



*The problem of the etiology of tumors, and in particular of carcinoma, has been investigated for a great many years, but has not yet been solved.*

[Yamagiwa K, Ichikawa K. Experimental Study of the Pathogenesis of Carcinoma. J Cancer Res 3:1-29 (1918).]

### What Is a Tumor Promoter?

The concept of the initiation, promotion, and progression of tumors as reflecting the natural history of neoplasia has been widely accepted and discussed for decades. The concept has been most often applied to the characterization of chemicals and other substances that induce tumors. The experimental basis of the concept was derived from the results of studies in which the application of low doses of a DNA reactive (also known as genotoxic) carcinogen did not result in the development of tumors until the animals were treated repetitively with another non-DNA reactive (also known as nongenotoxic) chemical that was itself known not to induce large numbers of tumors. The first chemical has been called an initiator and the second chemical referred to as the tumor promoter. The experimental paradigm came to be called two-stage carcinogenesis and became widely used to study the development of neoplasia. A hallmark of the model was that the effects of the initiator were irreversible, and application of the tumor promoter could be delayed many weeks or months. Also, the promoting substance did not induce tumors following limited exposures. The two-stage model has been adapted to several different tissues including the liver, thyroid, bladder, and, most often, the skin. In this editorial I present my perspective on how the identification of tumor promotion relates to the assessment of human health risk from environmental carcinogens. It is an important issue because few new chemicals are introduced into commerce or the environment today that are traditional genotoxic carcinogens.

In the skin, the early and predominant response is the development of benign squamous papillomas. In the liver, it is foci of cells with an altered pattern of proliferation reflected in the loss or gain of enzyme markers. In some cases these benign neoplasias could be associated with the subsequent development of malignant or invasive tumors such as squamous cell carcinomas of the skin or hepatocellular carcinomas. Further studies on the evolution of the malignancies led to the concept of tumor progression wherein repetitive exposure to a tumor promoter following initiation could result in the acquisition of independent growth potential for some cells associated with the benign neoplasms.

Subsequent characterization of many tumor-initiating agents showed them to be efficient mutagens in *in vitro* and *in vivo* genotoxicity assays. Papillomas induced by a two-stage skin protocol were often found to have characteristic mutations in the *c-Ha-ras* gene. Similar *c-Ha-ras* mutations were found in keratinocytes grown *in vitro* that were treated with an initiator; these keratinocytes developed into papillomas when implanted onto normal skin. In other two-stage models, e.g., rat liver, mouse bladder, thyroid, etc., such gene-specific mutations have not been identified, but the initiating agents are generally DNA reactive chemicals.

The role of the tumor-promoting agents has not been so specifically defined, even in the most well-studied mouse skin model. However, certain properties do appear to be shared by most promoters and are represented by the use of receptor-mediated pathways of induced gene expression. Induction of a variety of



biochemical pathways linked to the expression of individual genes have been identified and are associated with induced cell proliferation, exhibited as hyperplasia in the skin, or proliferative foci in the liver or thyroid.

Another common property is that most tumor promoters are non-DNA reactive. Most non-DNA reactive substances tested in

two-stage models have shown tumor-promoting potential. Few, if any, such chemicals have been reported to show no tumor-promoting potential. In addition, few, if any, DNA reactive or genotoxic substances are only tumor initiators. If applied repetitively, initiators have the capacity to both initiate and promote tumor development and also to cause progression to malignancy.

This is also the phenomenon seen in conventional 2-year carcinogenicity bioassays in mice and rats. Long-term, repetitive exposures to either DNA reactive or nonreactive substances can result in the initiation/promotion and progression of tumors. It can further be said that most of the agents which have shown tumor promotion capability in two-stage models have induced tumors alone when administered repetitively in conventional bioassays. The basis for classification of a chemical as a tumor promoter is therefore conditional and is done only within the context of a two-stage model protocol. These facts suggest that the mechanisms by which tumor promotion is achieved in a two-stage model are also intrinsic to the capacity of non-DNA reactive substances to induce tumors in conventional bioassays. The corollary to this is that the capacity of DNA reactive initiators to induce tumors in conventional assays is intrinsic to their capacity to also promote tumor development. For the vast majority of substances that are carcinogenic, repetitive exposures are required. There are relatively few DNA reactive complete carcinogens that have been shown to induce cancers following single exposures (e.g., X rays, urethane, ethyl nitrosourea). For virtually all other carcinogens, repetitive or prolonged exposures are necessary.

A concern regarding repetitive exposure is that for relatively weak carcinogens it is possible that the power of the assay (i.e., number of animals at risk per dose per unit of time) is inadequate to show a single dose/exposure effect. However, the combined results of over 400 bioassays conducted by the National Toxicology Program (NTP) show that exposures to a maximum tolerated dose (MTD) of the chemical for over two-thirds of the animal's life span is needed for even DNA reactive agents.

If repetitive exposures are required to manifest the carcinogenic potential of substances, the distinction between tumor initiator and tumor promoter becomes problematic. The action of the initiator appears to potentiate the tumor induction process through the induction of mutations in appropriate target cells, which are then provided with a proliferative stimulus by the actions of the promoter.

In conventional bioassays the capacity of an initiator to also promote the proliferation of initiated cells may be somewhat less, but it is still effective when exposures are repeated. The non-DNA reactive carcinogen or promoter may, conversely, be less efficient in creating cells that can be promoted into tumor development. Repetitive exposure to a promoter alone may result in mutations via indirect oxidative pathways or changes in gene regulation that provide cells with a proliferative advantage. The initiation step in the two-stage model thus potentiates the neoplastic process, which then develops over a period of many weeks upon repetitive exposure to the promoter, whereas the equivalent process in conventional bioassays requires a period of many months or years to develop.

The relationships described above have implications in drug and chemical safety assessment and risk assessment. There is a well-defined correlation between mutagenic or DNA reactive potential and carcinogenic potential. In the NTP database approximately 70% of the mutagens have been determined to be carcinogenic. However, even at the maximum tolerated dose, all of the substances have involved repetitive exposures, and many chemicals demonstrated carcinogenic potential only after 2 years of exposures and can only be observed after a detailed postmortem histopathologic examination. In addition, a large number of both DNA reactive and nonreactive chemicals were classified as carcinogens on the basis of a statistically significant increase in the incidence of tumors that have a high historical background frequency and inbred genetic bias in control animals. These agents may be promoting the development of genetically determined tumor cells via mechanisms or pathways that are different from those in two-stage tumor models. The classification of a chemical which causes an increase only in tumors that have a genetic bias in the test species is problematic from a risk assessment viewpoint. In these cases the action of the chemical may be to only indirectly affect the penetrance of the disease gene(s) and may have no consequence to other species or even to other strains of the same species. The interpretation of such effects as "clear evidence of carcinogenicity," however, makes these chemicals equivalent to other more potent carcinogens. Further, the presence of a high incidence of background tumors renders the effort to define the nature of the dose-response curve (i.e., linear vs. nonlinear) difficult and a risk assessment inherently problematic for species in which such susceptibility genes are absent.

The circumstances discussed above have now prevailed for decades and form the basis of most controversy in extrapolating the results of animal studies to humans. Despite the capability to identify tumor promotion potential, the data are not generally utilized in risk assessments. Progress is clearly needed on several fronts to improve our capacity to assess potential environmental cancer risks in humans. Beyond the use of the two-stage model to identify promotion activity, there is no information such as chemical structure or *in vitro* effects to reliably predict potential non-DNA reactive carcinogens. However, if the identification of non-DNA reactive carcinogens also identifies tumor promotion potential, the latter can be a surrogate. We have proposed that transgenic mouse models may be

useful in this effort. Two lines, the zeta globin *v-Ha-ras* (Tg.AC) and *c-Ha-ras* (Hras2) models, have been shown to possess a capability to respond to carcinogens. Both of these models, and the *p53* deficient line that preferentially identifies mutagenic carcinogens, are currently being evaluated in an international pharmaceutical collaborative project that is being managed by the International Life Sciences Institute (ILSI). The results of this effort, which includes other alternative models and 20 chemicals being tested in over 30 laboratories, will provide decisive data for the future use of these models. If true non-DNA reactive carcinogenic potential can be identified in transgenic bioassays of 6-month duration, it will be possible to derive dose-response and dose-rate data on a low to negligible background tumor incidence. Ultimately, it will also provide data for the development of possible structure-activity relationships for non-DNA reactive carcinogens. These systems will also allow for a direct exploration of the induced gene expression hypothesis. The explosive development of gene expression array technology makes it possible to systematically assess the patterns of temporal, spatial, and dose-dependent changes in gene expression in the tissues of mice. By direct comparison to concurrent controls, the genes for which induced expression is transgene dependent can be identified. It is also possible to compare them to changes in gene expression induced in the promotional phase of the two-stage model. Such an approach will identify critical genes and regulatory pathways that are common between the various models and thus identify genes whose induced expression may be a biomarker for non-DNA reactive carcinogens. It is also plausible that genes whose expression is required in highly conserved regulatory pathways for cell proliferation, differentiation, apoptosis, or injury may be identified and tested as surrogate biomarkers for drug and chemical safety in exposed humans. These approaches are speculative at this time, but a better understanding of the molecular consequences of chemical exposure are required if we are to move beyond the concepts that have guided us for the past decades. The availability of reliable alternative models and methods for large-scale analysis of gene expression mandate that we challenge those concepts.

To succinctly restate my proposition, tumor promoters are complete non-DNA reactive carcinogens that are identified in a shorter time by the two-stage assay. Such carcinogens can also be seen in an even shorter time using a transgenic mouse model. The mechanism(s) of carcinogenesis in all three situations involve induced and sustained specific gene expression as a key element. True non-DNA reactive carcinogens must be distinguished, however, from chemicals that show strain or species-specific responses in the long-term bioassays because the latter are unlikely to represent a threat to human health. Recent advances in biotechnology provide new tools with which to verify this hypothesis.

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