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Expert Review

Toxicogenomics in Drug Discovery and Drug Development: Potential Applications and Future Challenges

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Abstract. Despite the massive investments made by pharmaceutical companies on drug research and development, the number of new drug approvals has remained stagnant in the past decades. It is well known that developing safe and effective new drugs is a long, difficult, and expensive process. While the cost of developing new drugs is increasing rapidly, withdrawals of drugs from the marketplace due to adverse drug reactions (ADR) and/or toxicity is increasing concurrently. The recent advent of high-throughput *in silico* (computer softwares) and *in vitro* (cell cultures) screenings have somewhat alleviated some, but not all, of these challenges by providing an efficient and effective way for developing safer and better drugs. This emerging technology, known as toxicogenomics, has great potential to facilitate the development of methodologies that could predict the long-term toxic effects of compounds using relatively short-term bioassays. This review is aimed at discussing the potential applications and future challenges of toxicogenomics in drug discovery and drug development.

KEY WORDS: gene expression profiles; microarray; pharmacogenomics; signal transduction; toxicogenomics

INTRODUCTION

One of the biggest setbacks for the pharmaceutical industry in drug development is late-stage failures caused by a poor pharmacokinetic profile and/or toxicity of drugs (1). In fact, promising therapeutic drugs have been withdrawn from the marketplace because of unforeseen human toxicity. Therefore, information about the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of drugs is crucial to reduce the time and expense of drug development (2,3). A significant advancement in drug development is the application of the science of toxicogenomics. The concept of toxicogenomics was first introduced in 1999 (4) and can be defined 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" (5). The application of toxicogenomics provides an exceptional opportunity to identify the biological pathways and processes affected by exposure to pharmaceutical compounds and/or xenobiotics (exogenous agents) (6–11).

PREDICTIVE TOXICOLOGY

An early and reliable prediction of a drug candidate's induced toxicity represents one of the major challenges in drug development. Conventional methods for the evaluation of drug toxicity are often cost intensive and time-consuming. One of the major goals for toxicogenomics is to predict the long-term effects of compounds using short-term assays. Therefore, it is believed that toxicogenomics could accelerate the process of drug discovery and development. In this regard, global gene transcriptional profiling has the potential to predict toxic responses. It is assumed that compounds which induce toxicity through similar mechanisms will elicit characteristic gene expression patterns. By grouping the gene expression profiles of well-characterized model compounds and phenotypically anchoring these changes to conventional indices of toxicity, a gene expression signature or fingerprint related to specific organ toxicity could be generated and used to predict the toxicity of a candidate drug. The predictive capacity of gene expression profiling has been demonstrated in some recent studies. In fact, some pharmaceutical companies have started to build their own database in hopes of predicting the potential toxicity of compounds. For example, McMillian *et al.* (12) found that hepatotoxicants can be classified into macrophage activators, peroxisome proliferators, and oxidative stressors/reactive metabolites based on their gene expression profiles. Using the gene signature profiles for each of these classes of hepatotoxicants, this group has successfully categorized over 100 paradigm compounds based on oxidative stress induction in rat liver. Thukral *et al.* (13) have recently published their work on the prediction of nephrotoxicant action and identification of

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candidate toxicity-related biomarkers in rat kidney. Through the analysis of gene expression profiles, nephrotoxicants were clustered based on similarities in the severity and type of pathology in animals. The sensitivity and selectivity of this model in predicting the type of nephrotoxicity was then tested with a support vector machine (SVM)-based approach. This approach has successfully predicted the type of pathology of 28 test profiles with 100% selectivity and 82% sensitivity. Furthermore, a set of potential biomarkers showing a time- and dose-response with respect to the progression of proximal tubular toxicity was identified. Another study by Steiner *et al.* (14) demonstrated that by using a binary SMV model, it is possible to discriminate between hepatotoxic and non-toxic compounds. All vehicle-treated controls were precisely identified as non-toxic, while almost 90% of the toxic test samples were classified as toxic. Therefore, it is clear that the integration of gene expression profiling with supervised algorithms approaches, such as SMV, is highly beneficial for the prediction of toxicity, especially in the very early stages of drug development.

MECHANISTIC TOXICOLOGY

In addition to the classification of drugs based on the gene expression profiles, toxicogenomics could also provide valuable insights into the underlying mechanisms of toxicity. This mechanistic toxicological approach is very valuable, especially in risk assessment of candidate compounds during drug development. Many pharmaceutical compounds or xenobiotics can induce specific or non-specific cellular signal transduction events that activate various physiological and pharmacological responses, including homeostasis, proliferation, differentiation, apoptosis or necrosis, all of which can be detected at the transcriptional level. By examining alterations in gene expression in response to drugs, it is possible to generate hypotheses as to the underlying mechanisms of toxicity, which could be crucial for the identification of potential safety liabilities early in the drug development process. The application of toxicogenomics for mechanistic purposes could play an important role when the toxicity of candidate drugs is not associated with well-established biomarkers or significant morphological changes. One of the classical examples is testicular toxicity, which is almost undetectable as testicular changes are typically subtle in early stages. Numerous recent publications have demonstrated the ability of gene expression profiling to elucidate the molecular basis of testicular toxicity (15,16) and to detect early biomarkers of testicular toxicity (17). By using a semi-quantitative RT-PCR method, Lee *et al.* (16) found that administration of mono-(2-ethylhexyl) phthalate and 2,5-hexanedione, two widely-used Sertoli cell toxicants, resulted in the up-regulation of both FasL and Fas. They concluded that up-regulation of Fas is a common and critical step for the initiation of germ cell death. Likewise, Fukushima *et al.* (17) demonstrated that cDNA microarray might be a promising tool for evaluation of primary testicular toxicity. Six hours after the single dosing of one of four reproductive toxicants [2,5-hexanedione (Sertoli cells toxicant), ethylene glycol monomethyl ether (EGME; spermatocytes toxicant), cyclophosphamide (spermatogonia toxicant) and sulfasalazine] in male rats, gene expression in the testes was monitored by cDNA microarray and real-time RT-PCR, and the testes were histopathologically examined. They found that the expression of three spermatogenesis-related genes, heat shock protein 70–2, insulin growth factor binding protein 3, and glutathione S transferase pi, was altered by all of the compounds. These effects were detectable within a short period after dosing, prior to the appearance of obvious pathological changes, with the exception of slight degeneration of spermatocytes in the EGME-treated testes. Therefore, they proposed that these three spermatogenesis-related genes are potential biomarkers of testicular toxicity. It is obvious that such gene expression studies could provide rapid identification of mechanisms of toxicity, which would facilitate decision making in a lead compound's progression.

MAJOR ISSUES IN THE USE OF TOXICOGENOMIC STUDIES IN DRUG DEVELOPMENT

The withdrawal of established compounds, such as Vioxx (rofecoxib), from the market is a prominent reminder that there is still a dire need for improvement in the current industrial strategies used for the evaluation of drug safety during development. It is hoped that the application of toxicogenomics will not only reduce the time and cost of toxicity studies, but will also solve other problems of traditional methods, such as lack of sensitivity (10). However, there are still some major challenges and caveats that need to be resolved before this emerging new technology could be fully implemented. The success of a toxicogenomic study depends upon multiple factors, such as the use of different technologies (different type of arrays; data analysis software and tools) and the types of studies employed (*in vivo vs in vitro*; preclinical animal models *vs* human).

The Use of *in Vitro* Models in Toxicogenomic Studies

There are advantages and disadvantages of using *in vitro* data from toxicogenomics studies in drug discovery and development. Application of toxicogenomics using an *in vitro* system provides a high throughput, reproducible and cost effective method, especially in the early stages of drug development. Ideally, an *in vitro* system should allow pharmaceutical companies to screen for candidate compounds for potential safety liability using a relatively small amount of compound, and therefore, the number of *in vivo* studies needed in drug development can be significantly reduced. Several studies have demonstrated that it is feasible to distinguish compounds with different mechanisms of toxicity using *in vitro* systems (18–20). Waring *et al.* (18) compared the gene expression profiles of 15 well-characterized hepatotoxicants in isolated rat hepatocytes and found that, by using unsupervised hierarchical clustering, compounds which cause toxicity though different mechanisms can be successfully separated. Furthermore, they found that, in some cases, there is significant correlation between the genes regulated *in vivo* and *in vitro*. Obviously, gene expression profiling using *in vitro* systems is a very useful tool for understanding the mechanisms through which a compound exerts its toxicity. However, there are still some challenges for using *in vitro* systems for toxicogenomic studies. The predictive value of *in vitro* systems relies heavily on the selection of the optimal model for conducting toxicogenomic studies. For example,

hepatoxicity can be evaluated *in vitro* using either liver slices, isolated hepatocytes, or liver cell lines. In each of these models, the results, and the analysis and interpretation of those results, can differ substantially. A recent study conducted by Boess *et al.* (21) showed that, based on the gene expression profile, liver slices appeared to be the most similar to intact rat livers, followed by primary hepatocytes in culture. They also demonstrated that cultured liver cell lines expressed very low or undetectable levels of phase I metabolizing enzymes. Hence, it is possible that inappropriate selection of an *in vitro* model could lead to misinterpretation of results, especially when cell lines are used for predicting the toxicity of a compound that is due to the formation of reactive metabolites. Another limitation of applying *in vitro* systems in toxicogenomic studies for the prediction of chronic toxicity is loss of function with long term cultivation of primary cell/tissue culture or of cell lines (22). In addition, the local microenvironment of the tissues and complex interactions between adjacent tissues are difficult to be modeled in *in vitro* systems. Therefore, there are still circumstances in which animal models will be needed.

The Use of Preclinical Animal Models (Transgenic and Knockout Animals) for Toxicogenomic Studies

Preclinical animal models are essential in drug development for clarification of positive results of *in vitro* assays before candidate compounds can proceed to clinical trials. Indeed, most of the toxicogenomic studies performed so far are carried out using preclinical animal models (rats and mice). Preclinical animal models could offer additional value in cases where specific metabolic pathways cannot be implemented adequately in *in vitro* models. The recent advancements achieved in transgenic and knockout animal models have undoubtedly increased the value of applying preclinical animal models in toxicogenomic studies. The use of transgenic and/or knockout animals which contain specific human genetic characteristics of interest is crucial for gaining mechanistic information on candidate drugs. Our laboratory has recently studied the role of Nrf2 in the (−)-epigallocatechin-3-gallate (EGCG)-mediated gene regulation by using Nrf2 knockout mice (23). Nrf2 is a basic leucine zipper family transcription factor involved in the regulation of antioxidant response element (ARE)-mediated gene transcription (24). Nrf2 is believed to play an important role in detoxification as many phase II detoxification enzymes and antioxidant genes are main targets for Nrf2. On the other hand, EGCG is a green tea extract which is found to be a potent chemopreventive agent (25) and is currently under various clinical trials for cancer chemoprevention. By comparing the global gene expression profiles of Nrf2 knockout and wild type mice, Nrf-2 dependent genes regulated by EGCG were identified. The identification of these genes will give us some valuable insights in the potential role of Nrf2 in EGCG-mediated gene regulation. Similar studies have also been conducted with other cancer chemopreventive compounds, including curcumin (26), sulforaphane (27) and PEITC (unpublished observations), which are also under current clinical trials for cancer chemoprevention. However, future dose response studies, especially at higher dose levels that could elicit some toxicity, should provide some informative toxicogenomic data for the cancer chemopreventive compounds used.

In addition, mouse strains have been developed with knockouts (KO) of metabolic genes such as Cyp1a1, Cyp1a2 and arylhydrocarbon receptor (Ahr) to study the interaction between specific metabolic genes and carcinogen exposure (28–31). By using these knockout models, Talaska *et al.* (31) have demonstrated that when low doses of carcinogens are used, complete loss of these single metabolic enzymes results in little or no impact on the levels of DNA damage. On the other hand, a PPARα (peroxisome proliferator activated receptor alpha) KO mouse model has been used to study the role of PPARα ligands in rodent liver tumorigenic response to peroxisome proliferators by using microarray gene expression profiling of mRNA from wild type *versus* KO mice (32). Recently, a transgenic mouse model in which the human P450 enzyme CYP2A6 was expressed specifically in the liver (33) has been generated. This model can be valuable for studying the *in vivo* function of this polymorphic human enzyme in drug metabolism and toxicity.

Despite all of the promising concepts and studies discussed above, the application of preclinical animal models in drug development faces at least two major challenges. First of all, there are quantitative differences in dose-response relationships between animal models and humans. Although there is a certain degree of similarity in the biochemical and molecular pathways of different species, the biological response to drugs may certainly differ between the species. Therefore, it is important to find "bridging biomarkers" of damage that can be used to compare toxic responses among species (5). Secondly, in some extreme cases, the biological response to a given exposure may differ not only quantitatively, but also qualitatively, among species. The fact that only 71% of all human toxicities can be accurately predicted by using animal models indicates the existence of species-specific differences upon exposure to drugs (34,35). One of the examples is methapyrilene (MP), an antihistaminic compound used in over-the-counter cold and allergy medications as a sleep-aid component. MP was found to be carcinogenic in rat (36–38) and was subsequently withdrawn from the market. However, it was later determined that the carcinogenic effect was species-specific since carcinogenicity was not demonstrated in mice, guinea pigs, hamsters or humans (39–41). Likewise, there are marked species differences in the response to peroxisome proliferators. Peroxisome proliferators caused severe hepatic side effects including hepatomegaly and hepatic neoplasms in rats (42), but have appeared to be safe in primates and humans (43–45). Therefore, it is important to predict toxicity of candidate drugs across different species in order to minimize the risk of misinterpretation caused by species-derived differences in response to drug treatment.

Human Polymorphisms

Another major challenge for the pharmaceutical industry in drug development is the detection and prediction of idiosyncratic toxicity. Although the majority of drug candidates which cause toxicity are eliminated at the discovery or development stage, some of these drugs are not detected until they are introduced into the marketplace due to

idiosyncratic toxicity. Unexpected adverse drug reactions which occur randomly in a dose-independent fashion and are independent of pharmacological properties are referred as idiosyncratic effects (10). Many of these idiosyncratic reactions result from genetic variations (polymorphisms) in drug-metabolizing enzymes, immune-mediated responses to the drug (or one of its metabolites), the combination of drugs with low-level inflammatory reactions, and/or drug-induced mitochondrial toxicity (46). In addition to idiosyncratic toxicity, genetic variations also play an important role in cancer chemotherapy. Indeed, genetic polymorphisms have been extensively studied in oncology and cancer risk as well, and the therapeutic response appears to strongly depend upon the genetic background of individual patients (47). Therefore, the ability to identify genetic polymorphisms is not only critical for understanding mechanisms behind metabolic activation of potentially toxic and carcinogenic compounds, but also represents one of the major challenges in which toxicogenomics can be successfully implemented in drug development (48).

Databases and Data Analysis

The massive amount of genomics data generated from toxicogenomics studies has given scientists from all sectors of industry, academia and regulatory agencies a major challenge that has yet to be resolved. A comprehensive gene expression reference database and a robust software for data analysis play an important role in the interpretation of toxicogenomics data (49,50). Companies such as GeneLogic, CuraGen, Iconix and Phase I are some of the vendors who provide commercially available toxicogenomic databases for pharmaceutical companies. Indeed, most of the major pharmaceutical companies have started to build their internal toxicogenomics initiatives, which are normally not accessible by the public. Publicly available databases are currently being generated by some institutions. In 1999, under the coordination the ILSI Health and Environmental Sciences Institute (HESI), a consortium of academic, governmental and industrial representatives coordinated formed a committee on the use of genomics in mechanism-based assessment. This committee is currently working together with the European Bioinformatics Institute (EBI) to create a public domain for toxicogenomics data. The committee has also provided very useful guidelines on the application of toxicogenomics to risk assessment by standardizing the description and annotation of microarray data with the introduction of Minimal Information about Microarray Experiments (MIAME) standard. MIAME standard is an important step to enable inter-laboratory reproducibility of toxicogenomics data (51,52). MIAME guidelines have been recently modified (53) and are available at http://www.mged.org/index.html.

The massive amount of data generated from high-throughput toxicogenomics studies is complicated and often highly multivariate. Therefore, it is impossible to analyze these data without robust software. There are many statistical tools ranging from simple analysis to sophisticated software such as Eisen Clustering Tool (Stanford University), GeneSpring (Silicon Genetics), SIMCA-P (Umetrics) or Rosetta Resolver (Merck). Each of these software programs offers more than one analysis method, and the selection of the best method is always a major concern for scientists (54–56).

CONCLUSION AND FUTURE PERSPECTIVES

Toxicogenomics has emerged as a new and exciting technology that could potentially revolutionize drug discovery and development. Thus far, it has been shown that toxicogenomics could be successfully implemented to predict toxicity liability and the toxicity mechanisms in the drug discovery–development continuum. In addition, it is believed that toxicogenomics could offer additional added values compared to conventional toxicology methods (Fig. 1). However, there are still many caveats and challenges as described above which remain to be resolved before its full potential could be realized. Nevertheless, the proper exploitation of this technology, in conjunction with the current development of proteomics and metabolomics, appropriate

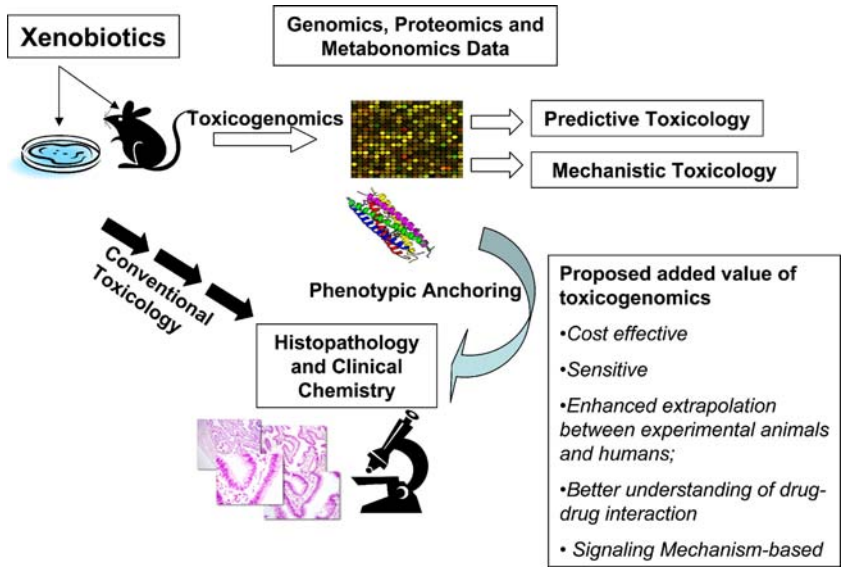

Fig. 1. Comparison of toxicogenomics and conventional toxicology.

clinicopathology biomarkers and pathological endpoints, could potentially offer a competitive advantage to pharmaceutical companies in their drug discovery and drug development paradigm.

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