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Toxicology and genetic toxicology in the new era of "toxicogenomics": impact of "-omics" technologies

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Abstract

The unprecedented advances in molecular biology during the last two decades have resulted in a dramatic increase in knowledge about gene structure and function, an immense database of genetic sequence information, and an impressive set of efficient new technologies for monitoring genetic sequences, genetic variation, and global functional gene expression. These advances have led to a new sub-discipline of toxicology: "toxicogenomics". We define toxicogenomics as "the study of the relationship between the structure and activity of the genome (the cellular complement of genes) and the adverse biological effects of exogenous agents". This broad definition encompasses most of the variations in the current usage of this term, and in its broadest sense includes studies of the cellular products controlled by the genome (messenger RNAs, proteins, metabolites, etc.). The new "global" methods of measuring families of cellular molecules, such as RNA, proteins, and intermediary metabolites have been termed "-omic" technologies, based on their ability to characterize all, or most, members of a family of molecules in a single analysis. With these new tools, we can now obtain complete assessments of the functional activity of biochemical pathways, and of the structural genetic (sequence) differences among individuals and species, that were previously unattainable. These powerful new methods of high-throughput and multi-endpoint analysis include gene expression arrays that will soon permit the simultaneous measurement of the expression of all human genes on a single "chip". Likewise, there are powerful new methods for protein analysis (proteomics: the study of the complement of proteins in the cell) and for analysis of cellular small molecules (metabonomics: the study of the cellular metabolites formed and degraded under genetic control). This will likely be extended in the near future to other important classes of biomolecules such as lipids, carbohydrates, etc. These assays provide a general capability for global assessment of many classes of cellular molecules, providing new approaches to assessing functional cellular alterations. These new methods have already facilitated significant advances in our understanding of the molecular responses to cell and tissue damage, and of perturbations in functional cellular systems.

As a result of this rapidly changing scientific environment, regulatory and industrial toxicology practice is poised to undergo dramatic change during the next decade. These advances present exciting opportunities for improved methods of identifying and evaluating potential human and environmental toxicants, and of monitoring the effects of exposures to these toxicants. These advances also present distinct challenges. For example, the significance of specific changes and the performance characteristics of new methods must be fully understood to avoid misinterpretation of data that could lead to inappropriate

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conclusions about the toxicity of a chemical or a mechanism of action. We discuss the likely impact of these advances on the fields of general and genetic toxicology, and risk assessment. We anticipate that these new technologies will (1) lead to new families of biomarkers that permit characterization and efficient monitoring of cellular perturbations, (2) provide an increased understanding of the influence of genetic variation on toxicological outcomes, and (3) allow definition of environmental causes of genetic alterations and their relationship to human disease. The broad application of these new approaches will likely erase the current distinctions among the fields of toxicology, pathology, genetic toxicology, and molecular genetics. Instead, a new integrated approach will likely emerge that involves a comprehensive understanding of genetic control of cellular functions, and of cellular responses to alterations in normal molecular structure and function. Published by Elsevier Science B.V.

Keywords: Genetic toxicology; Microarrays; Gene expression; Toxicogenomics; Biomarkers

1. Introduction

The advances in biotechnology that have been achieved in the past few decades are truly extraordinary. They have provided an understanding of biological processes and an array of molecular technologies that have dramatically impacted all fields of biological science. This knowledge, and the technologies upon which it is based, have already impacted the fields of genetic and general toxicology, and provide the opportunity for further major advances in these fields. The current generation of scientists has already witnessed an extraordinarily rapid progression of knowledge of cellular and molecular genetics—from the discovery in 1944 that DNA was the genetic "blueprint" of life [1], to elucidation of the molecular structure of DNA [2], unraveling of the genetic code [3], and establishment of methods to efficiently "engineer" the genetic information in organisms [4,5]. The complete genetic sequence of the human genome has now been determined [6], and the sequences of many other important species are now nearing completion. Even though the pace at which these accomplishments were achieved is extraordinary, the future promises even more dramatic change.

Among the advances in technology that have been achieved, methods that allow global analysis of cellular constituents are of particular importance to the fields of general and genetic toxicology. Of these global methods, nucleic acid microarrays are currently receiving the greatest attention by the toxicology community. These technologies have made it possible to monitor thousands of nucleic acid sequences simultaneously—either specific expressed RNAs or sequence variants (polymorphisms) in DNA [7,8]. The analysis of expressed mRNAs using microarrays is analogous to conducting thousands of Northern blot analyses concurrently, and offers the possibility to monitor expression of individual genes across the entire genome (i.e. global gene expression analysis, or transcriptomics). Thus, microarray technology provides a means of studying multiple pathways and mechanisms at the same time. Such a global analysis of gene expression has the potential to provide a more comprehensive view of toxicity than has been possible previously, since toxicity generally involves change not only in a single or few genes but rather is a cascade of gene interactions. This unbiased form of analysis is certain to result in a more comprehensive picture of toxicological mechanisms and to lead to the re-evaluation of many of our currently-held beliefs.

Although nucleic acid microarray technologies have received much attention recently, other powerful new tools for global analysis of cellular constituents are already available and will also have a major impact on the field of toxicology. These include technologies for global analysis of proteins and peptides (proteomics), and of cellular metabolites (metabonomics). Among these advances are improvements in classical 2-dimensional (2D) gel electrophoresis, the introduction of multidimensional liquid chromatography, tandem mass spectrometry, and database searching technologies (termed multidimensional protein identification technology, or MudPIT), and improved mass spectroscopic identification of protein sequences using matrix- or surface-enhanced laser desorption ionization (MALDI, SELDI) techniques that allow rapid characterization of proteins or protein fragments [9–13]. These proteomic methods allow for the analysis of the functional and structural proteins in a sample. Methods for simultaneously monitoring small molecules involved in intermediary

metabolic pathways (metabonomics) are also at hand [14–16]. The ability to monitor defense responses via proteomics or metabonomics in humans at sub-pathological doses is of particular importance because it will make possible human studies that could not be carried out at overtly toxic exposures. These technologies provide complementary information to gene expression data. Clearly, post-translational modifications of proteins, such as phosphorylation, will not be evident as changes in gene expression. Also, nucleic acids may not be available for analysis in all cases (e.g. invasive procedures would be needed to obtain samples from many human tissues), although proteins may be secreted or diffuse into accessible compartments or be more amenable to imaging techniques. The use of all of these tools will be important for obtaining a comprehensive picture of toxicological changes in cellular constituents.

Thus, we now have available new families of technologies that provide a comprehensive analysis of the complete, or near-complete, cellular complement of specific constituents, such as RNAs, DNAs, proteins, intermediary metabolites, etc. These have been termed "-omics" technologies, a terminology derived from the Latin suffix "-ome" which denotes a body or group—in the commonly-used sense of a complete body or group (e.g. the "biome"—the complement of living organisms in a particular environment, or the "genome"—the complete set of genes contained in the cellular complement of chromosomes) [17]. These now include genomics, transcriptomics, proteomics, and metabonomics. In the near future, we may expect extension of these technologies to include other classes of cellular molecules, such as lipids, carbohydrates, lipoproteins, etc. These technologies are extremely powerful new tools with which to study disturbances of cellular homeostasis or structural integrity at a molecular level.

Although the molecular technologies for studying the genetic basis of disease, responses to molecular damage, and variation in response to toxicants have advanced in parallel with the science of cellular genetics, the regulatory approach to safety evaluation of new products has undergone little change over the past few decades. For example, the strategy for evaluating cell and tissue damage during product development described by Lehman et al. [18] and Barnes and Denz [19] has changed little during the past 50 years. Likewise, the strategy of genotoxicity testing remains similar to that developed during the 1970s [20]. On the other hand, carcinogenicity testing and risk assessment have recently begun to incorporate new mechanistic knowledge of the genetic basis of cancer induction, and biotechnology is being employed to construct animal models with specific genetic characteristics relevant to human carcinogenesis [21–23]. Two examples of the impact on regulatory science are the 1996 EPA draft carcinogenicity risk assessment guidelines, which ask that a mechanistic approach be taken to cancer risk assessment [24], and the introduction of specific transgenic animal models for the evaluation of cancer risk from exposure to pharmaceuticals [22,25]. Thus, the potential of the powerful new tools that have facilitated the biotechnology revolution has just begun to be realized in the fields of regulatory toxicology, genetic toxicology, and carcinogenesis.

There is every reason to expect major change during the next decade, as new technologies and knowledge become incorporated into regulatory and industrial practice. Indeed, a new sub-discipline of "toxicogenomics" has already been recognized. We define toxicogenomics broadly—as the study of the relationship between the structure and activity of the genome (the cellular complement of genes) and the adverse biological effects of exogenous agents. This is consistent with the broad definition of pharmacogenomics recently proposed by Lesko and Woodcock [26]. Unlike other new approaches or methods in toxicology that have been adopted slowly, genomic, proteomic and metabonomic methods are being evaluated and adopted rapidly by industry, academia and regulatory agencies. This is evidence that the practice of toxicology has begun to change and that change can be expected to occur rapidly. As with most new toxicological methods and approaches, collaboration will be required to develop the data and approaches necessary to achieve worldwide acceptance and use. The implications for all involved in this field are profound. In the future, the marriage of the field of toxicology with genetics and molecular biologic techniques will likely lead to the eradication of sub-disciplines such as "genetic toxicology", "classical toxicology", or "toxicological pathology". Rather, the broad impact and application of the field of molecular genetics will transform the field of toxicology into a science based

on molecular biochemical knowledge and techniques. Further, the separation between toxicology measures and efficacy measures may no longer exist—as many of the same molecular endpoints will be used to assess both the efficacy and toxicity of new drugs and chemicals. In this article, we summarize the opportunities and challenges created by these impending changes, with special emphasis on genetic toxicology, regulatory science, and product development.

2. Monitoring of gene expression and alterations in cellular constituents in response to toxicants

The key concepts that underlie the application of new technologies for global analysis of cellular constituents to the field of toxicology originated with the recognition that application of these technologies could provide a more comprehensive view of cellular and molecular damage than was previously possible.

- It has been recognized that "... gene expression is altered either directly or indirectly as a result of toxicant exposure in almost all cases examined" [27]. Indeed, disturbance of normal function or structure essentially defines toxicity; hence, the ability to simultaneously monitor biochemical homeostasis in a wide variety of pathways provides a comprehensive means of identifying and monitoring toxic effects.
- Expression of certain genes is required to achieve pathological outcomes. It has even been claimed that "there (are) no toxicologically relevant outcomes in vitro or in vivo, with the possible exception of rapid necrosis, that do not require differential gene expression" [28]. Although this may be slightly overstated, it is, in fact, clear that most pathological processes are active events achieved under genetic control. Expression analysis provides a very powerful means of monitoring these processes.
- Changes in gene expression associated with toxicity are often more sensitive and characteristic of the toxic response than currently employed endpoints of pathology. See, e.g. the discussion by Nuwaysir et al. [29]. An ability to monitor defense responses and pre-pathological compensatory responses to cellular damage is certain to result in useful new biomarkers of sub-pathological cellular damage. Such biomarkers will be of particular importance because they will make possible human studies that could not be carried out at overtly toxic exposures.
- It is thought that patterns of gene expression, or "fingerprints", will be identified and these could be used as biomarkers of exposure and as a method of identifying mechanisms of toxicity [30]. Gene expression chips from many different species that permit thousands of genes to be monitored simultaneously are already available [31,32]. The next generation of expression chips can be expected to allow all expressed human genes to be monitored simultaneously. Analogous chips that can support similar monitoring in laboratory animal species are also expected. This capability of "global" monitoring of essentially all expressed genes provides the opportunity to characterize the patterns of gene expression associated with specific types of damage and/or specific classes of chemicals. Fig. 1 illustrates these concepts.

3. Application of "-omic" technologies—promises and pitfalls

The promises and challenges of some of the potential applications of global toxicogenomic approaches

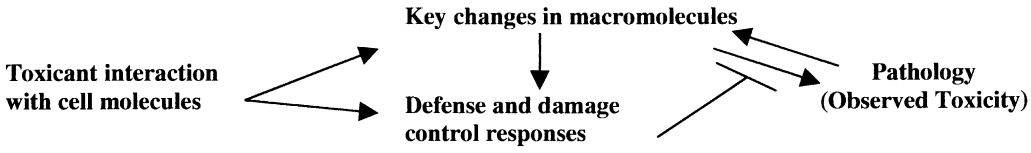

Fig. 1. Basis of toxicogenomics.

Fig. 2. Relationship between toxicity and development of fingerprints of gene/protein changes.

are described below. Certainly, uses beyond those mentioned here will develop as the field evolves.

3.1. Mode of action

Over the last several years, there has been an increased recognition by regulatory agencies of the value of mechanistic information for improving the risk assessment process. For instance, understanding the mode of action of a rodent carcinogen can help determine the relevance (or lack thereof) of carcinogenicity to humans or help in the quantitative extrapolation from high to low doses. The study of gene expression and genetically-controlled cellular constituents provides a new and powerful way of determining the mode of action. For example, association of a given toxic endpoint (e.g. carcinogenicity, genotoxicity, hepatoxicity) with a particular pattern of gene/protein expression, or a characteristic shift in cellular metabolites, may provide a "fingerprint" that is characteristic of a specific mechanism of induction of that toxicity (Fig. 2). For example, recent studies by Samson's laboratory of yeast exposed to alkylating agents, oxidizing agents, and ionizing radiation demonstrated novel regulatory pathways dealing with the induced damage [33]. Exposure to alkylating agents was found to induce genes involved in DNA repair that were co-regulated with genes involved with eliminating and replacing alkylated proteins [33,34]. This is an example of the type of information that can help determine which of the full profile of genes are involved in the mechanism of protecting cells against DNA damaging agents. Once a series of such fingerprints is defined for different mechanisms, the gene expression, protein, or metabolite pattern for a toxic chemical of unknown mechanism can then be compared to the established patterns for defined mechanisms. This provides a very powerful tool for categorization of toxicants according to mode of action. This is an important scientific advance, since defining the mechanism or mode of action of a chemical traditionally has required considerable time (often years), cost, and animal usage, and has included considerable uncertainty. Further, "fingerprints" of specific gene changes will provide the necessary information to design predictive toxicity screens.

It may take years to build a database of well-characterized toxicities and related gene expression changes that is comprehensive enough to represent the many different modes of action of toxic agents. Because of the importance and the magnitude of the task of building comprehensive databases of expression profiles, collaboration among toxicologists in industry, regulatory agencies, and academic institutions is needed. Some important collaborations have already begun, such as the International Life Sciences Institute–Health and Environmental Sciences Institute (ISLI–HESI) project on the application of genomics and proteomics to mechanism-based risk assessment [1] and the activities of the National Center for Toxicogenomics. [2] As discussed previously, this sharing of data and experience is expected to stimulate a rapid evolution of the field of toxicology.

3.2. Predictive toxicology

In addition to the capacity to define mechanisms, global analysis of gene/RNA/protein/metabolite patterns offers the potential to predict toxic responses (Fig. 3). Since changes in these cellular molecules are thought to precede toxic outcomes, appropriate changes may serve as early, sensitive indicators of a potential toxicity. As discussed above, it is expected that similar chemicals, as defined by mechanism of toxicity (e.g. DNA alkylating agents), will induce characteristic gene/protein expression patterns. Two recent studies [35,36] demonstrate this promise. In studies with genotoxic and non-genotoxic hepatoxins, patterns of gene expression changes were identified

[1] http://www.ilsi.org
[2] http://www.niehs.nih.gov/nct/

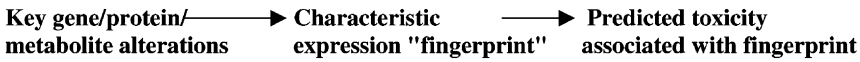

Fig. 3. Use of fingerprints of gene/protein expression changes to predict toxicity.

that may serve to distinguish chemicals with different mechanisms of action. Thus, it is expected that the profile of gene/protein expression changes will allow the discrimination among mechanisms of toxic damage, e.g. between a DNA alkylating agent, a microtubule inhibitor, and an agent that perturbs protein structure. These unique patterns may then lead to the development of high through-put screening tests for use in predicting specific toxicities and also molecular biomarkers, applicable in vivo, that signal particular toxic mechanisms. For some chemicals, the results of predictive studies may provide sufficient information to be able to eliminate some conventional tests, or may provide a basis for the design of an appropriate bioassay with relevant species. Because these predictive tools have the potential to provide considerable savings in terms of time, cost, and animal use relative to traditional methods, it is expected that commercial promise will help drive rapid development of practical assays.

The potential value of these new approaches to safety assessment has generated considerable excitement in the regulatory and industrial toxicology communities. However, it will be necessary to characterize multiple classes of agents with well-defined mechanisms of action before expression profiles for new biomarkers can be used reliably in regulatory decision-making [37]. It is critical that toxicologists in industry, regulatory agencies, and academic institutions develop a consensus, based on rigorous experimental data, about the reliability and interpretation of endpoints such as global gene expression patterns prior to use in regulatory and industrial settings.

3.3. Dose–response

A key aspect of the quantitative risk assessment process is understanding the effect of a chemical exposure at low doses typical of human exposure. Toxicity studies have traditionally been conducted at high doses, with various extrapolation methods used to estimate the effect at low doses. Quantitative differences in biochemical pathways and receptor affinities among species, coupled with the lack of biomarkers that respond to sub-pathological doses, has made it necessary to use exaggerated doses in order to minimize the chance of missing a toxic effect to which a particular species might be more resistant than is the human. Chemically-induced changes in gene/protein expression are expected to occur at doses of a chemical below those causing pathological alterations. Thus, by measuring gene/protein/metabolite changes at low doses, global analysis has the potential to provide critical information regarding biological effects at doses below those required for induction of the toxic endpoint. This should lead to improved extrapolations in the low dose range and a better determination of the effect of a chemical exposure to humans at low doses. It should also facilitate identification of threshold concentrations below which there is no significant risk.

A major challenge, of course, is to determine which of the molecular events that may change at low doses are necessary for pathological outcomes, versus those that are adaptive, beneficial, and/or unrelated to the development of pathologies. Molecular genetics has revealed the molecular basis of key functional systems within the cell, and it has also demonstrated the presence of defense mechanisms to protect these key functions (e.g. cell cycle control, structural integrity of proteins, control of free radicals associated with metabolism, and DNA repair mechanisms). The fact that these defenses have been highly conserved in evolution attests to their importance in limiting damage to these systems.

A specific example may serve to illustrate the various levels of understanding that are needed: the use of molecular responses to DNA damage as biomarkers of genetic toxicity. Maintenance of the fidelity of DNA replication is a prime example of the co-evolution of functional and defensive systems. The basic replicative DNA polymerase has co-evolved a proofreading function that corrects mispairings that occur during DNA replication. Additionally, specific damage-recognition and repair molecules have evolved to protect the integrity of DNA and insure the fidelity of its replication

(reviewed in [38]), and more than 125 genes directly involved in DNA repair have already been identified in humans [39]. These include pathways to prevent highly damaged cells from replicating under conditions that would induce extreme damage, such as *p53* and associated pathways. Thus, induction of genes associated with DNA repair is itself highly beneficial to the organism but is also a signal of the occurrence of DNA damage. If damage is extensive, then other responses may be induced, such as expression of *p53* and GADD-mediated processes that prevent replication of highly damaged cells. Ultimately, induction of the caspase cascade that leads to cell death in order to eliminate highly genetically aberrant cells may occur. The relationships between these defense systems, and their role in responding to various types and levels of DNA damage must be understood in order to appropriately interpret these responses in the context of safety assessment and product regulation.

Analogous to the above example of responses to DNA damage, the functional molecules that control protein folding and export, and protein destruction required for cell cycle control and tissue remodeling, antigen processing, and other functions, have co-evolved to respond to protein damage. Similar to their normal physiological roles, these molecules can function as molecular chaperones or proteasomes to refold or destroy structurally damaged proteins [40,41]. Likewise, in the case of cellular energetics, defense systems have evolved to scavenge potentially toxic oxidative by-products and respond to perturbations that increase oxidative species within cells. Now that global analyses are becoming practical, it may be expected that relationships among different classes of damage will be found. For example, protein damage has already been associated with agents and conditions that cause DNA damage. It is not surprising that DNA-damaging agents would also damage RNAs, and thereby cause transcriptional errors. Such an association between DNA and protein damage has been noted in a number of studies. For example, in a recent study of gene expression changes induced by DNA damaging agents, Jelinsky et al. [33] noted changes in genes involved in protein degradation and Radman and co-workers have noted that protein damage occurs both from transcriptional errors and also from protein structural alterations that lead to protein oxidation [42,43].

The biological relevance of gene expression changes, or of changes in functional levels of damage-response molecules, must be defined with respect to whether the gene(s) is pivotal in the pathway for a toxicity, if the change is reversible, and if the change in gene expression leads to altered cell or tissue function. It is important to guard against the temptation to classify every gene/protein expression change as adverse, as many changes will be physiological or reversible. Over-interpretation in the context of regulatory decision-making would result in inaccurate risk assessments and decision-making.

3.4. Species extrapolation

Because toxicity studies must often be conducted in animal models, extrapolation from results in animals to humans is needed to determine the potential human risk to a chemical exposure. The greatest single limitation of modern toxicological practice is probably the uncertainty of extrapolating from laboratory models to the human. Although the similarities in biochemistry and molecular biology among species has permitted a wide variety of useful laboratory models for the study of toxicological effects, quantitative differences often leave much uncertainty about differences in quantitative exposure–response relationships in the human compared with laboratory model systems. In almost all cases, quantitative differences in dose–response relationships exist between humans and model species, and in extreme cases biological responses to a given exposure may differ qualitatively. Thus, one of the great needs in the field is for “bridging biomarkers” of damage that can be used to compare toxic responses among species. In particular, human biomarkers that indicate that a given pathological condition is being approached—before it actually becomes manifest—are needed, so that it is ethically permissible to conduct human studies.

The increased understanding of cellular control and defense mechanisms, coupled with the powerful “-omic” technologies, have the potential to provide new bridging biomarkers that could greatly reduce this source of uncertainty in the risk assessment process (Fig. 4). Measurement of molecular events across a range of doses can be used to compare responses across species, including humans. The degree of similarity in gene expression pattern between different

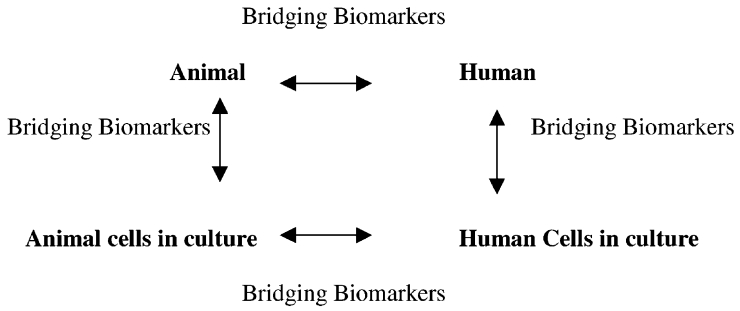

Fig. 4. Use of bridging biomarkers to extrapolate from laboratory models to humans.

species will provide a new tool to help determine whether results in one experimental species are relevant to another—i.e. a high degree of similarity in key genes in the gene expression fingerprint for a given exposure to a particular toxicant in a laboratory animal model compared to the expression pattern in a human (or human cells) should indicate that the molecular damage and response occurring in the animal model is similar to that in humans, and therefore, relevant to anticipated human health outcomes. Likewise, dissimilar patterns could provide support for the lack of relevance of a unique animal toxicity finding to human risk. A similarity in gene expression patterns could also be used to select the most appropriate animal model prior to the conduct of toxicity studies. Understanding critical gene/protein/metabolite changes will also be useful for extrapolating from in vitro test systems to animal models.

3.5. Human biomarkers of exposure and effect

Another key component of the risk assessment process is establishing the extent of human exposure to a hazardous chemical or drug. Biomarkers of change in molecular genes/proteins/metabolites, should allow assessment of response to exposure, as well as loss of cellular integrity or disturbance of homeostasis in cells or tissues. These new biomarkers will be identified and validated based on our increasing knowledge of the biochemistry and genetics of molecular damage response, host-defense cell signaling, and cell death, and will have to take into account the pathway from protection and defense to pathological changes.

To identify systematically and validate biomarkers of effects, two distinct strategies will likely prove useful. First, effects of specific well-characterized pathologies on gene/protein/small molecules within the cell will need to be characterized to determine the relationship between these potential markers and specific types of damage. Second, “fingerprints” of cellular responses to classes of chemicals with known common biological effects will need to be investigated to develop a “library” of chemical class-specific cellular perturbations. These two strategies should lead to an understanding of changes in gene products, proteins, and specific small molecules that are characteristic of specific types of pathology, and thus, to a new system of biological classification of chemicals based on similarities in their mechanisms of interaction with key cell receptors and response elements. Such studies will need to be conducted in important model species, beginning with well-established laboratory animal and cellular models, and ultimately undertaken in the human (such as during clinical trials) to understand differences and similarities in such responses among species. These biomarkers should allow monitoring and characterization of pathological damage as well as grouping of new chemicals into categories based on known responses to previously-studied chemicals.

3.6. Effect of multiple chemical exposures

Humans are rarely exposed to single chemicals at any given time. Most commercial products are a combination of chemicals. Nonetheless, because of practical technical limitations, most studies of toxicity evaluate one chemical at a time and risk assessments are traditionally conducted on single chemicals. It is virtually impossible to extrapolate results from a series of tests on individual chemicals to the effect

of exposure to a complex mixture. Using high throughput genetic and proteomic approaches to measure changes in gene/protein expression after exposure to mixtures of toxicants has the potential to allow for the assessment of interactions such as additivity, synergism, or antagonism. Additionally, comparing gene expression patterns of test substances containing contaminants to a library of patterns for known genotoxins could help identify the presence of minor contaminants in products, eliminating the often tedious analytical approaches currently used to identify toxic contaminants.

4. Influence of gene sequence variation (polymorphisms) on responses to toxicants

In addition to the study of gene expression/proteins/metabolites in response to exposure to toxicants, new methods of evaluating gene sequence variation (polymorphisms) are also available. Such methods are making possible a systematic evaluation of the effects of variant genetic sequences on responses to toxicants. Examples of genetic variants that affect sensitivity to adverse effects from chemical exposures have long been known [44–48]. However, one of the major findings of the human genome project is that variation in base sequence between individuals averages approximately 1 in every 1000 base pairs (bp) [6]. Thus, sequence variants among individuals will be very common in essentially all genes. Further, these genetic variants have been shown to be important in many human diseases [49–51]. Understanding the relationship of this genetic variability to human disease and sensitivity to chemical exposure will greatly facilitate individual health risk assessments and extrapolation of findings from laboratory models to human risk [52].

"Mapping" of sequence differences between individuals with and without diseases (most often single base differences known as "single nucleotide polymorphisms", or "SNPs") is now revealing a growing number of disease susceptibility genes, as well as polymorphisms that determine individual diversity in drug responses [53]. As the tools now available are applied to the field of genetic toxicology, two major advances can be anticipated: (1) identification of polymorphisms responsible for sensitivity to toxicity from particular agents, and (2) identification of chemical-induced genetic changes associated with particular diseases.

4.1. Examples of polymorphisms associated with susceptibility

Present examples of polymorphisms associated with individual susceptibility to chemical exposures consist mainly of metabolic polymorphisms that affect either activation or detoxification of particular toxicants [54,55]. A classical example is the genetically-determined rapid versus slow acetylator phenotype [56,57]. This polymorphism is responsible for the differences in sensitivity to isoniazide among subpopulations with these N-acetylase variants. Another example of the influence of genetic variation and toxicity is the sensitivity to fava bean toxicity among Mediterranean populations with glucose-6-phosphate dehydrogenase deficiency (G6PD), reviewed in [57]. Because of the very high frequency of base pair variation in the human genome, it is to be expected that genetic variability among individuals will occur at most, if not all, molecular targets for toxicants. It is also to be expected that this genetic variation will be found to be a major cause, or perhaps the major cause, for variation in susceptibility to toxicant exposure. This concept is supported by the exponentially growing list of spontaneous pathologies (diseases) associated with genetic variants, as many such variants would be expected to affect both spontaneous and chemically-induced pathologies. The knowledge that essentially every gene will vary in base sequence among individuals suggests that many examples of genetic determinants of variability in receptor conformation, in biochemical pathways, and in key defense molecules will be shown to be responsible for significant variations in sensitivity to different agents. Indeed, examples are already known—such as the polymorphisms in cardiac potassium channels that confer susceptibility to Q–T interval prolongation and susceptibility to fatal arrhythmias [53,58].

As the genetic basis of various diseases becomes better understood, it is expected that the contribution of chemically-induced genetic change to those diseases will likewise be elucidated. Such correlations are presently best understood in the case of carcinogenesis, where the genetic basis of cancer is finally becoming understood and the relationship

between chemically-induced genetic alterations and the development of carcinogenesis is well-established. The powerful genetic tools now available have made it possible to now consider studies of the relationship between chemical exposures and genetic predisposition to other diseases, such as aging, cardiovascular disease, and heritable biochemical diseases.

4.2. Identification of polymorphisms

Initially, practical applications of methods for identifying effects of genetic variation among individuals on responses to chemical agents may be expected for those classes of agents with mechanisms of action that are well characterized, such as pharmaceuticals. Interest in applying these technologies to pharmaceutical development has been stimulated by the occurrence of idiosyncratic toxic responses observed in humans, often after a drug is introduced into the marketplace. Many of these idiosyncratic reactions are likely to be due to genetic variations among individual patients. Sensitivities to adverse effects from many drugs are already known to result from modification of drug metabolism pathways (e.g. terfenidine, isoniazide [59–61]). As a result, gene arrays (chips) containing known variants of human enzymes for drug metabolism are being developed to identify these individuals (e.g. affymetrix CYP450 array, see www.affymetrix.com; eSensor^{TM}, see www.motorola.com/lifesciences). As gene-chip technology becomes more available, it is not far-fetched to expect that an individual's metabolic enzyme genotype might become a routine part of one's medical record, and be used in selecting appropriate drugs and adjusting doses on an individual basis.

These capabilities will also bring new ethical dilemmas to the field. A discussion of the ethical considerations of the application of genomics information to humans is beyond the scope of this paper, and is only mentioned here briefly to help illustrate the dilemma. Questions will arise, such as whether individuals with polymorphisms known to sensitize them to cardiac Q–T prolongation should be actively sought and included in clinical trials in order to better understand the potential for unexpected adverse reactions in the general population. To the contrary, should such individuals be actively excluded from clinical trials in order to minimize the risk to those in the trials at the expense of allowing the potential for adverse effects in the general population to remain relatively poorly defined? Ethically sound strategies that protect individual subjects but also allow the generation of scientific knowledge needed to protect the population at large need to be developed in conjunction with the regulatory application of new genetic knowledge and technologies.

4.3. Laboratory models with human genetic characteristics

Our increased understanding of the relationship between the nucleotide sequences of genes and the cellular functions that they control will certainly allow better interpretation of the significance to humans of laboratory findings in animal and cellular models. In addition to allowing a better understanding of laboratory findings, advances in the field of genetics have now made it possible to manipulate the genetic structure of laboratory models through transgenic and other technologies. These technologies have made it practical to construct laboratory animal and cellular models that contain specific human genetic characteristics of interest. Thus, it is now possible to construct "humanized" models that contain either normal or polymorphic forms of human genes and cellular macromolecules. As important human targets for drug and toxicant interactions are identified and characterized, we can now expect that analogous laboratory models that allow these interactions to be studied in animals, cells, and tissues will become available. Such "humanized" models have already been created, demonstrating the feasibility of this approach. Examples include animal models of sickle-cell disease [62], and cell lines engineered to express the human cytochrome P450 drug-metabolizing enzymes [63,64].

5. Other challenges

In addition to the issues discussed above, there are a number of general challenges to this new field of toxicogenomics. Genomic, proteomic and metabonomic methods are being evaluated or adopted by all sectors of industry, academia, and regulatory agencies at an unprecedented rate. These technologies are not yet standardized, and many formats are being

used in different laboratories. Many laboratories are developing their own custom microarrays, which are typically prepared with specific subsets of genes of interest to the investigator (e.g. toxicity related genes). The proprietary nature of some of these custom arrays prevents an evaluation of inter-laboratory reproducibility. This rapid adoption of new technologies will undoubtedly result in some publications of preliminary data without the appropriate validation and interpretation. This creates the need for caution in interpretation of data, until the strengths and limitations of these new tools are evaluated through carefully conducted studies in multiple laboratories.

A major issue with array technology is quality control and characterization of analytical performance. To obtain meaningful data, it is necessary that the sequences on the arrays are correctly identified and annotated. Further, the arrays need to be manufactured to a standard that yields reproducible results. Reproducibility, sensitivity, and robustness must be determined and controlled for each of the "spots" on the arrays that comprise many thousands of individual assays, and the biological meaning of alterations in specific expression patterns must also be determined.

Another challenge associated with the new "-omic" technologies is that of acquiring, storing, and analyzing the extensive amount of data that is generated by these studies. There is a need for sophisticated methods of storing and analyzing data, as well as standardized analysis approaches and algorithms that facilitate comparison of data among laboratories.

Soon, we may expect complete gene expression arrays that include all genes of a species being studied. However, at the present time most arrays are still incomplete, and are available for only a limited number of species. Thus, initial data will not be complete and only those genes available on the array for a few species will be measured. Of course, this is changing rapidly as updated arrays containing a large fraction of the genome are becoming available. It is anticipated that entire genomes of the species of most interest (e.g. human, mouse, rat, etc.) will be represented on the chips to appear in the near future.

Measurements of toxicity must be quantitative, because quantitative dose–response relationships are the basis of evaluating effects in "real-world" exposure situations and regulatory decision-making. Therefore, the use of these new methods of global analysis must also be quantitative. Thus, it is necessary first to assure that analytical results are quantitatively reliable, and second to determine the magnitude of changes in expression or cellular concentrations that are toxicologically relevant. The dynamic range of changes in individual genes, the reversibility of changes observed, and the role of particular changes in determining cellular pathologies are all important considerations. This, of course, requires an understanding of the kinetic and quantitative relationships among genetic pathways and their relevance to cell and tissue function. To avoid falsely indicting chemicals as toxic (or erroneously thinking they are safe), the scientific community must establish consensus on these key factors.

6. The future

As tools that enable simultaneous analysis of multiple targets and pathways are applied, a more comprehensive classification of cellular perturbations that result from toxicity will emerge. Already, toxicologists are starting to look at toxicity through the wider lens of toxicogenomic methods that provides a more complete view of complex networks of gene/protein changes. This is analogous to the difference between attempting to assess neurotoxicity by monitoring the activity of a single neuron at an arbitrary site versus simultaneously monitoring the function of thousands of neurons in multiple neural networks throughout the body. It is likely that these new technologies will reveal that many currently held beliefs are incomplete or incorrect. However, these new technologies, and those yet to be developed, will undoubtedly lead us to a better understanding of toxicity.

It is almost certain that global measures of gene structure, gene products, and small biomolecules will become inexpensive, and thus routine, as current methods are refined and additional ones introduced. Rather than analyzing endpoints of toxicity separately (e.g. genotoxicity, reproduction/teratology, allergenicity) and in isolation, relevant genes for all toxicities may eventually be formatted on an array to provide a far more comprehensive toxicity analysis. Further, toxicity and efficacy measures may be conducted concurrently, as the same genes and gene products are involved in both pharmacological and toxicolo-

gical responses. This will result in comprehensive, integrated, assessments of products with intended beneficial properties, such as pharmaceuticals and nutrients.

The toxicologist of the future will require a multidisciplinary understanding of genetics, cellular signal transduction and control, and conventional pathology. Through the fusion of these disciplines, a major beneficial transformation of the field is to be expected. The future of toxicology and genetic toxicology lies in the merger of these disciplines and the judicious application of genetic techniques and biomarkers to provide a more holistic understanding of the effects of chemicals on cellular alterations.

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