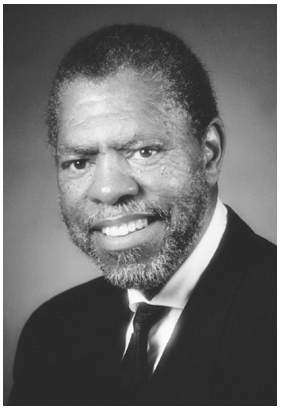
New opportunities in toxicology in the post-genomic era



'Toxicogenomics will have a profound impact on toxicology, drug development and the practice of medicine.'

Kenneth Olden, Director, National Institute of Environmental Health Sciences and the National Toxicology Program, National Institutes of Health

'Genes are only a small part of our makeup; the environment has a spectacular impact', as quoted by Eric Lander, Director, MIT Center for Genome Research at the Whitehead Institute for Biomedical Research, Cambridge, MA, USA in a Washington Fax dated 25 September 2000.

The evolution of the science of toxicology has been a slow process. The traditional methodologies that are available to researchers in the field are not adequate to investigate the complex biochemical and genetic interactions involved in the development of toxic injury. Therefore, toxicology studies have, out of necessity, focused on one chemical and one effect at a time. The development and application of the new genomic technologies will unshackle this important discipline, enabling global analysis of multiple pathways and molecular events simultaneously. Toxicology is now poised to have a leading role in biomedical research. In addition to the applied role in evaluating the toxicity of new products introduced into commerce (e.g. pharmaceuticals, pesticides), toxicologists have an important part in identifying environmental hazards and elucidating their role in the development of human diseases. These activities are enormously important both for the development of safe products and for sound environmental health regulatory decisions.

The recent publication of the first draft of the human genome sequence [1,2] has ushered in many new and exciting opportunities and challenges not only for geneticists but also for toxicologists. For example, using robust tools that are capable of elucidating mechanisms at the molecular level, toxicologists can now investigate the role of gene–environment interactions in the development of complex traits, diseases and toxic injury. These include approaches for the identification and characterization of both the genome and proteome of the mouse, rat and man. Such studies are crucial to establish a causal association between particular genotypes, specific environmental exposures and complex phenotypes, such as disease or toxic injury.

The surprise that the human genome consists of so few genes means that the environment might have a major role in human biology. With so few genes, the complexity of human growth, development and physiology must be programmed partially into gene–gene, gene–environment and protein–protein interactions. To assume that gene function can be inferred directly from phenotype is too simplistic. No single factor is responsible for the development of most complex phenotypes; genetic predisposition, exposure to environmental agents, behavior, gender, diet and nutrition all contribute. That is, genes exert their influence within certain environmental constraints. Genes might 'load the dice' in favor of, or against, the development of a specific phenotype but they do not dictate when, or if, the specific phenotype will be expressed. Therefore, to understand the development of complex phenotypes, we will require knowledge of the various interactions at the molecular level.

The availability of mouse, rat and human gene sequence databases has stimulated vigorous debate among toxicologists about how genomics can be applied to the field of toxicology [3–6]. Except in a general sense, it is clearly too early to predict what the ultimate impact of toxicogenomics will be. Nonetheless, one can be reasonably certain that genomics will have a major impact on five areas of toxicology: (1) the development of new and more informative test systems for toxicity or carcinogenicity assessment; (2) the elucidation of the genetic basis for differences in susceptibility or response to drugs or other environmental xenobiotics; (3) the development of biomarkers for use in quantification of exposure; (4) the elucidation of mechanisms and pathways involved in the development of disease or toxic injury; and (5) the development of tools and resources for use in population-based studies. However, for these outcomes to be realized genomics must be combined with proteomics and metabonomics. Thus, scientists

1359-6446/02/$ – see front matter PII: 1359-6446(02)02120-1

could conduct large-scale studies of the effects of toxicants on gene expression at the mRNA and protein levels and, at the same time, monitor metabolite profiles in body fluids and tissues to gain insight into the activity state of all relevant proteins. It will almost certainly be possible to predict toxicity and infer mechanisms of action on the basis of the combined use of these technologies. Using one approach alone is likely to be insufficient because most drugs and other environmental xenobiotics might act through multiple mechanisms depending on dose, timing and duration of exposure, and cell or tissue type. These sorts of analyses will lead to a better understanding of disease pathways and interacting networks that can be confirmed by conventional biochemical and genetic approaches. If successful, one of the 'grand goals' of toxicology – the characterization of the entire set of genes and proteins that are affected when animals are exposed to drugs and other environmental stresses – will have been achieved.

The objective in writing this editorial is to promote the use of genomic technologies and resources in the mechanistic and predictive toxicology assessment of drugs and other environmental xenobiotics. The genomic innovations reported here are already having a substantial influence on the kind of studies that are now being conducted by toxicologists. One can conclude that the 'post-genome' era will be put off until a later time.

Search for susceptibility genes

Now that the reference sequences of most human genes are available, the field of genomics has shifted from gene discovery to the study of gene function and elucidation of the genetic basis for differences in susceptibility to common diseases. Because gene structure and function can be influenced by the environment, efforts to understand the structure, function and diversity of the human genome and how it interacts with environmental factors, are the most challenging problems in biology. The identification and functional characterization of susceptibility alleles are crucial for understanding the pathways for development of human illness and for predicting risk to environmental exposures and response to pharmaceuticals.

Throughout life, man and other organisms are subjected to environmental insults on a continual basis. As a result, they have evolved sophisticated metabolic pathways to buffer against toxic injury. Collectively, these buffering pathways have been referred to as the 'environmental response machinery' [7]. All human genes, including those that encode protein components of the environmental response machinery, are subject to genetic variability (mutations or polymorphisms), that can result in outright failure or altered efficiency of a protective pathway. On average, five to six single nucleotide polymorphisms (SNPs) exist per gene, ~50% of which alter gene function [8]. Therefore, the risk for developing toxic injury (e.g. a disease) as a result of exposure to an environmental xenobiotic might be dependent on the efficiency of one's unique complement of response genes. These genes, for example, might determine how a person responds to, and metabolizes, drugs or carcinogenic compounds following exposure.

To date, very few environmental susceptibility genes have been identified, but with improvements in methods for gene discovery and genotyping, large-scale studies of the genetic basis for susceptibility to environmental exposures are now practical. In fact, strategies to characterize susceptibility polymorphisms, based on resequencing of candidate genes and association studies and scoring for SNPs, have been proposed with the announcement of such efforts as the Environmental Genome Project [9,10] and the Single Nucleotide Polymorphism Project [11]. Such variations have enormous implications for pharmacology, toxicology and susceptibility.

At present, environmental health regulatory agencies craft safety standards using arbitrary safety factors as if 'one-size-fits-all'. This is done because current toxicity assessments are performed in highly inbred, allelic-enriched animal models to limit the variety of possible responses. Although convenient experimentally, the extrapolation of results from such studies to genetically diverse humans is problematic. Therefore, knowledge of the prevalence of susceptibility alleles would take much of the guesswork out of human risk assessment.

Expression profiling of genes, proteins and metabolites

The vast majority of synthetic and natural chemicals in products used by man have never been thoroughly screened for toxicity. Also, the demand for toxicity assessment has increased dramatically over the past decade because of the rapid evolution of drug discovery science, fueled by combinatorial chemistry and recombinant DNA technology, and the build-up of chemical and physical pollutants in the environment resulting from human activity. Thus, more efficient and cost-effective toxicity screening methods must be developed. Because of this demand, toxicologists have embraced the new bioanalytical genomic technologies. The conventional approaches of exposing laboratory animals to high doses of the chemical are too slow, too expensive, use too many animals and are not very informative with respect to mechanisms of toxicity. Advances in genomics, proteomics and metabonomics offer promise for developing new test systems. In particular,

the capacity to array thousands of DNA fragments corresponding to specific genes on matrices and hybridization to mRNA or cDNA from the cells or tissues of animals or humans will enable toxicologists to globally assess chemical effects on biological systems.

Toxicogenomics will be applied to in-depth investigations of mechanisms that are difficult to achieve with conventional approaches and to establish relational databases in both humans and surrogate animal models for understanding interspecies differences and similarities. Although species differences in response to specific environmental exposures might occur as a result of genetic polymorphisms in genes coding for proteins that are responsible for buffering against environmental insults, it is probable that metabolic pathways that mediate toxicity are conserved or occur via mechanisms common to most species. Thus, alterations in gene expression signatures observed in rodents or other surrogate models are likely to have use as biomarkers for predicting toxicity in humans. Paucity of data on mechanisms and interspecies similarities and differences has impeded safety and risk assessment decisions.

Gene expression data can be used in conjunction with the search for specific susceptibility polymorphisms to improve drug development and predict individual response and likelihood of toxic injury from exposure to environmental xenobiotics. The future of toxicogenomics is predicated on the premise that chemicals give rise to unique expression signature patterns, which correspond to a select number of genes or proteins involved in specific metabolic pathways. However, for gene expression data to be useful for the characterization of chronic toxicity, the signature patterns must be correlated with conventional indices of toxicity. In fact, the application of gene and protein expression data can be analogous to diagnostic pathology once validated as predictors of adverse health outcomes.

Virtually any change in the environment will influence the expression of many genes. In fact, surveillance of the complete set of environmental response genes is just the starting point in searching for polymorphic alleles that are responsible for differences in susceptibility or mediation of toxicity. Therefore, toxicologists must first develop a knowledge base that will enable the discrimination between adaptive or pharmacological responses and toxicological effects.

The various methodologies for arraying genes and assessing mRNA expression will have to be validated, standardized and harmonized among laboratories before toxicogenomics can become a routine tool in toxicologic evaluation. Also, our ability to interpret the meaning of microarray analysis is limited. But, as mRNA and protein expression databases are expanded using a variety of chemicals, it will be possible to identify events that are crucial for the development of specific toxic endpoints and to link them to phenotypic markers of toxicity. However, patience and long-term commitment of resources will be required to achieve this scientific objective as hundreds of chemicals and many experimental variables will need to be examined.

Analysis of mRNA expression profiles is only one guide to assessing the effects of drugs or other environmental agents on cell physiology. Genes can be spliced to give rise to several proteins and peptides and posttranslational processing (e.g. proteolytic cleavage, phosphorylation, glycosylation) and cellular localization can be crucial to the function of gene products. Therefore, protein and metabolite analyses are a better reflection of the physiological state of the cell or organism. Promising new quantitative methodologies are emerging for protein and metabolite analysis including antibody arrays and matrix-assisted laser desorption ionization (MALDI) [12–14]. However, new and more-refined technologies are needed in this area of investigation.

Conclusions

Toxicogenomics will have a profound impact on the field of toxicology. It provides a toolbox of new technologies that can be used to address some of the intractable problems that have long characterized the field. These include intrinsic toxicity to humans, variation in susceptibility, crosstalk or interaction between agents in mixtures, and the type, pattern and magnitude of human exposure to chemicals. Application of these tools will enable hypothesis-driven research and examination of the exposure–disease relationship from either the exposure end or from the perspective of disease trends among populations. Analyses of exposure and susceptibility polymorphisms will provide the long awaited connection between traditional toxicology and epidemiology. Toxicogenomics has the power to revolutionize current regulatory decision-making, pharmaceutical and chemical product development and clinical matching of patients and dose regimens. Improved public health practice, as well as economic efficiencies, are the expected outcomes of toxicogenomics.

References

1 Venter, J.C. *et al.* (2001) The sequence of the human genome. *Science* 291, 1304–1351

2 Lander, E.S. *et al.* (2001) Initial sequencing and analysis of the human genome. *Nature* 409, 860–921

3 Nuwaysir, E.F. *et al.* (1999) Microarrays and toxicology: the advent of Toxicogenomics. *Mol. Carcinog.* 24, 153–159

4 Lovett, R.A. (2000) Toxicologists brace for genomics revolution. *Science* 289, 536–537

5 Olden, K. and Guthrie, J. (2001) Genomics: implications for toxicology. *Mutat. Res.* 473, 3–10

6 Tennant, R. (2002) The National Center for Toxicogenomics: using new technologies to inform mechanistic toxicology. *Environ. Health Perspect.* 110, A8–A10

7 Olden, K. and Wilson, S. (2000) Environmental health and genomics: visions and implications. *Nat. Rev. Genet.* 1, 149–153

8 Nebert, D.W. (2000) Extreme discordant phenotype methodology: an intuitive approach to clinical pharmacogenetics. *Eur. J. Pharmacol.* 410, 107–120

9 Kaiser, J. (1997) Environment institute lays plans for gene hunt. *Science* 278, 569–570

10 Brown, P.O. and Hartwell, L. (1998) Genomics and human diseases – variations on variation. *Nat. Genet.* 18, 91–93

11 Collins, F.S. *et al.* (1997) Variations on a theme: cataloging human DNA sequence variation. *Science* 278, 1580–1581

12 Rubin, R.B. and Merchant, M. (2000) A rapid protein profiling system that speeds study of cancer and other diseases. *Am. Clin. Lab.* 19, 28–29

13 Merchant, M. and Weinberger, S.R. (2000) Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry. *Electrophoresis* 21, 1164–1177

14 Huang, R. (2001) Detection of multiple proteins in an antibody-based protein microarray system. *J. Immunol. Methods* 255, 1–13

Kenneth Olden

Director, National Institute of Environmental Health Sciences and the National Toxicology Program
National Institutes of Health
Department of Health and Human Services
Research Triangle Park, NC 27709, USA
e-mail: olden@niehs.nih.gov