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Progress in applying genomics in drug development

Peter G. Lord*

Johnson & Johnson Pharmaceutical Research & Development LLC, 1000 Route 202, P.O. Box 300, Raritan, NJ 08869, USA

Abstract

Genomics has had an impact on two areas of drug development, "predictive" toxicology and mechanism-based risk assessment. Predictive toxicology studies are aimed at identifying the potential for a compound to be toxic. By developing databases of expression profiles for a wide variety of toxic compounds and toxic models it has been possible to create statistical and computational methods which provide an indication of the toxic potential of a drug from the pattern of gene expression changes it elicits in in vitro or in vivo systems. Because gene expression is central to many responses to xenobiotics, genomic approaches lend themselves very readily to mechanistic toxicology studies. By examining changes in gene expression in cells and tissues in response to drugs it is possible to generate hypotheses as to the underlying mechanism and in some cases it is possible to evaluate hypotheses of toxic mechanism. Some concerns remain about the use of the technology but toxicogenomics can no longer be regarded as "new" technology in drug development. The investments made in applying the technology are maturing and there is a determined effort to bring the full power of the technology into drug development.

Keywords: Toxicogenomics; Predictive toxicology; Mechanism-based risk assessment; Review

1. Introduction

Gene expression profiling lends itself readily to two highly topical areas of drug development: "predictive" toxicology and mechanism-based risk assessment. The acceleration of efforts in predictive toxicology in particular has been largely due to the technological and scientific advances made in the last decade in genomics research. Hence, the advent of arrayed gene platforms for gene expression analysis has led to much investment by drug companies, government agencies and technology providers in applying genomics-based approaches in drug development. Turning the promise of genomics into practice in drug development is not without its obstacles (Fielden and Zacharewski, 2001; Rockett et al., 1999; Smith, 2001). Technological advances in themselves do not guarantee success but they give faster access to biologically relevant data as well as to data that were previously difficult to obtain (Kozian and Kirschbaum, 1999). Prior to the development of DNA microarray technology, gene expression studies of toxicity have been limited to small numbers of genes (Garcia-Allan et al., 1997; Orton et al., 1996; Rumsby et al., 1994). Therefore, there is little information on gene expression changes as they relate to toxic responses, and only an embryonic understanding of the toxicological relevance of gene expression modulation by xenobiotics.

* Tel.: +1-908-927-2036; fax: +1-908-218-0668.
E-mail address: plord@prdus.jnj.com (P.G. Lord).

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Genomics research has very much been concentrated on the human genome, as a means of advancing our understanding of human disease, and on the mouse genome, as a means of understanding biology in a widely used laboratory species. Much less work has been done on the genomes of the laboratory species (rat, dog, monkey) used in toxicological studies. Full exploitation of genomics in toxicology awaits the sequencing of genes from these test species along with the accumulation, synthesis and interpretation of gene expression data from toxicity studies. Given the complexity of study designs for toxicity it is remarkable that positive progress has been achieved in the emergent field of toxicogenomics (Nuwaysir et al., 1999). To address drug toxicity, multiple dose levels, multiple species, multiple time-points, multiple organs and multiple biological and biochemical parameters need to be considered. Furthermore, the cellular response to xenobiotics and consequent pathogenesis represent a dynamic process, gene transcriptional responses being just one component part (see Fig. 1). A key challenge is to measure gene expression at time points (and dose levels) at which changes are meaningful for the response to a drug, for the adaptation to the response, and for the down-stream consequence of adverse drug reaction.

The high expectations for toxicogenomics coupled with the complexity of the task of putting genomics into practice in drug development has understandably raised a number of concerns within the drug industry and within drug regulatory agencies (Lesko et al., 2003; Petricoin et al., 2002). For the genomic data to gain basic acceptance there needs to be a confidence in the technology. As the field advances there then needs to be built confidence in the meaning of modulation of gene expression as it applies to assessment of toxicity. The huge data sets which are generated by toxicogenomic approaches create value in the wealth of information that can be gained. However, that abundance of information brings the danger of at least over-interpretation and at worst mis-interpretation of the data. The fears and concerns about using genomic data in risk assessment will only be dispelled with increasing experience. Fortunately there has been free and open debate about toxicogenomic applications as exemplified by a number of international collaborations between drug companies, regulatory agencies and academia.

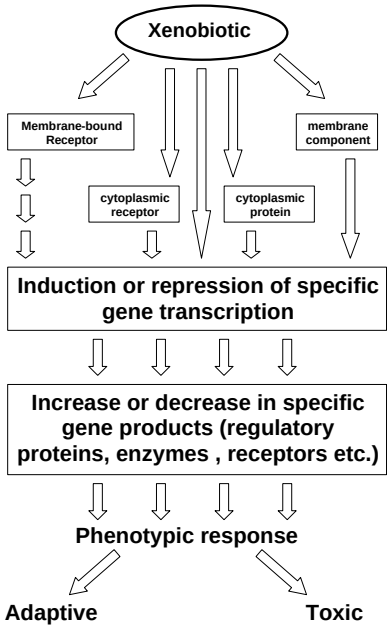

Fig. 1. The dynamics of a transcriptional response to xenobiotics. A xenobiotic may interact with a membrane-bound receptor which then signals through a signal transduction cascade to the nucleus leading to modulation of specific gene transcription. Alternatively it could act via a cytoplasmic receptor which translocates into the nucleus and modulates specific gene expression. Other possible interactions are with non-receptor components of the membrane or cytoplasm which feedback to the nucleus to induce or repress specific gene transcription. Some xenobiotics may interact directly with DNA to influence specific gene expression. However it occurs, the modulation of transcription translates to a modulation of any number of proteins involved in cellular homeostasis. This leads to a phenotypic response which may be adaptive or toxic. This diagram is not intended to show a complete picture of possible events but serves to illustrate that the response is a dynamic process involving several biochemical/molecular steps.

2. Predictive toxicology

The last few years have seen a lot of progress being made in linking the profiles of gene expression induced by drugs with their toxicities. By developing databases of expression profiles for a wide variety of toxic compounds and toxic models it has been possible to create statistical and computational methods which provide an indication of the toxic potential of a drug from the pattern of gene expression changes it elicits in in vitro or in vivo systems (Hamadeh et al., 2002; Harries et al., 2001; Thomas et al., 2001; Ulrich and Friend, 2002). The predictive toxicology field is evolving

rapidly and there is much debate about the predictive power of genomic approaches. One debate is whether information from the whole genome is essential for a prediction or whether predictive power is increased by focussing on small sets of genes whose function in toxic mechanisms is known. Waring et al. (2001), using an array representing around 1000 rat genes, were able to show patterns of rat liver gene expression that distinguished between 15 different hepatotoxins, directly from the data. They tried several statistical/informatic tools to analyse the data to reveal how the gene expression profiles fell into clusters. Each method highlighted similarities between compounds which were expected as well as a few that were not expected. The specific genes whose expression was modulated by the treatments were found to be indicative of several known toxic mechanisms. This initial study of a small set of compounds therefore showed utility for comparing and discriminating between compounds as well as for investigating underlying mechanism. Another early toxicogenomic study showed the utility of concentrating on specific genes to better discriminate between classes of toxicants. Burczynski et al. (2000) obtained differential gene expression data from a comprehensive study of 100 toxicants in HepG2 human hepatoma cells with a 250 gene cDNA microarray. Their first comparisons of the expression profiles were unable to discriminate between the toxicant classes represented by the 100 compounds. After examining gene expression data from further experiments of a small set of compounds they were able to select genes showing reproducible changes in expression with those compounds. By focussing the analysis of the data from the 100 compound study on this subset of (fingerprint) genes they found that the compounds clustered into the expected toxic classes.

As the knowledge base of toxicity related gene expression builds up it is becoming apparent that understanding of the toxicological relevance of specific genes helps to guide the predictive modelling. It remains to be seen whether the genomics-based predictive toxicity assays provide sufficient improvements on current cell-based or biochemical assays, but there is no doubt that such an approach is highly applicable to predictive toxicity screening. The approach shows powers in discrimination between classes of toxic agents and has the added advantage of providing mechanistic clues.

3. Mechanistic toxicology

In recent times, an understanding of the mechanism of toxicity of a new drug has become a major part of its risk assessment. Because gene expression is central to many responses to xenobiotics, genomic approaches are highly applicable to mechanistic toxicology studies (Macgregor et al., 1995; Farr and Dunn, 1999). A transcriptional response can give a preliminary indication of the biochemical or biological mechanism being affected by a xenobiotic. By examining changes in gene expression in cells and tissues in response to drugs it is possible to generate hypotheses as to the underlying mechanism. Used in this way, gene expression data should be viewed as starting points rather than as end-points in a toxicological examination. If the mechanism is unknown then the genomic data can help to identify more definitive end-points which may be proteomic or enzymatic in nature. For example Crosby et al. (2000) studied the gene expression pattern of mesothelial cells in vitro following exposure to the rat mesothelial, kidney, and thyroid carcinogen, potassium bromate ($KBrO_3$). They considered the biochemical pathways represented by the genes showing modulation of expression and visualised the pathways diagrammatically. From this they proposed that $KBrO_3$ generates a redox signal that activates p53 and results in transcriptional activation of oxidative stress and repair genes, dysregulation of growth control, and imperfect DNA repair. Thus they provide a hypothesis for how $KBrO_3$ leads to mesothelial cell carcinogenesis. In some cases it is possible to evaluate hypotheses of toxic mechanism. If a good correlation exists between gene expression and a toxic mechanism then the genomic data provide supportive evidence for that mechanism. In a study of drug-induced cardiac hypertrophy, Lord et al. (2001) took advantage of the literature to assemble a cDNA microarray of a selection of genes considered to be involved in cardiac enlargement. The results of the study showed the induction of several genes known to be induced during cardiac hypertrophy and more importantly showed that the expression more closely resembled preload- rather than afterload- induced hypertrophy. This was consistent with the hypothesis that the cardiac hypertrophy resulting from the drug treatment was preload-driven.

4. Concerns about the use of toxicogenomics

There are still a number of concerns around the use of gene expression data in drug risk assessment. There are technical concerns about the sensitivity and reliability of the methods. 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. These genes are not oncogenic in themselves but are found to be mutated or highly overexpressed in tumours (Varmus, 1985). 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. The availability of practically the whole genome for expression analysis also brings difficulties in interpretation. There just is not enough information in the literature to interpret the modulation of expression of every single gene. 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 ILSI Health and Environmental Sciences Institute (HESI). 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. A recent status report on the project can be found at the web-site http://hesi.ilsi.org/index.cfm?pubentityid=120. The data are being placed in the public domain (and a standard for such data submission, MIAME/Tox, is being developed) in partnership with the European Bioinformatics Institute. The committee aims to provide guidance on the application of toxicogenomics to risk assessment such that the technology can be applied in a pragmatic and realistic manner. The regulatory agencies have been active in encouraging the debate about the use of genomics in drug development not only through participation in the aforementioned ILSI/HESI committee but also through joint sponsorship of workshops and open debates (Lesko et al., 2003; Petricoin et al., 2002). The field of toxicogenomics can only benefit from these collaborations and initiatives.

5. Conclusions

Genomics, and more specifically toxicogenomics can no longer be regarded as "new" technology in drug development. The investments made in applying the technology are maturing and through open debate in the toxicological community, there is a determined effort to bring the full power of the technology into drug development, whether in choosing drug candidates or in assessing drug safety. Many of the initial concerns about the practical use of toxicogenomics have been addressed although several issues remain before such data are accepted with confidence in the Regulatory and Regulated environment. It is clear that transcriptional profiles can discriminate between classes of compound and some toxicities, hence showing value in predicting toxicity. These very preliminary data allow companies to make decisions about which drugs to develop and how to develop them. Some of these data may provide a foundation for studies aimed at risk assessment. Further toxicogenomic data may test hypotheses regarding drug toxicity when generated as part of an investigative study of the toxic mechanism. Expectations are high that these data will become an integral part of drug risk assessment. However there is much optimism. The technologies are maturing to the extent that there is a lot of experience in their use and there is much more awareness of the limits of sensitivity and reproducibility of the methods. With this experience comes an understanding of transcriptomic data and how they can be interpreted in the context of the pathology and other biological data from a toxicology study. The healthy debate amongst all the interested parties in applying toxicogenomics in drug risk assessment can only ensure its success.

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