Towards preventive medicine

High-throughput methods from molecular biology are about to change daily clinical practice

Hrvojka Bosnjak, Kresimir Pavelic & Sandra Kraljevic Pavelic

The sequencing of the human genome was an enormous achievement in more than one sense as both the annotated sequence and the bioinformatics tools developed have become enormously important to biomedical research. In addition, the technological advances made during the project have further promoted the new ‘-omics’ approach in molecular biology research; it is a global, systematic and comprehensive way of identifying and describing the molecular processes and pathways involved in physiological functions and pathological states.

Since the start of the Human Genome Project in 1990, its proponents have pointed out that the knowledge gained could lead to new cures or preventive measures for a wide range of diseases, as well as enormous benefits to general health. A recent white paper by the European Medical Research Council (Strasbourg, France) explicitly encourages the use of new -omics technologies, as well as systems biology, nanomedicine, regenerative medicine, and tissue and stem-cell banking, in order to improve clinical and medical practice (Billig *et al*, 2007).

Currently, the use of functional genomics includes methods to measure gene activity at the transcript level—differential display, expressed sequence tags, serial analysis of gene expression and microarrays—whereas new methods in proteomics help to analyse the protein content and composition of cells and tissues. These rely on instruments capable of the high throughput analysis of biological samples, which themselves generate a large amount of experimental data. This information must, in turn, be processed by powerful information systems and new computational methods to draw meaning from the primary data and generate new knowledge about molecular processes in cells and tissues.

These increasingly sensitive, efficient and precise high-throughput methods and the bioinformatics tools to handle the data, have paved a path on which biologists and clinicians can meet to achieve the ultimate goal: the successful use of these discoveries for both diagnosis and therapy in daily clinical practice (Table 1). Indeed, the various -omics approaches—including genomics, proteomics and metabolomics—will eventually allow physicians to practice truly preventive medicine, as well as individually tailoring therapies and generally personalizing medicine. We can already see the first applications in clinical practice, as this paper discusses. Nevertheless, the financial costs of personalized medicine are still prohibitively high and there are few medical centres that are able to afford or efficiently use the technology and the information gained from it. Yet, high-throughput methods have been successfully applied in various fields of clinical medicine. These methods have significantly affected patient management and the introduction of -omics in clinics has fuelled high expectations for a new era in which diagnostics will unite with therapy (Kling, 2007).

The main strength of high-throughput procedures is the ability to simultaneously monitor the activity of a large number of biomolecules. As such, the technology allows physicians to assess patients individually for disease risks even before symptoms emerge. Diagnosing a disease such as cancer at a very early stage markedly improves a patient's chances of survival and saves health-care resources spent on treatments for patients in the late stages of the disease. Moreover, functional genomics is already being applied to drug discovery with the aim of shortening and/or improving the overall process. Its expanded use, together with more detailed knowledge about pathological processes at

the molecular level, will probably improve drug discovery further, and hopefully lead to ‘smarter’ drugs with higher efficiencies and fewer side effects.

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Cancer, in particular, still presents an enormous challenge for public health. Despite the considerable efforts that have gone into cancer diagnostics and therapy, it is still one of the leading causes of death and disability in developed countries; it is also rapidly becoming a major public health problem in developing countries. As we lack specific therapeutics, many forms of cancer still cause high mortality and, perhaps more worryingly, many cases of cancer are still not diagnosed until an advanced stage; often too late for efficient treatment, particularly in the case of metastatic cancer. Most traditional methods—surgical removal of the primary tumour combined with chemotherapy and/or radiotherapy to kill any surviving cancer cells—often fail to cure the patient. High-throughput -omics methods could therefore lead to important improvements in cancer diagnosis and therapy, as they would allow physicians to detect the disease at an earlier stage and to analyse its molecular profile to select the most efficient therapy.

There are now various low-technology screening methods for detecting some forms of cancer at an early stage, but even these are rather primitive and are usually only applied to specific high-risk populations.

Among them is regular mammography, which is used to detect breast cancer and is recommended annually for every woman between 50 and 69 years of age. Women at a higher risk of developing breast cancer—those with mutations of the *BRCA1* or *BRCA2* genes, for example—should undergo a mammogram more frequently. Another important screening method for women is the so-called Pap test or cervical smear that is used to diagnose abnormal changes in the cervical epithelium, which could indicate the presence of cervical cancer. Similarly, the prostate-specific antigen (PSA) test that is used to diagnose prostate cancer at an early stage might have made a significant contribution to the declining mortality of the disease, although it is still not as widely recommended or used in screening programmes as mammograms or Pap tests.

Screening for early colon cancer includes the haemocult test to detect invisible traces of blood in the stool, digitorectal examination, sigmoidoscopy and colonoscopy. People older than 50 years and patients suffering from ulcerative colitis, Crohn's disease or colon polyposis are at a higher risk of developing colon cancer. For people over 50, a sigmoidoscopy every 5 years and a colonoscopy every 10 years could decrease their risk of developing colon cancer by as much as 75%.

These examples show that early detection and screening programmes are efficient at reducing the incidence and mortality of a few, select forms of cancer. But they also demonstrate the lack of efficacious methods and/or biomarkers available to detect cancer during the early asymptomatic stages of the disease, which represents a major bottleneck for the improvement of cancer diagnostics and therapy. The use of high-throughput methods to detect and analyse tumour biomarkers could provide a solution to this problem.

Until recently, the method of choice for detecting biomarkers was two-dimensional gel electrophoresis (2-DE PAGE). Although it proved effective for some specific uses, 2-DE PAGE is a time- and effort-consuming method that requires large sample volumes for adequate quantification. Furthermore, 2-DE PAGE cannot be used to analyse proteins with a molecular mass of less than 10 kDa, which excludes many important protein factors that are involved in a wide variety of regulatory processes in the cell.

Clinicians are also using several tumour markers in the blood for cancer diagnosis,

Table 1 | Randomly chosen examples of clinical studies using the -omics methods

Focus of article	Reference
Gene expression data in building prognostics models for early-stage lung cancer.	Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma <i>et al</i> (2008) Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. <i>Nat Med</i> 14 : 822–827
Gene expression in cytologically normal large-airway epithelial cells can act as a lung cancer biomarker, potentially owing to a cancer-specific airway-wide response to cigarette smoke.	Spira A <i>et al</i> (2007) Airway epithelial gene expression in the diagnostic evaluation of smokers with suspect lung cancer. <i>Nat Med</i> 13 : 361–366
Predicting medulloblastoma clinical outcome by gene expression profiling.	Korenberg MJ (2004) On predicting medulloblastoma metastasis by gene expression profiling. <i>J Proteome Res</i> 3 : 91–96
Discovery of bone metastasis genes. Metastasis requires the concerted action of multiple genes that belong to different functional classes.	Kang Y <i>et al</i> (2003) A multigenic program mediating breast cancer metastasis to bone. <i>Cancer Cell</i> 3 : 537–549
186 genes as the “invasion signature”.	Liu R <i>et al</i> (2007) The prognostic role of a gene signature from tumorigenic breast cancer cells. <i>N Engl J Med</i> 356 : 217–226
Prediction: which of the node-negative breast cancer patients would develop distant metastasis during the 5-year follow up period and which would remain disease-free?	Wang Y <i>et al</i> (2005) Proteomic profiling of urine for the detection of colon cancer. <i>Lancet</i> 365 : 671–679
Proteomic profiling of urine for the detection of colon cancer.	Ward DG <i>et al</i> (2008) Proteomic profiling of urine for the detection of colon cancer. <i>Proteome Sci</i> 6 : 19
Gene expression profiling allows reliable prediction of lymph node metastasis in cervical cancer.	Kim T-J <i>et al</i> (2008) Gene expression profiling for the prediction of lymph node metastasis in patients with cervical cancer. <i>Cancer Sci</i> 99 : 31–38
Pathological changes within an organ might be reflected in the proteomic pattern in serum. Proteomics analysis of serum could recognize ovarian cancer cases.	Petricoin E <i>et al</i> (2002) Use of proteomic patterns in serum /to identify ovarian cancer. <i>Lancet</i> 359 : 572–577

although they are not suitable for detecting early-stage cancer. Among them are the frequently used carcinoembryonic antigen (CEA), α -fetoprotein (AFP) and PSA, as mentioned earlier. Increased levels of CEA can indicate the presence of colon or pancreatic cancer, but the marker is not very specific as it is also elevated in patients suffering from ulcerative colitis, Crohn's disease or liver cirrhosis. However, CEA is a helpful marker for post-treatment monitoring and therapeutic efficacy: if the tumour resection is successful, CEA disappears from the patient's serum. Similarly, AFP is used for the early detection of hepatocellular carcinoma in patients with cirrhosis or α -1-antitrypsin deficiency, but this marker is also elevated in patients with hepatitis. PSA, together with other clinical parameters, is a biomarker for

prostate cancer, but can also be found in patients with benign prostate hypertrophy. Therefore, none of these markers are specific and they significantly fail to detect cancer at a very early stage.

Advanced high-throughput methods, based on the analysis of cell lysates, protein microarrays or the direct analysis of serum proteins by mass spectrometry, could therefore successfully complement the traditional methods outlined above. Their great advantage is that they can be used to monitor gene expression and protein content in parallel: a process known as molecular profiling. For example, during the past few years, SELDI-TOF mass spectrometry has been applied to the molecular profiling of cellular and serum protein content. The information gained should help scientists to develop a

new generation of biomarkers—named protein patterns—which will be more suitable for practical use in early cancer diagnostics. A protein pattern profile will not be a biomarker in a traditional sense; instead, it will detect characteristic patterns of protein content in various samples—such as serum, plasma or urine—as opposed to focusing on individual select proteins (Clarke *et al*, 2003; Petricoin *et al*, 2002a,b; Varnum *et al*, 2003). It is important that molecular biologists and clinicians accept this approach—to look for protein patterns instead of single proteins—because SELDI-TOF mass spectrometry indicates that there are not many single and specific biomarkers that indicate the occurrence of a particular disease.

Nonetheless, the main question remains whether it is possible to predict an individual's susceptibility to cancer based on protein patterns. This is an enormously important question to answer for public health, health-care and clinical practice, as many cancer patients are still not diagnosed until after the tumour has metastasized, which is usually too late for therapeutic intervention. Up to 60% of patients with breast, colon and lung carcinoma have microscopic or advanced disease at the time of diagnosis (Sahai, 2007), and more than 80% of patients with ovarian cancer are at an advanced clinical stage when they are diagnosed (Pantel & Brakenhoff, 2004). Many more patients might be cured if their cancer were detected earlier.

Modern cancer therapy therefore requires a personalized approach in which each patient is treated according to the specific genetic defects in his or her tumour

Pathological changes in an organ—such as cancerous growth—can be reflected in the protein pattern in the blood. Just 1 ml of human serum contains 60–80 mg of proteins and various small molecules, including salts, lipids, amino acids and sugars. Apart from the main protein constituents—albumin and immunoglobulins—the serum contains many other proteins that are synthesized and secreted or just shed from cells and tissues owing to necrosis, apoptosis and haemolysis. In addition, blood serum is easy to obtain through a minimally invasive procedure. Proteomic serum profiling therefore has the

potential to replace, or at least complement, the current methods of screening for tumour markers in early cancer detection.

In any case, blood serum is not the only sample that can be used for molecular profiling: the proteomic profiling of urine has already been successfully applied to the diagnosis of renal transplant rejection (Clarke *et al*, 2003), and new biomarkers for Alzheimer disease have been identified through the protein pattern profiling of cerebrospinal fluid (Carrette *et al*, 2003; Hampel *et al*, 2003; Lewczuk *et al*, 2003). Unfortunately, we do not yet have effective biomarkers for the early detection of cardiovascular diseases.

As mentioned above, the search for specific tumour markers analyses just one protein at a time, whereas protein patterns of human serum represent up to 15,000 proteins and/or protein fragments that are characterized by their mass-to-charge ratios (Kallioniemi, 2004). The sensitivity and specificity of these molecular profiles has exceeded 90% in lung cancer diagnostics and is approaching 100% in the detection of ovarian cancer. These impressive numbers indicate that proteomic pattern analysis can be developed into a reliable and effective method for the early diagnosis of these two types of cancer.

Currently, more than two-thirds of patients with ovarian cancer are not diagnosed until an advanced stage when the cancer cells have spread throughout the peritoneal cavity (Petricoin *et al*, 2002b). At this point, the success of therapeutic modalities is limited and the five-year survival rate drops to only 35–40%. If it were possible to detect ovarian cancer at an earlier stage, before dissemination, the effectiveness of conventional therapy would increase markedly and the five-year survival rate would be around 95% (Cohen *et al*, 2001). The application of high-throughput methods for early cancer detection—or at least detection in its pre-malignant state—could therefore have a tremendous impact on treatment outcomes. Moreover, a well-equipped clinical laboratory could perform hundreds of proteomic profiles per day, which would make it easier to screen a large number of people for disease markers.

Another problem that could be addressed by high-throughput analysis methods is the choice of an appropriate therapy according to the developmental stage of the tumour. Clinicians still rely on the micro-morphological analysis of

biopsy specimens under the microscope for disease prognosis. This is a subjective method of evaluation and empirical evidence indicates that tumours of similar histological appearance might have different clinical outcomes and therefore require alternative therapeutic approaches (Espina *et al*, 2004). More generally, each malignant tumour has its own unique genetic and molecular signature: there is no effective universal therapy to treat all malignant diseases. Modern cancer therapy therefore requires a personalized approach in which each patient is treated according to the specific genetic defects in his or her tumour.

It is therefore only a matter of time until high-throughput analysis spreads among clinics and, over time, changes clinical practice...

Functional genomics provides medicine with a range of new methods to monitor the development and progression of disease, and to select the correct treatment (Fig 1). However, it will not necessarily make therapeutic intervention any easier; the more scientists understand about cancer, the more they realize the complex nature of the disease. Nevertheless, combining our knowledge of the molecular processes in cancer development with high-throughput methodologies should lead to the identification of new biological indicators. In turn, these should help clinicians to make two important decisions: which patients to treat, based on prognostic biomarkers; and which therapy is most likely to be effective, based on predictive biomarkers (Van't Veer & Bernards, 2008). More specifically, prognostic biomarkers predict the clinical outcome for an untreated patient, whereas predictive biomarkers predict the outcome of a specific therapy for a given patient. Today, for example, more than 85% of women with early-stage, lymph-node-negative breast cancer are treated with adjuvant chemotherapy to prevent the recurrence of malignant cells. However, only 20–30% of the women with this type of cancer are likely to relapse after surgical removal of the tumour and localized radiation treatment. In the other cases, resection of the primary tumour by surgery is usually sufficient and harmful adjuvant chemotherapy is

therefore not necessary. The current conventional pathological parameters fail to distinguish adequately those patients who are likely to relapse from those who will not, and therefore do not require additional chemo- or radiotherapy.

High-throughput genomic techniques have already been used to analyse the unique molecular signatures of individual tumour types to classify patients into prognostic groups (Chung *et al*, 2002; Staudt, 2002). Microarray profiling of breast tumours, based on 70 informative genes, has revealed gene-expression signatures that relate to a good or a poor prognosis (van de Vijver *et al*, 2002). In this study, approximately 40% of the early-stage breast cancers were found to have had “a good signature” meaning that, although many of the patients received adjuvant chemotherapy, less than 1% of them were likely to benefit from it. An increasing number of researchers are therefore suggesting that adjuvant chemotherapy should be used only in those patients with an evident poor signature, as they are the ones who might actually benefit from the therapy.

Another group of patients who might benefit from individualized therapy are those diagnosed with diffuse large B-cell lymphoma (DLBCL). DLBCL is a subtype of non-Hodgkin lymphoma and it is possible to assign patients to prognostic subgroups according to their gene-expression profiles. Alizadeh and colleagues have developed so-called lymphochips (Alizadeh *et al*, 2000), which are specialized microarrays for the study of non-Hodgkin lymphoma that measure the activity of genes preferentially expressed in lymphoid cells, as well as genes with known or suspected roles in immunity or cancer. The study identified two distinct patient groups based on the gene-expression signature of the cancer with significant differences in survival rate: 76% of patients in the good signature group were still alive after five years compared with 16% in the poor signature group.

The completion of the Human Genome Project in 2003 revealed inherited genetic diversity between humans. As genetic diversity must lead to proteomic diversity, it is evidently necessary to adapt any therapy to an individual patient according to his or her unique genetic and proteomic constitution (Espina *et al*, 2004). As discussed above, high-throughput molecular profiling methods are particularly suited to revealing these

Fig 1 | The -omics approach to studying molecular mechanisms of disease and individualized therapy. Various samples (for example, cells and tissue) are ‘attacked’ by -omics procedures and processed by bioinformatics in order to learn about molecular basis of disease and early detection of disease.

individual genetic and proteomic patterns, which provide the basis for rational and individualized treatments.

Early detection and diagnosis, as well as individually tailored therapies, represent some of the most important applications of modern molecular medicine. Yet, there is still a great deal of work to be done in basic research and at the clinic. Given that diseases often result from both genetic and environmental factors, and that the genes that predispose an individual to getting a disease can sometimes overlap with other protective genes, the results of molecular profiling should be interpreted with caution. In addition, as most tissues are composed of many different cell types, there are unique and complex interactions between pathological and normal cells in any organ affected by disease. Furthermore, immune system function, nutritional status and the substances that mediate cell–cell interactions also have an important role (Liotta & Clair, 2000; Liotta, 2001; Liotta & Kohn, 2001). Differentiated human cells, whether diseased or not, are a product of their microenvironment and their genetic constitution. Cells with genetic defects therefore express various proteins depending on the particular defect, the microenvironment and their interactions with other cells. If one multiplies the environmental influences by the existing individual genetic variations, we

can see from the resulting number of possible interactions that using individual molecular profiles to inform therapy will remain a complex and complicated task.

Most drugs in use today do not act on a specific target, pathogen or cell type. Chemotherapeutics to treat malignant cancer, for example, attack all dividing cells in the body—not only tumour cells, but also normal body cells undergoing mitosis. Not surprisingly, these powerful cancer drugs have many serious side effects. Similarly, antibiotics do not only kill pathogenic bacteria, but also many beneficial ones.

Individualized therapies might therefore be more precise and effective with fewer side effects as they could make better use of existing drugs. On the basis of the molecular fingerprint of a disease—be it cancer, cardiovascular disease or an infection—doctors will be able to prescribe a therapy tailored to specific targets such as a molecular defect or invading pathogens (Kraljevic & Pavelic, 2005; Kraljevic *et al*, 2006). As a result, a disease could be efficiently cured with fewer side effects. The anti-cancer drug Herceptin® (trastuzumab; Roche, Basel, Switzerland), for example, is highly effective for treating late-stage breast cancer and metastases, but only in a subset

of patients whose cancer cells overexpress the HER2 surface receptor.

In addition, we are also likely to see more 'smart drugs', some of which are under development or are already on the market. These are small peptides or other molecules that tackle specific surface receptors on cells or induce specific changes in metabolism or gene expression. An example of such a 'smart drug' is the HIV protease inhibitor, which specifically targets the HIV protease to prevent maturation of the virus.

However, the development of highly specific drugs is an arduous task that requires much time, effort and investment—the cost of developing, testing and bringing a new drug to the market is constantly increasing, and is now between US\$800 million and US\$1 billion (Kraljevic *et al*, 2004). Pharmaceutical companies are already using high-throughput technologies for compound screening and target identification. Yet, we believe that the further development and application of new technologies from functional genomics and proteomics—such as microarrays or SELDI-TOF mass spectrometry—to the identification of new drug targets might accelerate the drug discovery process while lowering the overall costs.

The application of high-throughput methods from functional genomics and proteomics—and, in the longer term, metabolomics—will profoundly change all aspects of daily clinical practice, be it diagnostics, therapy or prevention. At present, however, most of these techniques are not sufficiently robust, efficient or easy to use, and therefore require further refinement and improvements to overcome these limitations. We think that the first applications to be used in clinical laboratories will be tools to monitor and analyse protein compositions in various biological samples, post-translational protein modifications and protein–protein interactions. At the same time, basic and biomedical research into functional genomics and proteomics are generating huge amounts of information that requires analysis and interpretation. We therefore need to develop new bioinformatics methods and tools for adequate and reliable data analysis both in basic research and in clinical applications.

Some of these new methods are already being used as diagnostic procedures at a few large academic medical centres—those that have the necessary expertise. But, it will take another 5–10 years until high-throughput analytical methods become available and

affordable for general clinical practice. In any case, functional genomics and proteomics are no longer confined to basic biomedical research laboratories; with further improvement and development, these tools and methods will become more robust, efficient and affordable. It is therefore only a matter of time until high-throughput analysis spreads among clinics and, over time, changes clinical practice from treating mainly symptomatic diseases towards preventive and prospective practice. Nearly 20 years after the Human Genome Project was started, it is beginning to deliver the promised benefits—not necessarily new cures, but certainly a revolutionary change in medical practice.

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Hrvojka Bosnjak (top left), Kresimir Pavelic (top right) & Sandra Kraljevic Pavelic are at the Rudjer Boskovic Institute, Division of Molecular Medicine, in Zagreb, Croatia. Kresimir Pavelic & Sandra Kraljevic Pavelic are also at the University of Rijeka, Department of Biotechnology in Rijeka, Croatia.
E-mails: hrvojka.bosnjak@zg.t-com.hr; pavelic@irb.hr; skraljevic@irb.hr

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