Toxicogenomics in Drug Discovery and Development — Making an Impact

Quin Wills and Cathrine Mitchell

SimuGen, London, UK.

Summary — As a branch of pharmacogenomics aimed at predicting drug safety concerns, toxicogenomics drew much excitement with the emergence of technologies such as gene expression microarrays. A few years down the line, the evidence is scant that current approaches to toxicogenomics are really making an impact in areas such as preclinical toxicology. It has been argued that there needs to be a re-focus of application toward high-throughput approaches which combine the best of tissue and genomic modelling. This commentary gives a brief introduction to *in vitro* toxicogenomics, drawn from the perspectives of the specialist toxicogenomics company, SimuGen.

Key words: cell culture, drug safety, *in vitro*, pharmacogenomics, toxicogenomics, toxicology.

Address for correspondence: *Q. Wills, SimuGen, Suite 405, International House Business Centre, 1–6 Yarmouth Place, London W1J 7BU, UK.*
E-mail: *quin.wills@simugen-global.com*

Introduction

At this meeting on *Speed and Safety in Drug Discovery* (1), academics, service providers and stakeholders were invited to present their views on *in silico*, *in vitro*, 'omic' and clinical trial trends, with the aim of improving the prediction of human drug safety. Much of the discussion centred on speeding up decisions and the reduction of drug development attrition rates — the 'fail early' paradigm highlighted in the US Food and Drug Administration's (FDA's) 2004 white paper on critical path challenges (2). Five years have passed since this FDA publication and the launch of the 'Critical Path Initiative' (3), which calls for some retrospection on how far we have come with 'omic' tools in drug safety.

Over the last few years, 'toxicogenomics' has stepped up as a term for 'omic' methods used to better understand and predict unwanted chemical and physical effects in organisms and ecosystems. In reality, its industrial application has largely been limited to the use of transcriptomic (gene expression) methods in animal trials, for the better understanding of small molecule toxicity in humans. With the current low levels of drug innovation, patents ending on a generation of blockbuster drugs, the notable development attrition, and market withdrawals of pharmaceuticals due to safety concerns, toxicogenomics has been touted as a 'better, cheaper and faster' option. In reality, the publication evidence does not support the claim that toxicogenomics makes decisions any faster, any less complicated or any more cost-effective. A study by Foster *et al.* (4) suggests that current advantages might be limited to overlaying traditional pre-clinical assessment with some mechanistic insights. Gene expression methods are no longer as immature as they were just a few years ago; whilst they still prove a technical challenge, it is these authors' opinion that much of the problem lies with how we have made use of them.

Toxicogenomics as a Tool

Being a fairly young, fast evolving, collection of sciences, 'omics' is sometimes driven by the latest technologies and what questions the technologies can help answer, rather than drawing from the questions that need be answered, and answering them in a way that best facilitates a decision. This remains a particular problem with large R & D pipelines, where different questions are asked of data that might not be appropriate for answering all of the questions as satisfactorily as possible. To the medicinal chemist, or other team members involved in drug discovery/development toxicology, a toxicogenomics tool would need to answer three questions in real-time: *What toxicity?, How toxic?*, and *What could be done to make the hit/lead less toxic*?

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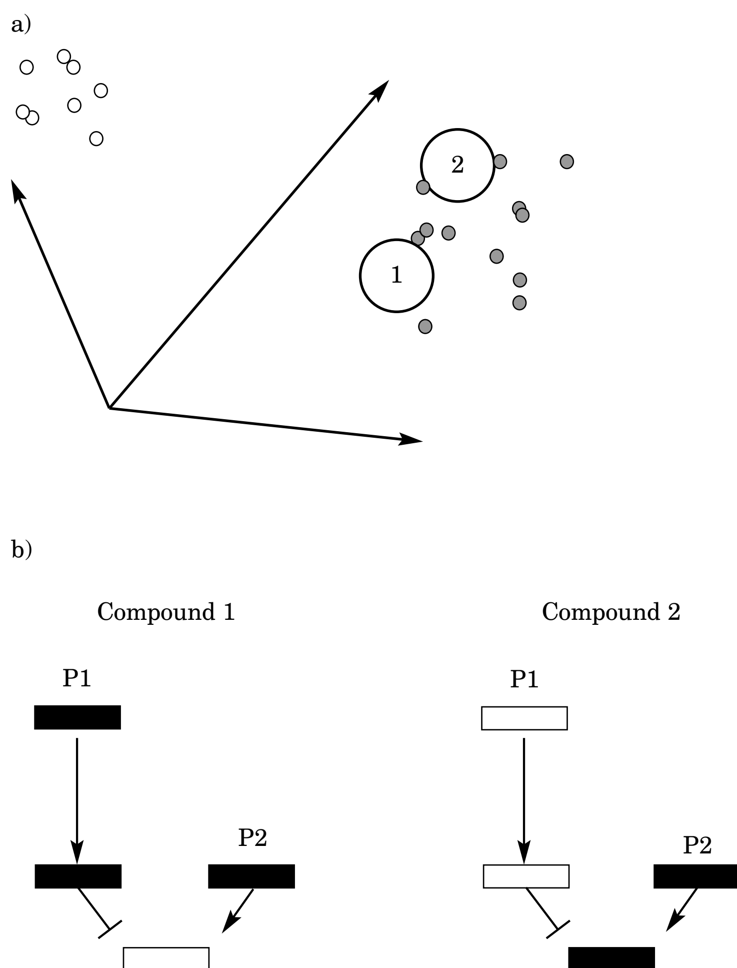
Today, there is no shortage of commercially-available gene expression microarrays for use in trying to answer these questions, including specialist microarrays and/or analysis software. For example, a market leader, Affymetrix Inc. (5), offers two products focused on drug metabolism and safety. Their DMET™ product focuses on pharmacogenetic markers for drug metabolism studies, featuring markers in FDA-validated genes and defined by pharmaADME (6). An analysis suite, ToxFX™, combining gene expression arrays from Affymetrix with the analysis software and a database from Entelos (7), is also available for preclinical work, based on signatures and pathways of toxicological interest in the rat genome. Using this type of gene expression data, two major analysis paradigms exist (Figure 1). The first would be to treat gene expression results as an exercise in pattern recognition. If a novel compound exhibits an expression pattern very similar to that of another,

Figure 1: The traditional toxicogenomic methods used when assessing compounds for toxic effects



a) Toxicity by association: traditional pattern recognition may cluster compounds 1 and 2 together with the 'signature' of other necrotic drugs in a database of compound-related gene expression data, but not provide any further information to allow confidence in this association. The figure shows a Principal Component Analysis with three variables. ○ = known non-necrotic drugs; ● = known necrotic drugs; 1 and 2 are drug candidates of unknown toxicity.

b) Understanding toxicity with bioinformatics: An example of four genes reacting to two compounds is shown. Black boxes represent genes being down-regulated (decreased expression), while the white boxes represent genes being up-regulated (increased expression), in two converging gene pathways (P1 and P2) associated with toxicity. The arrows denote known positive effects on downstream genes, while the T lines denote known inhibitory effects on downstream genes. The example here demonstrates that compound 1 inhibits P1 enough to allow increased expression of the gene common to the two pathways. Compound 2 has the reverse effect on P1 and the common gene, but the same effect on P2. Such results might be academically useful to begin understanding varying toxic mechanisms, but have proven to be of very little use as toxicity assays for routine industry use.

well-defined, compound in a database, then they might be assumed to cause similar toxicities (Figure 1a). Large databases, such as DrugMatrix® (7), have been used, in part, for this purpose. A second, increasingly popular, approach is to use bioinformatics-driven methods to mechanistically study molecular pathways thought to be central to toxic endpoints (Figure 1b). The first approach — practiced in the extreme — ignores biology, the second relies on a very poor understanding of biology. Both have traditionally ignored, or paid little attention to, the explicit modelling of dose–toxicity relationships; which is central to deciding acceptable toxicity.

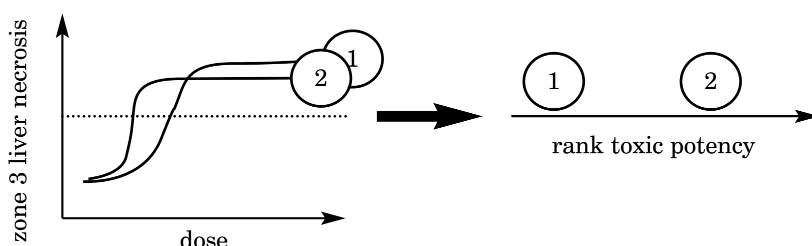
As suggested by Foster *et al.* (4), current methods may ultimately prove to be useful in pre-clinical work, but still do not address the 'fail early' needs of industry. It is our opinion that the most significant drug safety impact can be made by applying toxicogenomics to high-throughput *in vitro* assays, so that toxicity can be considered alongside early stage ADME. It is an opinion similarly expressed by some in industry (8), and will be the focus for the remainder of this commentary, with particular reference to liver toxicity. The approach advocated by SimuGen (9) is to slot toxicogenomics in with early stage human *in vitro* assays, to answer the questions of *What toxicity?*, *How toxic?* and *What could be done about it?*, at a stage when considering other predicted compound properties. With SimuGen's approach, compounds are added over multiple concentrations to human cell cultures, and the gene expression trends are used in dose–response models for particular relevant clinical toxic endpoints that have been validated against compounds of known human toxicity (Figure 2). In doing so, *in vitro* molecular events are mapped directly onto human toxic pathology. With the testing of a chemical series, rather than applying traditional pattern analysis to raw gene expression data, the parameters from the dose–response models can be used, and the results linked to compound structures, to identify any structure–toxicity relationships. This approach differs from many current *in vitro* toxicogenomic studies that measure gene expression changes in cells exposed to single, highly cytotoxic doses of compounds — arguably, far beyond a level from which to extrapolate normal biology.

The Use of *In Vitro* Models

In vitro studies reduce the reliance on preclinical testing (10), and outperform preclinical testing as high-throughput, more-reproducible models. It has been routinely argued that primary human hepatocytes represent the gold standard model for human *in vitro* studies on xenobiotic metabolism and liver toxicity (10). However, such arguments sometimes fail to take into account the basic sensitivity/specificity concerns of creating a good model: a model that is less biased from reality, but with a poor signal-to-noise ratio because of technical performance, does not make for a good model. Primary hepatocytes are associated with a number of limitations, such as scarce or unpredictable availability, limited growth activity and life-span, culture instability (early and unpredictable phenotypic alterations), and wide variations in functional activities from one hepatocyte population to another that needn't reflect sample genetic differences (10).

Cell lines derived from hepatocellular carcinomas, as an alternative, are easier to grow and

Figure 2: Combining toxicogenomics with dose–response modelling — the SimuGen approach



By combining gene expression trends with in vitro *assays to determine the extent of toxicity, over a wide range of compound concentrations, SimuGen is able to map the dose-response gene expression to the dose-response effect in humans. The figure uses zone 3 human liver necrosis as an example to show dose–response curves for two compounds, with compound 2 exhibiting higher toxic potency than compound 1. By using compounds with well-described human safety concerns to validate the model, SimuGen's approach enables the modelling of* in vitro *molecular events, to predict at which relative doses these events would become clinically significant in humans. The horizontal dotted line on the graph represents the threshold at which toxicity is established.*

maintain, which makes them useful for *in vitro* studies. The HepG2 cell line, for example, has been widely used to assess xenobiotic metabolism and toxicity. It is more stable than human primary hepatocytes, but has its limitations: the cells contain little Phase I drug metabolising activity, and do not mimic the regulation of gene expression observed in normal hepatocytes (10). A newer cell line, HepaRG®, exhibits liver parenchymal differentiation, greater metabolic competence for Phase I and II enzyme activities, greater culture stability than primary human hepatocytes, and can be cryopreserved without marked cell loss (11). HepaRG is proving increasingly popular as a scalable monolayer culture model, and is currently being assessed as a toxicogenomics tool in a project between SimuGen and the Centre for Proteomic and Genomic Research (9, 12).

The Future of *In Vitro* Toxicogenomics

With a focus on improving cell methods and genomic modelling, *in vitro* studies open up large possibilities for exploring mechanistic and predictive toxicology. Tissue engineering is pushing medium-throughput to high-throughput methods beyond current simple, single layer cultures, to complex co-cultures and 3-D cultures. For example, a 'lab-on-a-chip' specialist, the Hurel Corporation (13), are researching microfluidic circuits to permit the testing of multiple tissues together. The Griffith Lab at the Massachusetts Institute of Technology are also developing 3-D 'liver-on-a-chip' bioreactors, each only a few centimetres long (14). These comprise liver cells growing on silicon scaffolds, forming channels that resemble the capillary bed for the constant perfusion of nutrients. There are many such examples, with bodies such as the European Centre for the Validation of Alternative Methods (ECVAM), established to validate and list these newer culture approaches (15). Beyond tissue engineering, toward genomic engineering, *in vitro* gene expression can also be manipulated in a number of ways, to create functional variation more in line with human population variation, or to better understand toxicity mechanistically. Using RNA interference (RNAi), Dai *et al.* (16) have established an approach to suggest the causal sufficiency order network for liver hypertrophy in the rodent, based on the expression profiles in response to the small interfering RNAs against the gene for the PPARa receptor.

In vitro methods will also open up opportunities for the more sophisticated 'omic' study of toxic perturbation, such as drug–drug interactions and multiple dosing for chronic effects at the molecular level. Multiple drug regimens are often administered to a single patient, and drug–drug interactions occur during these regimens, resulting in adverse effects that may become life threatening. Rifampicin is a well known example, which is commonly used in clinical studies as a prototypical inducer of drug-metabolising enzymes, to test for effects with other drugs (17). Multi-dose, chronic studies in cell cultures are still complicated by the fact that many cell models become unstable when cultured for long periods of time. However, the HepaRG cell line again proves promising, by demonstrating relatively stable functional activities, weeks after confluency.

In summary, the field of toxicogenomics is likely to have a significant impact on drug discovery, if the fields of functional genomic and tissue culture modelling are brought together more successfully. It is with this goal that SimuGen models *in vitro* dose-response molecular events, mapping them directly to human toxic endpoints, rather than applying traditional pattern recognition or bioinformatics. This provides decision-focused, reliable computation models, bringing toxicogenomics into a high-throughput ADME toxicology pipeline.

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