Phenotypic Anchoring: Linking Cause and Effect

Toxicogenomics combines genetics, transcriptomics (genomic-scale mRNA expression), proteomics (cell- and tissuewide protein expression), metabonomics (metabolite profiling), and bioinformatics with conventional toxicology in an effort to understand the role of gene–environment interactions in disease. Central to the research strategy of the National Center for Toxicogenomics (NCT) at the NIEHS is the fundamental concept of "phenotypic anchoring," in which studies are designed to relate specific alterations in gene expression profiles to specific adverse effects of environmental stresses defined by conventional parameters of toxicity such as clinical chemistry and histopathology.

The Toxicology/Pathology (Tox/Path) Working Group of the NCT performs experiments designed to gain insight into mechanisms of toxicity, to establish signatures of effects, and to link the patterns of altered gene expression to specific parameters of well-defined indices of toxicity. Such proof-of-principle experiments can lead to specific applications of genomics, proteomics, and metabonomics technologies to toxicology.

The group's overall approach is guided by the fact that processes of injury and repair are highly conserved across many species. For example, necrosis, apoptosis, and DNA repair have been studied effectively in nonmammalian and nonprimate systems because there is such a high degree of conservation of the various genes involved in fundamental aspects of these effects. However, chemicals exert their adverse effects over dose and time, and there are modifier genes in various species or strains that can affect the action of a chemical through metabolism or during some of the basic processes of injury and repair.

Studies are therefore designed to cover a full range of dose-related effects—from pharmacologic, to beneficial, to irreversibly toxic. The Tox/Path researchers anticipate that these studies will enable them to both identify biomarkers of specific toxicity (by studying well-defined toxic end points) and apply these biomarkers at earlier times and lower doses in a predictive manner.

Phase One

Studies on transcriptome analysis make up the first phase of Tox/Path efforts. The group hopes these studies will lead to the identification of a minimum set of genes that may be candidate biomarkers for specific toxic effects. At the same time, tissue and serum are being collected from the proof-of-principle experiments for simultaneous proteomic analysis. The results of the transcriptome studies can serve as a guide in the search for specific proteins that could be used as biomarkers of incipient toxicity.

The ultimate linkage of candidate biomarkers to the actual causal processes that lead to specific toxic effects will be accomplished through studies involving *in situ* hybridization, immunohistochemistry, and the laser-capture microdissection of cells to relate the expression of the putative biomarkers to the specific cells that have undergone these adverse events.

Studies from the NCT have demonstrated the capability of identifying signature patterns of altered gene expression that can be used to predict the classes of chemicals that an animal was exposed to based on an initial training set of chemicals. Joint research by scientists at the NIEHS Microarray Center and Boehringer Ingelheim Pharmaceuticals performed on acutely exposed animals has shown that global gene expression profiles for chemicals from different mode-of-action classes can provide gene expression signatures of chemical exposures in male rats.

This work led to the hypothesis that it would be possible to define signature patterns of altered gene expression that indicate specific adverse effects of chemical, drug, or environmental exposures. The idea is that once signatures are identified using large-scale global microarray analysis, it will then be possible to develop smaller multichemical and multipathway arrays that can be used to assess the potential toxicity of chemicals in a rapid, prospective manner.

This phenotypic anchoring of gene expression data to toxicological and pathological indices removes some of the subjectivity of conventional molecular expression analyses. It also helps to distinguish the toxicological effect signal from other gene expression changes that may be unrelated to toxicity, such as the varied pharmacological or therapeutic effects of a compound. This distinction could mean better interspecies extrapolation, greater confidence in animal models, reduction in the number of animals needed for testing, faster testing, and, most important, insights into pathways of toxicity and disease processes and their mechanisms that have been heretofore unattainable.

Fine-tuning the Approach

In testing this hypothesis, the Tox/Path Working Group is taking a three-part strategic approach. The first component of the strategy is to select agents that induce specific types of toxicity in both rodent models and humans. The initial focus is on hepatotoxic substances. For example, experiments are being designed to correlate gene expression patterns with liver pathologies such as hepatocellular necrosis and inflammation. The group will also look for correlative patterns, for example with other classic parameters of hepatic toxicity such as changes in serum enzyme levels.

The second component of the strategy is to select several structurally and functionally diverse chemicals and drugs that also are hepatotoxic, in order to generate comparative data (no two chemicals produce exactly the same pattern of hepatotoxicity). The process of injury is itself extremely complex, from the initial point of exposure through metabolism, damage, repair, and cell death. The Tox/Path researchers believe that by selecting a number of agents and carefully defining the toxic effects of those agents through the application of bioinformatics tools, they can identify specific groups of genes whose expression is altered and causally related to the specific toxicity.

The third component of the strategy is to use nontoxic isomers of hepatotoxicants to analyze effects in target and nontarget tissues, for example by comparing the effects of hepatotoxic chemicals in both the liver and the kidney. Gene expression changes in the liver will also be compared with expression changes in surrogate tissues such as blood in a so-called matrix approach.

Opening Doors

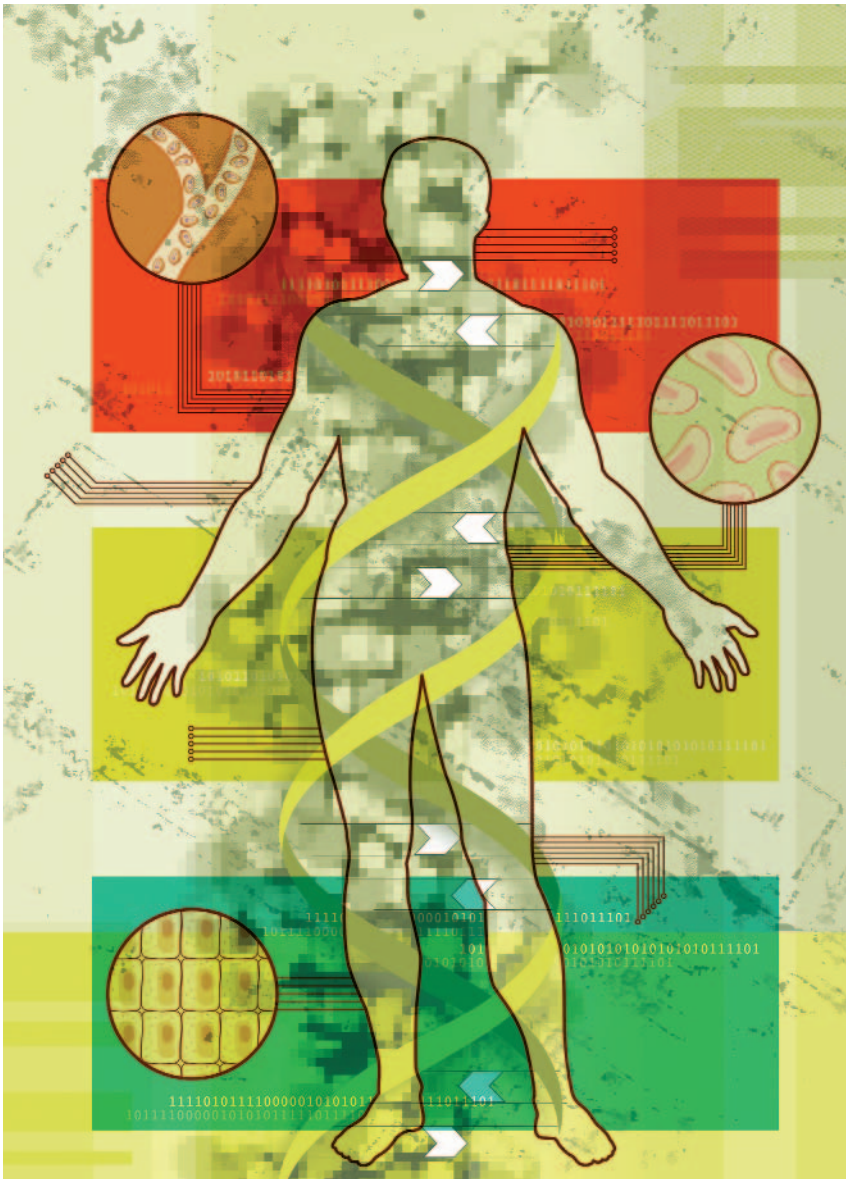
The philosophy of the Tox/Path Working Group is that only through a strategic, incremental study of specific agents and specific toxic effects can the most appropriate ways in which to use toxicogenomics technology in toxicology be identified. The group anticipates this will be a long-term process requiring a learning set of a substantial number of chemicals and drugs in order to achieve a meaningful array of candidate biomarkers. If such biomarkers can be established for processes such as hepatotoxicity, the approach may provide a strong stimulus for the search for biomarkers for other tissue- and organ-specific toxicities such as vasculitis, cardiotoxicity, and cancer, for which surrogate models provide poor predictive power.

According to the Tox/Path researchers, the application of global transcription analysis provides the opportunity for true discovery of novel entities such as potential biomarkers that cannot be hypothesized on the basis of the current state of knowledge. Such opportunities may revolutionize science's ability to characterize hazard. However, the challenge in the near future is to establish a body of available knowledge to serve as a foundation for applying the data generated by these new methods to risk assessment.

The scope of toxicology experimentation performed with toxicogenomics technologies is still relatively limited. Few data are publicly available, and broad consensus on the application and interpretation of them has not been reached in international regulatory and scientific arenas. There is significant need for a coordinated evaluation effort and a publicly available knowledge base.

Work in genomics will also result in a greater understanding of the mechanisms of chemical toxicity. This understanding will come through the determination of relationships between chemical exposure and changes in genomewide gene and protein expression patterns, or changes in patterns of metabolites. An understanding of the consequences of pattern changes is critically important in developing a complete understanding of toxicological processes, because gene expression is altered either directly or indirectly as a result of toxicant exposure in almost all cases examined. The spectrum of the altered genes or proteins then determines the type and outcome of the toxic response.

Viewed in this manner, patterns of gene and protein expression, or alterations in endogenous metabolism, can be used as markers of exposure and as methods for identifying mechanisms of toxicity. The simultaneous analysis of thousands of end points will allow toxicologists to take a new look at toxicological issues that cannot be completely understood using nongenomics techniques including mode of action, dose–response relationships, chemical interactions and hazard identification in chemical mixtures, and human exposure assessment.



Digital Vision, Artville, Matt Ray/EHP

The Tox/Path researchers hope that the combined and integrated data on gene, protein, and metabolite changes collected in the context of dose, time, target tissue, and phenotypic severity across species will provide the interpretive information needed to define the molecular basis for chemical toxicity and to model the resulting toxicological and pathological outcomes.

Future NCT studies will explore quantitative or absolute gene expression profiling and consider combining such an approach with physiologically based pharmacokinetic and pharmacodynamic modeling. Pharmacokinetic modeling can be used to derive a quantitative estimate of target tissue dose at any time after treatment, thus creating the possibility to anchor molecular expression profiles in internal dose, as well as in time and phenotypic severity. Relationships among gene, protein, and metabolite expression may then be described as a function of the applied dose of an agent and the ensuing kinetic and dynamic dose–response behavior in various tissue compartments. Scientists would then be able to search for evidence of exposure or injury prior to any clinical or pathological manifestation, facilitating identification of early biomarkers of exposure, toxic injury, and susceptibility.

Toxicogenomics research will likely lead to the identification, measurement, and evaluation of biomarkers that are more accurate, quantitative, and specific. These biomarkers will be recognized as important factors in a sequence of key events that will help to define the way in which specific chemicals and environmental exposures cause disease.

Researchers in the Tox/Path Working Group anticipate that understanding of the mechanisms of toxicity and disease will improve as new toxicogenomics methods are used more extensively and as toxicogenomics databases are developed more fully. The end result will be the emergence of toxicology as an information science that will enable thorough analysis, iterative modeling, and discovery across biological species and chemical classes.

–Richard Paules