Shock toxicology with transgenics

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Transgenic models for the short-term assessment of chemical and drug safety have provided alternative systems to traditional animal toxicology studies and ultimately may facilitate the adoption of in vitro assays. Several in vivo models are available for assessing the carcinogenic potential of genotoxic chemicals, but few are applicable to the toxicity testing of chemicals that do not interact with DNA. In this issue, Maria Sacco and colleagues[1] the Institute of Advanced Biomedical Technologies, National Research Council (Milan, Italy) have developed an in vivo bioassay that shows significant promise for the identification and evaluation of harmful nongenotoxic chemicals.

Compared with conventional bioassays, transgenic models allow toxicological studies to be accomplished in a significantly shorter period of time using fewer animals and resources. Models are based on the use of oncogenes, tumor suppressor genes, or DNA repair genes as the principal targets of chemical action. Toxicity is measured on the basis of the induction of tumors at various tissue sites or the use of reporter phenotypes, such as the induction of skin papillomas. Currently, many of these types of models are being assessed in different laboratories around the world, primarily for the evaluation of genotoxic chemicals. However, not all carcinogens are mutagens and many nongenotoxic compounds can exert carcinogenic and toxic effects. Moreover, the importance of nongenotoxic chemicals is now being recognized, particularly with the worldwide application of in vitro testing methods, such as the *Salmonella* mutagenesis assay, that eliminate potential mutagens from product development.

The vast majority of transgenic animals have been designed to specifically address disease syndromes thought to be of genetic origin, but a small number of transgenics have found another role in the field of chemical and drug safety assessment. Examples include *p53* mice[2], which are heterozygous for the *p53* gene; the H-ras model[3], comprising human *c-H-ras* with its endogenous promoter; and the xeroderma pigmentosum group A (XPA) deficient mouse model[4]. These systems are currently being used for the identification and evaluation of genotoxic carcinogens. One model—the Tg.AC transgenic mouse, designed by the Leder laboratory to contain a v-*Ha-ras* gene under the expression of a zeta-globin promoter—has potential in identifying both genotoxic and nongenotoxic carcinogens[5]. Other transgenic mice that are commercially available include the *LacI*-based Big Blue and Muta-mouse models, which are currently used to evaluate the in vivo tissue specific mutagenic potential of chemical compounds. Until now, however, we have lacked a model that is capable of responding to chemicals that are toxic, but not carcinogenic, and still pose a serious threat to human health.

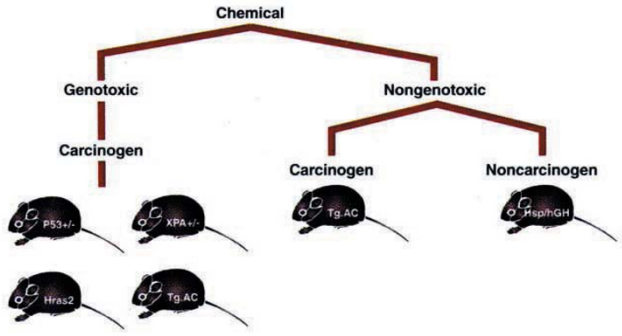

Figure 1. A scheme for the use of transgenic models in drug and chemical safety evaluation.

Enter the novel mouse model presented in this issue. Sacco et al. have engineered mice containing a human growth hormone (hGH) gene under the control of the human heat shock protein 70 (Hsp70) promoter. These mice, when treated with toxic inorganic compounds like arsenic and heavy metals, respond by expressing hGH in their blood. This represents the first transgenic mouse with the potential of evaluating a toxic response to this type of chemical.

Previous work has characterized the inductive effect of chemical stressors on heat shock protein expression. Molecular characterization of the promoters of heat shock protein genes has led to the identification and acquisition of DNA regions (sequences) regulating this response. With these control regions in hand, it was only a matter of time before they could be exploited in a toxicity assay. And by placing the hGH gene under the transcriptional control of the Hsp promoter, Sacco et al. have a readily detectable marker protein with which to evaluate heat shock protein induction.

The simplicity and brilliance of this model relies on the fact that following exposure to a toxic chemical the transgene encoded hGH is expressed and excreted into the peripheral blood for easy collection and analysis. The work, elegantly presented, confirms the construction of the transgenic, after which the authors address the predicted heat shock response. Analyses of primary cells derived from the transgenic were given a 44°C heat shock and were found to express both hGH mRNA and protein. Under wonderfully controlled experiments, the living mouse was shown to respond to heat shock induction by expressing hGH in the peripheral blood.

Having developed an animal that responds to heat stress in a predictive manner is gratifying, but would the mouse respond to toxic nongenotoxic inorganic compounds like arsenic and heavy metals? Carrying out intraperitoneal injection of various inorganic compounds like arsenic and heavy metals and measuring hGH in blood, the investigators demonstrated that this occurs not only in vitro, but also in vivo—hGH expression was induced a few hours after treatment. Over time, this expression subsided, but could be reestablished with a second challenging dose. The model thus may have immediate application to establishing dose parameters for long-term toxicology studies that currently require months of exposure. The authors have yet to challenge the model with a range of nontoxic agents.

The full understanding and utility of this mouse awaits further development. How sensitive and how discriminate is this response? To how many different classes of chemicals will it respond? Will it respond to other known toxicants? Can the effects be measured at a tissue-specific level? These are questions that will no doubt be on the minds of the principal investigators. Regardless, new ground has been broken and we anxiously await future reports.

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1. Sacco, M.G. et al. 1997. *Nature Biotechnology* **15:**1392–1397.
2. Donnehower, L.A. 1996. *Cancer Biol.* **1:**269–278.
3. Suzuki, H. et al. 1996. *Carcinogenesis* **17:**2455–2461.
4. deVries, A. et al. 1995. *Nature* **377:**169–173.
5. Tennant, R.W., French, J.E., and Spalding, J.W. 1995. *Environ. Health Perspect.* **103:**942–950.