Genetically Modified Mouse Models in Drug Discovery

Advances in the production of genetically modified mouse models – notably the ability to make multiple genetic changes in one mouse-line – has opened up new areas of application in drug discovery and development.

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The pharmaceutical industry is continually looking for new approaches to increase the efficiency of R&D and improve the quality of the innovative new medicines that they produce. In recent years, innovations in the ability to produce genetically modified mouse models have increased their use in drug discovery – both for the evaluation of the function of genetic targets \textit{in vivo}, and the predictive testing of the efficacy, pharmacokinetic and side effect profile of potential therapeutic agents.

THE GENERATION OF GENETICALLY MODIFIED MOUSE MODELS

Time and Cost

Historically, the production of a genetically modified mouse model has been a very long and expensive process. The large number of techniques and disciplines required (high quality molecular genetics, ES (embryonic stem) cell culture, cell injection techniques and mouse breeding) combined into a complex process that resulted in mouse model generation times of up to 24 months; this was far too long a time to allow a significant contribution to decision-making in drug discovery programmes. However, recent improvements in project management – as well as technical improvements in the time-consuming areas of vector construction, cell biology and mouse breeding – have reduced these times such that today all types of genetically modified mouse models can be generated within 12 months. Further improvements in technology – such as RNAi-induced gene knock-down \textit{in vivo} – have led to model generation times of less than four months (Figure 1). These times are now compatible with drug discovery programmes.

**Figure 1: Improvements in time to generate conditional knock-out mouse models from 1999 to 2004**

<table>
<thead>
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<th>Time (Months)</th>
<th>Year</th>
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<td>0</td>
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<td>3</td>
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<td>05</td>
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<td>24</td>
<td>06</td>
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</tbody>
</table>

Cell culture experiment

Conditional Knockout Mouse

Industrial production line process

Artemice® Platform

Designer mice

RNA

RNAi

Significant reductions in time and effort to generate genetically modified mouse models
and mean that data from advanced in vivo models can be made available in time to contribute to the decision-making process, and thereby make a significant contribution to increasing the efficiency and output of R&D drug discovery programmes.

**Early Limitations**

During the 1990s, genetic modification in a mouse was limited to the over-expression or knock-out of a gene from birth onwards. Experimenters introduced the modification into mouse germ-line or embryonic stem cells, which then developed into the genetically modified mouse model. This had the significant disadvantage that the gene of interest was modified in all mice from conception onwards. Associated problems – such as embryonic lethality (mice dying before birth due to the gene modification) or compensatory changes (changed expression of non-modified genes in the embryo compensating for the absence or over-expression of the gene of interest) – complicate the phenotypic analysis of such mice. For drug discovery teams who were interested in looking at the effects of gene modification in the adult, these techniques were – at best – only surrogates.

**Conditional Gene Modification**

In the mid 1990s, Professor Rajewsky and his group in Cologne, Germany, identified a method to allow the ‘conditional’ modification of gene expression in a mouse (1). The intention was to provide the investigator with the possibility of modifying gene expression in a mouse either in a particular cell of interest (leaving gene expression in all other cells unaffected), or at the time of interest (for instance only in the adult mouse). This technique was used during the 1990s in particular for the tissue-specific modification of gene expression. This allowed a closer dissection of genetic function for a particular tissue of interest. For drug discovery purposes, the more interesting and relevant application is an inducible gene modification. In order to achieve this, a suitable gene switch was required which, when introduced into a mouse, produced a high level of gene knock-down and only upon a particular chemical stimulus. It has recently become apparent that the gene switch of choice for this application is the CreER gene switch, which when introduced into a mouse can produce a suitable gene knock-down in all tissues of the body, only in the adult (2, 3).

These advances – together with the advances in timing described above – mean that for the first time drug discovery researchers have the ability to produce animal models, in a reasonable period of time, in which their gene of interest is only modified in the adult. This provides significant advantages in terms of the type and utility of the data obtained. In particular, it allows discovery teams to obtain direct information regarding drug target efficacy only in the adult and, if the target deletion is made once a disease state has developed, then direct information can be obtained about the therapeutic relevance of the target. In addition, discovery teams can obtain information about any non efficacy-related effects of the target, such as what degree of on-target toxicity could be expected from a future inhibitor. All of this data is obtained after gene modification only in the adult, and is therefore unencumbered by any compensatory or other effects which may complicate interpretation of the results if the gene expression is modified also in the embryo.

**RNAi Gene Knock-Down**

In recent years, RNAi knock-down in vitro has become a popular tool to study the effects of genetic modification. However, despite some early successes, it has not yet become widely usable in vivo. There are still many questions relating to repeatability and breadth of gene knock-down, as well as to the potential for off-target effects in vivo. Nevertheless, advances are being made in this field. The use of a targeted transgenesis approach in ES cells, together with generation of the mouse model via injection into tetraploid blastocysts, may have provided a breakthrough (4). Seibler’s group has shown that the incorporation of a single siRNA sequence into a specific genetic locus in the mouse leads to reproducible and broad deletion of the gene of interest (Figure 2). When combined with advanced methods of tetraploid

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**Figure 2: Efficient RNAi knock-down in vivo in the mouse**

<table>
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<tr>
<th>Relative expression level</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Muscle</th>
<th>Testis</th>
<th>Salivary Gland</th>
<th>Pancreas</th>
<th>Suprarenal Gland</th>
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injection, the group can produce adult mouse models within a four-month period. Potentially this can provide a revolution in the use of mouse models for discovery purposes. For the first time, investigators can obtain relevant models relatively cheaply and within a very short period of time. By reducing these twin constraints, more genetic targets can be validated in vivo earlier in the drug discovery process, thereby increasing the quality of decision-making and having a positive impact on R&D efficiency.

**Humanisation of Mouse Proteins In Vivo**

A further use of genetically modified mouse models, in addition to gene validation, is for drug testing. Recent advances in the technique of ‘humanisation’ have contributed to the utility of mouse models for drug testing applications. Using advanced cloning techniques such as ET cloning (5), it is now possible to easily modify large pieces of human DNA and build them into gene vectors. By homologous recombination, these can be introduced into the mouse genome at the appropriate site, and the resulting human protein can be expressed in the mouse. This protein will be active, expressed at the correct level and in the correct tissues. It will interact with other mouse genes in the pathway, thereby having the right physiological and pathological effects, but importantly it will respond to drug candidates as if it were a human protein. Using this technique, investigators can study the effects of drug candidates on the relevant human protein in vivo and obtain important information about their efficacy, pharmacokinetic and toxicological properties.

**LEAD SELECTION AND OPTIMISATION**

Traditionally, the major application of genetically modified mouse models in drug discovery has been in the areas of modelling human disease, target identification, target validation and the testing of candidate compounds to evaluate in vivo efficacy. Although standard rodent models are extensively used to study the safety and pharmacokinetic properties of compounds, the use of genetically modified mouse models has been relatively limited in this area.

This is due to a range of factors including: the generally more conservative nature of the drug development process as opposed to drug discovery; the extensive existing experience of using in-bred rodent species to evaluate risk to man; the preference in toxicological studies for the rat as a species, while the mouse is the preferred species for genetic modification; and the perceived difficulty in establishing with regulatory authorities the value of genetically modified animals in predicting the human situation.

However, around 40% of clinical drug failures are for pharmacokinetic reasons (6). As a result, pharmaceutical companies recognise that establishing improved predictive drug absorption, distribution, metabolism and elimination (ADME) testing early in the drug development process is a key element to improving the cost and time lost due to late-stage product attrition. Genetically modified mouse models are increasingly being considered as useful tools to assess the interaction of drug candidates with target proteins in vivo.
of compounds with genes important in drug metabolism and toxicology. Reporter gene systems, such as the Xenogen LPTA system (light producing transgenic animals) – where a bioluminescent signal that can be measured non-invasively is activated in response to the transcription of a specific gene – have been used to study the interaction of drugs with important metabolising enzymes such as Cytochrome P450 3A4 (7).

Genetic modification also provides a potential solution to some issues concerning the extrapolation of rodent data to humans. A key area where species differences can generate major problems is in ADME; the genes encoding key enzymes and factors relevant to ADME are subject to significant species differences in both their functions and regulation. Here, a relatively small sub-group of metabolising enzymes, drug transporters and transcription factors are responsible for the major pathways of transport and metabolism of a large proportion of drugs. Limited humanisation has already been achieved with the production of a mouse where the endogenous gene for mouse PXR (an important transcription factor that regulates certain metabolising enzymes) has been knocked out, and the human PXR gene has been introduced into the liver (8). This model does have limitations with regard to its restriction to the liver and its physiological control, indicating a need for further development in the field towards mouse models which incorporate key elements of human metabolic pathways.

A new research programme, announced in early 2005 by ITI Life Sciences (Dundee, Scotland), takes the concept of humanisation to a new level. This programme – in which both CXR Biosciences and Artemis Pharmaceuticals are participants – has the objective of creating a broad suite of humanised mouse models where entire pathways of metabolism and drug transport are humanised and under physiological control. Although humanised models have obvious benefits in determining the ADME properties of compounds, they can also play a key role in assessing the efficacy and toxicity of drugs. Species differences in drug metabolising enzymes or drug transporters not only result in changes in pharmacokinetic parameters, but can also have major consequences for efficacy and toxicity – either due to different patterns of drug disposition, or the production of different metabolites.

THE FUTURE

The creation of genetically modified mouse models has traditionally involved a major investment of time and money, and has been limited to the production of models with one or two genetic changes. The new technologies available today have radically changed the equation, and the production of new models is now a cost- and time-effective proposition. Perhaps more importantly for drug discovery and development, the ability to make multiple genetic changes in one mouse-line has opened up new areas of application where single gene changes are not sufficient to model the human situation.

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References


