151P TRANSGENIC MODELS OF HUMAN DISEASE

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Homologous recombination in embryonic stem cells can be used to generate mouse models of human diseases. Even when these models are not exact phenocopies of the human disease they are often sufficiently similar to be of great value in further research. These animals can be used to study the pathophysiology of the disorder including the identification of genetic factors that influence disease severity. They can also be used to test new treatment strategies such as gene therapy and to evaluate the therapeutic potential of substances in vivo. Murine models of cystic fibrosis (CF) will be used to illustrate these points.

Cystic fibrosis is a fatal autosomal recessive disorder affecting over 50,000 individuals who have an average life expectancy of around 27 years. The major cause of death is through gradual lung destruction brought about by microbial colonization of airway mucus. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encodes a cAMP activated Cl- channel located on the apical surface of epithelial cells. The most common mutation, found in 90% of CF patients, is loss of a single amino acid (DF508) which prevents trafficking of the CFTR protein to the cell surface. However, if the protein can reach the cell surface it retains most of its normal channel activity.

The development of treatment strategies for CF has been boosted by the generation of mouse models. We have made both null and DF508 CF mouse models which possess many of the characteristics of the disorder including the primary electrophysiological defect. The DF508 mice also show the CFTR trafficking defect which means that they can be used to evaluate substances for their ability to alter trafficking in a whole animal system.

These CF mice have also proved invaluable for testing the safety and efficacy of potential CF gene therapy treatments. We have shown that delivery of a liposome/CFTR expression plasmid complex into the lungs of CF mice can restore Cl- channel activity in the upper airways. Based on these pre-clinical studies we have successfully completed a double-blind, placebo controlled Phase I clinical trial to deliver a single dose of the CFTR gene to the nasal epithelium of twelve CF patients. On all criteria the procedure was well tolerated and delivery of a functional CF gene was demonstrated in six out of eight patients.

152P IMPACT OF GENE KNOCKOUT MODELS IN XENOBIOTIC METABOLISM AND CHEMICAL CARCINOGENESIS

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During the past ten years, considerable attention has been focused on the role of P450s in human cancer susceptibility. Polymorphisms in expression of P450s and transferases exist in humans and these might render increased susceptibility or resistance to cancer.

It is reasonably certain that P450s are required for carcinogen activation, as demonstrated by *in vitro* metabolism and by cultured cell transformation assays. However, only correlative studies suggest that these enzymes are essential for whole animal carcinogenesis.

Since there are marked species differences in expressions and catalytic activities of the multiple P450 forms that activate carcinogens, understanding how P450s participate in the carcinogenesis process is necessary in order to design and validate molecular epidemiology studies to investigate cancer susceptibility in humans and to develop rodent bioassays that accurately predict chemicals that might be human carcinogens.

To address the role of P450s in whole animal carcinogenesis, mice were produced that lack the P450s known to catalyze carcinogen activation. Mouse lines having disrupted genes encoding P450s CYP1A2 (activates arylamine and heterocyclic amines), CYP2E1 (activates low Mr nitrosamines, benzene, vinyl chloride and other suspect carcinogens) and CYP1B1 (activates polycyclic aromatic hydrocarbons) were established. Mice lacking the phase II enzymes epoxide hydrolase and NQO1 were also developed. These mice exhibit no grossly abnormal phenotypes, suggesting that some xenobiotic-metabolizing enzymes have no critical roles in mammalian development and physiological homeostasis.

However, these mice show differences in sensitivities to acute chemical toxicities, thus establishing the importance of these enzymes in activation/inactivation pathways that lead to toxicity and cell death.

Rodent bioassays using null mice and prototypical genotoxic carcinogens should establish whether xenobiotic-metabolizing are required for carcinogenesis in an intact animal model. These studies will also provide a framework for the production of transgenic mice and carcinogen bioassay protocols that may be more predictive for identifying human carcinogens.

153P AN INDUSTRIAL PERSPECTIVE ON THE USE OF TRANSGENIC MODELS IN DRUG DEVELOPMENT

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The advent of transgenic and targeted "knockout" technologies in the mouse over the last decade and a half has led to a very significant growth in our knowledge and understanding of complex biological systems. The potential of these technologies to produce rodent models with stable modifications in phenotype as a result of introduced genetic changes transmissible through the germline has also been utilized in drug development programmes.

Transgenic rodents are being used by the pharmaceutical industry in drug discovery and development in four main ways, i) target validation/evaluation, ii) provision of disease models, iii) functional analysis of novel genes emerging from genomics efforts and iv) safety evaluation of pharmaceutical agents. Examples from the literature and from our own research programmes will be used to help illustrate and evaluate the contribution of this technological approach to the drug discovery and development process.