Update on National Toxicology Program (NTP) Assays with Genetically Altered or "Transgenic" Mice

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The NTP is evaluating several lines of genetically altered mice for possible use in identifying and assessing carcinogens. The NIEHS/NTP programs and progress in this area were recently reviewed by the NTP Board of Scientific Counselors (BSC). A number of comments and concerns were raised. This commentary summarizes and responds to the BSC review and offers some thoughts on future directions for this line of research as well as possible ways genetically altered mice might be integrated into a comprehensive testing strategy. *Key words:* alternative cancer assays, genetically altered mice, transgenic mice, NTP Board of Scientific Counselors Review. *Environ Health Perspect* 106:619–621 (1998). [Online 2 September] *http://ehpnet1.niehs.nih.gov/docs/1998/106p619-621bucher/abstract.html*

Genetically altered mice have been proposed as adjuncts or replacements for conventional rodents in 2-year chemical carcinogenesis assays. Assays involving these mice hold promise for being more rapid and less expensive than traditional 2-year studies and in theory may be directly relevant to humans in that the mice are genetically engineered to possess alterations in genes known to be involved in many human cancers. For the past several years, an effort has been under way at the NIEHS to evaluate several promising transgenic or genetically altered mouse lines for carcinogen identification and assessment. Two lines have received particular attention; the *p53+/-* heterozygote possesses only one functional copy of the *p53* tumor suppressor gene in all cells. These mice rapidly develop tumors when exposed to mutagenic carcinogens. The other line, the Tg.AC, was produced by pronuclear injection of a v-Ha-*ras* gene under the control of the zeta-globin promoter. The presence of this oncogene confers on skin and certain other tissues an enhanced susceptibility to the development of tumors in response to physical wounding or to mutagenic or nonmutagenic chemical carcinogens. A third line (H-*ras*2), developed in Japan, has multiple copies of the human *ras* gene in all cells (*1*). This line has been evaluated for response to many known carcinogens and several noncarcinogens by Japanese researchers, and limited confirmatory studies are under way with this model at the NIEHS. Results from the NTP/NIEHS "transgenic" studies to date were reviewed by the NTP Board of Scientific Counselors (BSC) on 5 February 1998 (*2*). A report from this review raised several concerns and recommendations. It is important to address these and other issues that have arisen as the NTP attempts to appropriately integrate genetically altered mouse models into a scientifically valid research and testing strategy.

Many of the recent findings from NIEHS/NTP studies of these models are reported in a current issue of the journal *Toxicologic Pathology* (*3*). These reports cover the theoretical bases for the selection of these models as potentially useful chemical carcinogenesis screens, and examine the molecular events required to trigger tumorigenesis in the Tg.AC model. Descriptions of these reports and an update on the overall concordance between the results of 2-year rodent studies with results accumulated with the transgenic models were presented to the BSC.

At the time of the BSC review, results were available from NIEHS/NTP studies on 38 chemicals with the Tg.AC and/or *p53+/-* models, along with previously reported findings from Japan with 18 chemicals studied in the H*ras*2 model. For most studies, the doses used in the genetically altered mice were the same as those used in the 2-year assays. Concordance was defined as a positive result in any genetically altered model for a positive rodent or human carcinogen, or negative results in all genetically altered assays for a noncarcinogen. Not all chemicals were studied in all models, but of the 38 chemicals studied at the NIEHS/NTP, there were 13 results that were considered nonconcordant.

Examination of the 13 discordant results revealed some similarities that may illustrate some general properties of the current models under evaluation. There were 11 chemicals that produced tumors in rodents in the 2-year assays that were not detected as carcinogens in the genetically altered mouse assays. For two of the chemicals, the single tumor response in the 2-year study was hepatocellular neoplasms in the B6C3F$_1$ mouse. This tumor is frequently increased in chemical carcinogenesis studies and is a common spontaneous neoplasm in this strain. For four others, mouse liver tumors were accompanied by tumors of the rat or mouse kidney or mouse adrenal gland in the conventional bioassays. One chemical produced tumors in the rat nasal cavity and mouse kidney in 2-year studies. One nonmutagenic chemical was found negative in the *p53+/-* mouse, an expected result, and has not been studied in the Tg.AC or H*ras*2 models, which may respond to this carcinogen. Three multisite and/or multispecies carcinogens were also not detected by the genetically altered models, and the reasons for these discordant results are under further study. In two cases, a tumorigenic response was obtained in a genetically altered mouse model with a chemical considered negative in traditional rodent studies (seven chemicals were studied). One of these chemicals produced a lesion of questionable neoplastic character in the Tg.AC mouse (myelodysplasia). Perhaps the most important result from this data review was that of the eight chemicals tested that are recognized as known human carcinogens, all eight were identified as carcinogens in the genetically altered mice.

The conclusions from this review were that the new models performed largely according to predictions; they identified all known human carcinogens and most of the multisite/multispecies rodent carcinogens; they consistently failed to identify rodent carcinogens that induced tumors at only a few selected sites in 2-year studies; and finally, while false positives did occur, the overall tendency to miss certain chemicals, which were identified as rodent carcinogens in the 2-year assay, did not support the notion that the genetically altered mice were overly sensitive to chemical carcinogens.

The BSC response to the results and interpretations of these studies was mixed. While the BSC believed that it was appropriate for the NTP to attempt to integrate new models into a research and testing framework and that the selection of the *p53+/-* model was justified, the use of the Tg.AC model was questioned. Responding to research findings demonstrating that chemicals producing a neoplastic response in this mouse do so through activation of a zeta-globin promoter region on the v-Ha *ras* transgene, board members questioned the

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conceptual relationship between the ability to activate this specific promoter, with the broader ability to induce cancer. The Tg.AC was described as widely perceived as a tumor promotion model and, if true, would be unable to distinguish between complete carcinogens and tumor promoters. Other concerns raised by the BSC included the general lack of information on dose response in these initial assessments, the lack of comparative toxicokinetic data in genetically altered versus wild type mice, and the lack of a more complete understanding for the reasons why chemicals inducing tumors in certain tissue sites in the 2-year assay were missed by the new models.

The BSC also recommended that the NTP should continue to develop a research strategy for the next phase of development of these and other transgenic models that may emerge as promising general chemical carcinogen screens or as models for human cancers occurring at sites which are infrequently seen in traditional 2-year rodent studies. Noting that only three chemicals have been shown to induce brain tumors and no chemicals have induced prostate tumors in rodents in the NTP/NCI database, the BSC suggested that models incorporating disabled genes commonly found in human tumors at these sites, and perhaps others such as the colon, may be a useful part of an overall chemical testing strategy.

An additional BSC charge to the NTP was to begin to address how the findings from genetically altered mouse models could be used in risk assessment. Cancer risk assessment models may need to be modified to accommodate the responses in genetically altered mice. Clearly the characteristics of tumor response would be expected to differ in animals harboring a lesion in an early (*H-ras*?) versus late (*p53*?) cancer gene. It is conceivable that certain of these new models will mimic sensitive human subpopulations and, as such, represent a superior tool with which unique human risks could be identified and judged.

Many of the concerns of the BSC are shared by the NIEHS/NTP and are being addressed in ongoing studies. For example, the findings that TCDD and diethylstilbestrol (DES) induced skin papillomas in the Tg.AC mouse are being followed up with studies examining in detail the dose–response relationships for this response. Tumor dose–response characteristics in the Tg.AC mouse can be directly compared with the results from traditional bioassays and with the doses of DES known to result in human cancers. These kinds of studies will begin to reveal how these models can be used in risk assessment. Other chemicals with dioxinlike activity are being evaluated in the Tg.AC model in hopes that cancer potency can be quantitatively related to other dioxinlike actions and that additive, synergistic, or antagonistic tumor responses to mixtures of these chemicals can be assessed. It is likely that the tumor responses to TCDD and perhaps DES in genetically altered mice are through receptor activation, similar to their actions in conventional animals. The dose response for activation of the aryl hydrocarbon and estrogen receptors in comparison to activation of the zeta-globin promoter in the Tg.AC mouse need to be examined. Interesting studies could also be performed with DES in a possible cross between the Tg.AC and the ERKO (estrogen receptor knock out) mouse line developed by K.S. Korach at the NIEHS. The possibilities presented by the Tg.AC mouse for studying the interactions of signaling pathways in receptor mediated tumorigenesis appear great.

Efforts are also under way in response to the question of comparative toxicokinetics in genetically altered and wild-type mice. There is little reason to assume that the *p53+/-* or Tg.AC models would differ in basic metabolic capacities from normal mice, and in fact studies showing similarity in many enzyme activities central to chemical metabolism in genetically altered and wild-type mice have recently been published in abstract form (*4*).

The NIEHS/NTP believes it is very important to further study and understand the apparent inability of the current models to detect all of the rodent carcinogens tested and why the tested models seem to be "blind" to carcinogens producing tumors at certain sites. This seeming deficiency of the new models may actually be an advantage if the tumors missed are, for any reason, not predictive of human responses. Alternatively, the findings may suggest critical insensitivities of the new models to potentially important human health risks. A number of possibilities are being considered. For example, although the *ras* and *p53* genes are clearly altered in tumors in many organs in rodents and humans, many tumors arise without alterations in these genes, and chemicals that act through pathways independent of *ras* or *p53* may not cause an accelerated tumor onset in these models. Another important consideration involves the degree to which strain-specific characteristics of tumor development may influence the response to expression of neoplasia in a genetically altered mouse. A presumption underlying the development of these models was that the strain-specific susceptibility or resistance to expression of spontaneous tumors, or to chemically induced tumors that appear strain- or species-specific, would be avoided in genetically altered mouse assays (*5*). This was held as an advantage of the models and was based on two assumptions. First, the 6-month duration of the studies would be too short for expression of tumors unrelated to the genetic alteration, and second, strain-specific influences on the expression of oncogene or suppressor gene-related tumors would be minimal. The fact that certain multisite, multispecies rodent carcinogens were not found to be tumorigenic in genetically altered mice suggests that these assumptions may not be entirely correct.

Harvey et al. (*6*) showed that strains of mice of both low and high spontaneous lymphoma background, when made

Conclusions from the NTP evaluation of "transgenic" mouse models for carcinogen identification and assessment

- Models performed largely according to predictions
- Models identified all known human carcinogens
- Models failed to identify rodent carcinogens that induced tumors at multiple sites in 2-year studies
- False positives did occur, but the genetically altered mice were also more sensitive to chemical carcinogens

Comments of the NTP Board of Scientific Counselors

- The NTP should integrate new models into a research and testing framework
- Use of the *p53* model is justified
- Use of the Tg.AC is questioned
- More information is needed on
  - Dose response
  - Comparative toxicokinetics
  - Why chemicals inducing tumors in certain tissue sites in the 2-year assay were missed by the new models
- A research strategy should be developed for the next phase of development of these and other transgenic models
- How can the findings from genetically altered mouse models be used in risk assessment?

homozygous for the *p53* null allele had enhanced spontaneous expression of lymphoma compared to wild-type mice. Another tumor type, teratocarcinoma, which is uncommon in any strain of mice, developed rapidly in *p53-/-* mice of one strain but not the other. This experiment gave evidence for a suppressor gene-related effect outweighing strain-specific influences on lymphoma incidence, as well as strain-specific influences enhancing or completely repressing the development of teratocarcinomas. We are unaware of similar studies of strain-specific influences on chemically induced tumors in genetically altered mice. Until these studies are done, it is not possible to determine whether the rodent carcinogens missed by the new mouse models represent strain-specific influences or simply were due to use of an improper study duration or dose.

Clearly genetically altered mouse models hold great promise in carcinogenesis research and testing. The challenge facing the NTP is to design studies that address the concerns and opportunities outlined above while preserving sufficient resources to provide information from traditional prechronic and 2-year rodent assays. The program is currently looking into ways in which genetically altered mouse assays could replace certain prechronic studies. In this way, if the results from the genetically altered mice were not sufficient to draw conclusions on the carcinogenic potential of a chemical or to fully characterize risk, information would at least be available to allow selection of doses for a conventional 2-year assay, which could begin immediately if needed.

Many other studies using genetically altered mouse models are under way at the NIEHS/NTP. These, along with studies being done as part of a cooperative International Life Sciences Institute-coordinated program within the pharmaceutical industry, in partnership with government agencies, should soon provide a much expanded dataset of alternative assay results on which decisions about their further use can be based. Many NIEHS staff have contributed to these efforts and deserve much credit for bringing these new mechanistically based models to where they are today (J.C. Barrett, R. Cannon, R. Chhabra, J. Dunnick, W. Eastin, J. French, G. Lucier, R. Maronpot, J. Mahler, G.N. Rao, M. Shelby, J. Spalding, R. Tennant, K. Tindall, S. Stasiewicz, and M. Vallant).

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