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Toxicogenomics - Applications and Future Perspectives

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ABSTRACT As one reflects back through the past 50 years of scientific research, a significant accomplishment was the advance into the genomic era. Basic research scientists have uncovered the genetic code and the foundation of the most fundamental building blocks for the molecular activity that supports biological structure and function. Accompanying these structural and functional discoveries is the advance of techniques and technologies to probe molecular events, in time, across environmental and chemical exposures, within individuals, and across species. The field of toxicology has kept pace with advances in molecular study, and the past 50 years recognizes significant growth and explosive understanding of the impact of the compounds and environment to basic cellular and molecular machinery. The advancement of molecular techniques applied in a whole-genomic capacity to the study of toxicant effects, toxicogenomics, is no doubt a significant milestone for toxicological research. Toxicogenomics has also provided an avenue for advancing a joining of multidisciplinary sciences including engineering and informatics in traditional toxicological research. This review is aimed at discussing the potential applications and future challenges of toxicogenomics in drug discovery and drug development.

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 (Waterbeemd and Gifford 2003). In fact, promising therapeutic drugs have been withdrawn from the marketplace because of unforeseen human toxicity.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 (Ekins et al. 2005; Davis and Riley 2004). A significant advancement in drug development is the application of the science of toxicogenomics.

WHAT IS TOXICOGENOMICS?

The concept of toxicogenomics was first introduced in 1999 (Nuwaysir et al. 1999) 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" (Aardema and MacGregor 2002). 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) (Fielden and Zacharewski 2001; Hayes and Bradfield 2005).

The biggest improvement needed in the drug development process is in the field of toxicology, which is the point where most developmental bottlenecks occur. One promising area of advancement is the new field of toxicogenomics. Detection of changes at the molecular level provides insight into a toxicant's mechanism-of-action and its potential to cause human toxicity. Toxicogenomics has grown quickly with the number of articles published approximately tripling in 2009, (estimated) over that of 2005. However, the field is still in its infancy. As toxicogenomics data grows, a developing challenge is the analysis of large datasets and the building of predictive toxicogenomic databases (Cynthia et al. 2011).

THE EVOLUTION OF TOXIOGENOMICS

The history of molecular biology is rooted back to the discovery of DNA structure by Watson and Crick (1953) nearly 60 years ago. However, the ability to fully translate the code to function is an ongoing challenge for scientists today.Understanding the translation of the genetic code to clear revelation of the function

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of proteins, cells, organs, and organisms will require many more advances in technology,data knowledge integration, and collaborative science. That said, substantial progress is being made, and the advance of molecular biology integration to toxicology is providing the foundation for the translation of molecular perturbations to cellular, organ, and organismal health more rapid and higher throughput methods that require very small amounts of sample material and enable the tracking of molecular events at a whole-genomic level across multiple doses and time points.The most enabling technology for such assessments is the microarray chip. First published in the mid-1990's, DNA microarrays of two main types of platforms emerged. One platform, borrowing technology from the semiconductor industry, was produced with ''on-chip synthesis'' of sets of short oligo sequences that spanned each gene transcript with compilation of the individual gene probe sets to cover a whole genome. The other platform involved-deposition of longer length complementary DNA ''spots''generated, a priori, by chemical synthesis or PCR onto specially coated glass slides. The result for either platform was a miniature array that could ideally allow the probing of the whole-genomic transcript profile or monitor the expression of a host of functionally related genes for any biological sample RNA that was hybridized to it. The application of array technology in toxicology experiments provided the basis for the emergence of a new field, toxicogenomics. Today, the term toxicogenomics represents the interface of multiple functional genomics approaches as applied to understand mechanisms of toxicity.

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 hope of predicting the potential toxicity of compounds. (McMillian et al. 2005) 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. (2005) have recently published their work on the prediction of nephrotoxicant action and identification of 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. 2004) demonstrated that by using a binary SMV model, it is possible to discriminate between hepatotoxic and non-toxic compounds.

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 phar-

maceutical 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 (Boekelheide et al. 1998; Lee et al. 1999) and to detect early biomarkers of testicular toxicity(Fukushima et al. 2005). By using a semi-quantitative RT-PCR method (Lee et al. 1999) 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.

TOXICOGENOMICS: UNDERSTANDING THE VISUALIZATION OF COMPLEX DATA

First discussed in the public literature (Nuwaysir et al. 1999), toxicogenomics was first described as a term to illustrate the integration of toxicological research with the emerging new technologies designed to broadly interrogate the functional genome (that is, RNA, protein, metabolite profiling, and polymorphisms/functional DNA mutations). Since then, there has been a steady adoption of the principles and technologies relevant to toxicogenomics throughout academic and industry laboratories, and there have been many scientific advances in various toxicology-related disciplines since.

IMPACT ON THE PHARMACEUTICAL INDUSTRY

The potential benefits of a successful toxicogenomics program have been described briefly. Treatment with animals or cells in culture with new chemical entities (NCE) and examination of the resulting gene expression profiles can influence several areas of drug development (Schena1996; Collings and Vaidya 2008). First, it can impact the quality of drug development pipelines by improving the science of toxicology, providing more specific information as to the mechanisms of drug pathologies and providing it earlier in the discovery– development continuum (Altman and Raychaudhuri 2001). Second, it can improve the efficiency of the process because toxicogenomics information complements genomic target identification and characterization methods used in discovery and leads to reduced attrition during drug development for unfavourable compounds (Tseng et al. 2001; Reilly et al. 2005). Toxicogenomics can be applied at any stage in the drug development process, but appears to have greatest potential use when used in one or more of the following settings:

The risk to a pharmaceutical company for misunderstanding incurred with toxicogenomics approaches will depend on multiple factors, such as the technology and the type of studies employed (that is, *invivo* and *in vitro*, examining reactions in animals or humans) (Stevens et al. 2006; Rusyn et al. 2010). In general, *in vivo* studies using global gene expression profiling platforms with compounds that have already advanced into clinical trials are considered to have the highest risk of uncovering some unexplained or uninterpretable toxicogenomics data (Olson et al. 2000). The least amount of risk to drug development would be seen with *in vitro* studies using only model compounds from the literature and clinical trial failures or less potent analogs from the discovery program of interest . Alternatively, *in vivo* or *in vitro* systems that use a targeted approach in which only a few genes of known function are measured should be of low risk.

CONCERN ABOUT THE USE OF TOXICOGENOMICS

There are still a number of concerns around the use of gene expression data in drug risk

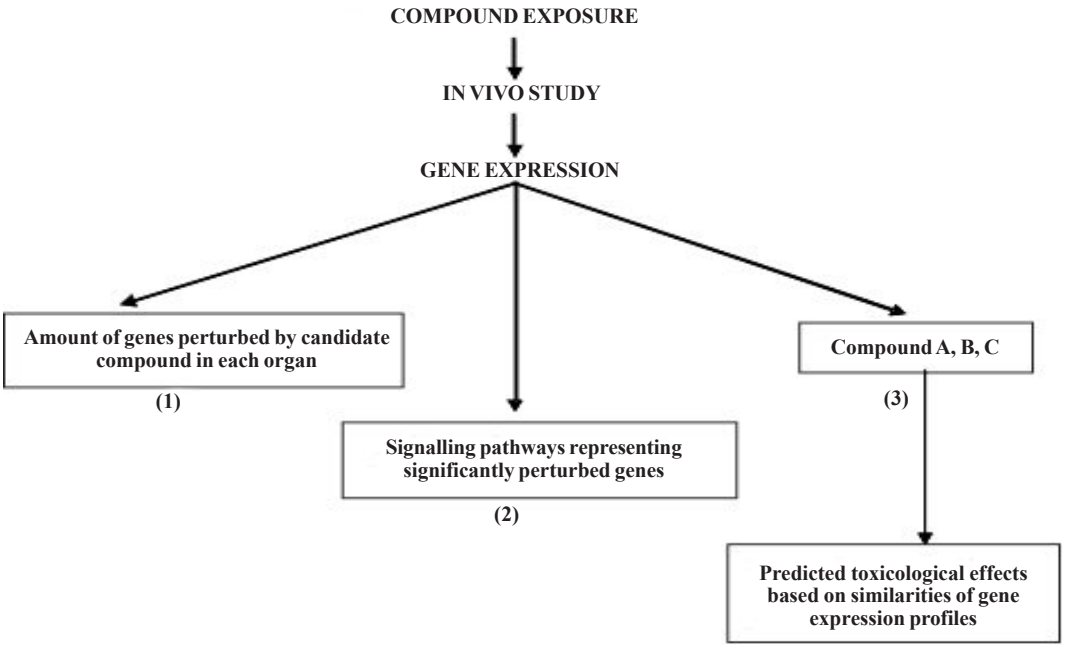

Fig. 1. Example of toxicogenomics flow scheme. Calculations are made to (1) determine the significantly altered genes in each sample and (2) map these gene changes into annotated pathways. This allows for initial assessment of a view to potential mechanisms of tissue response to compound perturbation. As illustrated by (3), expression files may also be mapped against archived files to determine similarity of compound action/response to other compounds that have been previously studied in the database. It should be noted that analyses may be conducted on individual dose/time profiles or across dose and time response with an assessment of "trend."

assessment. There are technical concerns about the sensitivity and reliability of the methods (Ambroise and McLachlan 2002). There are also concerns about the interpretation of the data, especially if genomic data are taken out of context. For example, genes such as c-myc, c-fos and c-Ha-ras which are associated with carcinogenesis may be found to have increased expression (Altman and Raychaudhuri 2001; Ganter et al. 2008). These genes are not oncogenic by nature butare found to be mutated or highly overexpressed in tumours (Crosby et al. 2000). The increased expression in response to drug treatment may simply reflect an acute, and probably benign, stress response. They are, after all, genes for normal cellular functions in cell growth and viability (Smith 2001; Guillouzo and Guguen-Guillouzo 2008). The availability of practically the whole genome for expression analysis also brings difficulties in interpretation. There is not enough information in the literature to interpret the modulation of expression of every single gene (Collings and Vaidya 2008). Until the knowledge base is complete, it must be accepted that toxicogenomic data will provide a starting point for further investigations and not necessarily give definitive answers. To address these concerns (with particular attention to using genomic data in the regulatory environment) a consortium of academic, governmental and industrial representatives formed a committee on the use of genomics in mechanism based risk assessment coordinated by the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) (DeRisi and Penland1996; Olson et al. 2000). The committee's findings have shed much light on the technical issues and have shown the relevance of the data in understanding several mechanisms of toxicity.

APPLICATIONS

Examples of Toxicogenomics Applications

- Clustering of compounds in similar mechanistic classes
- Generation of hypotheses regarding compound action

- Revelation of mechanisms of compound action
- Classification of blinded compounds
- Clustering of compounds by elicited toxicant phenotype
- Ranking and categorization of drug candidates by toxicogenomics signature
- Discerning no effect level for compound transcript effect
- Discovery of biomarkers of toxicity
- Discovery of exposure biomarkers
- Validation/qualification of biomarker signatures

Challenges for Toxicogenomics Applications

- Gene annotation. Example: Public genome projects
- Cross-species extrapolation. Example: Public genome projects
- Technical standards for evolving platforms. Example: National Institute of Standards, MIAME and MAQC consortiums
- Standards for data sharing. Example: NCBI, MIAME, and MAQC consortiums
- Signature/biomarker qualification. Example: Critical Path Institute, FDA, Environment Protection Agency and European Regulatory Groups
- Translation of assays for regulatory purposes. Example: FDA Critical Path Initiative, ICH
- Ethical, Legal, Social Issues. Example: National Institutes of Health, NHGRI

Note. MIAME, Minimal Information About a Microarray Experiment; MAQC, Microarray Quality Control; NCBI, National Center for Biotechnology Information; FDA, Food and Drug Agency; ICH, International Conference on Harmonization; NHGRI, National Human Genome Research Institute.

ADVANCING TOXICOGENOMICS IN FUTURE YEARS

As toxicogenomics continues to move forward, it will likely seem at times as if progression is standing still and at other times advancing quickly. There are a number of challenges that need to be continuously pursued by the field in order to ensure future progression (Fig. 1). In order to advance, key milestones will require coordination across fields and disciplines, so progress is likely to be incremental. Within the next 5 years, it is likely that toxicogenomics will move slowly forward. Biologists will continue to impact the field of informatics, and what constitutes a pathway will be better defined and begun to be standardized universally. In addition, the work of discovery and validation of prodromal biomarkers for a variety of toxicities and diseases will continue to evolve. Ten to 20 years from now, toxicogenomics will likely recognize progress and establishment of uniform technical measure and definition of gene expression events toward exquisite quantitation (that is, possibly taking advantage of techniques such as laser capture micro dissection to look at single cells on platforms for transcript counting, such as NextGen sequencing). The advance in technology will no doubt come with reduced cost per sample for analysis and will enable simultaneous probing of genetic, genomic, proteomic,and metabolomic events. In the regulatory environment, toxicogenomics biomarker data will routinely be used to better inform the risk assessment from *in vitro* and *in vivo* test systems. The acceptance of modified test systems will eventually lead to an impact that minimizes animal testing and allows efficient modelling from human in vitro–based assays and ultra low-dose testing of human subjects to extrapolate and inform toxicity predictions. These models will eventually lead way to predictive in silico models that can help reduce use of animals and cost of experiments conducted to assess hazard and risk (Cynthia et al. 2011)

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. 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 comparison of toxicogenomics and con-

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

REFERENCES

Aardema M J, MacGregor J T 2002. Toxicology and genetic toxicology in the new era of Btoxicogenomic: Impact of B-omics technologies. *Mutat Res,* 499: 13-25.

Afshari CA, Hamadeh HK, Bushel RP 2011. The evolution of bioinformatics in toxicology: Advancing toxicogenomics. *Toxicological Sciences,* 120(S1): S225-S237.

Altman RB, Raychaudhuri S 2001. Whole-genome expression analysis: Challenges beyond clustering. *Curr Opin Struct Biol*, 11: 340–347.

Ambroise C, McLachlan GL 2002. Selection bias in gene extraction on the basis of microarray gene-expression data. *Proc Natl Acad Sci USA*, 99: 6562–6566.

Boekelheide K, Lee J, Shipp EB, Richburg JH, Li G 1998. Expression of Fas system-related genes in the testis during development and after toxicant exposure. *Toxicol Lett*, 102 –103: 503-508.

Collings FB, Vaidya VS 2008. Novel technologies for the discovery and quantitation of biomarkers of toxicity. *Toxicol*, 245: 167-174.

Crosby LM, Hyder KS, DeAngelo AB, Kepler TP, Gaskill B, Benavides GR, Yoon R, Morgan KT 2000. Morphologic analysis correlates with gene expression changes in cultured F344 rat mesothelial cells. *Toxicol Appl Pharmacol*, 169: 205–221.

Davis AM, Riley R J, 2004. Predictive ADMET studies, the challenges and the opportunities. *Curr Opin Chem Biol*, 8: 378-386.

DeRisi JL, Penland PO, Brown ML, Bittner PS, Meltzer M, Ray Y, Chen YA, Trent JM 1996. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet*, 14: 457–460.

Ekins S, Nikolsky Y, Nikolskaya T, 2005. Techniques: Application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends Pharmacol Sci*, 26: 202-209.

Fielden MR, Zacharewski TR 2001. Challenges and limitations of gene expression profiling in mechanistic and predictive toxicology. *Toxicol Sci*, 60: 6-10.

Fukushima T, Yamamoto T, Kikkawa R, Hamada Y, Komiyama M, Mori C, Horii I 2005. Effects of male reproductive toxicants on gene expression in rat testes. *J Toxicol Sci*, 30: 195-206.

Ganter B, Zidek N, Hewitt PR, Müller D, Vladimirova A 2008. Pathway analysis tools and toxicogenomics reference databases for risk assessment. *Pharmacogenom*, 9: 35-54.

Guillouzo A, Guguen-Guillouzo C 2008. Evolving concepts in liver tissue modeling and implications for in vitro toxicology. *Expert Opin Drug Metab Toxicol*, 4: 1279-1294.

Hayes KR, Bradfield CA 2005. Advances in toxicogenomics. *Chem Res Toxicol*, 18: 403-414.

Khor TO, Ibrahim S, Kong ANT 2006. Toxicogenomics in drug discovery and drug development: Potential applications and future challenges, *Pharmaceutical Research,* 23 (8): DOI: 10.1007/s11095-006-9003-8.

Lee J, Richburg JH, Shipp EB, Meistrich ML, Boekelheide K 1999. The Fas system, a regulator of testicular germ cell apoptosis, is differentially up-regulated in Sertoli cell versus germ cell injury of the testis. *Endocrinology,* 140: 852-858.

McMillian M, Nie A, Parker JB, Leone A, Kemmerer AM, Bryant S, Herlich J, Yieh L , Bittner A, Liu X, Wan J, Johnson MD, Lord P 2005. Drug-induced oxidative stress in rat liver from a toxicogenomics perspective. *Toxicol Appl Pharmacol*, 207: 171-178.

Nuwaysir EF, Bittner M, Trent J, Barrett JC, Afshari CA 2009. Microarrays and toxicology: The advent of toxicogenomics. *Mol Carcinog,* 24: 153-159.

Olson H, Betton G, Robinson D 2000. Concordance of the toxicity of pharmaceuticals in humans and animals. *Regul Toxicol Pharmacol*, 32: 56-67.

Reilly TP, Bourdi M, Brady JN 2005. Expression profiling of acetominophen liver toxicity in mice using microarray technology. *Biochem Biophys Res Common*, 27: 321-328.

Rusyn I, Gatti DM, Wilshire T, Kleeberger SR, Threadgill DW 2010. Toxicogenetics: Population-based testing of drug and chemical safety in mouse models. *Pharmacogenomics J*, 10(4): 247-257

Schena M 1996. Genome analysis with gene expression Microarray.*V Bioessays*, 18: 427-431.

Smith LL 2001. Key challenges for toxicologists in the 21st century. *Trends Pharmacol Sci*, 22: 281–285.

Steiner G, Suter L, Boess F, Gasser R, Verade C, Albertini S, Ruepp S 2004. Discriminating different classes of toxicants by transcript profiling. *Environ Health Perspect*, 112: 1236Y1248

Stevens JL, Liu H, Halleck M, Bower RC, Chen QM, van De WB 2006. Linking gene expression to mechanisms of toxicity. *Toxicol Lett*, 112-113: 479-486.

Thukral SK, Nordone P J, Hu R, Sullivan L, Galambos E, Fitzpatrick VD, Healy L, Bass MB, Cosenza ME, Afshari CA 2005. Prediction of nephrotoxicant action and identification of candidate toxicity-related biomarkers. *Toxicol Pathol*, 33: 343-355.

Tseng GC, Rohlin L, Liao JS, Wong WH 2001. Issues in Cdna microarray analysis: Quality filtering, channel normalization, models of variations and assessment of gene effects. *Nucleic Acids Res*, 29: 2549–2557.

Waterbeemd HVD, Gifford E 2003. ADMET in silico modelling: Towards prediction paradise? *Nat Rev Drug Discov*, 2: 192Y204.