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Application of Toxicogenomic Technology for the Improvement of Risk Assessment

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Abstract

Recently, there has been scientific discussion on the utility of -omics techniques such as genomics, proteomics, and metabolomics within toxicological research and mechanism-based risk assessment. Toxicogenomics is a novel approach integrating the expression analysis of genes (genomic) or proteins (proteomic) with traditional toxicological methods. Since 1999, the toxicogenomic approach has been extensively applied for regulatory purposes in order to understand the potential toxic mechanisms that result from chemical compound exposures. Therefore, this article's purpose was to consider the utility of toxicogenomic profiles for improved risk assessment, explore the current limitations in applying toxicogenomics to regulation, and finally, to rationalize possible avenues to resolve some of the major challenges. Based on many recent works, the significant impact toxicogenomic techniques would have on human health risk assessment is better identification of toxicity pathways or mode-of-actions (MOAs). In addition, the application of toxicogenomics in risk assessment and regulation has proven to be cost effective in terms of screening unknown toxicants prior to more extensive and costly experimental evaluation. However, to maximize the utility of these techniques in regulation, researchers and regulators must resolve many parallel challenges with regard to data collection, integration, and interpretation. Furthermore, standard guidance has to be prepared for researchers and assessors on the scientifically appropriate use of toxicogenomic profiles in risk assessment. The National Institute of Toxicological Research (NITR) looks forward to an ongoing role as leader in addressing the challenges associated with the scientifically sound use of toxicogenomics data in risk assessment.

Keywords: Risk assessment, Toxicogenomics, Toxic information

Government agencies charged with protecting public health are required to review, quantify, and regulate hazardous materials in a manner in which the public's health will be guarded and enhanced. Increasing environmental contamination by thousands of chemical compounds is an important concern for human health worldwide. Although many chemicals are suspected to have hazardous effects, evaluating their toxicity remains difficult and challenging. The goal of risk assessment is to obtain a reasonable likelihood estimate of the harm associated with a toxic chemical exposure on the basis of four steps: i) hazard identification, ii) hazard characterization (or dose-response assessment), iii) exposure assessment, and iv) risk characterization (Figure 1). Whenever possible, risk assessors must evaluate every source of scientific information to draw conclusions about the human health concerns of a chemical exposure. Risk assessments are frequently criticized for their dependence on default values used to deal with uncertainties in the absence of relevant data[1]. Therefore, a better definition of the mode-of-action (MOA) of a chemical could provide information that is critical to reducing the uncertainty within risk assessment. If the molecular level effects of a chemical exposure are related to pathophysiological changes in an organism, these methodologies could be used as an alternative approach for predicting toxicity. New scientific technologies that might resolve crucial data gaps and data inconsistencies could potentially improve risk assessment by: providing additional data on toxic effects, increasing understanding on mechanisms and modes of toxic action, and enhancing the reliability of dose-response extrapolation. -Omics technologies such as genomics, proteomics,and metabolomics hold promise for obtain-

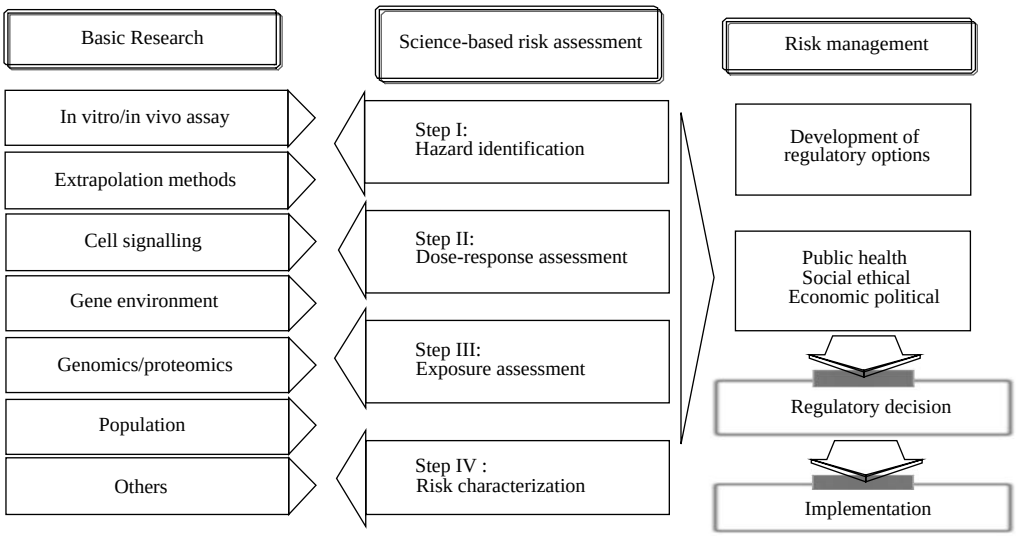

Figure 1. Framework of conventional risk assessment to strengthen decision-making for public health protection.

ing new information that is relevant to the scientific requirements of risk assessment. In this article, we begin with an overview of the utility of toxicogenomic profiles for improved risk assessment and discuss how findings from toxicogenomic technologies inform risk assessment. Finally, we rationalize possible avenues to resolve some of the major challenges.

Information Gaps and Data Limitations Considered in Risk Assessment

Concerns on hazardous substances were first issued a few centuries ago with the intention of improving human well-being. Today, more than 100,000 environmental and industrial chemicals have been identified, and nearly 2,000 new chemicals per year are introduced to human life. However, there is little information for determining the safety of these chemicals to human health. In addition, one of the difficulties in evaluating safety is the presence of chemicals that are reported to have adverse effects on organisms despite having negative results in traditional animal toxicity tests.

The three principal components for predicting and assessing potential human health risks are: (1) the diverse structure and physicochemical properties of existing or new chemicals, (2) the time and dose parameters that define the relationship between exposure and toxic response, and (3) the genetic diversity of organisms used as surrogates to determine adverse effects. Many regulatory agencies worldwide have focused on science-based decision-making for public health protection. Risk assessment is a systematic and science-based process to assess human health effects. An understanding of basal mechanisms of toxicity could assist risk assessment, but such mechanisms have unfortunately remained elusive. Table 1 describes the most crucial information gaps and inconsistencies that limit the accuracy of risk assessment. Many uncertainties are generally recognized as affecting the confidence of risk assessments; all are mainly dependent on either the amount of the data set for risk assessment or the accuracy of the data set for the chemically-induced critical endpoint. By identifying gene or protein expression changes that correlate with toxicity, one can acquire better understanding of the molecular mechanisms of toxicologic changes, which in turn helps to establish human relevance as well as safety margin estimates.

Utilization of Toxicogenomic Profiles for the Improvement of Risk Assessment

Cell-based screening methods could be devised based on acquired knowledge of molecular mechanisms, toxic pathways, and biomarkers of toxicity. -Omics technologies, which have emerged as an attractive approach to uncovering the critical molecular events altered by xenobiotic exposure, can simultaneously measure the expression of thousands of genes, proteins, or metabolites, providing the potential clue in defining toxicant pathways[2-4]. These findings, by the use of -omics technologies, can be utilized to predict the toxicity of unknown compounds after comparing their molecular fingerprints with those obtained from compounds of known toxicity, and can also

Table 1. The limitation of datasets and inconsistencies that complicate risk assessment.

Limitation in risk assessment datasets
• Examples on the lack of data for evaluating the potential adverse effect or carcinogenicity of a chemical: - Lack of sufficient screening data of untested compounds using inexpensive short-term indicator methods. - Lack of data for evaluating potential hazardous effects such as in vitro or animal toxicity tests or epidemiologic data. - Lack of experimental data; for example, quantitative structure-activity relationships. - Lack of information on the relevance of animal data to humans. - Lack of information on the relationship between dose and response, especially low doses. - Lack of exposure data obtained at different life stages. - Lack of data on the impact of co-exposures to other chemicals. - Lack of information on human variability in susceptibility.
Inconsistencies
• Examples of inconsistencies in animal data: - Species differences, such as between rats and mice. - Gender differences. - Different target organ responses in species. • Insufficiencies and uncertainties in human data, including exposure levels.

provide mechanistic insight into the mode of toxicity. Furthermore, this new approach could contribute to improvements in risk assessment by enhancing our ability to identify molecular mechanisms of action and to possibly better assess dose-response curves. In the future, it is expected that the impact of -omics technologies on toxicology will translate into fundamental changes within regulatory risk assessment and decision making.

Because some risk assessors may not be sufficiently familiar with toxicogenomics to see its eventual impact on their work, it may not be clear for some time exactly how toxicogenomic findings will be incorporated into the regulatory process. Furthermore, the concepts of how toxicogenomics might be incorporated into risk assessment may be unclear to researchers not working directly in risk assessment. In an attempt to bridge that gap and to provide an opportunity for researchers interested in regulatory issues, this article discusses how toxicogenomics has been applied in risk assessment based on the following topics:

(1) The application of toxicogenomics towards predicting the mechanisms of toxicological changes.
(2) The application of toxicogenomics to understand the human relevance of toxicologic changes observed in animals during risk assessment processes.
(3) The identification of selective gene expression signatures that could be used as sensitive biomarkers.

The Application toward Predictive Toxicology

Many studies demonstrate that toxicogenomic data are useful for the predictive toxicology of unknown toxicants[5]. One commonly used approach within the toxicologic prediction of unknown toxicants is quantitative structure-activity relationships (QSARs)[6,7]. QSARs models have been used to infer likely effects on poorly tested or untested compounds; however, any QSAR model produces some degree of error[7]. This is partially due to the inherent limitation in predicting toxicological activity based solely on a chemical's structure. The current limitations of QSARs could be lowered by using gene expression patterns generated by chemicals that have relatively well-known adverse effects. The exact interpretation of change patterns in toxicogenomic profiles with relation to other biological end points will provide the critical context for determining the suitability of a data set for risk evaluation.

Approximately half of all compounds tested in rodents are carcinogenic, and most of these are nongenotoxic carcinogenes[8]. While various genotoxicity assays are predictive for DNA-damage-induced carcinogenicity in rodents, nongenotoxic carcinogens have proven more difficult to accurately predict in these short-term assays. Nongenotoxic carcinogenes refer to the lack of a direct effect on DNA, and their continued administration is often required for tumor development. The nongenotoxic carcinogens have been postulated to act via a number of mechanisms such as increased mitogenesis, decreased apoptosis, interference with gap junction intercellular communication, interference with tubulin polymerization, and cellular property changes by the accumulation of cellular injury[8]. It was reported that microarrays and expression profiling were used to characterize nongenotoxic carcinogens, and the gene expression signatures determined for the nongenotoxic compounds were over 80% accurate in classifying samples[9]. In terms of the prediction accuracy of nongenotoxic carcinogens, it was

Table 2. The application of toxicogenomic approaches in various fields.

The major applications in toxicologic assessment:
- To predict toxic mechanisms of potential human health hazard materials that lack data sets to elicit a toxic response. - To understand the human relevance of toxicologic or pathologic changes observed in preclinical species. - To understand the relationship between exposures and human disease susceptibility. - To identify useful biomarkers of disease and exposure to toxic substances. - To improve computational methods for understanding the biological consequences of exposure and responses to exposure. - To create a public database of environmental effects of toxic substances in biological systems.
The major applications in drug discovery and clinical experiments:
- As a useful tool to identify safer drugs in a faster, more cost-effective manner. - To be used early in drug discovery to identify candidates with potential toxic liabilities. - For diagnosis of disease using sensitive gene expression markers.

expected that the available whole rat genome microarray and the final validity of those signatures would enable us to find additional as well as superior genes to predict nongenotoxic carcinogens.

During drug development, a key regulatory issue is the identification of target organ toxicity induced by a drug[10]. Since the development of -omics technologies, toxicogenomic profiling stands as a complementary alternative approach to the traditional histopathological approach in preclinical drug safety evaluations. Identifying safety in terms of drug-induced organ damage is of great importance to pharmaceutical companies during drug development. Identifying drug safety at the preclinical stages would not only reduce the cost of drug development, but also improve the accuracy of pre-marketing risk assessment. For example, toxicogenomics was recently attempted in predicting and diagnosing preclinical renal tubular toxicity[11]. The kidney is a major organ for filtration, secretion, reabsorption, and ultimately, the excretion of drugs or drug metabolites. Due to its primary function, the kidney is especially vulnerable to toxic insults by various drugs, and thus, nephrotoxicity is a major concern in preclinical safety evaluations. Recently, an attempt was made to predict the prognosis of kidney toxicity using the toxicogenomics data of tissue treated with known kidney toxicants[12]. These results achieved very good performance (sensitivity of 80% or above and specificity of 90% or above) for toxicity subtype prediction, and this is much better than traditional approaches that often have no significant prediction values. Therefore, toxicogenomics may be generally applicable in the diagnosis of drug-induced organ toxicities. On the other hand, gene expression changes associated with toxicological changes typically reflect a large number of complex pharmacological, physiological, and biochemical processes, and most of them interact with each other. Therefore, prior to being used for risk assessment, toxicogenomic profiles related to toxicity need to be identified and separated from those that are adaptive, beneficial, or unrelated to the development of the toxicological change.

Tool for Bridging Animal to Human Toxicity Studies

Toxicologic changes occurring in animal toxicity testing are not necessarily relevant to humans due to differences in species in their responses to changes induced by chemicals[13]. On the other hand, the use of human data, such as results of epidemiologic studies, eliminates the uncertainties in extrapolating effects from other animals to humans during risk assessment. However, such epidemiologic studies of chemical exposure are lacking or have various limitations. Some studies have reported that toxicogenomic techniques could lead to better understanding on the relevance of animal data to humans[14,15]. For example, some toxicity mechanisms are quite well-conserved across species, whereas others are not. Toxicogenomic techniques could be used to define these similarities and dissimilarities across species. Based on many carcinogenicity tests conducted by the National Toxicology Program (NTP), level evidence for carcinogenic activity was different according to species or gender. According to an NTP technical report, these differences in response to a chemical are critical for 30% of tested chemicals. Toxicogenomic data would provide more accurate information on these differences. In addition, comparing the unique patterns of gene-expression or protein expression changes within the target organs of rats and mice could help in determining the differential sensitivity in tumor incidence exposure levels of humans. These patterns could be compared with those in humans, helping to determine whether humans more closely resemble rats or mice for chemical sensitivity. Thereby, a better understanding of the molecular mechanisms of toxicologic changes can be used to improve the accuracy of extrapolation from experimental animals to humans, and consequent-

ly, reduce this source of uncertainty in risk assessment. For instance, hepatic toxicity induced by the fibrate class of cholesterol-lowering drugs has marked species differences and is directly correlated with the number of hepatic peroxisome proliferator-activated receptor α (PPARα)[15]. Genomics technology can be used to further understand the molecular mechanisms associated with the various effects of peroxisome proliferators[6,9]. In the case of arsenic, humans appear to be more sensitive in terms of carcinogenic effects than laboratory animals; but of the tumors observed in humans, the involved MOAs are not known[16,17]. If unique patterns of gene- or protein-expression changes were not observed in animals exposed to arsenic, but were detected in humans exposed to arsenic, risk assessors might confirm the proposed MOAs or evidence provided in the human carcinogenicity of arsenic. In the USA, genes and proteins in which expression reverses in lower arsenic-exposed populations, have been used as indicators of the cellular processes involved in arsenic's effects.

Identification of Biomarkers

The biomarkers specific to toxicologic changes within preclinical studies are useful for monitoring in clinical trials and longer-term preclinical studies. For the practical use of a biomarker in risk assessment, it should be sensitive, specific for the toxicologic change, and detectable in easily accessible tissue samples. The use of *in vitro* or *in vivo* toxicogenomics approaches may be a powerful tool in identifying new gene expression changes that highly correlate with toxicity, and in finding possible biomarkers of early response[15]. Genes that respond transcriptionally to a certain toxicant in an organism may not only provide important clues on the toxic mechanisms of the chemical, but also on potential biomarkers. There are a number of reported examples of the application of toxicogenomics towards biomarker development in the literature. For example, microarray-based toxicogenomics approaches may be useful for identifying target genes and biomarkers of developmental toxicity. The use of DNA microarrays is represented as a potentially suitable assay system by identifying responsive genes upon exposure to a teratogen such as valproic acid in cell models, which could be readily used in a tiered testing system for developmental toxicity testing[4]. In another example, the quinolone and trovafloxacin regulated a number of mitochondrial genes that were not regulated by the other quinolone compounds; this toxicity has been purported as a possible mechanism for the serious liver toxicity resulting from trovafloxacin[18-20]. Yet, despite these examples, the true value of sensitive biomarkers is not yet demonstrated in the risk assessment of many toxicants.

Preparation for the Future Regulation Submission of Toxicogenomic Data

Although toxicogenomics has seen considerable progress in many of the areas mentioned above, its routine application to preclinical risk assessment is not yet universally accepted. Some researchers have expressed concern that the capacity to rapidly obtain large amounts of data on chemical effects by using these technologies could result in inappropriate decisions regarding the potential for chemical-induced adverse effects[21,22]. Toxicogenomics data alone are currently insufficient as a basis for risk assessment and management decisions. In risk assessment, vast amounts of toxicogenomics data must be analyzed and correctly interpreted in order to help identify, evaluate, and validate toxic materials for hazard identification. Incomplete understanding of the findings may lead to adverse regulatory outcomes.

In order for toxicogenomic techniques to be used in risk assessment, and than accepted by regulatory agencies, their limitations of use need to be identified. Ultimately, the success of a toxicogenomics approach within risk assessment depends on the assessor's ability to interpret the data in relation to existing information. Due to the potential of toxicogenomic profiling to improve the risk assessment of new chemical compounds, regulatory agencies must prepare to appropriately evaluate future submissions of toxicogenomic data in the drug application review process. For example, the US FDA has issued the publication: "Guidance for Industry: Pharmacogenomic Data Submission"[19,23,24]; the guidance provides recommendations to industry on when to submit pharmacogenomics data to the FDA during the drug or biological drug product development and review process, and it includes what format and the content to provide for submission, as well as how and when the data will be used in regulatory decision making. Presently, Korea regulatory agencies have to prepare the scientific foundations to address the challenges associated with the integration of toxicogenomic data into risk assessment. These will include the preparation of guidelines for determining how and when toxicogenomic data should be suitable for regulatory decision making, the establishment of a publicly available toxicogenomics database, validation of available technologies, future discussions on the scientifically appropriate use of toxicogenomic data in risk assessment, and the nonscientific challenges involved in using such information. Many practical and conceptual challenges remain before the utility of toxicogenomics can be achieved in regulatory activities. Within toxicogenomic analy-

sis, the application of strict quality criteria provides the main explanation for the limited identification of significant changes.

Discussion

In the hazard characterization as first step in the risk assessment, scientific studies are reviewed to determine what effects a chemical can cause, including identification of the target organ and identification of the critical effect. Consideration of weight of evidence is important for all hazard characterization. In general, hazard characterization review the available epidemiology data, chronic animal toxicity data, other short -term screening bioassays, mechanistic studies, genotoxicity test, metabolic and pharmacokinetic properties, and physicochemical properties. Moreover, the sequence of events by which a chemical causes cancer can provide specific information for the carcinogenic risk assessment.

Toxicogenomic technology has increasing utility in a broad range of scientific areas[5,10,14,24], including risk assessment to confirm the MOA of hazardous chemicals, screening within the drug discovery process, and in making the decision to refrain from further toxicity testing of new chemicals. In addition, a successful transition of toxicogenomics into testing programs should markedly decrease resource and animal use. Since 2005, the NITR has been conducting a project to develop a long-range vision for a strengthened risk assessment system, as well as a strategic plan for implementing that vision. This article focused on the knowledge gap within current risk assessment, and how to incorporate the recent advantages of new technologies such as toxicogenomic technology to improve risk assessment. The successful incorporation of toxicogenomics into regulatory framework may someday be regarded as the most important intellectual and practical contribution the current generation of toxicologists.

Currently, the application of toxicogenomics is rapidly being embraced in various scientific fields (Table 2). To address this issue, it is important for regulatory agencies to develop scientific framework. And well-trained experts and advanced capabilities are needed for toxicogenomic data analysis. The predictive accuracy and confidence of the toxicogenomic technique for different unknown chemicals, varies according to how well the training set represents the unknown chemicals, and how robust the technique is in extrapolating beyond the biological space defined by the training set. To maximize utility of these techniques in regulation, researchers and regulators must resolve many parallel challenges with regard to data collection, integration, and interpretation. Furthermore, more complete reference databases and improved quality control of laboratory procedures would allow more exact data interpretation, thereby improving risk assessment for public health decision-making. Also things to be considered in the framework should be a better-defined process to validate biomarkers, and guidelines for the regulatory submission of toxicogenomic data.

The NITR looks forward to an ongoing role as leader in addressing the challenges associated with the scientifically sound use of toxicogenomics data in regulatory risk assessment processes. We will effort to develop collaborative partnerships with scientists in academia, company, other governmental organizations, and private institutes to create the integrated knowledge base that will be a scientific resource for risk assessment.

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