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Essential Concepts in Toxicogenomics

Edited by

Donna L. Mendrick
William B. Mattes

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Preface

The field of toxicogenomics is moving rapidly, so it is impossible at the time of this writing to compile a classic methods textbook. Instead, we chose to identify experts in all aspects of this field and challenged them to write reviews, opinion pieces, and case studies. This book covers the main areas important to the study and use of toxicogenomics. Chapter 1 speaks to the convergence of classic approaches alongside toxicogenomics. Chapter 2 deals with the usefulness of toxicogenomics to identify the mechanism of toxicity. Chapter 3 calls attention to the issues that affect the quality of toxicogenomics experiments, as well as the implications of using microarrays as diagnostic devices. The need for appropriate statistical approaches to genomic data is discussed in Chapter 4, and Chapters 5 and 6 describe the use of genomic data to build toxicogenomic models and provide insights from the approaches of two companies. The important topic of storing the data generated in such experiments and the correct annotation that must accompany such data is considered in Chapter 7. The discussion in Chapter 8 speaks to the use of toxicogenomics to identify species similarities and differences. Chapters 9 and 10 deal with the use of genomics to identify biomarkers within the preclinical and clinical arenas. Biomarkers will only be useful if the community at large accepts them as meaningful. Consortia are important to drive this function, and Chapter 11 discusses current efforts in this area. Last but not least, Chapter 12 presents a perspective on the regulatory implications of toxicogenomic data and some of the hurdles that can be seen in its implication in GLP studies. Although this book tends to focus on pharmaceuticals, the issues facing toxicology are shared by the chemical manufacturers, the tobacco industry, and their regulators. We want to thank our contributors for their generous time and energy in providing their insights. Sadly, we must note the unexpected passing of one of our authors, Dr. Joseph Hackett of the FDA. Joe's contribution serves as a testimony to his accomplishments in this field, and his insight will be missed in the years to come.

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Toxicogenomics and Classic Toxicology: How to Improve Prediction and Mechanistic Understanding of Human Toxicity

Donna L. Mendrick

Summary

The field of toxicogenomics has been advancing during the past decade or so since its origin. Most pharmaceutical companies are using it in one or more ways to improve their productivity and supplement their classic toxicology studies. Acceptance of toxicogenomics will continue to grow as regulatory concerns are addressed, proof of concept studies are disseminated more fully, and internal case studies show value for the use of this new technology in concert with classic testing.

Key Words: hepatocytes; hepatotoxicity; idiosyncratic; phenotypic anchoring; toxicogenomics; toxicology.

1. Introduction

The challenges facing the field of toxicology are growing as companies demand more productivity from their drug pipelines. The intent of this chapter is to identify the issues facing classic approaches to nonclinical toxicity testing, the cause of the deficiencies, and ways in which toxicogenomics can improve current *in vitro* and *in vivo* testing paradigms. The public at large continues to exert pressure on the pharmaceutical industry to develop new drugs yet are intolerant of safety issues and the high cost of drugs when they reach the market. This is setting up a "perfect storm" with a recognized decrease in productivity in this industry, continual increase in costs of developing new drugs, and rising attrition rates due to nonclinical and clinical safety failures ***(1–4)***.

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2. Classic Toxicology

Those in the field of drug and chemical development know of the multitude of compounds to which humans were never exposed either in the clinic or in the environment because of obvious toxicity seen in preclinical species. However, it is well-known that classic testing in animals is not infallible. A study done by a group within the Institutional Life Sciences Institute (ILSI) illustrates the problem *(5)*. Twelve companies contributed data on 150 compounds that have shown toxicity in humans of a significant enough nature to warrant one of four actions: (1) termination, (2) limitation of dosage, (3) need to monitor drug level, or (4) restriction of target population. The group compared the human toxicities with the results of the classic toxicity employed for each drug. They found that only ~70% of these toxicities could be predicted in classic animal testing even when multiple species, primarily the rat (rodent) and dog (nonrodent), were employed. The dog was better than the rat in predicting human toxicity (63% vs. 43%, respectively), with the success rate varying depending on the human target organ. However, escalating concerns regarding the use of animals in medical research, the amounts of compound required for such large animals, and the cost of such studies prevents this species from being used as the first species or in sufficient numbers to detect subtle toxicities. The exact failure rate due to toxicity and the time of its detection continues to be the subject of study because only by understanding the problem can one begin to propose solutions. Authors tend to report somewhat different findings. The drugs terminated because of human toxicities evaluated in the ILSI study *(5)* failed most often during Phase II (**Fig. 1**). Suter et al. at Roche

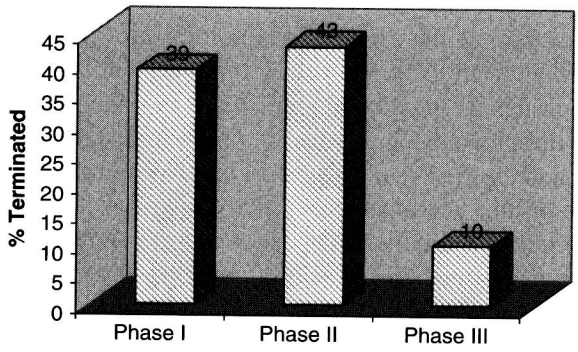

Fig. 1. Data illustrating the termination rate of compounds due to human toxicity during clinical trials. (Data adapted from Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., et al. 2000. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol.* **32**, 56–67.)

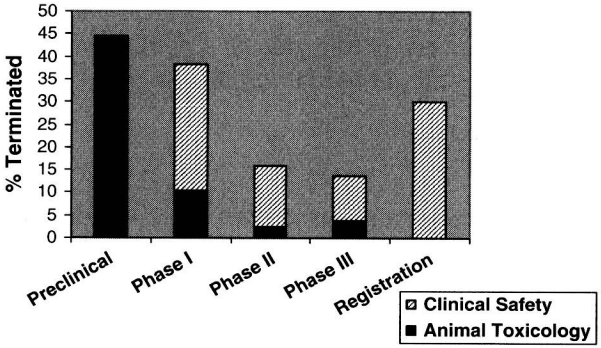

Fig. 2. Failure rate due to animal toxicity and human safety during the development pipeline. (Data adapted from Suter, L., Babiss, L.E., and Wheeldon, E.B. 2004. Toxicogenomics in predictive toxicology in drug development. *Chem. Biol.* **11**, 161–171.)

(6) examined the failure rate of compounds from preclinical to registration and divided safety failures into animal toxicity and human toxicities. Their work found the highest failure rates due to human safety in Phase I and registration (**Fig. 2**). Note that both studies found a high rate of failure in Phase II or beyond, a costly scenario. Dimasi and colleagues have examined financial models of drug development and have estimated the savings of terminating unsafe compounds earlier within the clinical trial paradigm *(7)*. For example, if 25% of the drugs that will fail in Phase II were discontinued in Phase I, the clinical cost savings alone per approved drug would be $13 million to $38 million dollars. Obviously, the cost savings will be greater if one could prevent such a drug from even entering clinical trials by improving preclinical detection and/or by failing it earlier within the clinical testing phase (e.g., Phase I vs. Phase III). The Food and Drug Administration's (FDA) Critical Path Initiative (www.fda.gov/oc/initiatives/criticalpath/) quotes one company executive as saying clinical failures due to hepatotoxicity had cost the company more than $200 million per year over the past decade. Clearly, there are many financial incentives to address the issue of safety.

3. Toxicogenomics

Many in the field have written excellent opinion pieces and reviews on the use of toxicogenomics in drug discovery and development and in the chemical/agrochemical sectors. Toxicogenomics is used in three areas: predictive applications for compound prioritization, mechanistic analyses for compounds with observed toxicity, and biomarker identification for future

screens or to develop biomarkers useful in preclinical and/or clinical studies. Though impractical to list all of the relevant publications, a few excellent articles on toxicogenomics are provided *(2,3,6,8–15)*.

3.1. Study Designs

As with all scientific endeavors, to answer the questions being posed it is important to have an optimal study design. Genomics tends to be somewhat expensive, so understandably some try to downsize the experimental setting. Unfortunately, that may prevent hypothesis generation or evaluation of a preexisting theory. As an example, if one is trying to form a hypothesis as to the mechanism of injury induced by a compound, sampling tissue only at the time of such damage may prevent evaluation of the underlying events that started the pathologic processes. Similarly, sampling only one time point will inhibit the fullest evaluation of the dynamic processes of injury and repair. In classic toxicology testing, one would not claim with certainty that a compound is not hepatotoxic if one saw no elevation of serum alanine aminotransferase (ALT) or histologic change in the liver in a snapshot incorporating only one time point and dose level. Likewise, one does not pool blood from all animals and perform clinical pathology on such. Unfortunately, some have approached toxicogenomics in this manner (using restrictive study designs and pooled RNA samples) and then felt betrayed by the lack of information. This does not mean to suggest that all toxicogenomic studies must be all-encompassing as long as the investigator understands beforehand the limits of his or her chosen design. One approach might be to collect samples from multiple time points and doses and triage the gene expression profiling to determine the most important study groups within that experimental setting. Establishment of the appropriate dose is important as well. Classic toxicology endeavors use dose escalation until one sees a phenotypic adverse event such as changes in classic clinical pathology, histology, body weight, and so forth. An anchoring of the dose used for toxicogenomic studies also must be employed and contextual effects of such doses understood. Doses that severely affect body weight likely induce great stress upon the animal, and this must be taken into account if this phenotypic anchor is followed. A recent paper by Shioda et al. studied effects of xenoestrogens in cell culture and explored the relationship of doses to transcriptional profiles *(16)*. This work suggests that doses chosen for equivalent cellular responses highlight the differences between compounds while those selected based on the compounds' action on a particular gene reveal mostly similarities between the compounds. Additional work remains to be done to determine if this conclusion can be extrapolated to other compound types and *in vivo* environments, but, at a minimum, this report reinforces the

need to understand the chosen study design and fully explain criteria used for dose selection.

3.2. Genomic Approaches Can Clarify Basic Husbandry Issues

Genomics enables detection of toxicity parameters as well as differences in animal husbandry. In many cases, the study design may call for food restriction or animals may be accidentally deprived of food. Genomic analysis can detect such events as shown in **Fig. 3** and **Fig. 4**. Studies in Gene Logic's (Gaithersburg, MD) ToxExpress® database were used for the analysis. In **Fig. 3**, the data from the probe sets (~8800) on the Affymetrix Rat Genome U34A GeneChip® microarray (Santa Clara, CA) were subjected to a principal components analysis (PCA). Such a test illustrates underlying differences in the data in a multidimensional picture. For ease in viewing, two-dimensional graphical representations are provided. In **Fig. 3**, the data from all probe sets were used, and, even with the accompanying noise when so many parameters are measured, one can see differentiation of the groups particularly if one combines the x and y axes, accounting for 39% of the gene expression variability.

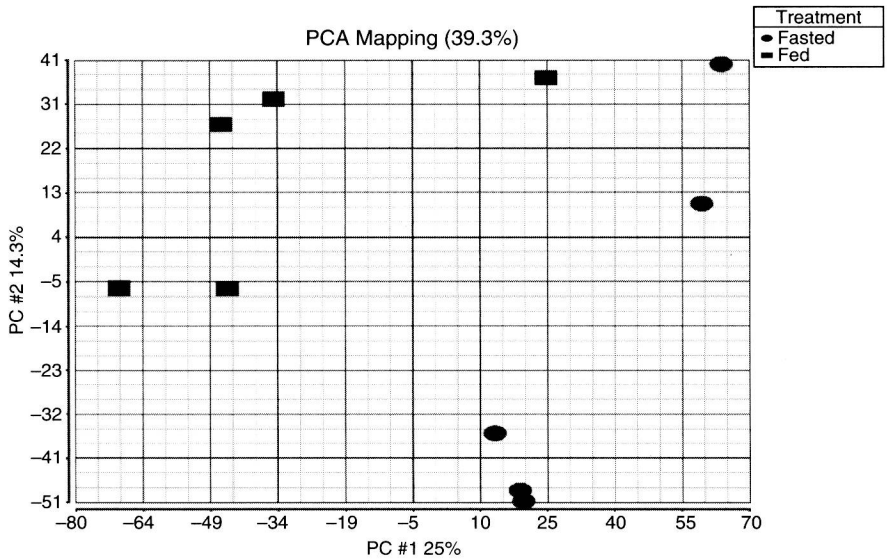

Fig. 3. A PCA using all genes on the Rat Genome U34A microarray illustrates the differentiation on a genomic basis between rats fasted for 24 h versus those rats that had food *ad libitum*. Use of all genes on the array is accompanied by noise and yet one obtains reasonable separation using the x axis and better discrimination if one employs both x and y axes. Such findings illustrate the ability of gene expression to provide insight into animal husbandry.

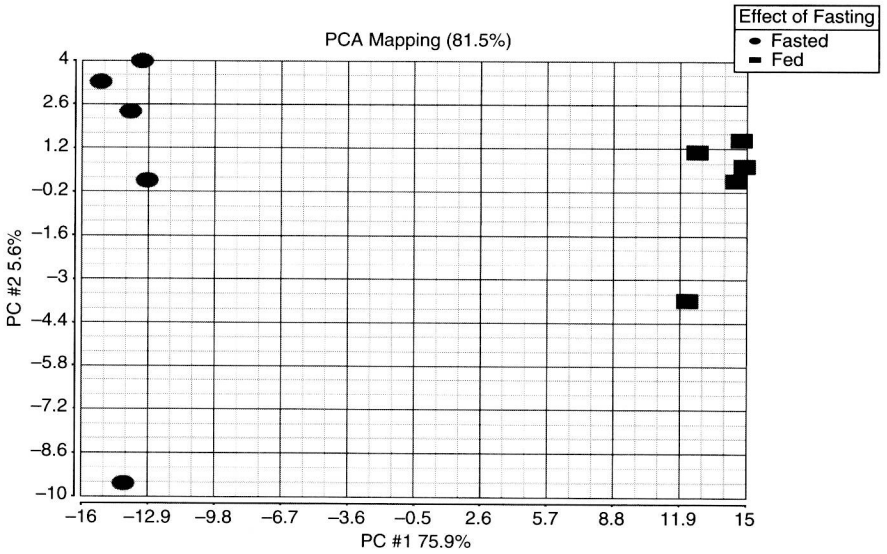

Fig. 4. An analysis filter was applied to identify differentially regulated genes. The filter has a cutoff as follows: fold-change ≥1.8 with *t*-test *p*-value <.05 and ≥90% present in reference or experimental group with mean avg. diff. >40. This resulted in 281 genes identified as dysregulated between fed and fasted rats. Almost all of the variation is captured in PC #1 (75.9%) and the groups are more clearly separated than seen when all genes were used as shown in **Fig. 3**.

When genes that were differentially regulated among the fed and fasted animals were chosen, the gene list was reduced from >5000 to 281. The results in **Fig. 4** demonstrate a complete discrimination of these rats with the x axis accounting for 76% of the variability.

Genomics can be used to discriminate strain and gender as well. In the former case, female rats of Sprague-Dawley (SD) or Wistar origin were compared. Although evaluation using all genes discriminated these strains (data not shown), selection of differentially regulated genes resulted in a clearer separation as shown in **Fig. 5**. Because both strains are albino, one could envision using a genomics approach should there be a potential mix-up of strains in the animal room.

What is likely less surprising is the ability to categorize gender based on gene expression findings. Although it is usually easy to identify the gender of rats by physical examination alone, one could envision the use of a genomic approach to study the feminization of male rats under drug treatment or vice versa. As shown in **Fig. 6**, a PCA employing all genes discriminates between genders although the first two axes capture only ~23% of the variation suggesting there