beforehand. This is extremely important when clinical applications are considered in which hundreds of thousands of patients might be screened for a particular cancer. In fact, it is possible to generate not just one, but multiple combinations of discriminating proteomic patterns from a single mass spectral training set, each pattern combination readjusting as the models get better in the adaptive mode. This is exactly what has been observed as the expanding ovarian cancer patient sera set has now given rise to many combinations of patterns that are, together, 100% sensitive and specific.

The adaptation of SELDI-TOF-based protein chips to mass spectrometry instruments with much higher resolution — for example, the hybrid QqTOF — might offer even more robust models with spectra that are consistently invariant over many months and between machines. This will be crucial as endeavours are made to bring this type of technology to the clinic.



detection and to the problem of ovarian cancer diagnosis[53]. More than two-thirds of ovarian cancer cases are detected at advanced stages, when the cancer cells have already spread away from the ovary surface and disseminated throughout the peritoneal cavity. Even though the disease at this stage is advanced, it rarely produces specific diagnostic symptoms[54–58]. Most treatments for advanced ovarian cancer have limited efficacy, and the resulting 5-year survival is just 35–40%. By contrast, if ovarian cancer is detected when it is still confined to the ovary (stage I), conventional therapy produces a high 5-year survival rate (95%). So, early detection of ovarian cancer, by itself, could have a profound impact on the successful treatment of this disease (FIG. 3). In the study, a discriminatory pattern that distinguished normal from ovarian cancer was developed from a training set of mass spectra, which was derived from sera of women with a

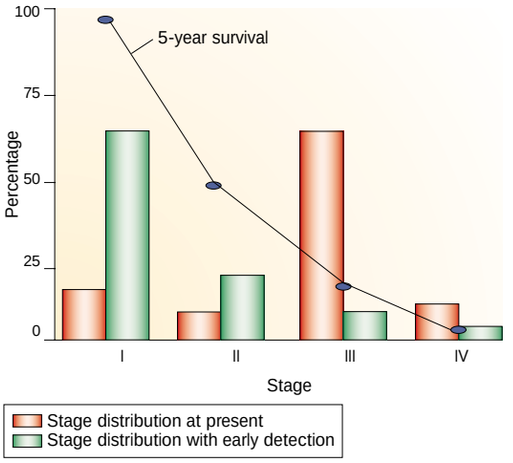


Figure 3 | **The potential impact of proteomic pattern diagnostics for the early detection of ovarian cancer on 5-year survival statistics.** Today, most ovarian cancer cases are diagnosed at advanced stages when the prognosis for 5-year survival is poor, whereas those women diagnosed with Stage I cancer have a more than 90% chance of 5-year survival. Implementation of a highly sensitive and specific test for the early detection of cancer could significantly increase the number of ovarian cancer cases detected at early stages and have a marked impact on the 5-year survival statistics for this disease.

diagnosis of ovarian cancer and unaffected women. This diagnostic pattern was then applied to a blinded set of samples from both cancer patients and unaffected women. The algorithm correctly identified 100% of ovarian cancers, including 18 samples with stage I disease, and assigned 95% of the healthy and benign controls correctly. These controls included women with non-gynaecological diseases (for example, sinusitis and arthritis), and non-malignant gynaecological disease (for example, ovarian cysts and endometriosis). Intriguingly, when this model was tested with serum from individuals with other types of cancer such as prostate cancer, it was unable to correctly classify them, indicating that disease-specific models can be generated[53]. The hope is that after further validation, serum proteomic pattern diagnostics will be applied in screening clinics as a valuable supplement to diagnostic work-up and assessment.

Since this initial report and discovery, the use of proteomic pattern diagnostics has been confirmed in other types of cancer as well. For example, mass spectral proteomic profiling of blood serum has been combined with bioinformatics tools to detect breast cancer[59]. A pattern consisting of three mass spectral ions was found to distinguish stage 0–I, as well as stage II–III, breast cancer patients from normal controls with significantly greater sensitivity and specificity than those with single biomarkers. In the diagnosis of prostate cancer, testing for elevated levels of prostate-specific antigen (PSA) combined with manual digital rectal examination represents the gold standard for early detection of disease[60]. However, these tests require a biopsy to confirm the presence of cancer or BENIGN PROSTATIC HYPERPLASIA, which can also cause elevation of PSA levels. A number of recent studies have focused on proteomic pattern diagnostics in serum as a potential means to diagnose prostate cancer more accurately[61–63]. These studies used various bioinformatics tools to identify patterns within the serum proteomic signature that could discriminate normal sera from that taken from patients with benign disease and normal sera from that taken from patients with cancer[61,62]. In one study, a decision tree classification system was used to identify a proteomic pattern that discriminated between prostate cancer and non-cancer cohorts. This pattern was able to classify a test set of 60 serums from healthy/benign controls and patients with prostate cancer with a sensitivity of 83% and a specificity of 97% (REF. 61). In subsequent analyses, this same group used a boosting method of iterative analysis of the same data over and over to increase the sensitivity and specificity of their models to 100% (REF. 62). Another study focused on using serum proteomic patterns that could discriminate between cases of benign disease and cancer, particularly in patients whose PSA levels are moderately elevated (4–10 ng/ml), with the goal of preventing biopsies in all men with elevated PSA[63]. This algorithm was able to correctly classify 70% (107 of 153) of sera from patients with benign disease and PSA levels of >4 ng/ml, and could accurately predict the presence of cancer in 95% of the patients tested, including 18 of 21 men in the diagnostic grey zone of PSA.

Interestingly, among the benign sera that were incorrectly classified as cancer, follow-up information indicated that seven of those patients developed cancer within 5 years, showing that not all incorrect classifications were false positives. Although these specificities do not support serum proteomic pattern analysis as a replacement for biopsy in prostate cancer diagnosis, it does have the potential to complement current medical decisions and to develop new testing diagnostics to evaluate who should get a biopsy when PSA is slightly elevated. It could, ultimately, affect treatment by identifying a serum proteomic pattern that could discriminate who might have aggressive or indolent prostate cancer once the biopsy is performed.

Future implications/conclusions

Clinical applications of proteomic research are an exciting component of the proteomics field. Improvements and miniaturization in the area of multidimensional separations promise to reinforce the importance of discovery-based proteomics projects for biomarker identification[40–45,64] (TABLE 1). The continuing development of protein-based microarray technologies, antibody arrays and multiplexed on-chip enzyme arrays represents a versatile advancement in the throughput of the traditional ELISA assay[65–71] (TABLE 1). Although many protein microarray technologies are limited by the requirement for highly specific, high-affinity antibodies, two-site approaches and/or sensitive detection and signal amplification systems, they have the advantage of being an excellent means for high-throughput, simultaneous analysis of potentially hundreds of analytes at once in a wide variety of formats[23].

BENIGN PROSTATIC HYPERPLASIA
A non-cancerous condition in which an overgrowth of prostate tissue pushes against the urethra and the bladder, blocking the flow of urine.

'DISRUPTIVE' OR 'NON-LINEAR' TECHNOLOGY
A technology that represents a significant, unexpected change in an existing model that does not progress in a straightforward linear fashion, thereby polarizing the existing infrastructure.

The development of proteomic pattern diagnostics might represent a revolution in the field of molecular medicine in that it not only represents a new model for disease detection, but it is also clinically feasible. This is certainly an example of a 'DISRUPTIVE' OR 'NON-LINEAR' TECHNOLOGY. The overarching clinical impact of proteomic pattern diagnostics remains untested and the early, yet highly accurate, results have not yet been validated in larger trials. However, mass spectrometry platforms — already capable of reporting tens of thousands of events in less than a few minutes from a microlitre of blood — are advancing rapidly with even greater speed, throughput, sensitivity and direct protein identification capabilities.

By coupling these advances in instrumentation with new adaptive and vigilant bioinformatic pattern-recognition tools, it is possible to see the potential that these new methods have for markedly changing how disease is detected and followed beyond the existing immunoassay-based approaches. Importantly, because it will ultimately be regulatory agencies that evaluate the entire method and process of proteomic pattern diagnostics — as opposed to just the results obtained — a number of important issues regarding its performance and use must be addressed over the next several months to few years for this technology to have real clinical impact. Before proteomic pattern diagnostics can be incorporated into routine clinical practice and receive regulatory approval, standard operating procedures must be established for sample handling and processing. Reproducibility standards for proteomic patterns and a universal reference standard for quality control of mass spectrometry instruments must also be developed. Equivalent reproducibility and quality control/quality assurance release specifications, spectral quality measures, machine-to-machine, lab-to-lab and process-driven variability measures must be identified and controlled for. Because of the high cost of instrumentation, the likelihood that specialized core competencies will be required for performing the process, and the reagents that this type of testing requires, routine use will probably lie in large reference labs and centralized testing facilities, not unlike most of the diagnostic tests that are available at present for patient care. Consequently, the ultimate cost to the patients might be driven lower by these same centralized approaches and cost/benefit analysis over existing poorer-performing single analyte tests.

Because of the significant clinical potential proteomic pattern diagnostics has over traditional biomarker testing for early cancer detection, National-Cancer-Institute-based clinical trials to evaluate proteomic pattern diagnostics are planned during the next year for ovarian cancer followed by other cancers, and large reference labs have now begun evaluating the eventual implementation of proteomic pattern diagnostics in their routine practice.

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