The Role of Cell Proliferation in Multistage Carcinogenesis (43307)

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Carcinogenesis is a complex process in which it is believed that normal cellular growth control genes are altered by sequential mutational events, with subsequent clonal growth of the resulting precancerous or cancerous cells (1, 2). The induction of mutations and the preferential clonal growth of the resulting prenalignant or malignant cells, thus, become critical events in the stages of initiation, promotion, and progression in chemical carcinogenesis (Fig. 1). One class of chemical carcinogens are the genotoxicants. These compounds or their metabolites are DNA reactive and directly induce mutations or clastogenic changes. The observation that most mutagens are also carcinogenic is the basis for many current predictive assays and risk assessment models. However, there are also different classes of nongenotoxic carcinogens that do not interact with the DNA. The class designated as mitogens directly induces cell proliferation in the target tissue. Another class, the cytotoxicants, produces cell death followed by regenerative cell proliferation. For the mitogens and cytotoxicants, differential toxicity and growth stimulation may provide a preferential growth advantage to spontaneous or chemically induced precancerous or cancerous cells. Furthermore, mutagens are much more effective as carcinogens at doses that also induce cell proliferation. Mutational activity may occur as an event secondary to cell proliferation caused by the mitogens or cytotoxicants. Thus, chemically induced cell proliferation is an important mechanistic consideration for the genotoxic and nongenotoxic carcinogens. Both genotoxic activity and induced cell proliferation have been associated with the known human chemical carcinogens (3, 4). Knowledge of the relationship of induced cell proliferation to carcinogenic activity would be valuable in setting doses for cancer bioassays, classifying chemical carcinogens, and providing more realistic carcinogenic risk assessments.

Initiation

Initiation represents initial or early events in the carcinogenic process that predispose a cell to malignant transformation. The predominant view of initiation is that normal growth control genes are either mutated or their expression is altered to produce an active oncogene. Initiation might also result from the loss or inactivation of a tumor suppressor gene(s) (1, 2, 5, 6). However, the term "initiation" is somewhat vague, because so many different mutations or combinations thereof are involved in cancer and because these changes are frequently assessed in tumors, rather than in the earliest stages of tumor development.

Cell Replication Enhances the Effectiveness of Mutagens. Cell proliferation is an integral part of the process of converting DNA adducts to permanent mutations. Therefore, a genotoxic chemical administered at a toxic dose that also induces cell proliferation will be far more effective as a mutagen and as a carcinogen than when given at a low dose that does not induce cell proliferation (7, 8). The increased effectiveness of a genotoxicant in the initiation process has also been demonstrated (i) when the agent is administered to weanling animals in which the cells of the target tissue are proliferating, or (ii) following induced cell proliferation in the target organ, such as partial hepatectomy in the liver. In a population of proliferating cells, there will be less time for DNA repair processes to remove DNA adducts before cell replication converts adducts to mutations (9).

Mutations Occur as Events Secondary to Cell Proliferation. Enhanced cell replication may increase the frequency of spontaneous and chemically associated mutations either by (i) errors in replication or (ii) by conversion of endogenous or exogenous DNA adducts to mutations before DNA repair can occur (10). Events of nondisjunction and mitotic recombination that can occur during cell division are effective in converting a heterozygous mutation to homozygosity or hemizygosity. Increased rates of cell replication associated with clonal expansion of lymphocyte populations in humans have been demonstrated to increase the mutant frequency in those clones (11). The potential for mutational events in growth control genes is enhanced during replication, because many of those genes are being transcribed during cell replication and may experience

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0037-9727/91/1982-0683$3.00/0

greater exposure to endogenous and exogenous mutagens (12–15).

Promotion

Promotion describes the activity of a large and diverse group of agents that accelerate tumor formation by enhancing clonal expansion of preneoplastic or neoplastic cells (16, 17). An extensive amount of literature deals with tumor promotion (18). In contrast to the process of initiation, the action of most promoters can be reversible to some point, and continuous application over extended periods is usually required to effect carcinogenic activity (19). The mechanistic interpretation of promotion is difficult to define because of the complex nature of the process, the possibility that different chemicals promote via different or multiple mechanisms, and the variety of experimental systems used.

Mitogen-Induced Cell Proliferation in Promotion. Mitogenic agents have the ability to directly stimulate cell proliferation and often act through a cellular receptor. Liver mitogens such as phenobarbital and α-hexachlorocyclohexane induce a hyperplastic increase in liver weight that is maintained with continued administration of the compound (20–23). Homeostasis in normal tissue and accelerated growth of preneoplastic foci are characterized by the ratio of the rates of cell birth and death. Evidence to date indicates that mitogenic agents can cause the preferential growth of preneoplastic foci by altering those rates (24).

Regenerative Cell Proliferation in Promotion. Regenerative cell proliferation induced by cytotoxicants may also act as a stimulus for the preferential growth of precancerous or cancerous cells. Simple abrasion of the skin with a felt wheel to remove epidermal cells results in cell proliferation and is a strong promoting stimulus in the two-stage mouse skin carcinogenesis model (25, 26). Surgical removal of part of the liver (partial hepatectomy) results in regrowth of the tissue with a concomitant increase in spontaneous tumor formation in B6C3F1 mice (27). A greater susceptibility of normal cells to the cytotoxic effects of a compound relative to preneoplastic cells would also result in the preferential growth of these preneoplastic cells (28).

Progression

The term "progression" describes later stages of cancer development characterized by changes in the number and arrangements of chromosomes associated with increased growth rate, invasiveness, and metastases. Experimental models have been described to study progressive lesion development in various organs, especially in skin and the rodent liver (1, 2, 18, 19, 29). The same secondary mutagenic and selective growth processes associated with induced cell proliferation in initiation and promotion would also apply to progression.

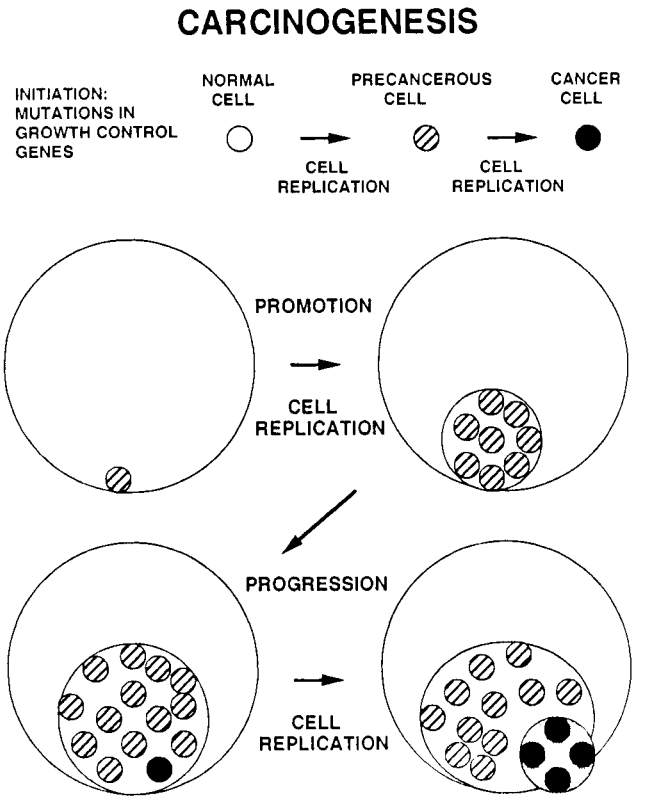

Figure 1. Schematic diagram of the multistep process of carcinogenesis.

Classification of Carcinogens

As new information about the carcinogenic process becomes available, it is increasingly difficult to rigidly define a chemical as an initiator or promoter. It is more instructive to consider the manner in which mutations are induced, or the way in which clonal growth of preneoplastic cells is affected. Initiation, promotion, and progression processes are highly dependent on the dose rate and total dose delivered to the target tissue, as well as on the particular experimental model and species under study. An example of the importance of dose rate delivered to the target tissue in relation to its carcinogenicity is that of chloroform. Administration of very high doses of chloroform, 238 mg/kg/day, by gavage in a corn oil solution 5 days a week for 2 years yielded an 82% incidence of liver tumors in female mice (30). However, there was no tumor response in female mice when doses of up to 263 mg/kg/day of chloroform were given in the drinking water (31). Chloroform is not genotoxic, as judged by a variety of short-term tests (32), and preliminary evidence indicates that there was induced cell proliferation in the gavage, but not the drinking water, study (33). One hypothesis is that a certain dose delivery rate and corresponding metabolic rate must be exceeded in order to kill hepatocytes (33, 34).

The dependence of the perceived activity of a

chemical on the experimental model chosen can be illustrated with 12-*O*-tetradecanoylphorbol 13-acetate (TPA), which is defined as a potent promoter because it accelerates tumor formation in the two-stage SENCAR mouse skin-painting carcinogenesis model. Yet, the same chemical exhibits no activity with the identical protocol when tested on a different strain of mouse (35). It becomes vital to know the characteristics of the animal model used, the pharmacokinetics of the chemical in the body (including the dose and time dimensions over which it reaches the target organ), and the mechanism by which biological changes occur. Because the process of chemical carcinogenesis is complex, a useful starting point for classifying chemical carcinogens is by biological activity (36–40). Suggested classifications are the genotoxicants and the nongenotoxic mitogens and cytotoxicants (Table I).

Setting Bioassay Doses

With so few animals on test in a bioassay, it is important to maximize the ability of these experiments to detect potential carcinogens by including the highest reasonable dose of test compound. This dose is referred to as the maximum tolerated dose (MTD) and has been defined as a dose that has no life-shortening toxicity or more than a 10% decrease in body weight gain (41). Ironically, bioassays are faulted and often required to be repeated at higher doses if *no* toxicity is seen in the study. It is important to be aware of situations in which high doses of the test chemical compromise the health of the animal, overwhelm natural detoxification mechanisms, or yield tumors secondary to excessive organ specific toxicity.

Gathering chemically induced cell proliferation data as part of the 90-day study that precedes a cancer bioassay could provide valuable information to aid in the rational selection of both the high and low doses for long-term studies, and help in the eventual interpretation of the final outcome. If one biological property of a chemical to be tested is the induction of cell proliferation, then the shape of the cell proliferation dose-response curve might be one consideration in setting the MTD. Similarly, selection of at least one dose that does not induce cell proliferation would greatly aid in the interpretation of the results of the bioassay. This may be particularly valuable for the nongenotoxic cytotoxicants that yield tumors only when there is preceding chemically induced cytotoxicity and cell proliferation.

Table I. Classification of Carcinogens According to Mode of Action

Genotoxic carcinogens
DNA reactive or DNA reactive metabolites
Direct interaction to alter chromosome structure or number
May also be mitogenic or cytotoxic
Nongenotoxic carcinogens
Mitogens
Mitogenic stimulation of growth
Mutations may occur secondary to cell proliferation
May cause the preferential growth of preneoplastic cells
Cytotoxicants
Cytolethal
Induce regenerative growth
Mutations may occur secondary to cell proliferation
May cause the preferential growth of preneoplastic cells

Induced Cell Proliferation in Risk Assessment

The most frequently used mathematical models for assessing risk treat all carcinogens as mutagens and assume that no matter how low the dose, there remains the possibility that even a single DNA-reactive molecule might mutate a critical site on the DNA. Regulatory policy is often directly dependent on these models. The challenge for cancer risk assessment is to develop more realistic models that incorporate the roles of pharmacokinetics and mode of action, and both the induction of mutations and the induction of cell proliferation (42).

Over half of the compounds tested by the National Toxicology Program at the MTD are carcinogenic in rodents. Some have raised the concern that many of these responses could be secondary to organ-specific toxicity seen only at the MTD and question the relevance of these observations for predicting carcinogenicity in people at low levels of exposure (43–45). Thus, the mode of action of a carcinogen becomes an important consideration in risk assessments. The biological activity of genotoxicants will be dependent on the time course and dose delivered to the target tissue. Because DNA reactivity is an *intrinsic* property of these chemicals, even small amounts of such chemicals may be assumed to react with the DNA, if delivered to the target cell nucleus. The biological activity of nongenotoxicants will also be dependent on the time course and dose delivered to the target tissue. In contrast to the genotoxicants, however, the carcinogenic activity of the cytotoxic non-DNA-reactive carcinogens may be *conditional* on doses high enough to produce cell death and subsequent cell proliferation. In the special case of those nongenotoxic chemicals that induce regenerative cell proliferation in the target organ secondary to cytotoxicity, the level of concern for potential carcinogenic activity may be decreased below doses that do not induce the cytotoxic-proliferative response. It is difficult to imagine how a single molecule, were it to escape detoxification, might kill a cell. A no observed effect level for induced cytotoxicity-proliferation with a safety

factor would be one alternative risk assessment model for such carcinogens.

Carcinogenesis is exceedingly complex and there are many ways that its onset and progress may be affected by xenobiotics. Chemical carcinogenesis is also a dynamic process that is influenced both by target tissue exposure rate as well as total exposure. The challenges for the toxicologist are to define useful general principles without reliance on simplistic models, to judiciously evaluate key chemical and biological interactions without under- or overestimation of their importance, and to base estimates of risk on all available information without dogmatic adherence to one theoretical approach.

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