

# DRUG RESISTANCE AND MICROARRAYS

How far are we from personalized cancer treatment?

BY CHUNG-HAE LEE AND PASCALE F. MACGREGOR

**T**he development of high-throughput global expression profiling techniques—with their unprecedented capability for measuring and comparing the expression levels of virtually every gene in the human genome—was expected to be followed closely by advances in medical research. Great hopes were held that microarrays would break the code of cancer cells—from their origin to their adaptation to the tumor environment and ultimately their supremacy over normal cells—and lead to better detection, as well as more accurate diagnosis and prognosis. Visions blossomed of personalized medicine, better response rates, decreased side effects, less drug resistance, and, eventually, higher cancer cure rates.

But thousands of microarray papers later, what have we learned, and where are the success stories?

## DRUG RESISTANCE

Despite significant advances in surgery, radiation therapy, and anticancer treatment in the past 30 years, chemotherapy resistance remains a major obstacle to improving a cancer patient's outcome. Because there are presently no proven predictors of a patient's response to chemotherapy, all cancer patients selected for chemotherapy receive the same treatment. Resistance to chemotherapy can be observed either at the onset of treatment, when a patient fails to show clinical response (intrinsic resistance), or at a later time, when the disease recurs despite an initially successful response (acquired resistance). Along with severely compromising patient outcome, chemotherapy resistance greatly limits the range of possibilities for subsequent treatments, because some tumors become resistant not only to the initial drug but also to new therapeutic agents with different mechanisms of action.

Drug resistance in cancer arises from a complex range of biochemical and molecular events, which ultimately result in the tumor cells escaping death. Virtually any of these events—from drug uptake, absorption, and metabolism to drug efflux/influx and acti-



**Figure 1. Exploring drug resistance.** Monique Albert, a researcher at Toronto's Clinical Genomics Centre, prepares human tissue samples for microarray analysis.

vation/inactivation, as well as DNA repair mechanisms—can be targeted to tip the balance in the cancer cell from tumor growth to apoptosis. Regardless, a better understanding of the molecular mechanisms involved is paramount.

Global genomic approaches offer the advantage of attacking anticancer drug resistance on several fronts. Identifying key genes and gene pathways involved in the molecular mechanisms of resistance can establish new drug targets and enable the rational design of new anticancer drugs, moving the drug discovery process away from serendipity. In addition, new strategies to overcome drug resistance can be envisioned by developing compounds that could be administered alongside traditional treatment to limit or modulate resistance. Arguably, the most attractive result would be to discover molecular markers of resistance, because this would open the door to stratifying cancer patients before therapy into potential responders or non-responders.

## STUDYING DRUG RESISTANCE

Studies of anticancer drug resistance are generally carried out either *in vitro* on cell line model systems, or *in vivo* on patient samples (Figure 1).

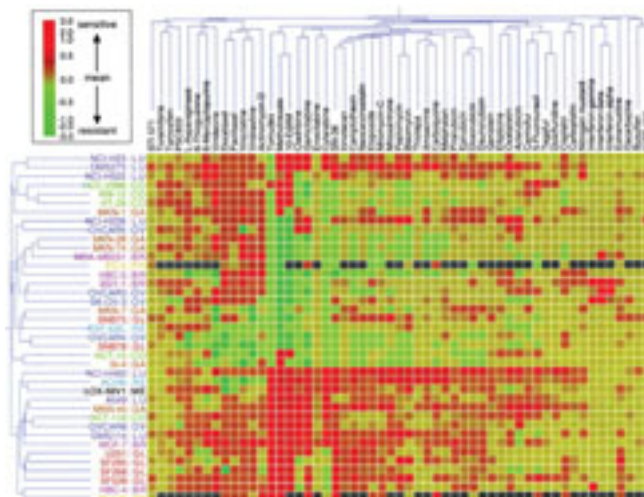
Numerous immortalized cancer cell lines are commercially available, and by exposing these cells to increasing concentrations of an anticancer drug of interest, researchers can isolate drug-resistant clones. They can then perform global expression profiling, as well as more traditional assays, on the drug-resistant cells and compare results to ones generated with drug-sensitive cells. Many groups have used this approach in microarray studies to better comprehend important molecular events. In a few large-scale, landmark microarray studies, research groups led by the National Cancer Institute's Sally Amundson (1) and John Weinstein (2), and the Japanese Foundation for Cancer Research's Takao Yamori (3), established global gene expression patterns for a panel of 60 drug-naïve human cell lines and attempted to correlate these patterns with the cell lines' sensitivity to chemotherapeutic drugs (Figure 2).

However, the major flaw of cell-line-based studies resides in their very nature. Several observations suggest cell lines represent an oversimplified model of drug resistance in human cancer. Isolation of drug-resistant clones often requires exposing cancer cells to clinically irrelevant doses of anticancer agents. Long-term cell culture lines, although heavily used, have been shown to be genomically unstable and to develop a significant degree of variation, thereby weakening the interpretation of whole-genome experimental strategies.

In addition, such models primarily address acquired drug resistance and do not provide insights into the expression and genomic alterations impacting overlapping pathways associated with intrinsic drug resistance. More importantly, cell line models might not accurately reflect the *in vivo* situation of patients treated with anticancer drugs, and thus would hamper the translation of *in vitro* results from the laboratory to the clinic. In particular, drug resistance in patients is likely to involve the *in vivo* tumor microenvironment, a parameter not easily accounted for in cancer cell cultures, although Kevin Hicks and colleagues at the University of Auckland recently attempted to do so (4).

## IN VIVO ANALYSIS

Although using patient samples to study anticancer drug resistance should lead to more clinically relevant findings, this strategy comes with its own challenges. Even when high-quality, fully consented, ethics board-approved patient samples are accessible, large-scale profiling generally requires a minimum sample-cohort size for sta-



**Figure 2. Cells and sensitivity.** Based on changes in gene expression, researchers correlated the resistance patterns of different cancer cell types to various therapeutic agents. (Adapted with permission from Dan, S.; et al. *Cancer Res.* **2002**, *62*, 1139–1147.)

tistically significant results. Although this problem is not unique to microarray studies, it is particularly important in whole-genome screening studies, where tens of thousands of genes are interrogated simultaneously in a limited number of samples, and the danger of data overfitting, in which results appear to be significant but are actually just noise, is greatly increased.

When assembling a cohort of samples, clinical homogeneity of the specimens selected is crucial. Microarray technology involves many discrete steps, from microarray production to experiment to

data analysis, and meaningful results can easily be buried under a large amount of data noise. Extracting results specific to the biological variation of interest can be extremely challenging if the sample cohort itself varies widely in other parameters, such as tumor stage, grade, or histology. Although patient samples ideally should differ only in their response to chemotherapy, assembling such cohorts is very difficult in practice. This has resulted in many studies on heterogeneous specimens, where interpretation of the genomic differences observed is confounded by clinical differences between tumor samples. In addition, an ideal study group would include tumor material collected from patients before and after chemotherapy, which for practical reasons is rarely feasible. In limited cases, such as ovarian cancer, it is possible to circumvent this problem by collecting other biological samples, such as ascitic fluid, after chemotherapy.

Finally, defining end points for chemotherapy resistance studies *in vivo* is still controversial. For example, anticancer drug resistance remains a major hurdle to decreasing the overall mortality rate in ovarian cancer. Although most ovarian cancer patients respond well to standard carboplatin–paclitaxel combination chemotherapy, drug resistance often develops quickly, and tumors recur in most cases within two years, leading to a median patient survival of only two to three years. Several groups, including ours at the Clinical Genomics Centre in Toronto, use microarray expression profiling on patient samples to identify genes or gene pathways responsible for chemotherapy resistance in ovarian cancer. However, defining a response to chemotherapy for the purpose of patient classification is a complex issue.

At the clinical management level, clinicians often define resistance on the basis of patient symptoms and radiological evidence, and the disease-free interval is traditionally used as the clinical end point to classify patients into sensitive and resistant groups. More recently, Gordon Rustin and colleagues at the Mount Vernon Centre for Cancer Treatment have proposed using levels of the tumor marker CA125 as a more accurate surrogate indicator of

chemotherapy response. Rising CA125 levels have been shown to predate clinical relapse by a median of four months in 70% of ovarian cancer patients (5). Our research group at the Clinical Genomics Centre also believes that CA125 levels might provide more accurate and clinically relevant end points in microarray studies for classifying ovarian cancer patients into resistant and sensitive groups.

### STUMBLING BLOCKS

To publish microarray data, most journal editors and reviewers require that the results be validated by another independent method—originally Northern blots or, more recently, real-time RT-PCR. More importantly, this is also a first step for translating microarray results from the laboratory to the clinic. Still, the issue of corroborating microarray data is challenging.

To facilitate the creation and sharing of microarray databases, researchers formed an international initiative called the Microarray Gene Expression Data (MGED) Society in 1999, and established standards for microarray experiment annotation called the minimum information about a microarray experiment, or MIAME. However, no such standard has been proposed yet for validating microarray results, and most scientific journals still evaluate the need for it on a case-by-case basis. Although many publications present some form of expression-level validation, the formats in which these results are displayed vary widely. In many cases, particularly when real-time RT-PCR results are reported, the data lack the minimum amount of information required to conclude whether validation has been successful.

For example, researchers liberally use so-called housekeeping genes, such as *GAPDH* or *beta-actin*, as internal standards, often without any verification that these genes do indeed show consistent expression levels among the samples studied. In ovarian cancer, for example, Michél Schummer and colleagues have reported differential expression of *beta-actin* (6), while our group at the Clinical Genomics Centre has recently observed that *GAPDH* expression levels vary significantly between serous epithelial specimens.

The necessity and feasibility of corroborating microarray results by an independent technology have even been questioned recently, as has the choice of real-time RT-PCR as the “gold standard” for measuring absolute gene expression levels. Regardless of the outcome of this debate and the ultimate decision made by science journal editors on the matter, it is highly probable that a lack of correlation between microarray results and other methods has considerably delayed movement from the laboratory to the clinic.

Another stumbling block in the gene discovery process has undoubtedly been the annotation of the human genome. Although excitement and publicity have surrounded its sequencing, much annotation work remains to be done, and many microarray projects (particularly those measuring not only expression levels but also DNA copy numbers) and subsequent validation efforts are stalled by delays in completing gene annotation.

Finally, even after biomarkers of drug resistance have been identified by microarray studies and the results corroborated by

an independent technique, such as quantitative RT-PCR, and the genes appropriately annotated and mapped, these markers will need to undergo clinical validation. This final step is likely to require yet another high-throughput technology or at least one that can simultaneously measure multiple gene expression levels on several patient samples. Ideally, this final validation will be carried out on even larger sets of clinical samples, different from those used in the discovery process. Because this process is expensive and time-consuming, very few such studies have been undertaken.

## THE FUTURE IS NOW

Although microarray use has not yet had any significant impact at the clinical level on the selection of chemotherapy for cancer patients, the technology has allowed for a better understanding of the molecular events implicated in drug resistance. An increasing number of microarray users have overcome the many hurdles inherent in using any new technology. New data-mining and analysis software is available. Meanwhile, downstream validation technologies, such as Luminex Corp.'s bead-based platforms, are being developed and should soon facilitate translating microarray findings from the laboratory to the clinic.

Despite the challenges of moving research from bench to clinic and back, and the extraordinary complexity of the medical and technical problems at hand, genomic research is plowing its way through roadblocks, and concrete results have finally started to appear. Although not focused specifically on chemotherapy, two patient profiling tests based on gene expression hit the market at the beginning of 2004, breaking ground for similar tests to emerge and further test the power of genomics in medicine.

Not surprisingly, these first tests are geared toward breast cancer, which offers a substantial potential market. The tests, Oncotype DX, launched in January by the California-based company Genomic Health, and Mammaprint, commercialized by the Dutch company Agendia, are expression-based and are aimed at determining a patient's risk of developing metastases and, thus, potentially reducing the rate of unnecessary chemotherapy. Because Oncotype DX is sold as a lab service and not a diagnostic test, it has not been subjected to FDA review. These two breakthroughs will undoubtedly be highly scrutinized as test cases for genomic medicine.

Even if these tests have limited commercial success, they have provided proof of principle. Thus, despite apparent obstacles, genomic medicine offers promise, and other applications to cancer patient management, such as predicting chemotherapy response, are moving within reach.

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