

Commentary

Oncogene Activation in Experimental Carcinogenesis: The Role of Carcinogen and Tissue Specificity

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Techniques of molecular biology have been used to determine the relationship of cellular oncogenes to mechanisms of experimental carcinogenesis. Model systems involving three direct-acting alkylating carcinogens, two organ sites, and two species have been employed to elucidate the relationships between carcinogenic etiology, tissue specificity, and activation of known and novel oncogenes. Dimethylcarbamyl chloride, a reactive acylating agent, induces tumors of both the rat nasal mucosa and mouse skin whose DNA is devoid of NIH 3T3 transforming activity. β -propiolactone-induced rat nasal carcinomas contain a novel oncogene, 6 to 9 kb in size, whereas a mouse skin carcinoma induced by this agent possesses an H-*ras* oncogene activated by a 61st codon A to T transversion mutation. The novel oncogene activated in rat nasal tumors induced by β -propiolactone is distinct from one found in methylmethane sulfonate-induced tumors. The implications of these findings for understanding how oncogenes fit into general mechanism of carcinogenesis are discussed.

Introduction

Research into experimental carcinogenesis has provided major insights related to the biology of cancer since the beginning of the century. This field of research has always been quick to adapt concepts and technologies from other areas of biomedical research in order to advance understanding of the carcinogenic process in laboratory animals and humans. The most recent example is the use of molecular biology to address some of the most basic questions concerning the mechanisms by which normal cells can be altered to express the malignant phenotype. Earlier work in experimental tumor virology demonstrating the existence of transforming oncogenes (1) has been extended more recently to discoveries of cellular oncogenes and their activation by environmental carcinogens, such as radiation and chemicals (2).

The concept that all cancers induced by viral, chemical, or physical agents, as well as those due to genetic susceptibility, or of spontaneous origin share a common pathway involving activation of a specific set of genes is an attractive idea for the unification of formerly diverse mechanistic theories. Verification of this hypothesis requires evi-

dence that cellular oncogenes are in fact targets for environmental carcinogens. Moreover, the data from research into cellular oncogene activation must be correlated with the wealth of biological and biochemical data amassed from experimental cancer research during the past decades. Several laboratories have successfully approached these critical issues.

In the context of two-stage carcinogenesis, Balmain and co-workers have shown that activation of H-*ras* is an early event, probably coincidental with the initiation step (3). Furthermore, this group has found that an active H-*ras* gene can serve as an initiator *in vivo* in the mouse skin system (4). We have found evidence that *ras* gene transformation of NIH 3T3 cells *in vitro* may also be a multistage process and that events analogous to promotion are required for complete transformation even after transfection with a purified, active oncogene (5).

Barbacid and his colleagues (6,7) have convincingly demonstrated mutational activation of the H-*ras* oncogene in a manner consistent with the predicted chemical reactivity of the carcinogens used to produce rat mammary tumors. Other laboratories have explored the influences of carcinogen and mouse strain on oncogene activation in murine lymphomas (8,9) and compared patterns of activating *ras* mutations in spontaneous and chemically induced mouse liver tumors (10).

We have been investigating the issues of carcinogen and tissue specificity in experimental carcinogenesis using direct-acting alkylating agents (11). This class of chemical carcinogens does not require metabolic activa-

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tion, and generally produces tumors in diverse tissues of several species. The carcinogens β -propiolactone (BPL), methylmethane sulfonate (MMS), and dimethylcarbamyl chloride (DMCC) produce squamous cell carcinomas of the rat nasal mucosa after inhalation (12) and in mouse skin after topical administration.

Results and Discussion

None of the rat nasal carcinomas induced by any agent contained activated *ras* oncogenes, although DNAs from many of the BPL- and MMS-induced tumors were positive in the NIH 3T3 transfection focus assay. In contrast, none of the DMCC-induced tumors contained DNA with NIH 3T3 transforming activity. It appeared initially that BPL and MMS, which are both SN2 alkylating agents, might be activating a common oncogene. The highly reactive acylating agent DMCC produces a different pattern of DNA adducts according to an SN1 reaction mechanism (13,14). By the technique of comparing restriction endonuclease sensitivity patterns of the transforming activity from these tumors, we found that a common novel oncogene was activated in the BPL-induced tumors. However, this gene was distinct from another novel oncogene reproducibly activated in the MMS-induced tumors. Further characterization of the activated gene from BPL-induced tumors indicated its size to be between 6 and 9 kb (15). This was determined using transformant DNA originally derived from a BPL-induced tumor, digested with Pst 1 (an enzyme previously shown not to alter the gene's transforming activity). The digested DNA was separated on a preparative agarose gel and purified size fractions were used in a transfection experiment. Only the fraction migrating to the region on the gel corresponding to the 6- to 9-kb range possessed transforming activity. We have begun further rounds of transfection using a ligated, selectable marker in order to clone and further characterize this novel oncogene.

The carcinogen specificity in oncogene activation demonstrated by BPL-, MMS-, and DMCC-induced rat nasal carcinomas is strengthened by the data from DMCC-induced mouse skin carcinomas and fibrosarcomas. As in the rat nasal model, none of the more than 20 mouse skin carcinomas or fibrosarcomas induced by DMCC contained DNA with any transforming activity in the NIH 3T3 focus assay. This highly carcinogen-specific effect of DMCC is the first report of consistently negative DNAs from tumors of diverse tissues and species induced by the same agent. As opposed to our experience with DMCC, the role tissue specificity in oncogene activation is illustrated by our results using BPL-induced tumors. As discussed earlier, a novel oncogene of 6 to 9 kb in size was consistently activated in BPL-induced rat nasal carcinomas. However, in the mouse skin model, one of two carcinomas examined contained an activated H-*ras* oncogene (11). This gene proved to be activated by a codon 61 A to T transversion mutation (15), as has been found in other mouse skin tumors induced by aromatic hydrocarbons (16,17). Thus, in the case of BPL, as with other carcinogens such as *N*-methylnitrosourea (7), but in contrast to

DMCC, tissue specificity appears to play a dominant role in the determination of the identity and mechanisms of activation of cellular oncogenes.

The implications of this research are that activation of any single particular oncogene is not sufficient to produce a transformed cell and that several tissue-specific events must occur for transformation to proceed. This conclusion is consistent with data accumulated during years of research into the biology of carcinogenesis. A useful working hypothesis is that the nature of the oncogene(s) involved with cellular transformation is a function of both tissue and carcinogen specificity, and that alternative sets or pathways of oncogenes may contribute to transformation to varying degrees depending on cell type and identity of the carcinogen used.

A model based on the probabilities of particular genes being activated in specific cell types, and the impact of carcinogens on these probabilities has been developed (18). Future efforts at elucidation of the molecular parameters of mechanisms of experimental carcinogenesis involving oncogene activation, as well as other related events, will very likely lead ultimately to a deeper understanding of the common pathways associated with cancer induction in experimental animals, as well as in man.

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