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Advantages and limitations of microarray technology in human cancer

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Cancer is a highly variable disease with multiple heterogeneous genetic and epigenetic changes. Functional studies are essential to understanding the complexity and polymorphisms of cancer. The final deciphering of the complete human genome, together with the improvement of high throughput technologies, is causing a fundamental transformation in cancer research. Microarray is a new powerful tool for studying the molecular basis of interactions on a scale that is impossible using conventional analysis. This technique makes it possible to examine the expression of thousands of genes simultaneously. This technology promises to lead to improvements in developing rational approaches to therapy as well as to improvements in cancer diagnosis and prognosis, assuring its entry into clinical practice in specialist centers and hospitals within the next few years. Predicting who will develop cancer and how this disease will behave and respond to therapy after diagnosis will be one of the potential benefits of this technology within the next decade. In this review, we highlight some of the recent developments and results in microarray technology in cancer research, discuss potentially problematic areas associated with it, describe the eventual use of microarray technology for clinical applications and comment on future trends and issues.
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Introduction

In April of this year, we witnessed one of the most monumental achievements in biology: the complete sequencing of the human genome. The decoding and database deposition of billions of bases of sequence is the starting point of postsequence functional genomics. The discovery of the Periodic Table had an important impact on chemistry. So too, the complete deciphering of the human genome will have impressive effects on human health and quality of life. Currently, we understand the function of only a limited number of human genes. To study all human genes function is a technological challenge. To face this challenge, new high-throughput tools have been developed. The microarray assay is a powerful molecular technology that allows the simultaneous study of the expression of thousands of genes or their RNA products, giving an accurate picture of gene expression in the cell or the sample at the time of the study.

For example, the expression of all the genes for drug resistance and metabolism or all the known oncogenes in a cell can be detected and measured in the same timeframe (Brown and Botstein, 1999; Collins, 1999; Lander, 1999). The microarray can be defined as an ordered collection of microspots (the probes), each spot containing a single species of a nucleic acid and representing the genes of interest. This technology is based on hybridization between labeled free targets derived from a biological sample and an array of many DNA probes that are immobilized on a matrix (Southern *et al*., 1999). The targets are produced by reverse transcription and the simultaneous labeling of RNA extracts from a biological sample hybridized with DNA fragment probes. The hybridization signal produced on each probe is the mRNA expression level of the corresponding gene in the sample at the time of the study. The signals are detected, quantified, integrated and normalized with dedicated software and reflect the 'gene expression profile' or 'molecular portrait' for each biological sample.

Many thousands or tens of thousands of distinct spots can be printed on a silicon or glass slide or a nylon solid-state base. There are mainly two variants of microarrays: cDNA and oligonucleotide microarrays (Schena *et al*., 1995, 1996; Lockhart *et al*., 1996). Although both types of microarray are used to analyse gene expression patterns, these variants are fundamentally different (Lipshutz *et al*., 1999). In cDNA microarrays, relatively long DNA molecules are immobilized on a solid surface. This type of microarray is mostly used for large-scale screening and expression studies. The oligonucleotide microarray is fabricated by *in situ* light-directed chemical synthesis or by conventional synthesis followed by immobilization on a glass matrix. This microarray is used for detection of mutations, gene mapping and expression studies and allows for the differential detection of gene family members or alternative transcripts that are not distinguishable by cDNA microarrays.

The chemistry of the microarray in itself is not new, since hybridization technology has been well established for decades. However, the simultaneous study of

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thousands of genes transforms the microarray technique into a powerful whole system analytical tool. Almost 10 years have passed since the first microarrays were created, and yet this technology is still improving and advancing. Since its initial introduction, the number of microarray applications has expanded (Figure 1). Starting from their use in gene screening and target identification, this technology is finding new applications such as developmental biology, disease classification, pathway studies, drug discovery and toxicology. The technology involved in the production and use of the microarray is beyond the scope of this review, but has been extensively reviewed elsewhere (Schena *et al*., 1995; Niemeyer and Blohm, 1999; Bowtell, 1999; Brown and Botstein, 1999; Celis *et al*., 2000; Cheung *et al*., 1999; Duggan *et al*., 1999; Graves, 1999; Khan *et al*., 1999; Hegde *et al*., 2000; Meldrum, 2000). We describe here some of the recent developments and results in microarray technology in cancer research, discuss potential problems, describe clinical applications and comment on the future of this technology.

The importance of measuring global gene expression in human cancers

Characterizing the population of transcribed genes has led to the creation of a new term, the *transcriptome* (Su *et al*., 2002). This concept defines the complete set of transcribed genes expressed as messenger RNAs for a particular species. The transcriptome, therefore, represents the universe of RNA messengers that may code for proteins. Only approximately 5% of genes are active in a particular cell at any given point in time. Most of the genes are repressed, and this control may occur at either the transcriptional or the translational level. Since the regulation of protein expression at the level of transcription is more efficient, most control takes place at this level. The gene expression profile of a cell determines its function, phenotype and response to external stimuli. Therefore, gene expression profiles can help to elucidate cellular functions, biochemical pathways and regulatory mechanisms. In addition, gene expression profiles of disease cells/tissues, compared with normal controls, may promote the understanding of disease pathology and identify new therapeutic points of intervention, improving diagnosis and clarifying prognosis.

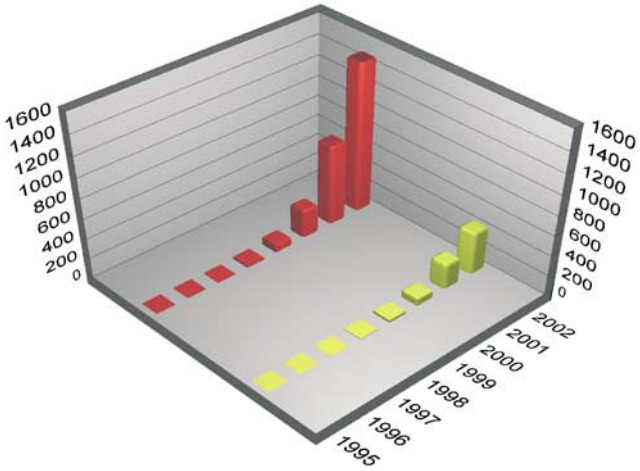

Figure 1 Number of citations found in MEDLINE by inserting 'microarray' (yellow bar) or 'microarray + cancer' (red bar) for a PubMed search. Articles were published between 1995 and 2002

During the past few years, several gene expression profiling methods have emerged and have been applied successfully to cancer research. These include differential display, serial analysis of gene expression and microarrays (Velculescu *et al*., 1995; Granjeaud *et al*., 1999; Cheng *et al*., 2002). The microarrays have become important because they are easier to use, do not require large-scale DNA sequencing and allow the parallel quantification of thousands of genes from multiple samples. Gene expression profiling of cancers represents the largest category of research using microarray technology and appears to be the most comprehensive approach to characterize cancer molecularly. Although the cancer phenotype is only partially determined by its transcriptome, it still provides a clear picture of a cell's physiological state. The power of this approach has been demonstrated in studies performed on an extensive variety of malignancies, including cancers of the breast, head and neck, liver, lung, ovary, pancreas, prostate and stomach (Bhattacharjee *et al*., 2001; Dhanasekaran *et al*., 2001; Garber *et al*., 2001; Tonin *et al*., 2001; Al Moustafa *et al*., 2002; Belbin *et al*., 2002; Chen *et al*., 2002; Han *et al*., 2002; Hedenfalk *et al*., 2002; Hippo *et al*., 2002; Luo *et al*., 2002a).

Several studies of cancer profiling by microarray analysis have used different strategies such as tumor *versus* control, in which the tumor gene expression profile is compared with its corresponding control sample in order to measure the differences and similarities between both phenotypes, cancer stratification, in which the gene expression profiles from different samples of the same cancer type are compared to reveal distinct subgroups to better define molecular classification of a common histological type of cancer, and finally temporal evaluation of the tumor, in which the gene expression patterns from tumor samples derived from different stages of progression are compared to elucidate the differences between the early and advanced stages of the disease. Although many studies of microarray analysis in human disease have been published, we present here some of those having a clinical interest for oncology.

Microarray and prostate cancer

Several studies using microarrays to characterize prostate cancer gene expression profiles have recently been published. These studies have used microarray technology as a gene discovery tool to identify genetic markers that discriminate between normal and cancerous prostate tissues. A simple microarray study has been performed using spotted membrane arrays to analyse normal and cancerous tissues and cell lines (Bull *et al*., 2001). Membrane microarray findings are limited by the

relative insensitivity of this technique to detect transcripts expressed at low levels and the small number of spots that can be placed on the membranes; however, this study has yielded candidate markers of prostate cancer for further evaluation. Five published studies have analysed gene expression profiles in several thousand genes in normal and prostate tissues and used unsupervised hierarchical clustering analysis to sort specimens (Dhanasekaran *et al*., 2001; Luo *et al*., 2001, 2002b; Welsh *et al*., 2001a; Singh *et al*., 2002). Dhanasekaran *et al*. (2001) were able to distinguish normal prostate, benign prostatic hyperplasia (BPH), localized prostate cancer and metastatic prostate cancer samples using 9,984 element-spotted microarrays. Using hierarchical clustering analysis, Luo *et al*. (2001) were able to discriminate 16 prostate cancer samples from nine BPH specimens on the basis of differences in gene expression profiles as measured on 6,500 element-spotted cDNA microarrays. Welsh *et al*. (2001a) reported a similar sorting of normal and malignant prostate tissue samples using oligonucleotide microarrays. Interestingly, all the five groups identified the transmembrane serine protease hepsin as displaying significantly increased expression in malignant tissues as compared with that of normal prostate tissue (Dhanasekaran *et al*., 2001; Luo *et al*., 2001, 2002b; Welsh *et al*., 2001a; Singh *et al*., 2002). Many other candidate markers of prostate cancer such as the proto-oncogene *PIM1* have emerged from other studies and are being further investigated as potential diagnostic markers. The diminished *PIM1* expression on the immunohistochemistry of prostate tumor samples conferred an increased risk of recurrence after surgery (Dhanasekaran *et al*., 2001). Other groups using combinations of subtractive hybridization and microarray analysis have identified several potential candidates for prostate cancer immunomodulatory therapy including prostein (Xu *et al*., 2001), STEAP (Hubert *et al*., 1999) and p504S/Alpha-Methylacyl-CoA-Racemase (Jiang *et al*., 2001). In a very recent study, Virolle *et al*. (2003) used a prostate cancer cell line that expresses a high constitutive level of Egr1 protein, a transcription factor overexpressed in the majority of aggressive tumorigenic prostate cancer cells. They assessed *Egr1* transcriptional regulation, by performing an oligonucleotide microarray analysis using cells rendered deficient in *Egr1* as the comparison sample for the identification of Egr1 target genes. For the first time in prostate tissues, this study confirmed the growth-enhancer role of Egr1 previously observed in other cellular systems, and identified several new target genes specifically controlling growth, cell cycle progression and apoptotic pathways.

Microarray and oral cancer

To date, only a few microarray studies relevant to oral cancer have been published. Chang *et al*. (1998) illustrated the use of cDNA microarrays to characterize transformation-related genes in oral cancer. Villaret *et al*. used a combination of complementary DNA subtraction and microarray analysis to evaluate unique genes specific for squamous cell carcinoma of the head and neck (HNSCC) as potential tumor markers and vaccine candidates. Nine known genes were found to be significantly overexpressed in HNSCC as compared to normal tissue. In addition, four novel genes were overexpressed in a subset of tumors (Villaret *et al*., 2000). Alevizos *et al*. (2001) analysed the transcriptome in oral cavity squamous cell carcinoma. They found about 600 candidate genes (oncogenes, tumor suppressors, transcription factors, differentiation markers, metastatic proteins and xenobiotic enzymes) that were differentially expressed in oral cancer, validating only three of these genes by PCR.

Lu *et al*. (2001) used the microarray approach to evaluate gene expression profile changes during the initiation and progression of squamous cell carcinoma of the esophagus. They examined gene expression profiles in different stages of the initiation and progression of esophageal cancer in order to identify genes differentially expressed between these stages. Frierson *et al*. (2002) used oligonucleotide microarray analysis to study the expression of 8,920 different human genes in 15 adenoid cystic carcinomas (ACCs), one ACC cell line and five normal major salivary glands. Among genes with altered expression in ACC were those encoding the transcription factors SOX4 and AP-2 gamma, casein kinase 1 as well as epsilon and frizzled-7, both of which are members of the Wnt/beta-catenin signaling pathway. In a very recent study, Leethanakul *et al*. (2003) generated high-complexity cDNA libraries from laser capture microdissected normal and cancerous squamous epithelium. In this study, the authors surveyed the available sequence information using bioinformatic tools and identified 168 novel genes differentially expressed in normal and malignant epithelium. Moreover, using cDNA arrays, they obtained evidence that a subset of these new genes might be highly expressed in HNSCC.

Microarray and breast cancer

Given the clinical heterogeneity of breast cancer, microarray technology may be an ideal instrument to establish a more accurate classification. Initial studies using microarray-based expression profiling demonstrated its ability to correctly classify estrogen receptor-negative and estrogen receptor-positive breast cancers (Perou *et al*., 2000; West *et al*., 2001) and to differentiate BRCA1-related tumors from BRCA2-related and sporadic tumors (Hedenfalk *et al*., 2001; van't Veer *et al*., 2002).

The study of van't Veer *et al*. has been one of the most extensive and informative studies performed to date. The authors examined 117 primary breast samples by microarray-based gene expression profiling to develop prognostic profiles and compare these with known prognostic markers in breast cancer. Out of the 5,000 genes with variable expression profiles, 70 were identified for optimal accuracy in predicting recurrent disease. Using this classification, the authors correctly predicted the actual outcome of disease for 65 out of the 78

patients. Five patients with good prognosis and eight patients with poor prognosis were incorrectly assigned. Standard prognostic markers in breast cancer were used to estimate the risk of cancer recurrence and help to make decisions about adjuvant therapy. Unfortunately, current prognostic markers do not adequately identify the most correct therapy for the patient. The predictive power of the microarray approach is much greater than that of currently used approaches, but it needs to be validated in more prospective clinical studies. If the prognostic value of this approach was confirmed, the expression-profiling classifier would result in about a four-fold decrease in patients receiving adjuvant therapy unnecessarily (Caldas and Aparicio, 2002).

Martin *et al*. (2001) described a method of identifying circulating breast cancer by a two-step process of differential display and high-sensitivity array-based expression profiling. Even if the potential of this technique is promising, its sensitivity and specificity still need to be improved and more work is required to determine the clinical significance of gene expression profile detection in the peripheral blood. Some articles have now demonstrated a link between tumor expression profiles using microarray technology and clinical outcome. For example, Sorlie *et al*. (2001) demonstrated that tumor subclasses defined by expression profiling can predict disease-free and overall survival, and Sotiriou *et al*. (2002) showed that pretreatment expression profiles predicted clinical response to chemotherapy in a small sample of breast tumors. Although the study of Sorlie *et al*. was very provocative, the authors did not compare the prognostic value of the groups identified by hierarchical clustering with currently used prognostic factors in breast cancer. Since drug resistance in cancer is a major obstacle to successful chemotherapy, the feasibility of obtaining a potential molecular profile or fingerprint of anticancer drugs in cancer cells by microarray technology is critical to predict chemotherapy response. Kudoh *et al*. (2000) demonstrated this ability to define changes in gene expression profiles in a breast cancer cell line treated with chemotherapy. They monitored the expression profiles of MCF-7 breast cancer cells that were either transiently treated with doxorubicin or selected for resistance to doxorubicin. This study showed that transient treatment with doxorubicin altered the expression of a diverse group of genes in a time-dependent manner.

Microarray and ovarian cancer

Over the past few years, several investigators have published interesting studies regarding expression profiling of ovarian cancers. Martoglio *et al*. (2000) analysed the gene expression profiles of five normal ovaries and four poorly differentiated serous papillary ovarian adenocarcinoma samples. Using a small 'in-house' nylon membrane cDNA microarray, they found an overall increase in angiogenesis-related markers (e.g. angiopoietin-1, VEGF), apoptotic and neoplastic markers, immune response mediators and novel potential markers of ovarian cancer (e.g. cofilin, moesin and neuron-restrictive silencer factor protein) in the cancer tissue. The study was intriguing because they used a low-cost cDNA array tailored for studies of specific pathways, such as angiogenesis and tumorigenesis. Since it is problematic to access an adequate quantity of early ovarian tumor tissue, the researchers used different strategies to circumvent the need of tissue amounts typically required by microarray analysis. For example, Ismail *et al*. (2000) reported a study of 864 DNA elements screened against 10 ovarian cancer cells lines and five normal epithelial cell lines using short-term cell culture to expand the ovarian surface epithelium before RNA extraction. Other investigators purified ovarian epithelium by *in vitro* procedures, such as adherence to glass or immunomagnetic enrichment (Ono *et al*., 2000; Welsh *et al*., 2001b). However, these two approaches may introduce biases in observed gene expression. In fact, the first approach (Ismail *et al*., 2000) uses cultured cancer cells, which may not reflect *in vivo* cancers because of the possibility of secondary gene expression changes occurring *in vitro* as a result of culture conditions. The second strategy (Ono *et al*., 2000; Welsh *et al*., 2001b) is very long and may result in the degradation of less stable RNA messengers. To avoid possible biases inherent in *in vitro* cultures used in some studies (Ismail *et al*., 2000; Matei *et al*., 2002), other investigators have studied gene expression patterns directly from surgically resected tumors (Shridhar *et al*., 2001). Small, specialized microarrays have several practical advantages and can reveal information that may be lost in larger microarrays. Sawiris *et al*. (2002) used a highly specialized cDNA microarray named 'Ovachip', and found this microarray extremely sensitive at differentiating ovarian cancer from colon cancer based on gene expression patterns. Screening biomarkers for ovarian cancer are very important because of the late stage at diagnosis and poor survival associated with this type of cancer. Recently, two studies used microarray technology to identify two overexpressed potential ovarian cancer serum markers called osteopontin and prostasin, and reported preliminary validation of their use for the early detection of the disease (Mok *et al*., 2001; Kim *et al*., 2002).

Microarray and other cancers

The application of microarray technology to other human cancers is rapidly expanding. The pioneering study of Golub *et al*. (1999) demonstrated the possibility to distinguish acute myeloid leukemia and acute lymphoblastic leukemia (ALL) based on gene expression monitoring and how, in a simulated situation 'blinded' to the histological diagnosis, the two classes could have been discovered by the gene expression patterns alone. Alizadeh *et al*. (2000) identified the two forms of diffuse large B-cell lymphoma (DLBCL) on the basis of gene expression profiles that are indicative of different stages of B-cell differentiation. Interestingly, this molecular classification has prognostic value independent of stratification by the usual clinical grading. To study

gene expression in lymphoid malignancies, a large collaborative group created a specialized microarray, named 'Lymphochip', which is enriched in genes that are selectively expressed in lymphocytes and in genes regulating lymphocyte function (Alizadeh *et al*., 1999). This group used this microarray to examine DLBCL and found two molecularly different forms of this tumor. Furthermore, they demonstrated that the DLBCL subgroups defined a subgroup of patients with a distinct clinical prognosis. To test the hypothesis that B-cell chronic lymphocytic leukemia (CLL) is more than one disease, Rosenwald *et al*. (2001) related the gene expression patterns of CLL to their Ig mutational status and to other types of normal and malignant B cells. Interestingly, the genes identified as highly expressed in CLL compared to DLBCL were expressed equivalently in all CLL samples regardless of their Ig mutational status. This study suggested that all CLL cases shared a common mechanism of transformation and/or cell of origin. A recent study (Stratowa *et al*., 2001) has proposed a list of potential new prognostic markers involved in lymphocyte trafficking and associated with disease staging and/or patient survival.

In a very recent study, Gariboldi *et al*. (2003) analysed the gene expression profiles in the normal tissue of skin tumor-susceptible and -resistant mice in order to identify genes that play a functional role in genetic susceptibility. This study has suggested a role of *Scca2* gene, a member of the serine protease inhibitor superfamily, in the genetic predisposition to skin tumors.

Microarray technology has also been used in analysing melanoma (Bittner *et al*., 2000). This study suggested that gene expression profiles within an individual patient's tissue may be remarkably conserved over time and that global transcript analysis can identify unrecognized subtypes of cutaneous melanoma and predict experimentally verifiable phenotypical characteristics.

Studies on colon cancer cells and tissues demonstrated significant suppression of the kinase gene, *WEE1Hu* (Backert *et al*., 1999).

Many transcriptomes change after specific overexpression of tumor-related genes. For example, we have utilized an adenovirus-mediated expression system of the *RB2/p130* tumor-suppressor gene in a non-small lung cancer cell line in order to identify specific genes that are regulated by pRb2/p130 (Russo *et al*., 2003). Our microarray results have identified a variety of genes involved in many cellular processes including cell division, cell signaling/cell communication, cell structure/motility and gene expression and metabolism. These results suggest new potential therapeutic biomarkers in lung carcinoma. Furthermore, the results of another cDNA microarray study indicate that the overexpression of the tumor-suppressor gene *PTEN* may inhibit lung cancer invasion by downregulating a panel of genes (Hong *et al*., 2000). In the light of the above data, it is clear that the microarray approach is very important in the analysis of a variety of tumor types.

Microarray-derived technologies and cancer

Besides DNA microarray, other array-based technologies have been developed. Here we review two of them, tissue microarray and proteome microarray, and their applications in cancer research.

Tissue microarray

Using this technology, small tissue cylinders are acquired from hundreds of formalin-fixed tissues and are arrayed at a high density to form a single paraffin block. Typical tissue microarrays contain up to 1,000 tissue specimens, and they are used for simultaneous *in situ* analysis of DNA, RNA and protein targets. The idea of bringing multiple tissue specimens into a single slide is not new (Battifora, 1986), but this technology uses a different sampling approach maximizing the number of parameters that can be analysed from each specimen and providing a higher level of sophistication. The first paper about tissue microarray described the potential of this technology to detect six gene amplifications as well as *p53* and estrogen receptor expression in breast cancer (Kononen *et al*., 1998). So far, typical tissue microarray technologies include multitumor microarrays, progression, prognosis and cryomicroarrays.

A multitumor microarray is composed of samples from multiple histological tumor types. The first example is the study of Schraml *et al*. (1999), which used a tissue microarray containing 397 samples from 17 different tumor types. The authors used this array technology to screen for amplifications of the oncogenes *CCND1, MYC* and *HER2* by FISH analysis and found high concordance with the previous literature.

Progression microarray contains samples of different stages of tumor progression within a given organ such as the breast, kidney or prostate (Kononen *et al*., 1998; Bubendorf *et al*., 1999a,b; Moch *et al*., 1999).

Prognosis microarrays contain tumor samples from patients for whom clinical follow-up data are available. Several studies comparing molecular data with clinical end points have been published (Moch *et al*., 1999; Barlund *et al*., 2000a,b; Richter *et al*., 2000; Kallioniemi *et al*., 2001; Simon *et al*., 2001, 2002; Torhorst *et al*., 2001). The published studies have confirmed or newly discovered a prognostic significance of estrogen receptor, progesterone receptor, *p53*, *HER2* and S6-kinase expression/amplification in breast cancer; cyclin E amplification/overexpression and Ki67 labeling index in urinary bladder cancer and vimentin expression in kidney cancer. In several studies, multiple samples from each tumor were analysed in four replicas (Nocito *et al*., 2001; Simon *et al*., 2001; Torhorst *et al*., 2001). Interestingly, all associations with prognosis were always identified on each individual replica, suggesting that most associations between molecular changes and clinical end points may be detected on tissue microarrays containing just a single specimen per tumor.

Frozen tissues are superior to formalin-fixed tissues in terms of RNA and protein integrity. Many antibodies

that do not work in formalin-fixed tissues work readily in frozen tissues. One approach with cryomicroarray technology has been described by Fejzo and colleagues (Fejzo and Slamon, 2001). This array utilizes frozen tissues embedded in an optimal cutting temperature compound. Even if the number of specimens that can be analysed per array is lower than that with typical tissue microarrays, this 'cold' technology may overcome the limitations of other tissue microarrays. It may also be used for the analysis of tissues from which RNA is used for DNA microarray, allowing a direct comparison of gene expression profiles obtained from both technologies. Owing to the novel character of this technology, more studies are necessary to standardize and optimize the quality of this array.

Owing to the small size of the arrayed samples, some authors have questioned whether the specimens are truly representative of the entire tumor. Several studies compared results obtained from tissue microarray analysis with results from the corresponding conventional large sections. It was demonstrated that the tissue microarray generally works well and that it can be further enhanced if multiple tissue punches of every sample are included (Sallinen *et al*., 2000; Hoos *et al*., 2001; Torhorst *et al*., 2001). However, it must be kept in mind that large sections represent only a very small fraction of the whole tumor, and that tissue microarray may fully repeat the known associations between molecular alterations and tumor phenotype or clinical end points. Moreover, tissue microarrays are a population-level screening tool, and this technique was not designed to characterize every detail of a particular tumor tissue.

Proteome microarray

A complementary technology to DNA microarrays for monitoring gene expression is provided by *proteomics*, a term generally used to encapsulate the quantification of all proteins, the determination of their post-translational modifications and how these are dependent on cell state and environmental influences. Proteins are frequently functional molecules and therefore provide a better view to understanding gene function and reflecting the post-transcriptional regulation level. Some messengers are transcribed but not translated, and the number of mRNA copies does not necessarily reflect the number of functional protein molecules.

Proteome array-based methodologies have been divided into two classes based upon the nature of the arrayed molecules: proteins or protein-binding particles (DNA, RNA, antibody, ribozymes and modified binding proteins). These two types of proteome microarrays are named protein function array and protein-detecting array, respectively (Kodadek, 2001). In protein function array, a large amount of protein is spotted on a solid support at a defined location and used to characterize either a molecular interaction or a biochemical activity. The protein-detecting array uses a set of protein ligands to analyse gene expression patterns indicative of the cellular state. To date, three different supports have been optimized: glass slides, pieces of polyacrylamide gel on glass slides and microwell plates.

A proteome microarray known as 'ProteinChip' was successfully applied to study head and neck, ovarian and prostate cancers (Senior, 1999; von Eggeling *et al*., 2000). These studies revealed the involvement of proteins in carcinogenic processes and specifically identified cancer biomarkers. Knezevic *et al*. (2001) analysed protein expression in tissue derived from squamous cell carcinomas of the oral cavity through an antibody microarray approach. Using laser-capture microdissection to obtain total proteins from specific microscopic cellular populations, they found differential expression of multiple proteins in stromal cells surrounding and adjacent to regions of diseased epithelium that correlated directly with tumor progression of the epithelium. Paweletz *et al*. (2001) used an interesting reverse system of proteome microarray in which a pool of extracted proteins from a patient sample was printed onto a support, allowing biochemical screening of signaling pathways in prostate cancer.

A very recent study identified autoantibodies against a number of intracellular and surface antigens that were detectable in the sera from patients with different cancer types (Hanash, 2003). Another approach for detecting tumor antigens that induce an immune response using a random peptide-library system was recently published (Mintz *et al*., 2003).

Microarray diagnosis, screening and prognosis

The idea of using microarrays in cancer diagnosis was initially suggested by Khan *et al*. (1998). It was then reinforced by a study by Golub *et al*. (1999) that distinguished between acute myeloid leukemia and ALL by gene expression profiles. It was encouraging that the diagnostic power of this technology was being demonstrated on a range of other cancers known to appear similar on routine histology. Khan *et al*. (2001) were able to correctly classify the four types of small round blue-cell tumors of childhood cancer samples, analysing the gene expression patterns of 83 samples and artificial neural networks. These four distinct categories often represent diagnostic dilemmas in clinical practice. Similarly, Gordon described a classifier of eight genes whose expression was capable of accurately distinguishing between malignant pleural mesothelioma and adenocarcinoma of the lung (Gordon *et al*., 2002). Alizadeh identified two new subclasses of diffuse large B-cell lymphoma derived from different stages of B-cell maturation by gene expression profiling (Alizadeh *et al*., 2000). Bittner identified two subclasses of cutaneous malignant melanoma that further showed distinct aggressive potential (Bittner *et al*., 2000).

Another application of microarray technology is the identification of diagnostic markers through the screening of thousands of tested genes and then comparing gene expression profiles from normal, premalignant and malignant tissues from the same organ. For example, a comparison of gene expression profiles of prostate

samples from different tissue types identified α-methylacyl-CoA racemase as a potential cancer marker (Zhou *et al*., 2002). Another example is the comparison of gene expression patterns of blood samples from women with breast cancer *versus* healthy volunteers (Martin *et al*., 2001). DNA arrays may also contribute to the diagnosis of metastases by identifying the tumor tissue origin (Khan *et al*., 1998; Giordano *et al*., 2001; Ramaswamy *et al*., 2001; Su *et al*., 2001). These studies showed that metastases generally conserve the expression profile from the tissue of origin, thus suggesting the potential of technology to identify the tissue origin of unknown primary site carcinoma.

Another advantage of microarray technology is the identification of gene sets associated with metastasis or response to treatment. In a recent study, Zajchowski identified 24 genes differentially expressed between weakly and highly invasive breast cancer cell lines and showed that their RNA expression profiles were sufficient to predict the aggressiveness of previously uncharacterized cell lines. Other studies have demonstrated correlations between gene expression patterns and chemosensitivity profiles from a panel of NCI60 cancer cell lines to hundreds or thousands of tested chemical compounds (Scherf *et al*., 2000; Staunton *et al*., 2001). Retrospective studies on pretreatment tissue samples have suggested the utility of gene expression profiles in the prognostic classification of solid tumors (Garber *et al*., 2001; Sorlie *et al*., 2001; Takahashi *et al*., 2001; Ahr *et al*., 2002; Beer *et al*., 2002; Bertucci *et al*., 2002; Singh *et al*., 2002; van't Veer *et al*., 2002) and hematological malignancies (Alizadeh *et al*., 2000; Devilard *et al*., 2002; Hofmann *et al*., 2002; Rosenwald *et al*., 2002; Shipp *et al*., 2002). Most of these studies analysed lymphomas and breast cancers. A very encouraging study was performed on DLBCL from 40 patients treated with anthracycline-based chemotherapy (Alizadeh *et al*., 2000). The tumors with a gene expression profile close to that of germinal center B cells had a significantly better survival than the tumors with expression patterns corresponding to activated B cells. This prognostic stratification was confirmed and refined by a study of 240 DLBCL samples (Rosenwald *et al*., 2002).

Comparing the lists of identified genes in several published breast cancer studies (Sorlie *et al*., 2001; Ahr *et al*., 2002; Bertucci *et al*., 2002; van't Veer *et al*., 2002), 26 genes can be found in at least in two lists, despite the different methodologies. Some of these genes have a known prognostic value, while others are new candidates for novel therapeutic targets. Using these strategies, the number of genes needed to diagnose a disease can be narrowed down to only those that act as clear indicators of that disease state.

One tumor one microarray

With the capacity to evaluate thousands of genes and/or proteins in a single experiment by using microarray technology, the potential of this approach in clinical research is enormous. Recently, different groups have begun to develop sophisticated molecular classifiers for a broad range of human cancers (Alizadeh *et al*., 2001; Giordano *et al*., 2001; Ramaswamy *et al*., 2001; Su *et al*., 2001; Agrawal *et al*., 2002). Currently, microarray technology is being used to derive gene expression profiles that predict the accurate tissue origin of a particular tumor. For the first time, it is possible to make the diagnosis of a particular cancer and cancer subsets, without examining the histology. This technology may not only eliminate the diagnostic category of the unknown primary cancer, but may also improve the diagnostic accuracy of current approaches by using immunohistochemical analyses combined with classic histopathological techniques. Moreover, it is now possible to predict clinical outcome on the basis of gene expression patterns (Bhattacharjee *et al*., 2001; Takahashi *et al*., 2001; Shipp *et al*., 2002). The microarray technology will be applied clinically to predict accurate diagnosis, prognosis and possibly even therapeutic options. It may even be possible to predict which patients will actually benefit from extirpative surgical procedures. Finally, gene expression signatures may be used to predict the clinical response to both conventional and targeted therapeutics.

Limitations, conclusions and future directions

While microarray technology represents a wonderful opportunity for clinical research, significant issues must be considered. Although microarray-related studies can monitor global changes in gene expression, these studies are limited by access and cost. Since microarray experiments need the physical disruption of a cell to gain access to its gene expression patterns; this technology can be considered as an example of 'destructive testing'. There is also a lack of rigorous standards for data collection, analysis and validation. These technical aspects are the 'Achilles' heel' of current microarray technology.

The quality and amount of RNA remains a major challenge in the microarray experiments. The amount of obtained tissue and the complexity of the tissue sample itself limit the quality and quantity of RNA that can be isolated. Therefore, clinical studies that are published using the microarray approach are performed in settings where biological samples are abundant and easily obtainable. Processing tissue rapidly to maintain RNA integrity is crucial. False microarray data can be generated from degraded mRNA. Owing to the numerous error-prone steps in microarray experiments, the experiments need to be replicated in order to eliminate sources of error. Most human tissue samples are a mixture of different cell types. Therefore, changes in gene expression patterns, when comparing two different tissue biopsy samples, are a manifestation of all the cell types present in that sample. This issue can render the analysis inaccurate. Methods such as laser capture microdissection, which permits the isolation of specific cells, can be useful but still are limited technologically

(Simone *et al*., 1998; Brail *et al*., 1999; Luo *et al*., 1999; Best and Emmert-Buck, 2001). Furthermore, the small size of many clinical specimens from early diagnoses is another critical problem. Efforts are under way to reduce the amount of sample required for analysis. Most microarray platforms work with a few micrograms of mRNA, except for nylon microarrays, which use only a few nanograms of nucleic acid (Bertucci *et al*., 1999). One possible solution is to amplify the mRNA sample using linear amplification methods before labeling (Luo *et al*., 1999). More challenges need to be faced such as the modification of cell-based technologies into single-cell platforms, permitting a more natural approach to biological research.

An additional limitation of this technology is that each microarray can only provide information about the genes that are included on the array. Another goal will be the miniaturization and integration of the microarray technology into single pieces of equipment that are easy to use.

Different studies in gene expression profiling of tumor samples have revealed the great transcriptional heterogeneity of cancer. Altogether, they have shown the advantages of the microarray to discriminate between RNA expression levels of different genes. Furthermore, among classically indistinguishable tumors, these studies have allowed the classification of new clinically and biologically important subclasses that may represent different diseases requiring different strategies. However, technical limitations must not be ignored. One of the key factors that will eventually determine the future success of the microarray is standardization, not only in an experimental sense but also in a computational sense. Significant advances have been made toward introducing standardization criteria, but so far researchers have not successfully compared results from different laboratories using different microarray technologies. Nevertheless, the recent advances in gene expression profiling technology and the important contributions of microarray technology to cancer research (the detection of new tumor markers, the capacity for predicting the survival of patients with breast cancer, lung cancer, brain cancer and acute lymphocytic leukemia), have now made it possible to suggest that within this decade this technology will be routinely used in cancer diagnosis and for monitoring desired and adverse outcomes of therapeutic interventions. A new era is beginning in which the integration of large data sets (biochemical, histological and molecular) has given birth to the concept of systems biology, a new multidisciplinary approach to accurately determine diagnosis, prognosis and effective treatment regimens for each patient. The challenges of translating the benefits of sequencing the human genome to clinical medicine will require the combined help of molecular biologists, pathologists, surgeons, chemists, mathematicians and computer scientists to be successful. Currently, the use of microarray technology in cancer research is limited by the tissue sample, available arrays and the analysis of data generated from this technology. Clinical use of microarray technology is still in its infancy. In the not too distant future, advancing automation in microarray analysis will largely expand the use of this technology in cancer investigation, ultimately leading to improvements in developing rational approaches to therapy as well as to improvements in diagnosis and prognosis of cancer.

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References

Agrawal D, Chen T, Irby R, Quackenbush J, Chambers AF, Szabo M, Cantor A, Coppola D and Yeatman TJ. (2002). *J. Natl. Cancer Inst.,* **94,** 513–521.

Ahr A, Karn T, Solbach C, Seiter T, Strebhardt K, Holtrich U and Kaufmann M. (2002). *Lancet,* **359,** 131–132.

Al Moustafa AE, Alaoui-Jamali MA, Batist G, Hernandez-Perez M, Serruya C, Alpert L, Black MJ, Sladek R and Foulkes WD. (2002). *Oncogene,* **21,** 2634–2640.

Alevizos I, Mahadevappa M, Zhang X, Ohyama H, Kohno Y, Posner M, Gallagher GT, Varvares M, Cohen D, Kim D, Kent R, Donoff RB, Todd R, Yung CM, Warrington JA and Wong DT. (2001). *Oncogene,* **20,** 6196–6204.

Alizadeh A, Eisen M, Davis RE, Ma C, Sabet H, Tran T, Powell JI, Yang L, Marti GE, Moore DT, Hudson Jr JR, Chan WC, Greiner T, Weisenburger D, Armitage JO, Lossos I, Levy R, Botstein D, Brown PO and Staudt LM. (1999). *Cold Spring Harb. Symp. Quant. Biol.,* **64,** 71–78.

Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson Jr J, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Staudt LM *et al*. (2000). *Nature,* **403,** 503–511.

Alizadeh AA, Ross DT, Perou CM and van de Rijn M. (2001). *J. Pathol.,* **195,** 41–52.

Backert S, Gelos M, Kobalz U, Hanski ML, Bohm C, Mann B, Lovin N, Gratchev A, Mansmann U, Moyer MP, Riecken EO and Hanski C. (1999). *Int. J. Cancer,* **82,** 868–874.

Barlund M, Forozan F, Kononen J, Bubendorf L, Chen Y, Bittner ML, Torhorst J, Haas P, Bucher C, Sauter G, Kallioniemi OP and Kallioniemi A. (2000a). *J. Natl. Cancer. Inst.,* **92,** 1252–1259.

Barlund M, Monni O, Kononen J, Cornelison R, Torhorst J, Sauter G, Kallioniemi O-P and Kallioniemi A. (2000b). *Cancer Res.,* **60,** 5340–5344.

Battifora H. (1986). *Lab. Invest.,* **55,** 244–248.

Beer DG, Kardia SL, Huang CC, Giordano TJ, Levin AM, Misek DE, Lin L, Chen G, Gharib TG, Thomas DG, Lizyness ML, Kuick R, Hayasaka S, Taylor JM, Iannettoni MD, Orringer MB and Hanash S. (2002). *Nat. Med.,* **8,** 816–824.

Belbin TJ, Singh B, Barber I, Socci N, Wenig B, Smith R, Prystowsky MB and Childs G. (2002). *Cancer Res.,* **62,** 1184–1190.

Bertucci F, Bernard K, Loriod B, Chang YC, Granjeaud S, Birnbaum D, Nguyen C, Peck K and Jordan BR. (1999). *Hum. Mol. Genet.,* **8,** 1715–1722.

Bertucci F, Nasser V, Granjeaud S, Eisinger F, Adelaide J, Tagett R, Loriod B, Giaconia A, Benziane A, Devilard E, Jacquemier J, Viens P, Nguyen C, Birnbaum D and Houlgatte R. (2002). *Hum. Mol. Genet.,* **11,** 863–872.

Best CJ and Emmert-Buck MR. (2001). *Expert Rev. Mol. Diagn.,* **1,** 53–60.

Bhattacharjee A, Richards WG, Staunton J, Li C, Monti S, Vasa P, Ladd C, Beheshti J, Bueno R, Gillette M, Loda M, Weber G, Mark EJ, Lander ES, Wong W, Johnson BE, Golub TR, Sugarbaker DJ and Meyerson M. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 13790–13795.

Bittner M, Meltzer P, Chen Y, Jiang Y, Seftor E, Hendrix M, Radmacher M, Simon R, Yakhini Z, Ben-Dor A, Sampas N, Dougherty E, Wang E, Marincola F, Gooden C, Lueders J, Glatfelter A, Pollock P, Carpten J, Gillanders E, Leja D, Dietrich K, Beaudry C, Berens M, Alberts D and Sondak V. (2000). *Nature,* **406,** 536–540.

Bowtell DD. (1999). *Nat. Genet.,* **21,** 25–32.

Brail LH, Jang A, Billia F, Iscove NN, Klamut HJ and Hill RP. (1999). *Mutat. Res.,* **406,** 45–54.

Brown PO and Botstein D. (1999). *Nat. Genet.,* **21,** 33–37.

Bubendorf L, Kolmer M, Kononen J, Koivisto P, Mousses S, Chen Y, Mahlamaki E, Schraml P, Moch H, Willi N, Elkahloun AG, Pretlow TG, Gasser TC, Mihatsch MJ, Sauter G and Kallioniemi OP. (1999a). *J. Natl. Cancer Inst.,* **91,** 1758–1764.

Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, Willi N, Mihatsch MJ, Sauter G and Kallioniemi OP. (1999b). *Cancer Res.,* **59,** 803–806.

Bull JH, Ellison G, Patel A, Muir G, Walker M, Underwood M, Khan F and Paskins L. (2001). *Br. J. Cancer,* **84,** 1512–1519.

Caldas C and Aparicio SA. (2002). *Nature,* **415,** 484–485.

Celis JE, Kruhoffer M, Gromova I, Frederiksen C, Ostergaard M, Thykjaer T, Gromov P, Yu J, Palsdottir H, Magnusson N and Orntoft TF. (2000). *FEBS Lett.,* **480,** 2–16.

Chang DD, Park NH, Denny CT, Nelson SF and Pe M. (1998). *Oncogene,* **16,** 1921–1930.

Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO, Van De Rijn M, Botstein D and Brown PO. (2002). *Mol. Biol. Cell,* **13,** 1929–1939.

Cheng Q, Lau WM, Tay SK, Chew SH, Ho TH and Hui KM. (2002). *Int. J. Cancer,* **98,** 419–426.

Cheung VG, Morley M, Aguilar F, Massimi A, Kucherlapati R and Childs G. (1999). *Nat. Genet.,* **21,** 15–19.

Collins FS. (1999). *Nat. Genet.,* **21,** 2.

Devilard E, Bertucci F, Trempat P, Bouabdallah R, Loriod B, Giaconia A, Brousset P, Granjeaud S, Nguyen C, Birnbaum D, Birg F, Houlgatte R and Xerri L. (2002). *Oncogene,* **21,** 3095–3102.

Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta KJ, Rubin MA and Chinnaiyan AM. (2001). *Nature,* **412,** 822–826.

Duggan DJ, Bittner M, Chen Y, Meltzer P and Trent JM. (1999). *Nat. Genet.,* **21,** 10–14.

Fejzo MS and Slamon DJ. (2001). *Am. J. Pathol.,* **159,** 1645–1650.

Frierson Jr HF, El-Naggar AK, Welsh JB, Sapinoso LM, Su AI, Cheng J, Saku T, Moskaluk CA and Hampton GM. (2002). *Am. J. Pathol.,* **161,** 1315–1323.

Garber ME, Troyanskaya OG, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, van de Rijn M, Rosen GD, Perou CM, Whyte RI, Altman RB, Brown PO, Botstein D and Petersen I. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 13784–13789.

Gariboldi M, Peissel B, Fabbri A, Saran A, Zaffaroni D, Falvella FS, Spinola M, Tanuma J, Pazzaglia S, Mancuso MT, Maurichi A, Bartoli C, Cataltepe S, Silverman GA, Pilotti S, Hayashizaki Y, Okazaki Y and Dragani TA. (2003). *Cancer Res.,* **63,** 1871–1875.

Giordano TJ, Shedden KA, Schwartz DR, Kuick R, Taylor JM, Lee N, Misek DE, Greenson JK, Kardia SL, Beer DG, Rennert G, Cho KR, Gruber SB, Fearon ER and Hanash S. (2001). *Am. J. Pathol.,* **159,** 1231–1238.

Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD and Lander ES. (1999). *Science,* **286,** 531–537.

Gordon GJ, Jensen RV, Hsiao LL, Gullans SR, Blumenstock JE, Ramaswamy S, Richards WG, Sugarbaker DJ and Bueno R. (2002). *Cancer Res.,* **62,** 4963–4967.

Granjeaud S, Bertucci F and Jordan BR. (1999). *Bioessays,* **21,** 781–790.

Graves DJ. (1999). *Trends Biotechnol.,* **17,** 127–134.

Han H, Bearss DJ, Browne LW, Calaluce R, Nagle RB and Von Hoff DD. (2002). *Cancer Res.,* **62,** 2890–2896.

Hanash S. (2003). *Nat. Biotechnol.,* **21,** 37–38.

Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, Meltzer P, Gusterson B, Esteller M, Kallioniemi OP, Wilfond B, Borg A and Trent J. (2001). *N. Engl. J. Med.,* **344,** 539–548.

Hedenfalk IA, Ringner M, Trent JM and Borg A. (2002). *Adv. Cancer Res.,* **84,** 1–34.

Hegde P, Qi R, Abernathy K, Gay C, Dharap S, Gaspard R, Hughes JE, Snesrud E, Lee N and Quackenbush J. (2000). *Biotechniques,* **29,** 548–550 552–554, 556 passim.

Hippo Y, Taniguchi H, Tsutsumi S, Machida N, Chong JM, Fukayama M, Kodama T and Aburatani H. (2002). *Cancer Res.,* **62,** 233–240.

Hofmann WK, de Vos S, Elashoff D, Gschaidmeier H, Hoelzer D, Koeffler HP and Ottmann OG. (2002). *Lancet,* **359,** 481–486.

Hong TM, Yang PC, Peck K, Chen JJ, Yang SC, Chen YC and Wu CW. (2000). *Am. J. Respir. Cell Mol. Biol.,* **23,** 355–363.

Hoos A, Urist MJ, Stojadinovic A, Mastorides S, Dudas ME, Leung DH, Kuo D, Brennan MF, Lewis JJ and Cordon-Cardo C. (2001). *Am. J. Pathol.,* **158,** 1245–1251.

Hubert RS, Vivanco I, Chen E, Rastegar S, Leong K, Mitchell SC, Madraswala R, Zhou Y, Kuo J, Raitano AB, Jakobovits A, Saffran DC and Afar DE. (1999). *Proc. Natl. Acad. Sci. USA,* **96,** 14523–14528.

Ismail RS, Baldwin RL, Fang J, Browning D, Karlan BY, Gasson JC and Chang DD. (2000). *Cancer Res.,* **60,** 6744–6749.

Jiang Z, Woda BA, Rock KL, Xu Y, Savas L, Khan A, Pihan G, Cai F, Babcook JS, Rathanaswami P, Reed SG, Xu J and Fanger GR. (2001). *Am. J. Surg. Pathol.,* **25,** 1397–1404.

Kallioniemi OP, Wagner U, Kononen J and Sauter G. (2001). *Hum. Mol. Genet.,* **10,** 657–662.

Khan J, Saal LH, Bittner ML, Chen Y, Trent JM and Meltzer PS. (1999). *Electrophoresis,* **20,** 223–229.

Khan J, Simon R, Bittner M, Chen Y, Leighton SB, Pohida T, Smith PD, Jiang Y, Gooden GC, Trent JM. and Meltzer PS. (1998). *Cancer Res.,* **58,** 5009–5013.

Khan J, Wei JS, Ringner M, Saal LH, Ladanyi M, Westermann F, Berthold F, Schwab M, Antonescu CR, Peterson C and Meltzer PS. (2001). *Nat. Med.,* **7,** 673–679.

Kim JH, Skates SJ, Uede T, Wong Kk, KK, Schorge JO, Feltmate CM, Berkowitz RS, Cramer DW and Mok SC. (2002). *JAMA,* **287,** 1671–1679.

Knezevic V, Leethanakul C, Bichsel VE, Worth JM, Prabhu VV, Gutkind JS, Liotta LA, Munson PJ, Petricoin III EF and Krizman DB. (2001). *Proteomics,* **1,** 1271–1278.

Kodadek T. (2001). *Chem. Biol.,* **8,** 105–115.

Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G and Kallioniemi OP. (1998). *Nat. Med.,* **4,** 844–847.

Kudoh K, Ramanna M, Ravatn R, Elkahloun AG, Bittner ML, Meltzer PS, Trent JM, Dalton WS and Chin KV. (2000). *Cancer Res.,* **60,** 4161–4166.

Lander ES. (1999). *Nat. Genet.,* **21,** 3–4.

Leethanakul C, Knezevic V, Patel V, Amornphimoltham P, Gillespie J, Shillitoe EJ, Emko P, Park MH, Emmert-Buck MR, Strausberg RL, Krizman DB and Gutkind JS. (2003). *Oral Oncol.,* **39,** 248–258.

Lipshutz RJ, Fodor SP, Gingeras TR and Lockhart DJ. (1999). *Nat. Genet.,* **21,** 20–24.

Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H and Brown EL. (1996). *Nat. Biotechnol.,* **14,** 1675–1680.

Lu J, Liu Z, Xiong M, Wang Q, Wang X, Yang G, Zhao L, Qiu Z, Zhou C and Wu M. (2001). *Int. J. Cancer,* **91,** 288–294.

Luo J, Duggan DJ, Chen Y, Sauvageot J, Ewing CM, Bittner ML, Trent JM and Isaacs WB. (2001). *Cancer Res.,* **61,** 4683–4688.

Luo J, Dunn T, Ewing C, Sauvageot J, Chen Y, Trent J and Isaacs W. (2002a). *Prostate,* **51,** 189–200.

Luo JH, Yu YP, Cieply K, Lin F, Deflavia P, Dhir R, Finkelstein S, Michalopoulos G and Becich M. (2002b). *Mol. Carcinogen,* **33,** 25–35.

Luo L, Salunga RC, Guo H, Bittner A, Joy KC, Galindo JE, Xiao H, Rogers KE, Wan JS, Jackson MR and Erlander MG. (1999). *Nat. Med.,* **5,** 117–122.

Martin KJ, Graner E, Li Y, Price LM, Kritzman BM, Fournier MV, Rhei E and Pardee AB. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 2646–2651.

Martoglio AM, Tom BD, Starkey M, Corps AN, Charnock-Jones DS and Smith SK. (2000). *Mol. Med.,* **6,** 750–765.

Matei D, Graeber TG, Baldwin RL, Karlan BY, Rao J and Chang DD. (2002). *Oncogene,* **21,** 6289–6298.

Meldrum D. (2000). *Genome Res.,* **10,** 1288–1303.

Mintz PJ, Kim J, Do KA, Wang X, Zinner RG, Cristofanilli M, Arap MA, Hong WK, Troncoso P, Logothetis CJ, Pasqualini R and Arap W. (2003). *Nat. Biotechnol.,* **21,** 57–63.

Moch H, Schraml P, Bubendorf L, Mirlacher M, Kononen J, Gasser T, Mihatsch MJ, Kallioniemi OP and Sauter G. (1999). *Am. J. Pathol.,* **154,** 981–986.

Mok SC, Chao J, Skates S, Wong K, Yiu GK, Muto MG, Berkowitz RS and Cramer DW. (2001). *J. Natl. Cancer Inst.,* **93,** 1458–1464.

Niemeyer CM and Blohm D. (1999). *Angew Chem. Int. Ed. Engl.,* **38,** 2865–2869.

Nocito A, Bubendorf L, Maria Tinner E, Suess K, Wagner U, Forster T, Kononen J, Fijan A, Bruderer J, Schmid U, Ackermann D, Maurer R, Alund G, Knonagel H, Rist M, Anabitarte M, Hering F, Hardmeier T, Schoenenberger AJ, Flury R, Jager P, Luc Fehr J, Schraml P, Moch H, Mihatsch MJ, Gasser T and Sauter G. (2001). *J. Pathol.,* **194,** 349–357.

Ono K, Tanaka T, Tsunoda T, Kitahara O, Kihara C, Okamoto A, Ochiai K, Takagi T and Nakamura Y. (2000). *Cancer Res.,* **60,** 5007–5011.

Paweletz CP, Charboneau L, Bichsel VE, Simone NL, Chen T, Gillespie JW, Emmert-Buck MR, Roth MJ, Petricoin IE and Liotta LA. (2001). *Oncogene,* **20,** 1981–1989.

Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO and Botstein D. (2000). *Nature,* **406,** 747–752.

Ramaswamy S, Tamayo P, Rifkin R, Mukherjee S, Yeang CH, Angelo M, Ladd C, Reich M, Latulippe E, Mesirov JP, Poggio T, Gerald W, Loda M, Lander ES and Golub TR. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 15149–15154.

Richter J, Wagner U, Kononen J, Fijan A, Bruderer J, Schmid U, Ackermann D, Maurer R, Alund G, Knonagel H, Rist M, Wilber K, Anabitarte M, Hering F, Hardmeier T, Schonenberger A, Flury R, Jager P, Fehr JL, Schraml P, Moch H, Mihatsch MJ, Gasser T, Kallioniemi OP and Sauter G. (2000). *Am. J. Pathol.,* **157,** 787–794.

Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, Yang L, Pickeral OK, Rassenti LZ, Powell J, Botstein D, Byrd JC, Grever MR, Cheson BD, Chiorazzi N, Wilson WH, Kipps TJ, Brown PO and Staudt LM. (2001). *J. Exp. Med.,* **194,** 1639–1647.

Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, Lopez-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T and Staudt LM. (2002). *N. Engl. J. Med,* **346,** 1937–1947.

Russo G, Claudio PP, Fu Y, Stiegler P, Yu ZL and Macaluso M and Giordano A. (2003). *Oncogene* (in press).

Sallinen SL, Sallinen PK, Haapasalo HK, Helin HJ, Helen PT, Schraml P, Kallioniemi OP and Kononen J. (2000). *Cancer Res.,* **60,** 6617–6622.

Sawiris GP, Sherman-Baust CA, Becker KG, Cheadle C, Teichberg D and Morin PJ. (2002). *Cancer Res.,* **62,** 2923–2928.

Schena M, Shalon D, Davis RW and Brown PO. (1995). *Science,* **270,** 467–470.

Schena M, Shalon D, Heller R, Chai A, Brown PO and Davis RW. (1996). *Proc. Natl. Acad. Sci. USA,* **93,** 10614–10619.

Scherf U, Ross DT, Waltham M, Smith LH, Lee JK, Tanabe L, Kohn KW, Reinhold WC, Myers TG, Andrews DT, Scudiero DA, Eisen MB, Sausville EA, Pommier Y, Botstein D, Brown PO and Weinstein JN. (2000). *Nat. Genet.,* **24,** 236–244.

Schraml P, Kononen J, Bubendorf L, Moch H, Bissig H, Nocito A, Mihatsch MJ, Kallioniemi OP and Sauter G. (1999). *Clin. Cancer Res.,* **5,** 1966–1975.

Senior K. (1999). *Mol. Med. Today,* **5,** 326–327.

Shipp MA, Ross KN, Tamayo P, Weng AP, Kutok JL, Aguiar RC, Gaasenbeek M, Angelo M, Reich M, Pinkus GS, Ray TS, Koval MA, Last KW, Norton A, Lister TA, Mesirov J, Neuberg DS, Lander ES, Aster JC and Golub TR. (2002). *Nat. Med.,* **8,** 68–74.

Shridhar V, Lee J, Pandita A, Iturria S, Avula R, Staub J, Morrissey M, Calhoun E, Sen A, Kalli K, Keeney G, Roche P, Cliby W, Lu K, Schmandt R, Mills GB, Bast Jr RC, James CD, Couch FJ, Hartmann LC, Lillie J and Smith DI. (2001). *Cancer Res.,* **61,** 5895–5904.

Simon R, Nocito A, Hubscher T, Bucher C, Torhorst J, Schraml P, Bubendorf L, Mihatsch MM, Moch H, Wilber K, Schotzau A, Kononen J and Sauter G. (2001). *J. Natl. Cancer Inst.,* **93,** 1141–1146.

Simon R, Struckmann K, Schraml P, Wagner U, Forster T, Moch H, Fijan A, Bruderer J, Wilber K, Mihatsch MJ, Gasser T and Sauter G. (2002). *Oncogene,* **21,** 2476–2483.

Simone NL, Bonner RF, Gillespie JW, Emmert-Buck MR and Liotta LA. (1998). *Trends Genet.,* **14,** 272–276.

Singh D, Febbo PG, Ross K, Jackson DG, Manola J., Tamayo P, Renshaw AA, D'Amico AV, Richie JP, Lander ES, Loda M, Kantoff PW, Golub TR and Sellers WR. (2002). *Cancer Cell,* **1,** 203–209.

Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lonning P and Borresen-Dale AL. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 10869–10874.

Sotiriou C, Powles TJ, Dowsett M, Jazaeri AA, Feldman AL, Assersohn L, Gadisetti C, Libutti SK and Liu ET. (2002). *Breast Cancer Res.,* **4,** R3.

Southern E, Mir K and Shchepinov M. (1999). *Nat. Genet.,* **21,** 5–9.

Staunton JE, Slonim DK, Coller HA, Tamayo P, Angelo MJ, Park J, Scherf U, Lee JK, Reinhold WO, Weinstein JN, Mesirov JP, Lander ES and Golub TR. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 10787–10792.

Stratowa C, Loffler G, Lichter P, Stilgenbauer S, Haberl P, Schweifer N, Dohner H and Wilgenbus KK. (2001). *Int. J. Cancer,* **91,** 474–480.

Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, Orth AP, Vega RG, Sapinoso LM, Moqrich A, Patapoutian A, Hampton GM, Schultz PG and Hogenesch JB. (2002). *Proc. Natl. Acad. Sci. USA,* **99,** 4465–4470.

Su AI, Welsh JB, Sapinoso LM, Kern SG, Dimitrov P, Lapp H, Schultz PG, Powell SM, Moskaluk CA, Frierson Jr HF and Hampton GM. (2001). *Cancer Res.,* **61,** 7388–7393.

Takahashi M, Rhodes DR, Furge KA, Kanayama H, Kagawa S, Haab BB and Teh BT. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 9754–9759.

Tonin PN, Hudson TJ, Rodier F, Bossolasco M, Lee PD, Novak J, Manderson EN, Provencher D and Mes-Masson AM. (2001). *Oncogene,* **20,** 6617–6626.

Torhorst J, Bucher C, Kononen J, Haas P, Zuber M, Kochli OR, Mross F, Dieterich H, Moch H, Mihatsch M, Kallioniemi OP and Sauter G. (2001). *Am. J. Pathol.,* **159,** 2249–2256.

van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R and Friend SH. (2002). *Nature,* **415,** 530–536.

Velculescu VE, Zhang L, Vogelstein B and Kinzler KW. (1995). *Science,* **270,** 484–487.

Villaret DB, Wang T, Dillon D, Xu J, Sivam D, Cheever MA and Reed SG. (2000). *Laryngoscope,* **110,** 374–381.

Virolle T, Krones-Herzig A, Baron V, De Gregorio G, Adamson ED and Mercola D. (2003). *J. Biol. Chem.,* **278,** 11802–11810.

von Eggeling F, Davies H, Lomas L, Fiedler W, Junker K, Claussen U and Ernst G. (2000). *Biotechniques,* **29,** 1066–1070.

Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson Jr HF and Hampton GM. (2001a). *Cancer Res.,* **61,** 5974–5978.

Welsh JB, Zarrinkar PP, Sapinoso LM, Kern SG, Behling CA, Monk BJ, Lockhart DJ, Burger RA and Hampton GM. (2001b). *Proc. Natl. Acad. Sci. USA,* **98,** 1176–1181.

West M, Blanchette C, Dressman H, Huang E, Ishida S, Spang R, Zuzan H, Olson Jr JA, Marks JR and Nevins JR. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 11462–11467.

Xu J, Kalos M, Stolk JA, Zasloff EJ, Zhang X, Houghton RL, Filho AM, Nolasco M, Badaro R and Reed SG. (2001). *Cancer Res.,* **61,** 1563–1568.

Zhou M, Chinnaiyan AM, Kleer CG, Lucas PC and Rubin MA. (2002). *Am. J. Surg. Pathol.,* **26,** 926–931.