

## Promising Next Generation Technology in Toxicology-Toxicogenomics

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### Abstract

Toxicology is a multidisciplinary field, and an important science that impacts both environmental health regulation and the development and practice of medicine. The rapid progress in cellular and molecular biology, like many other branches of biomedical research, toxicology is now experiencing a renaissance fueled by the application of “omic” technologies to gain a better understanding of the biological basis of toxicology of drugs and other environmental factors. In this review on current progress on toxicology, the future perspective, concept, approaches and applications of toxicogenomics as next generation promising technology in toxicology field will be described.

**Keywords:** toxicogenomics, transcriptomics, proteomics, metabolomics, toxicoinformatics

The detection and the regulation of man-made synthetic chemicals and the establishment of toxicity that may pose a genetic hazard in our environment are subjects of great concern because of its close correlation between environmental contamination and human health. Since the tens of thousands of man-made chemicals that have been introduced into the environment in the last few decades must also be tested for their damaging effect on DNA, the agents that cause this damage must be identified.

Toxicology has been defined as the qualitative and quantitative study of the adverse effects of xenobiotics on living organisms. Moreover, modern toxicology

goes beyond the study of the adverse effects of exogenous agents to the study of cellular and molecular effects of toxicants using molecular biological tools. Toxicology is a multidisciplinary field, and an important science that impacts both environmental health regulation and the development and practice of medicine<sup>1</sup>. Classical toxicological tools require hundreds of animals and provide little information with respect to mechanism (s). For example, descriptive studies in genetically inbred animals do not explain genetic and biological differences in the human population that influence individual response to drugs and environmental xenobiotics.

Recently, several new methods such as single cell gel electrophoresis (comet) assay, mouse lymphoma thymidine kinase forward gene mutation assay, transgenic animal and cell line model as a parameter of lac I (Big Blue) or lac Z (Muta mouse), and *in vivo* survival micronucleus assay with peripheral reticulocytes using acridine orange fluorescent staining for the detection of genetic damages *in vitro* and *in vivo* were introduced according to the rapid progress in toxicology combined with cellular and molecular biology<sup>2-4</sup>. Moreover, the rapid progress in cellular and molecular biology, like many other branches of biomedical research, toxicology is now experiencing a renaissance fueled by the application of “omic” technologies to gain a better understanding of the biological basis of toxicology of drugs and other environmental factors<sup>5-7</sup>.

In this review on current progress on toxicology, the future perspective, concept, approaches and applications of toxicogenomics as next generation promising technology in toxicology field will be described.

The recent completion of the human genome sequencing project and the push to finish the mouse genome have raised the stakes in science with predictions of disease cures, more effective and safer pharmaceuticals, and a greater understanding of environmental effects on human health. The impact of human genome projects on toxicological research is high, heralding the emerging technologies of toxicogenomics, proteomics, and bioinformatics<sup>8,9</sup> for the future use of these technologies and their impact on drug discovery, safety evaluation, elucidation of pathways of toxicity, and risk assessment. The NIEHS established the National Center for Toxicogenomics (NCT) in September 2000. According to the center's

mission statement, its goal is “to use the methodologies and information of genomics science to significantly improve our understanding of basic biological responses to environmental stressors/toxicants.” Paul Gilman, who is assistant administrator for EPA, states that toxicogenomics is a powerful tool with great promise for risk assessment<sup>10</sup>. And also, he stressed that EPA encourages and supports continued genomics research as a powerful tool for understanding the molecular basis of toxicity and developing biomarkers of exposure, effects, and susceptibility. He also mentioned that genomics information is useful in a weight-of-the-evidence approach for human health and ecological risk assessments on a case-by-case basis at this time. By combining these new “omics” approaches with classical and/or conventional toxicological methods, it is possible to develop the experimental models and strategies to evaluate 1) the diverse structure and properties of various chemicals; 2) the relationship between the time of exposure, dose, and health outcomes; 3) the influence of genetics and behavioral factors; 4) interaction between multiple components of biological systems in development of toxic responses, and 5) intrinsic biological health responses with extremely low concentration for long time exposure. 6) toxicological responses of compounds at an early stage of the drug discovery process and health risk assessment.

### What is Toxicogenomics?

Toxicogenomics, in a broader sense, is defined as a study of the response of a genome to hazardous substances, using: i) Genomic-scale mRNA expression

(transcriptomics) ii) Cell and tissue wide protein expression (proteomics), and iii) Metabolite profiling (metabonomics) in combination with bioinformatic methods and conventional toxicology (In a narrow sense, it refers to the use of transcriptomics). In relation to chemical hazard/risk assessment, this emerging science could provide tools for improving the understanding of mechanism of toxicity, identification of biomarkers for prediction of toxicity and exposure, and possibly alternative methods for chemical screening, hazard and toxicity identification, characterization, classification. The basis of toxicogenomics in our sense is summarized in Fig. 1.

### Approaches and Application of Toxicogenomics

The utilization of these new technologies along with more established genetic approaches such as quantitative real time polymerase chain reaction (QRT-PCR), and the use of genetically altered animals will dramatically move the field of toxicology forward. As you know well, the recent remarkable advances in genomics, proteomics, and metabonomics, the interactions between multiple genes, proteins, and pathways can now be investigated with more easy and time-saving ways<sup>11</sup>. cDNA and oligonucleotide microarrays and high-throughput 2-D electrophoresis systems have quickly emerged as the premier tools to enable genomewide analysis of gene expression at the RNA and protein level. These new technologies are heavily influencing drug discovery and preclinical safety in the biotechnology and pharmaceutical industry<sup>12,13</sup>. Toxicologists are also pro-

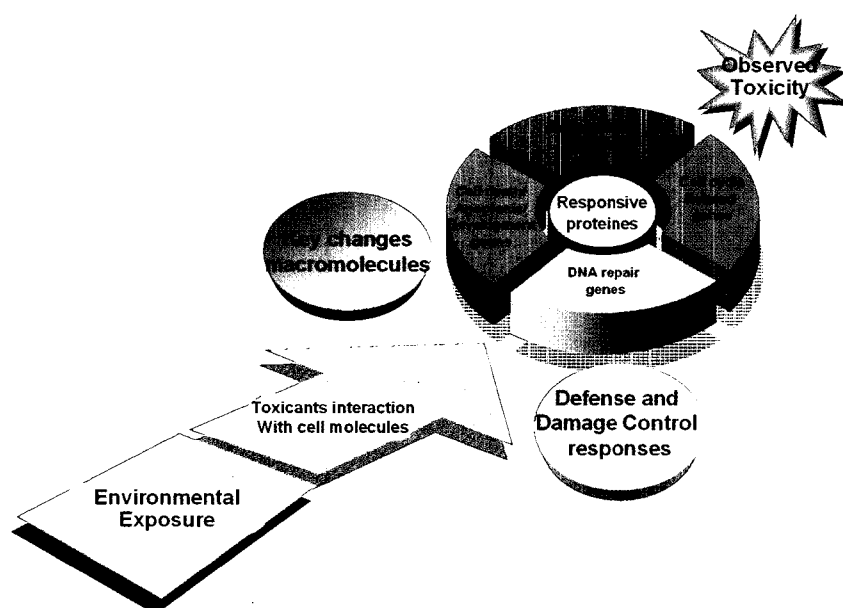


Fig. 1. The Basis of Toxicogenomics

moting genomic expression technologies as a superior alternative to traditional rodent bioassays to identify and assess the safety of chemicals and drug candidates for human safety<sup>14-17</sup>. It is expected that gene expression profiling will identify mechanisms of action that underlie the potential toxicity of chemicals and drug candidates. Ultimately, toxicogenomics (the integration of genomics, bioinformatics, and toxicology) is expected to accelerate drug development<sup>18,19</sup>, and aid environment ecological<sup>20</sup> and health risk assessment.

In the laboratory, these are some examples of questions that researchers will be able to address by toxicogenomics as follows; Which genes are regulated upwards or downwards (apoptosis, cellular proliferation, metabolism, communication and cell adhesion, etc.) following an exposure to mercury, PCBs or triazines pesticides? Is the response similar in the liver, lung, testes, ovaries and brain? Are the responses of animal and cell models comparable to humans? The network is also particularly interested in the genetic factors responsible for environmental contaminant-induced breast cancers, unfavorable pregnancy outcomes, and children's health.

The approaches in the commercial basis, one of examples, TNO pharma<sup>21</sup> has taken up the challenge of toxicogenomics. A research program is initiated with the following features;

- Embedding in "classical" toxicology: comparison of multiple gene expression and proteome changes induced *in vivo* as a function of time and dose level.
- Use of *in vitro* systems to underscore *in vivo* findings (but not the other way around!).
- Integrated genomics and proteomics analysis of target organs.
- Incorporation of kinetics and metabolism in target organ toxicity assessment.
- Evaluation of possible polymorphisms in the mechanism of toxicity, and the consequences for extrapolation to the human situation.
- Combination of tissue microdissection and gene expression analysis in order to relate pathology and genomics. Comparison of gene expression changes to a toxicogenomics database to allow for classification of the effects.
- A proprietary pathway related bioinformatics system aiding the toxicologist in understanding the data.
- Complex data analysis based on pattern recognition and proprietary principle component analysis.
- First grade technology ("home-made" high density DNA arrays, 2D-proteomics with MALDI-TOF and nanospray MS).

## Advantages and Limitations

There are both advantages and limitations to the use of gene microarray and proteomics technologies in toxicological screening. The main advantage is a global approach to understanding the complex mechanisms involved in toxicology. Gene microarrays have been costly and limited in availability, but the past year has shown a commitment by the scientific community to the general use and availability of gene microarrays. Consequently, cost has been reduced by increased supply and demand. Furthermore, the availability and cost is substantially improving with many universities and research centers establishing genomic and proteomic facilities. Also recent experiments applied to cancer genetics have demonstrated the potential of gene expression profiling to accurately classify disease phenotypes<sup>22,23</sup>, thus lending hope that expression profiling may classify and thus predict phenotypes of toxicity. Despite these expectations, it is still uncertain how gene expression profiling experiments will ultimately contribute to our understanding of toxicity and allow us to realize the full potential of this new technology.<sup>16,17,24</sup> have also discussed the possibilities and caveats of gene expression profiling in the context of mechanistic and predictive toxicology and have addressed the certainty, biological relevance. Therefore, toxicogenomics and proteomics will certainly become generally used technologies in the near future.

## Health Risk Assessment and Biomarkers

Biomarkers will play an important role in early detection of environmentally induced disease, since routine surveillance programs in both human and animals in suspected environmentally hazardous areas could be instituted. Toxicogenomics and proteomics are also providing new biomarkers for use in human studies. It is now possible to envision schemes for integrating the results of molecular epidemiological investigations into the general toxicological evaluations of environmental agents. These will allow intermediate endpoints to be used for making realistic human health assessments and for elucidating pathogenic mechanisms that identify targets for intervention, all with the goal of preventing environmentally mediated human disease. Also, select biomarker responses that predict the likelihood of disease occurrence will find application in the interpretation of individual medical diagnostic tests, with the goal of improving cancer detection and management. The objective is to determine whether gene, protein or metabolite expression profiles or "signatures" can serve as markers to predict toxicity. Current efforts are underway to establish "best choices and practic-

es" and perform proof-of principle experiments to phenotypically anchor altered patterns of expression to conventional parameters of toxicity. These trials are more closely define the time and dose relationships to express "signatures" that develop tools of predictive toxicology and elucidate common mechanism of toxicity and drug action. Early detection of toxic exposures is a developing art, but many groups have already successfully classified chemical exposures based on profiling of mRNA from treated animals<sup>25-30</sup>. This kind of information might be useful for risk assessment in that significant changes in expression in a small set of highly discriminatory genes can together act as a biomarker of toxic mechanisms or endpoints.

### Toxicoinformatics

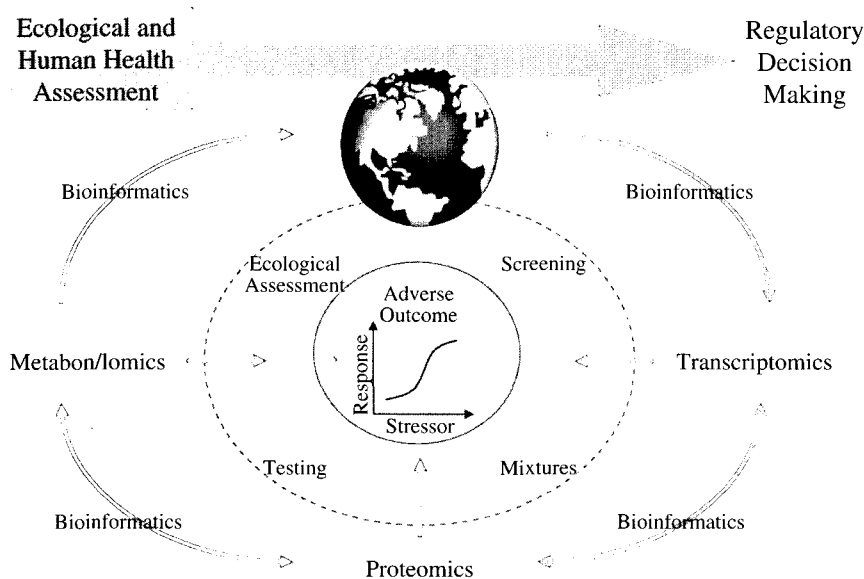
There is more specified fields such as toxicoinformatics. Toxicoinformatics is the important factor of toxicogenomics field. Toxicoinformatics are essential computational tools for the analysis of time- and dose-dependent changes in patterns of toxicant-induced gene expression. Development of these capabilities will require a database that complies high quality data from diverse sources involving different gene expression platforms, assay methods, validation results and diverse drugs, chemicals or environmental agents. Complete analysis will require linkage between and among additional databases that provide most current sequence identity can annotate gene identify and function, chemical structure, toxicity, pathology, pharmacokinetics/biodistribution, and genotoxicity<sup>31-34</sup>.

### International Harmonization and QA/QC

To promote the further development and application of the "omic" technologies to toxicology and environmental health risk assessment, recently, on Oct 12-13, Toxicogenomics International Forum<sup>35</sup> was held, and continuously, OECD/IPCS organized a special workshop for toxicogenomics focusing ecotoxicogenomics at Kyoto, Japan on Oct 13-15, 2004. In this OECD/IPCS workshop<sup>36</sup>, 4 group sessions open to discuss the problems and future plan of toxicogenomics, especially focused on ecotoxicogenomics (Fig. 2).

One is biological breakout group and they summarized that toxicogenomic technologies have unique opportunities to address ecological and human health concerns, such as; i) offering possibilities to reduce, refine and replace costly animal intensive methods for chemical screening and testing, ii) understanding how and why species and subgroups differ in sensitivity and response to chemical stress, and create a stronger scientific foundation for the safety factors. This will allow effective policies to be developed in order to protect endangered and importance species iii) assessing the effects of chemical mixtures and combination of stressors. Previously, appropriate methods have been lacking. iv) reduced uncertainty in assessment of ecological conditions. These will allow effective policies to be developed to protect endangered and important species: For these reasons, it is important that these new tools are evaluated and implemented for chemical risk assessment.

In the technical group, several useful technical tools such as global oligo array, global cDNA array,



**Fig. 2.** Proposed Toxicogenomics Research with Coordinated International Research Program by OECD/IPCS Workshop, Oct. 13-15, 2004, Kyoto, Japan

targeted oligo array, targeted cDNA array, Q-PCR and SAGE for transcriptomics, and 2D-Maldi, 2D-MS/MS, Ciphergen-Maldi, Protein arrays, LC-MS/MS, ICAT-Maldi, ICAT-MS/MS and ELISA for proteomics, and NMR, targeted MS-based, LC-electrochemical for metabolomics were discussed.

The regulatory usefulness of toxicogenomic tools is very important<sup>37</sup>. In this respect, in the regulatory group, expected outcomes are a road map for development, validation and regulatory use of genomic-based tools, proposals for further activities related to the use of genomic-based methods in chemical assessment and promotion of related research to be undertaken within the OECD Environment, Health and Safety Programme, and proposals for mechanism of international co-ordination for the development, validation and regulatory use of genome-based tools.

The bioinformatics group adopted general recommendations such as 1) stable funding and adequate funding must be provided to a national and international level for standardization initiatives, database and tool development and long-term maintenance, 2) provide training in data requirements and bioinformatics issues relating to ecotoxicogenomics for risk assessors, 3) governments should promote synergy between the toxicology and ecotoxicology communities, including a dialogue between scientists, regulators and bioinformaticians on an international scale, 4) in the broader context of ecotoxicogenomics studies, initial efforts should be centered on a few species representative of the ecological complexity and 5) establish a task force/working group to implement these recommendations and coordinate future actions.

The ultimate promise of toxicogenomics lies in its potential ability; (i) to identify sources of interindividual variability in response to drugs and environmental xenobiotics, both in terms of efficacy and toxicity; This area of research requires genetics knowledge regarding individual variation which determines person's responses to drugs and xenobiotics; (ii) to provide a database for the development of high-throughput and low-cost platforms for screening substances for toxicity, and (iii) to improve the process of discovering new targets for drug action. However, the high degree of QA/QC on microarray chips to produce more reliable data will be remained to be solve at present.

As every human being on the earth are unique in their genetic make up, there are huge variations in responses against outer environmental factors and also in susceptibility to a disease. As the medical research is getting into the arena of personalized medicine which applies individual's genetic information in disease treatment and selection of drugs,

toxicogenomic research also have to look into the individual genetic differences. A genetic variation which is involved in metabolism of toxicants and chemicals can answer the questions of why some people react more sensitively to same environments than others. Careful cataloging of association between genetic background and responses to toxic substances will serve as a powerful tool to understand toxicological phenomenon. This way, one can possibly assess each individual's risks in a given environment and design a plan to avoid any toxic effects. Consequently, toxicogenomics will be a great promising next generation technology<sup>38</sup> in the fields of health risk assessment, drug safety, food safety and forensic toxicology etc.

Additionally, there will a new paradigm that is systems biology. The confluence of omics, bioinformatics, mechanistic studies at the molecular level, and computational biology has yielded a new discipline called systems biology<sup>39</sup>. Systems biology is a new field of biology that aims to develop a system-level understanding of biological systems<sup>40</sup>. It requires collective efforts from multiple research areas, such as molecular biology (genomics), high-precision measurement, computer science, control theory and other scientific and engineering fields.

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