Of mouse and man – what is the value of the mouse in predicting gene expression in humans?

'The validity of mouse or other animal species as a human surrogate should not be assumed.'

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The mouse is the mainstay of the drug research scientist; it is small but not too small, durable, tractable, intelligent, it breeds readily, has a short life span and costs little. Although genomically the mouse is virtually indistinguishable from human, anatomically, physiologically and pathologically, it differs significantly. If scientists were aiming to discover novel drugs to treat murine disease, it would be logical to experiment on mice, and the use of any other species would be rightly questioned. To follow this logic, to develop better drugs to treat human disease, humans should be used as experimental subjects. Herein is the problem. It is unethical and even illegal to do so – at least it is without a substantial body of supportive information on the safety and likely efficacy of the drug – hence our reliance on surrogate species. However, experimental animals are surrogates, and the results obtained should not necessarily be taken at face value.

The enthusiasm for using mice as experimental subjects has been fuelled recently by the completion of the mapping of the mouse genome [1], and the realization that, at least in terms of their genomes, mice and humans are remarkably similar. Furthermore, in its Model Organisms Guide, the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/About/model/) described the mouse as 'the closest model organism to man', and goes on to state that 'elucidation of the mouse genomic sequence will also provide a system for studying and understanding human disease, as well as a mechanism for investigating new treatment strategies in ways that cannot be done in humans'. Is such unerring faith well founded? Mouse models have provided valuable information about some human diseases, for example, Huntington's chorea, diabetes and Alzheimer's disease [2,3], but for other diseases, such as asthma, cystic fibrosis (CF) and many cancers [4–6], mouse models have been less helpful – or even misleading.

In modelling CF, the most common monogenic recessive disease known in humans, which is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), the mouse is rather disappointing. CF is marked by widespread pathology, which is particularly severe in lung and gut. However, attempts to model the disease in mice by knocking out the gene encoding CFTR result in a pathology characterized by serious gastrointestinal (GI) problems, but with little or no effect on the lung [6]. This might be due to the co-expression in mouse lung of an alternative, albeit uncharacterized, Cl^- transporter that can functionally replace the absent CFTR [7].

Genomics in mouse and human – is it all that matters?

So why are mice and humans similar and yet distinct? We are, of course, largely products of our genomes, but the genome is not everything. A caterpillar and a butterfly are genomically identical, but are anatomically and physiologically distinct. It is not just the genome *per se* that dictates what and who we are, but how that genome is expressed. So although mice and humans are apparently at least 95% identical at the genomic level, this does not prevent our respective phenotypes from being different.

Direct comparisons of gene expression in mice and humans

Because the generation of mRNA is the most immediate marker of gene expression, a comparison of specific mRNA levels for particular genes of therapeutic relevance can

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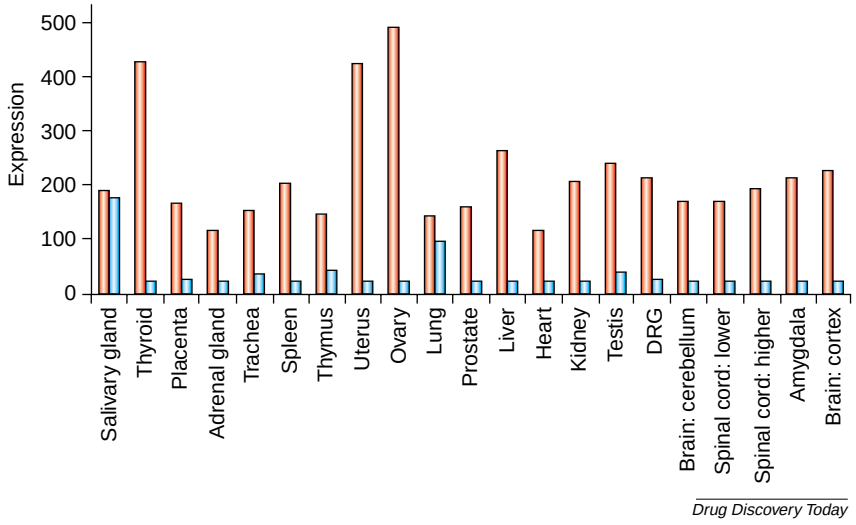

Figure 1. Gene expression profile based on gene-chip technology for the relative expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in 21 tissue types from human (blue) and mouse (red). Data obtained from http://expression.gnf.org. Abbreviation: DRG, dorsal root ganglion.

provide valuable information regarding the credentials of the mouse as a human surrogate in testing therapeutic hypotheses.

Although there are few reports in the literature comparing patterns of gene expression in mice and humans, the recent publication of the gene expression profiles of these species by Novartis (http://expression.gnf.org) using gene-chip array technology has provided a wealth of data from which to draw comparisons. Although they probed different selections of genes in different selections of tissues in the two species, there is sufficient overlap to make useful comparisons. To date, we have performed only a limited analysis of their data, but this has revealed some interesting findings. For example, in humans, expression of the gene encoding the CFTR is predominant in the salivary gland and the lung, consistent with the known functional role of this transporter in these tissues, whereas in mouse, expression in salivary gland and lung is relatively low (Fig. 1). This result is particularly significant when one considers that in humans, CF is predominantly seen as a disease of the lung, whereas in mouse CFTR knock-outs, the effects on lung function are minimal [6]. By contrast, in the mouse, CFTR gene expression is relatively high in thyroid, uterus and ovary, all low-expressing tissues in humans. By far the highest expressor in the human tissue set was the pancreas, but unfortunately this tissue was not included in the mouse set, so no comparison could be made. In the mouse, by far the highest level of CFTR expression was in the intestine, but again unfortunately, GI tissues were not included in the human data set. Several other genes also showed a similar lack of correlation between mouse and human, such as the 5-HT_{2B}-receptor, the prostanoid EP_3-receptor and inducible nitric oxide synthase (iNOS).

Although the results are interesting, the Novartis data were not generated with a view to comparing mouse with human gene expression profiles, and there are several problems. First, the accuracy of some of the sequences on the mouse chip (U74A) has been questioned [8]; second, gene-chip array technology has only moderate sensitivity, and thus can provide misleading data when studying low-expression genes; and finally, array technology requires a compromise in the hybridization conditions for different genes, and thus cannot be optimal for all.

Therefore, to investigate patterns of gene expression in mice and humans more directly, we collaborated with Lark Technologies Inc. (http://www.lark.com) to perform a study using TaqMan® quantitative real-time (QRT)-PCR technology to compare the expression patterns of a range of 32 genes of interest in 20 tissues from mouse and human. The selected genes included examples of G-protein-coupled receptors (GPCRs), nuclear receptors, ion and water channels, cytokines, integrins and enzymes involved in signal transduction, and the selected tissues represented the cardiovascular system, the GI tract, the CNS, the lung, the genito-urinary systems, spleen and skin (Fig. 2).

Several general observations were made:

- The basic patterns of gene expression differed; some genes (31%) were expressed at similar levels in all tissues studied in each species, others (59%) were expressed in virtually all tissues but at greatly differing levels, and for the remainder, measurable expression was only detected in a minority of tissues.
- In 56% of the genes, maximum expressed levels of specific mRNA were similar in tissues of both species. For most of the others (38%), expression levels in the human tissues were higher (3–60-fold) than those in mouse tissues. For two genes (6%), levels in mouse tissues were approximately threefold higher than those in human tissues.
- There was no general rule regarding the similarity between the whole-body expression profiles between the

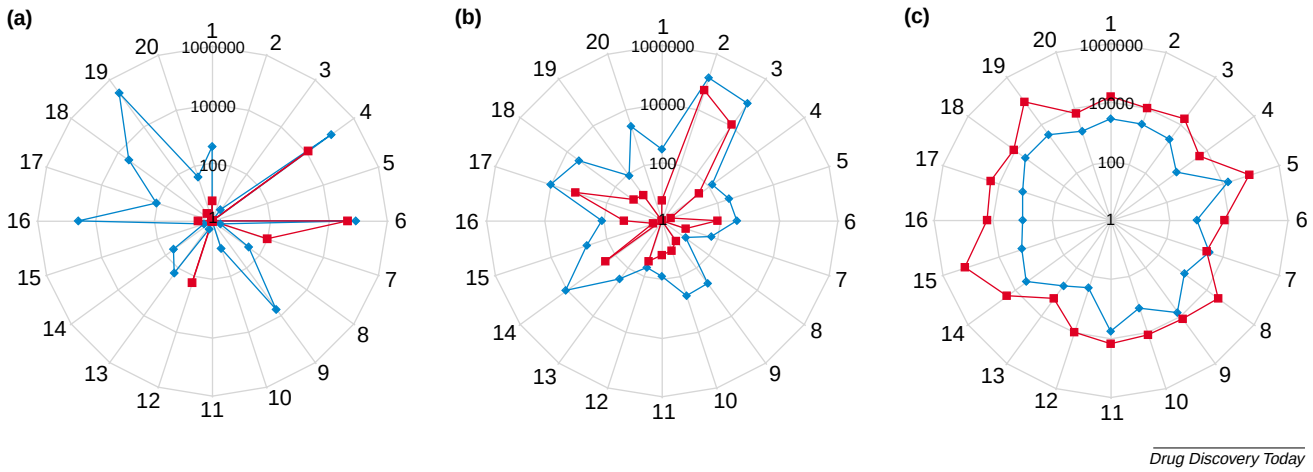

Figure 2. Comparison of quantitative real-time(QRT)-PCR expression of **(a)** CLCA1 chloride channel, **(b)** KV3.1 potassium channel and **(c)** inducible nitric oxide synthase (iNOS) in human and mouse. Each numbered radial arm represents a different tissue type, and concentric circles represent the magnitude of gene expression in copy number per 100 ng total RNA. Data points (human is blue and mouse is red) are mean values from three independent experiments (i.e. generated from three samples of each tissue type, each obtained from a separate donor animal).

species; for some, the expression profiles were similar (34%), for others they differed markedly in specific tissues (47%) and in others, the profiles appeared completely different (19%) (Fig.2).

Summary

The study provides a limited but quantitative snapshot of mouse and human gene expression patterns. Unsurprisingly, the results demonstrated that many, but not all, gene expression patterns of human and mouse are similar. Therefore, if an experimental model is needed as a predictor of human biology and disease, the mouse can be useful. However, the validity of mouse or other animal species as a human surrogate should not be assumed, and some attempt should be made to establish its suitability for this purpose. Comparative gene expression studies provide a good starting point.

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