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Animal models and human genome diversity: the pitfalls of inbred mice

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Modern medicine and the pharmaceutical industry rely heavily on animal models for drug-target validation. Preclinical drug development is performed almost exclusively in inbred mice and rats. This affords substantial savings by increasing data reproducibility because of the small genetic heterogeneity of the animals. However, these savings are often offset by failed clinical trials, sometimes reflecting the inadequacy of inbred animals to model genetic variation in humans. The solution could include using mouse strains closely related to wild mice and more elaborate comparative genomics of the natural variations of new drug-target genes in humans and mice.

Animal models, in particular mouse models, for human diseases have made a significant contribution to modern medicine. During the second half of the preceding century, mice have become the most widely used species in biomedical research. It would be impossible to envisage modern medicine without the availability of transgenic mice, knockout mice and inbred strains of mice with defined traits, such as obesity, susceptibility to stroke or seizures, and so on.

Inbred strains of animals are generated by mating male and female siblings of >21 generations. The founders for a new strain are sometimes selected for a particular trait, such as susceptibility to a certain disease. This ensures that descendants of the original pair are almost homogenous genetically and, therefore, respond similarly to treatments provoking the onset of disease (such as diet) as well as to drugs designed to combat it. Indeed, marked strain differences exist in the susceptibly of mice to atherosclerosis[1], autoimmune diseases[2], stroke[3] and asthma[4].

Inbred mice: insufficient representation for natural genome diversity

Although the advantages of the genetic homogeneity of inbred mice for medical and pharmacological research are obvious, the pitfalls are sometimes overlooked. Complex diseases involve numerous modifying genes that govern disease severity, age of onset, progression rate and the efficacy of available drugs. The most common monogenic disease, cystic fibrosis, is highly variable even in patients carrying the same specific mutation, demonstrating the significant role of modifying genes in its etiology[5]. The contribution of different genes to phenotypic expression and the progression of complex diseases reflects the patient's share of the natural human-genome diversity, in addition to the contribution of non-genomic factors.

Using a single strain of inbred mice cannot reflect the natural variation of the human patient population. For example, using genetically homogenous mice for studying complex human disorders, such as cancer, asthma and diabetes, might obscure the input of crucial human modifying genes. Such pitfalls are also evident when using transgenic and knockout mouse models, as these are typically produced on specific backgrounds, such as 129/Sv or C57BL6[1]. This might interfere with the identification of new drug targets or, worse, lead to heavy investments in drugs that eventually fail in clinical trials. Indeed, <10% of new drugs tested in clinical trials receive Food and Drug Administration (FDA) approval. Many failures are related to unwanted side effects, sometimes reflecting the different drug metabolism in mice and humans; however, some failures are simply a result of lack of efficacy. This might, in some cases, reflect the inadequacy of animal models to mirror human genome variation, and contribute to the escalating cost of new drugs. A study of drug efficacy using a disease model in a single inbred mouse strain could be compared with a clinical drug trial performed in an isolated South Pacific island population. Clearly, such trials in a non-representative selected sample cannot depict the huge intricacy and heterogeneity of human complex diseases. Indeed, Phase III trials ideally involve several medical centers in different countries to allow, among other factors, generalization for different ethnic backgrounds. Likewise, disease models using a single strain of inbred mice cannot reflect the natural variation of the mouse genome faithfully, and the full spectrum of interactions between products of the major disease-related genes and the disease-modifying genes.

The need for comparative genomics in humans and mice

The realization that our genome is highly variable is changing modern medicine[6]. Humans are much more homogenous than other mammal species because all

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living human populations probably evolved from a small founder population 100,000–200,000 years ago. Yet, our genome includes at least three-million SNPs in addition to thousands of larger polymorphic alleles, such as deletions, insertions and variable-length repeats[7]. Many of these SNPs and polymorphic alleles might contribute to the expression of complex diseases and drug efficacy. Cataloging and understanding the large human-genome diversity and its role in complex diseases remains the most challenging issue for medicine in the aftermath of the Human Genome Project[8].

The mouse genome apparently evolved at a much higher level of natural diversity during more than one million years of separate evolution of *Mus musculus* and *Mus domesticus*. For some homologous human and mouse genes the natural diversity is surprisingly similar[9–12], whereas for other genes entirely different polymorphic patterns have evolved[13]. At present, it is unfeasible to evaluate what fraction of the human and mouse genomes contain similar polymorphic patterns. Certain homologous regions in the mouse and human genomes might contain similar polymorphic alleles (for example, variable length repeats) although they evolved separately; other genes might have evolved completely dissimilar polymorphic alleles.

The prevalence of human and mouse genes that share a similar natural variation pattern is unknown. Resolving this will require scanning large portions of the human and the mouse genomes for SNPs, the most common form of genome diversity. Until such studies are accomplished, this could be performed on a small scale by studying the natural diversity of selected analogous genes in several human ethnic groups and in wild mice (*Mus domesticus*) and/or in a large number of unrelated mouse strains (e.g. strains originated from wild mice from different locations). If such similarities are found, we should consider a more elaborate 'mouse genome diversity project' to supplement the on-going effort of cataloging natural human-genome diversity[7]. Such genome-wide comparative genomic studies will allow the identification of new drug targets that share similar natural variations in mice and humans and, thus, are more suitable for studies in mouse models for human diseases. When such drug-targets are found and the interacting drug demonstrates efficacy in genetically heterogeneous mice, it will be more likely to be effective for humans. For drug targets showing entirely different natural variations in humans and mice, it might be more difficult to extrapolate from mouse data to human disease, but at least we shall be aware of such information when evaluating drug data in mouse models. The cataloging of drug-target genes with similar or dissimilar natural diversities in humans and mice will allow the selection of the most suitable research tools, and eventually the identification of the best drug targets.

Interim solutions

Cataloging the entire natural diversity of the mouse genome would require a dedicated consortium and could take many years. The annotation of the mouse genome itself lags considerably behind the Human Genome Project[14]. So, what can be done in the meantime? Once a new drug target is identified, the natural diversity of its gene-related target should be studied in parallel in humans and mice. Such comparisons should include, in addition to the drug-target gene, genes whose products directly interact with the new target. If the relevant mouse and human genes are found to exhibit comparable variation, the new drug target should be studied, preferably, in parallel in several mice strains that could partially resemble the genetic polymorphism of humans. This might be compared with the advantage of studying drug efficacy in parallel in patients from different ethnic origins, rather than only in Caucasians, as often happens in clinical trials. Indeed, several well-known drugs, such as fluoxetine[15], angiotensin-converting enzyme (ACE) inhibitors[16] and corticosteroids[17], were found to be less effective for African-American patients than for Caucasians. Such observations demonstrate the complexity of drug response in genetically heterogeneous populations.

Another approach for studying polymorphic drug targets is to prepare transgenic mice expressing the spectrum of the human allele repertoire. This method proved successful for studies of the contribution of the various apolipoprotein-E gene alleles in Alzheimer's disease[18], and the adenomatous polyposis coli (APC) gene in colon cancer[19]. Another interim solution, which is valid as long as the drug targets remain unidentified, is to increase the use of mouse strains

Box 1. Outstanding questions

- How well do current mouse models reflect the contribution of human genetic diversity to the etiology of complex diseases?
- Can such models predict which drugs will show efficacy in a larger proportion of patients?
- Can animal models be designed to reproduce, more faithfully, disease-related aspects of human genetic diversity?
- How similar are the allelic polymorphisms of the human and mouse genomes?
- Can we improve mouse models for drug-target validation by analyzing mouse genome diversity?
- What other measures can ensure that new drugs will be efficacious for diverse human populations?

showing higher levels of natural polymorphism compared with classical inbred strains, such as the PWD/Ph and PWK/Ph strains that are more closely related to wild mice[20].

Even with such limitations, mouse and rat models for human disease will continue to be a major resource for drug-target validation in the foreseeable future. However, we should be aware of the limitations of such models to correctly imitate human diseases; particularly when considering the genetic variation of disease-related and disease-modifying genes in humans versus mice. We should keep in mind that drugs showing efficacy in various mouse strains, and in particular in mouse strains more closely related to wild mice, could potentially be more likely to show efficacy for diverse patient populations. When the natural variation of the human drug-target genes is found to be completely unrelated to the homologous mouse genes, more care will be needed for choosing new candidate drug-targets for clinical trials. Ultimately, this knowledge will allow better drug-target selection for complex diseases and an improved success rate for new drugs in diverse human populations.

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What do YOU think about the appropriateness of our general use of inbred animals for research?

Do you think that using strains more closely related to wild-type strains would significantly help reduce the costs associated with high failure rates of drugs in the clinical trial stages?

Or do you think we have to use inbred animals to avoid the complications associated with not using a homogenous group of animals?

Please send your comments to Dr Rebecca Lawrence, News & Features Editor, *Drug Discovery Today*, e-mail: Rebecca.Lawrence@drugdiscoverytoday.com

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