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*Review Article*

# Application of DNA Microarrays in Pharmacogenomics and Toxicogenomics[1]

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Many drugs or xenobiotics can induce specific or nonspecific cellular signal transduction events that activate various physiologic and pharmacologic responses including homeostasis, proliferation, differentiation, apoptosis, and necrosis. To minimize the insults caused by these xenobiotics, tissues and organs are equipped with protective mechanisms that either pump drugs out of the cells (e.g., the multidrug-resistant, *mdr*, family of proteins) or increase the level of detoxifying enzymes such as phase I and II drug-metabolizing enzymes (DMEs), after exposure to xenobiotics. This review discusses the molecular analysis of pharmaco- or toxicogenomic gene expression profiles following exposure to cancer chemotherapeutic and chemopreventive agents. We present the development of DNA microarray technology and its use in expression profiling of possible signal transduction events elicited by these compounds, and its potential future applications in drug discovery and development in the pharmaceutical industry.

**KEY WORDS:** microarray; pharmacogenomics; toxicogenomics; signal transduction; apoptosis.

## INTRODUCTION

Completion of the human genome draft sequence predicts the existence of approximately 30,000 to 40,000 genes (1,2). Although the precise number of genes in the genome remains controversial (3,4), it is believed that the 80,000–90,000 unigene clusters, each potentially representing a unique transcript, correspond to their respective genes and splice variants, thus confounding the issue of gene counting of the human genome. Nevertheless, bioinformatics and the available collection of cDNA clones can be immediately applied to (a) the discovery of new genes, (b) functional genomic analysis of various diseases, (c) distinguishing responders and nonresponders to a given drug, (d) pharmacogenomics (identification of genes that are involved in determining drug responsiveness), and (e) toxicogenomics (characterization of potential genes involved in toxicity and adverse effects of drugs). The recent advent of DNA microarray technology should revolutionize future pharmacologic investigations, making it possible to examine the expression of all genes in the human genome in a single experiment. Therefore, monitoring gene expression profiles may provide insights into (a) the molecular fingerprints of different diseases including cancer, diseases of the central nervous system (CNS), and the cardiovascular system, (b) therapeutic treatments, (c) environmental agents, and, most importantly, (d) the prevention of these diseases.

Publication of the draft human genome sequence will undoubtedly fuel the discovery of novel genes and their corresponding protein products that may have roles in determining pharmacologic responses to pharmaceutic agents. In an attempt to associate genes or proteins with drug responses, DNA microarrays can be used to analyze genome-wide changes in gene expression patterns (5). Either cDNA microarrays or oligonucleotide-based gene chips can be used for gene expression analysis. A cDNA microarray contains a large number of genes or expressed sequence tags (ESTs) that are physically spotted on glass slides or nylon membranes (5). Similarly, oligonucleotides complementary to known genes or ESTs can be synthesized *in situ* on a miniature matrix using a photolithographic process to produce oligonucleotide-based microarrays (6). Oligonucleotide-based DNA chips can also be used to screen individuals for DNA mutations and polymorphisms by analyzing variations in genomic DNA (7).

The traditional approach to assessing gene expression induced by pharmaceutic compounds includes Northern blotting of mRNAs, reverse-transcription-coupled polymerase chain reaction (RT-PCR) of mRNAs, Western blotting of proteins, and various enzymatic assays. However, these techniques allow evaluation of only a few to tens or at most hundreds of genes/gene products per study. With the advent of DNA microarray technology, expression of thousands or tens of thousands of genes can be queried simultaneously, including various phase I and II DME genes and a battery of genes

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that may be pharmacologically (as potential therapeutic targets) or toxicologically (leading to undesirable or toxic effects of the drugs) relevant to drug administration in humans. Recently, microarray data have been successfully used to analyze gene expression changes in response to environmental toxins, chemotherapeutic agents, and cytokines (8–10).

The use of DNA microarrays to interrogate the gene expression patterns of cells exposed to pharmacologic small molecules and environmental toxicants may yield insights into the mechanisms of drug- or chemical-induced toxicity and potential carcinogenesis. We have previously studied gene expression changes in cells exposed to the anticancer chemotherapeutic agent doxorubicin or the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) using DNA microarrays (9,11). Changes in gene expression are coordinated, hierarchic, and consistent with the phenotypic and biochemical response to these agents. We have also shown that naturally occurring cancer chemopreventive agents can activate multiple biochemical pathways including phase II detoxifying enzymes and various signal transduction cascades (12). Therefore, characterization of these genetic and biochemical changes in response to pharmaceutical agents and environmental toxicants may yield potential biomarkers and/or identify molecular targets for drug intervention and prevention.

## ACCESS TO MICROARRAY TECHNOLOGY

Despite the enthusiasm generated by the promise of DNA microarrays for genome-wide expression analysis, access to and routine application of this technology in laboratories have not become widespread, especially in academia. One of the primary reasons for this shortfall is the prohibitive cost of acquiring the DNA microarray from commercial sources for expression analysis.

There are currently two DNA array formats available for gene expression profiling studies, the GeneChip® array, produced by the combined processes of photolithography and *in situ* solid-phase oligonucleotide DNA synthesis, and custom printed arrays that contain either PCR-amplified products of cDNA inserts derived from ESTs or presynthesized oligonucleotides deposited on a matrix surface using high-speed robotics. Both GeneChip® probe arrays and custom printed arrays can be acquired commercially. However, commercially prepared arrays can be very expensive, which prevents the routine use of this technology in general laboratory applications for gene expression analysis. As a result, microarray core or shared facilities have been established in academic centers where custom arrays are fabricated and distributed to investigators at a substantial discount to provide broad access to microarrays for expression-profiling studies.

Establishing a microarray facility can be a challenging task but not an insurmountable one. The process generally involves amplification of cDNA inserts from bacterial clones harboring the respective ESTs, followed by purification of the PCR products and their arraying onto either glass slides or nylon membranes (Fig. 1). The resultant arrays can be hybridized with either fluorescent-tagged (for glass slide arrays) or radiolabeled (for nylon membrane arrays) targets from the appropriate RNA sources. The collection of up to 40,000 EST clones, a high-throughput arrayer, multiblock thermocycler for PCR, laser scanner (for glass slide) and phosphorimager (for nylon membrane), and other related equipment are all commercially available for fabrication of custom arrays. Various quality control issues and concerns have been addressed; for example, EST clones can be validated by gel electrophoresis after PCR amplification to ascertain their molecular size based on data in a published database. To circumvent the many problems associated with cDNA arrays, presynthesized oligonucleotides corresponding to transcripts of various model organisms, typically 40 to 80 bases in length, have become commercially available for the production of custom printed arrays. Unlike EST clones, which can be cultured and replenished, the source of these synthetic oligonucleotides is nonrenewable, and the cost of initial and subsequent acquisitions can be substantial.

Software suites for image processing and array data analysis can either be downloaded at no cost from the internet

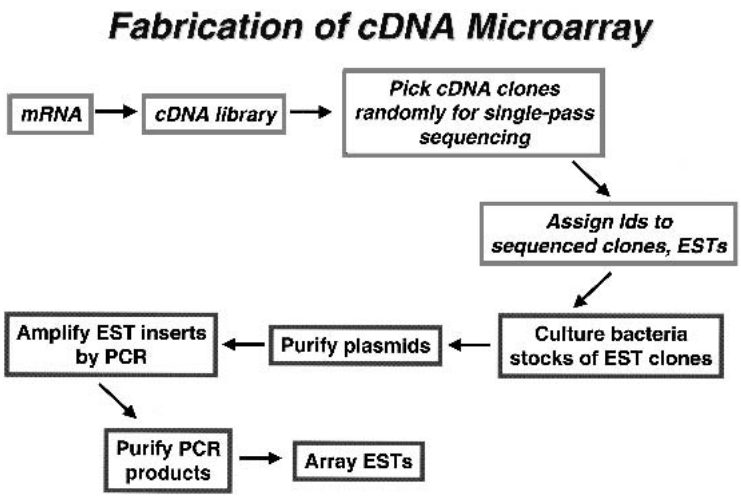


**Fig. 1.** Linear scheme for fabricating a custom microarray. Typically PCR amplifications are either conducted with plasmids isolated from freshly cultured bacterial stocks containing the respective ESTs or amplified directly from frozen stocks of hypotonically lysed bacteria. PCR products are purified and then arrayed onto glass slides or nylon matrices using high-speed robotics.

or purchased through commercial vendors. Biostatistical support for experimental design and data analysis is important, and such resources are usually drawn from services or collaborations offered by biostatistics and biometry departments associated with academic centers. Standard statistical software suites including SAS and more advanced custom software such as S-Plus are used for statistical analysis. Permutation tests, classification analysis, regression modeling, and other algorithms can be applied to the analysis of array results. Additional data analysis using cluster analysis software developed by Eisen *et al.* (13) can be downloaded from *http://rana.stanford.edu/* and can be used to analyze patterns of gene expression. This analysis uses standard statistical algorithms to organize genes according to similarity in expression patterns, and the output is displayed graphically using TreeView (also downloadable at *http://rana.stanford.edu/*) to convey the clustering and underlying expression data simultaneously. Additionally, various bioinformatics tools and resources for array data mining provide access to annotated gene information for genes and are freely available over the internet with the appropriate uniform resource locator (URL) links including UNIGENE, GeneCards, the Kyoto Encyclopedia for Genes and Genomes, and other databases.

High-density arrays with approximately 10,000 targets that correspond to known genes in the GenBank database can be rapidly produced in a single day using the above approach. Scaling up the number of targets on the array to include anonymous ESTs as well as ESTs with homologies to known genes and to functional orthologs of other model organisms can be insightful for novel gene and drug target discovery. Nonetheless, these low-cost arrays can be useful for investigators interested in expression profiling of various environmental toxicants in monitoring environmental exposure; treatments with various chemotherapeutic anticancer drugs as well as naturally occurring chemopreventive compounds. The expression data can be compared to the published database such as those obtained using the more established Affymetrix GeneChip® array (14).

## GENOMICS AND GENE EXPRESSION PROFILING OF CHEMOTHERAPEUTIC DRUGS

Using the above approach of fabricating custom arrays, we have been able to conduct microarray studies with affordably priced printed arrays to investigate mechanisms of drug resistance development in cancer. It has become clear that drug resistance in cancer cannot be attributed solely to the overexpression of P-glycoprotein (ABCB1/MDR1) or other members of the ATP-binding cassette (ABC) transporter family. Moreover, cancer chemotherapeutic treatment is empiric, often based on the outcome of clinical trials using combinations of highly toxic anticancer drugs approved for oncology practice; some of which have been in clinical use for a while. This approach to cancer treatment has resulted in some cures. Often in relapse and metastasis, most cancers fail to respond to further treatment, and the subsequent development of drug resistance leads to a high rate of mortality. The development of drug resistance has also been associated with genetic alterations in multiple oncogenes and tumor suppressor genes during tumorigenesis. Therefore, drug resistance in cancer is a major obstacle to successful chemotherapy. Cancer cells exposed to chemotherapeutic drugs may be directly induced to express a subset of genes that could confer resistance, thus allowing some cells to escape killing and form the relapsed resistant tumor. Alternatively, some cancer cells may express an array of genes that could confer intrinsic resistance, and exposure to cytotoxic drugs merely selects for the survival of these cells that form the relapsed tumor.

We have used DNA microarrays to monitor the expression profiles of human breast cancer MCF-7 cells that are either transiently treated with doxorubicin or selected for doxorubicin resistance (9). Our results showed that transient doxorubicin treatment induced temporal changes in the expression of a large number of genes in MCF-7 cells (Fig. 2). A subset of these induced genes was also found to be constitutively overexpressed in cells selected for doxorubicin resistance. Some of these genes, including microsomal epoxide hydrolase 1, the 26S proteasome regulatory subunit 4, and XRCC1 (involved in repair of DNA strand breaks following exposure to ionizing radiation or alkylating agents) may be functionally relevant for drug resistance. Because doxorubicin causes DNA double strand breaks by trapping topoisomerase II in a ternary complex of drug/enzyme/DNA that prevents religation of the transient break generated by topoisomerase II, up-regulation or constitutive overexpression of XRCC1 may enhance the repair of such strand breaks and confer drug resistance in tumor cells. We speculate that the up-regulation of the drug-metabolizing enzyme microsomal epoxide hydrolase 1 may enhance the metabolism of doxorubicin (15), leading to decreased availability of drug for interaction with topoisomerase II and a resulting decrease in DNA strand breaks. It has also been shown that topoisomerase II is targeted for degradation via the ubiquitin/26S proteasome pathway (16). Increased expression of the 26S proteasome regulatory subunit 4 may enhance the proteolysis of topoisomerase II and reveal double strand breaks previously concealed by the protein, thus facilitating XRCC1-mediated repair of these breaks and increasing drug resistance in tumor cells. These results suggest that multiple factors may act in concert to confer multidrug resistance in cancer.

It has been demonstrated recently that expression profiling analysis of human breast cancer samples, together with the use of a supervised hierarchic clustering algorithm, can predict the clinical outcome and determine the course of treatment in these patients (17). Gene expression profiles have also been successfully used to identify prognostically important leukemia subtypes and patients who would eventually fail therapy (18); to formulate a gene-based molecular predictor that can forecast survival after chemotherapy in patients with diffuse large-B-cell lymphoma (19); and to predict high- and low-risk groups among lung adenocarcinoma patients; survival in early-stage disease allows delineation of a high-risk group that may benefit from adjuvant therapy (20). Our studies of drug resistance demonstrate the feasibility of obtaining molecular profiles or fingerprints from cancer cells following treatment with anticancer drugs that might predict tumor resistance, yield insights into the mechanisms of drug resistance, and suggest alternative methods of treatment.

## GENOMICS AND GENE EXPRESSION PROFILING OF CHEMOPREVENTIVE AGENTS

Naturally occurring compounds in dietary constituents that contribute to cancer chemoprevention have been in-

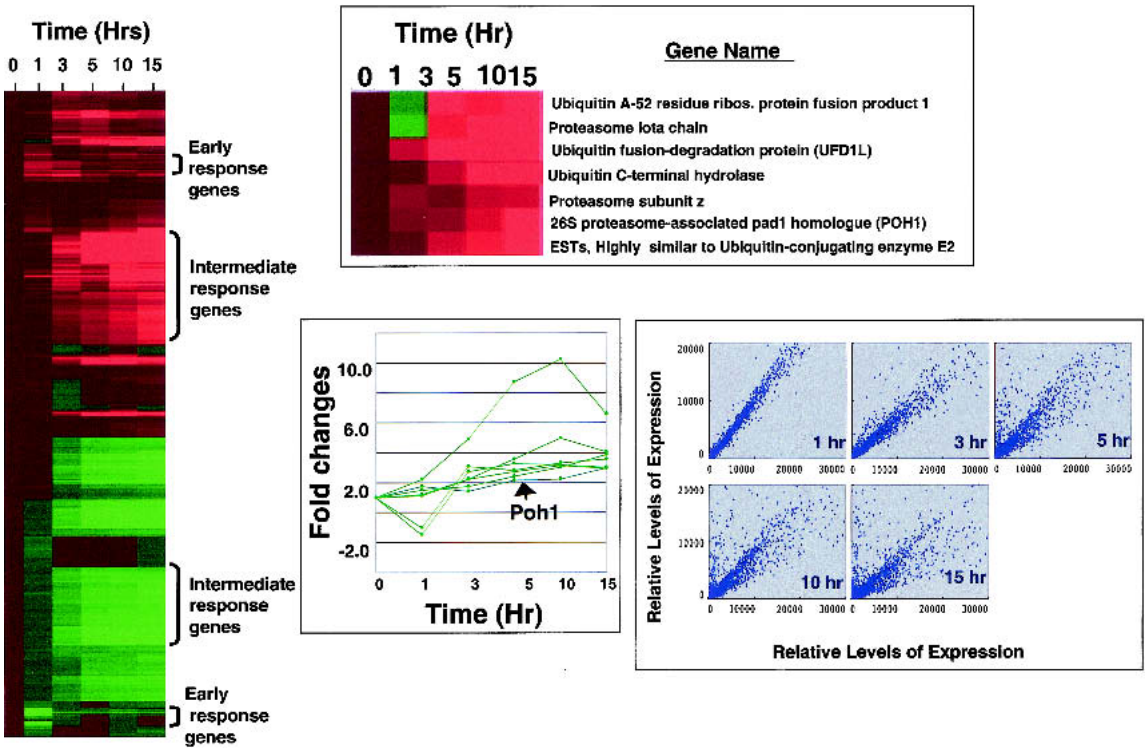


**Fig. 2.** Cluster analysis of temporal changes in gene expression. MCF-7 cells were treated with doxorubicin in a time-course study. Distinct temporal changes in gene expression were observed and functionally clustered together. **A,** Clustergram of the time course analysis of doxorubicin-induced gene expression. Distinct temporal changes in expression of gene clusters are categorized as early- and intermediate-response genes. **B,** Clustergram of functionally related genes in the ubiquitin-proteasome pathway induced by doxorubicin. **C,** Line graph showing time-dependent changes in the expression of ubiquitin-proteasome pathway genes in **B. D,** Expression profiles of the untreated control (x-axis) and doxorubicin-treated (y-axis) MCF-7 cells are shown as bivariate scatterplots of 5,180 genes from the microarray.

tensely investigated in recent years. However, the mechanisms by which these compounds act are still not fully understood. Ironically, to elucidate the mechanisms of action of these chemopreventive agents, some lessons can be learned from cellular exposure to xenobiotics or toxic insults such as environmental pollutants. These toxins alter the expression of various cellular defensive proteins including xenobiotic-metabolizing enzymes or DMEs. One consequence of inducing these genes is that the body can remove the "toxic insults" very rapidly [reviewed by Rushmore and Kong (14)]. For instance, the induction of various cytochrome P450 (CYP) genes by different xenobiotics is mediated by specific ligand–receptor interactions at the promoter/enhancer elements of CYP and/or other responsive genes. On the other hand, induction of phase II DMEs and other cellular defensive genes by chemicals and oxidative stresses can be mediated by non-receptor signaling mechanisms including the mitogen-activated protein kinase (21), protein kinase C (22), and phosphoinositol-3-kinase (23) pathways and the subsequent activation of members of the basic leucine zipper (bZIP) family of transcription factors such as Nrf1 (24), Nrf2 (25,26), and small Maf proteins (27). Transactivation of target genes by these transcription factors may occur via nucleic acid/protein interactions on *cis*-regulatory elements within the target gene promoters, and some of these consensus elements include the antioxidant response element (ARE)/electrophile response element (EpRE) (28). The mechanism of activation of the Nrf2/Maf complex is presently unclear, possibly involving transcription factor phosphorylation and/or modulation of the cytosolic protein Keap1, which has been proposed to suppress Nrf2 transcriptional activity by retaining Nrf2 in the cytoplasm (29). Surprisingly, microarray analysis of some chemopreventive compounds, including green tea polyphenol (–)-epigallocatechin gallate (EGCG) and isothiocyanates, also showed induction of phase II DMEs, cellular defensive genes, as well as cell death genes (30). It has been postulated (12) that at low concentrations, these compounds can modulate signaling proteins such as the MAPK pathway, which in turn induce Nrf2/ARE-target genes, a potential pharmacogenomic response. However, higher concentrations of these compounds activate the caspase pathway and induce cell death genes, leading to apoptotic cell death, a potential toxicogenomic effect. Therefore, the use of microarray expression profiling to further dissect the various pathways that are directly or indirectly affected by these chemopreventive compounds will provide insights into the mechanisms by which these agents act to prevent cancer. Ultimately, genomic profiles could pave the way for clinical applications of these agents.

## POTENTIAL APPLICATIONS OF GENOMICS TO DRUG DISCOVERY AND DEVELOPMENT

The genomic studies described above detailing the effects of chemotherapeutic and chemopreventive agents could be potentially extended to pharmaceutical drug discovery and

development. It is also clear that pharmaco- and toxicogenomic profiles based on expression patterns alone may reveal many complex cellular events but may have limited applications. The advent of "combinatorial chemistry" and the development of highly specific receptor/enzyme will enable the use of high-density microarrays for chemical genomics, in which hundreds and thousands of compounds can be screened simultaneously for interactions with their molecular targets. This high-throughput process, coupled with the application of pharmacogenomics and toxicogenomics to the early discovery phase of the drug developmental process, may yield insights into potentially beneficial vs. cytotoxic effects of the compounds before reaching clinical trials. Therefore, the combination of chemical genomics and signature expression profiles provide a wealth of information on any compounds selected from the screen, which may include their solubility, absorption profile (either substrates of the MDR family of proteins and or highly metabolized by DMEs), induction and or inhibition of DMEs (potentially resulting in drug–drug interactions), and toxicity (specific receptor-mediated or nonspecific via various signal transduction pathways). Such an integrated approach to drug discovery will undoubtedly produce global as well as specific information regarding pharmacologic and toxicologic responses and a comprehensive understanding of the biologic response to the therapeutic effects of candidate small molecules (Fig. 3). In conjunction with further understanding of the physical pharmacy (formulation properties), pharmacokinetics (ADME, absorption, distribution, metabolism, and excretion), interspecies differences between preclinical animal model systems and humans, and pharmacodynamics (handling of the drugs), the combined use of chemical genomics and expression array will increase the efficiency of drug discovery.

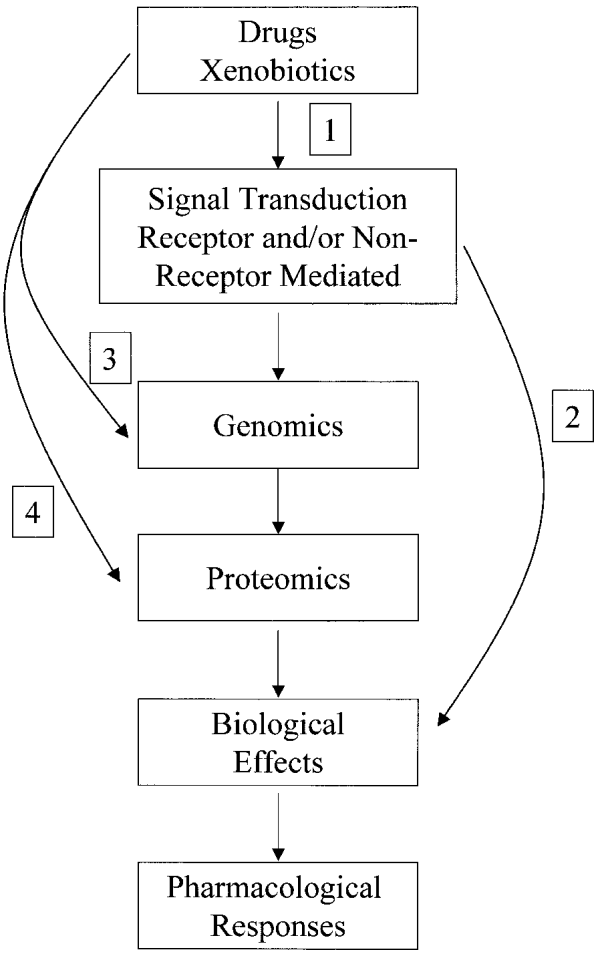


**Fig. 3.** A schematic model to illustrate the flow of information from the exposure of the cells, tissues, or organs to the potential drug candidates or xenobiotics with the modulation of signal transduction events (receptor- or non-receptor-mediated) resulting in genomics (gene expression profiles) followed by proteomics (protein expression profiles) leading to biologic effects and ultimately the coordinated pharmacologic responses (**1**). However, other combinations of information flow could also be possible, such as direct drug action on proteomics (receptors/membrane transporters/ion channels) and produces biologic effects (**2**). The drug can also affect gene expression directly (posttranscriptional; **3**) or protein (posttranslational; **4**), leading to biologic effects. Ultimately, these biologic effects would produce pharmacologic responses, sometimes beneficial but other times deleterious.

## DISCUSSION AND FUTURE PERSPECTIVE

The development of DNA microarray technology has enabled researchers to interrogate the expression of tens of thousands of genes simultaneously in a single experiment. High-density arrays that include all the known human genes provide the platform to further assess the induction of genes such as phase I and II DMEs that have important roles in drug metabolism, as well as groups of functionally related genes that provide signature pharmacologic (potential therapeutic targets) or toxicologic (undesirable adverse or toxic effects of the drugs) profiles of therapeutic molecules. Microarrays have been successfully used to analyze cellular responses to environmental toxicants, chemotherapeutic agents, and cytokines (8–10). It seems that exposure to xenobiotics, including chemotherapeutic and chemopreventive agents, may trigger cellular "stress" responses. These responses can then lead to increased expression of many "stress response genes" such as DMEs, which enhance the elimination and clearance of the xenobiotics and/or harmful intermediate reactive oxygen species (ROS). Consequently, this homeostatic cellular response plays a central role in protecting the organism against "environmental" insults.

Completion of the human genome sequence coupled with advances in DNA microarray and proteomic technologies will ultimately allow quantitative assessment of expression profiles of all the genes and their products. Our ability to predict biologic and pharmacologic/toxicologic outcomes from gene expression profiles is currently in its infancy. Additional computational and modeling tools (bioinformatics) will be required to decipher, from reams of microarray data, the genetic and biochemical changes that define disease states and the gene expression changes in response to drug treatment. Integration of the expression database with *in vivo* pharmacokinetic/pharmacodynamic studies and modeling/simulation data will yield insights into the mechanisms of drug action and will help expedite the process of drug discovery and development.

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