Feature Article

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Designer Mice: Transgenic and Knockout Mice in Toxicology Research

The molecular revolution in science has had an enormous impact on animal facilities. Research shifted from studies in living animals to studies in human tissues and cultured cells, and, most recently to the production of "designer mice." These mice are answering important scientific questions, refining hazard evaluations, and changing the nature of our animal facilities as researchers clamor for increasingly more sophisticated models of disease.

We are all familiar with mutant mice and their importance to research. Nude and SCID mice, for example, are used in tissue transplantation and immunology studies[1,2]. Produced by chance events, these mice are being preserved for their value to science. In contrast, the intentional insertion or activation of genetic material (transgenic mice), or the intentional inactivation of a specific gene or genes (knockout mice) produces most new mutant mice.

The genetic changes in these mice are the result of the experimental design, rather than chance. For example, investigators have produced mice with defects in the gene that causes cystic fibrosis in humans[3–5]. The technology has become sophisticated enough that transgene expression may be tied to tissue-specific functions and, therefore, present only in those specific tissues[6,7]. Mice can be constructed so that although the gene is inactivated (knockout mice), the gene can be turned back on at a predetermined point in mouse growth or development, or in association with other specific events, such as changes associated with the development of cancer (these are called "knock-in" mice[8]). Finally, we can construct mice with more than one transgene or with the loss of function of more than one gene[9]. This ability permits us to study the effects of multiple genes on the development of disease, especially cancer, which is typically considered a consequence of defects in multiple genes.

In toxicology research, transgenic and knockout mice are of value for hazard evaluation, mechanistic studies, and studies of therapeutic intervention in genetic disease. The use of transgenic and knockout mice in toxicology is the subject of several extensive reviews[10–12].

Hazard Evaluation

The commonly held view is that cancer develops through a multistage process involving an early genetic mutation, known as initiation. The initiated cell population expands and develops additional genetic changes leading to cancer[13]. Some of the genetic changes that lead to cancer are the activation of specific cancer-associated genes, the oncogenes. Other genetic changes that lead to cancer are the inactivation of genes that tend to inhibit the development of cancer, the tumor suppressor genes. The gene most frequently mutated in human cancer is the p53 tumor suppressor gene[14]. Mutational activation of the *ras* oncogene plays an important role in both animal and human tumors[13–16].

Taking advantage of this knowledge, the Strategene Big Blue mouse and the Hazleton Muta™ Mouse have bacterial genes incorporated into their own DNA[12]. Because these bacterial genes can be

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reintroduced into bacterial systems by DNA isolation and bacteriophage packaging, mutations in these bacterial genes are easier to quantitate than in normal mammalian genes. These bacterial genes code for enzymes that cause color changes in the appropriate bacterial assay systems. Since they are part of the mouse, however, the effects of the living animal will be reflected in the number of mutations occurring in these genes. In other words, the mutation frequency in a specific organ will reflect the absorption of the toxic agent, the metabolism of the agent, and its effects in a specific organ. These mice are expected to play a role in simplifying the detection of those early initiating events of cancer. On the other hand, the inserted bacterial genes seem to acquire more spontaneous mutations than endogenous genes[17]. Factors that could cause differences in the detection of mutations in endogenous genes and bacterial transgenes, such as differences in DNA repair of bacterial genes, clarify the relevance of each model. Mice with easily detected mutations will probably be developed in the future.

Cancer studies in mice usually take a long time. Transgenic mice containing activated oncogenes, such as *ras* and knockout mice without one p53 tumor suppressor gene, develop tumors earlier than normal mice[9,18]. In the future, these mice may be useful for *in vivo* screening designed to detect potential human carcinogens and for providing dose-response data that will be useful in risk assessment. Provided that these models are both validated and display the anticipated increased sensitivity to carcinogens, their use may significantly reduce the number of animals needed for carcinogenicity testing in the future.

Mechanistic Studies

An explosion in knowledge of the mechanisms leading to toxic injury is largely due to these designer mice. Before transgenic and knockout mice, studying the role of a biological molecule in a response often meant depleting that molecule. The necessary manipulations potentially affected the experimental results. Now, we can increase or decrease the expression of a variety of biological molecules, or change the biological molecule, through transgenic and knockout technology. Researchers are using transgenic and knockout mice to improve the understanding of how chemicals cause cancer[9], how injured lungs can be repaired or scarred after chemical injury[19], and factors involved in the development of alcoholism[20].

Using toxic and pharmacologic agents, researchers are gaining additional insight into some diseases modeled by transgenic mice. For example, human papilloma virus type 16 is an important cause of cervical cancer in women. Researchers constructed mice that express the oncogenes of this virus. Estrogen treatment increased the development of cervical cancer in these mice[21]. This finding is certainly important in the management of women with a history of this virus and could lead to follow-up human epidemiology studies.

Therapeutic Intervention Research

The discovery that mice with genetic defects similar to humans often do not develop the same disease that we see in people is an unexpected result of transgenic and knockout mouse research[22]. For example, inhibition of an alternate biochemical pathway normally functioning in the mouse has improved the mouse model of Lesch-Nyhan syndrome so it more closely mimics the human condition[24]. As the mouse models are perfected, therapies can be investigated for efficacy and safety in mice with diseases analogous to the human disease.

In Alzheimer's disease research, a major goal has been reached with the development of a transgenic mouse that develops the β-amyloid deposits characteristic of Alzheimer's disease[24,25]. These mice can be used in pharmacology and toxicology studies for the development of agents that interfere with the development of these plaques or which promote their removal[26]. This example is just one of the uses of transgenic mice for *in vivo* efficacy studies that investigators would necessarily have previously first conducted on human patients.

Impact on Animal Facilities

Development of these mice in barrier facilities is the exception rather than the rule. This situation is of concern because toxicologists and pharmacologists often bring animals carrying a variety of disease-producing agents into facilities. Some of the designer mice have unusual responses to microbiological agents, and it is possible to see disease in these mice when only seroconversion would be apparent with normal mice.

Importantly, some designer mice, as well as the more traditional nude mice, can become transmitters of rodent and human diseases. This possibility is a concern not only for toxicology research facilities, but for all research facilities using mice with altered immune systems, and is best demonstrated with lymphocytic choriomeningitis in nude mice, a disease which can be transmitted to humans[27]. Recent research has shown that knockout mice with decreased T-cell function are potential carriers of lymphocytic choriomeningitis[28]. In addition, cultured cells can become contaminated with rodent viruses and Mycoplasma[29]. The technology used to construct these mice involves cultured cells, and it is important that the laboratory animal staff work with the researchers to prevent the contamination of cultured cells and the animal colony. It is critical that tumor cells inoculated into mice and embryonic stem cells used to construct designer mice remain free of rodent viruses and Mycoplasma.

Finally, international shipment of knockout and transgenic mice can potentially introduce foreign rodent diseases into our animal facilities. Of particular concern is the demonstration that T-cell deficient nude mice persistently shed Hantaan virus[30], the cause of a potentially fatal human disease which laboratory rats can carry[31]. Facility managers and laboratory animal veterinarians play an important role in preventing the accidental introduction of such agents. By working to prevent risks to research and facility personnel by serosurveillance directed towards these viruses, it is possible to control both the risk and fear of such diseases. An important caveat to serosurveillance in potentially immunosuppressed design-

er and mutant mice is that the room surveillance animals must themselves be capable of mounting an immune response, but must not introduce pathogens into the room.

A widespread concern for animal facilities will be the potential introduction of rodent diseases and the increased susceptibility of some of these mice to diseases that may already be in your facility. It is important to remember that the manner in which these animals are created means they are not "normal." We should not expect that their response to disease will be the same as traditional mice.

For laboratory animal personnel, this difference means an increased awareness of surveillance and isolation procedures. Embryo and cesarian rederivation may be necessary to prevent the introduction of undesirable pathogens into a facility. Pathogens that have not previously caused a problem in your facility may cause significant disease in transgenic and knockout mice. In short, these unique new residents of our facilities are going to present challenges to the facility management that are at least equal in magnitude to the opportunities they offer the toxicologists.

As we begin to respond to the needs of the transgenic mice, we also need to be aware of future directions suggested by this technology. Mice are small, and many studies are difficult to complete in such small animals. In addition, toxicologists need to study species that are metabolizing a toxic agent using the same pathways as humans. Sometimes a species other than the mouse is needed. The technology for the production of transgenic rats already exists[32]. We may well be on the brink of adding many new species of transgenic animals into our animal facilities as these animal models are increasingly refined to answer important questions in toxicology.

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