Cell Proliferation and Chemical Carcinogenesis: Symposium Overview

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Cancer, by definition, is a proliferative disease. The fundamental scientific issue explored at the international symposium “Cell Proliferation and Chemical Carcinogenesis” was the impact of chemically enhanced cell proliferation on the dynamic carcinogenic processes. This conference, held at the National Institute of Environmental Health Sciences January 14–16, 1992, provided an open forum for the exchange of new results, information, and ideas in four areas: a) general principles of cell division and carcinogenesis, b) critical evaluation of cell proliferation methodologies, c) cell proliferation and modeling of organ-specific carcinogenesis, and d) cell proliferation and human carcinogenesis. This overview summarizes key findings from that symposium. The general view expressed was that although cell proliferation is involved inextricably in the development of cancers, chemically enhanced cell division does not reliably predict carcinogenicity. Our knowledge of the multistep nature of carcinogenesis has advanced substantially during recent years; however, much still needs to be learned. A greater understanding of the cellular and molecular events in chemical carcinogenesis should improve all aspects of the overall risk assessment process, including extrapolations based on dose, species, and interindividual differences.

Introduction

Cell proliferation has long been recognized as an important factor in human and experimental carcinogenesis. The fixation of carcinogen-induced promutagenic DNA damage into heritable mutations and the clonal expansion of initiated cells are two of the steps in the carcinogenesis process that are likely enhanced by cell proliferative stimuli. Partly because cell proliferation is an integral component of the carcinogenic process, controversy has emerged over how and to what extent chemically induced cell proliferation influences the carcinogenic process. In one view, an increased rate of cell division may lead to carcinogenesis; in the other, cell proliferation is considered to be one of many factors involved. An extension of this debate is the opinion that if cell proliferation per se is a primary mechanism of carcinogenesis for some chemicals that do not appear to react with DNA, there would be no increased cancer risk for those chemicals at exposures that do not cause a sustained proliferative response.

An earlier conference, “Chemically Induced Cell Proliferation: Implications for Risk” (1), held in 1989, drew attention and scientific interest to the possible importance of cell proliferation data in the evaluation of animal carcinogenicity studies. Since that time, new data have been generated, and hypotheses related to the role of cell proliferation in chemical carcinogenesis have been refined.

The present symposium was organized to provide a forum for open exchange of results and ideas related to how chemically induced cell proliferation affects the carcinogenic process and on whether cell proliferation data would be useful in cancer risk assessments. The results of scientific debates on this topic are particularly important because they may be used to formulate national and international public health policies.

Participants in this conference comprised a wide range of scientific backgrounds and interests and included cellular and molecular biologists and biochemists active in the area of cell cycle control and gene expression, toxicologists and experimental pathologists studying chemically induced cell proliferation, statisticians attempting to model chemical car-

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cinogenesis, and representatives of regulatory and research agencies responsible for estimating human cancer risk. The conference was held at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina, January 14–16, 1992, and was sponsored by the NIEHS, the Chemical Industry Institute of Toxicology (CIIT), the International Life Sciences Institute-Risk Science Institute (ILSI-RSI), and the American Industrial Health Council (AIHC). Approximately 500 people attended, with participants from Austria, Canada, France, Germany, Great Britain, Italy, Japan, Sweden, and the United States. This symposium overview was authored by members of the scientific program committee.

The conference was organized to address three major questions related to cell proliferation and chemical carcinogenesis: a) Can the stimulation of cell division alone lead to a carcinogenic response? b) What is the mechanistic role of cell proliferation in the carcinogenic process? If chemically induced cell proliferation is detected in an organ that had an increased incidence of tumors, we need to determine whether enhanced cell replication was involved in the process or was primarily responsible for the cancer. c) Should cell proliferation data be used in cancer risk assessment; if so, how?

To address these questions, the conference was organized into four platform sessions consisting of 27 separate presentations, two conference summations with discussions of future directions, plus 38 poster presentations. The sessions were a) general principles of cell division and carcinogenesis, chaired by H. Yamasaki (International Agency for Research on Cancer) and J. C. Barrett (NIEHS), b) critical evaluation of cell proliferation methodologies, chaired by R. Maronpot (NIEHS), c) cell proliferation and modeling of organ-specific carcinogenesis, chaired by J. Popp (CIIT) and G. Lucier (NIEHS), d) cell proliferation and human carcinogenesis, chaired by A. Upton (New York University), e) summation and future directions, presented by J. Swenberg (University of North Carolina) and I. B. Weinstein (Columbia University).

A brief summary and overview of the data and key findings presented at the symposium are given here. More details and extended discussions can be obtained from the individual papers that comprise these proceedings.

**General Principles of Cell Division and Carcinogenesis**

To help understand relationships between cell proliferation and chemical carcinogenesis, it is important to recognize and understand the complex cellular processes involved in cell cycle control (2) and the relationships between DNA damage, DNA repair, and cell-cycle progression (3). It has been postulated that perturbations of cell-cycle controls may cause genetic instability that can increase spontaneous DNA damage and lead to tumor initiation. However, multiple check points exist during the growth arrest phases of the cell cycle that presumably permit repair of DNA damage before cell division. DNA damage at cell-cycle check points (e.g., mutations of the p53 gene may be critical in carcinogenesis by establishing cellular lineages capable of undergoing replicative DNA synthesis with a damaged template (4).

Other factors important in understanding the process of chemical carcinogenesis include a) identifying the cell from which a particular cancer originates [e.g., maturation-arrested stem cell or dedifferentiated mature cell (5)]; b) clarifying the role and relationship of gap junctional intercellular communication and cell proliferation in tumor promotion and progression (6); and c) determining factors involved in regulation of programmed cell death (7). An increase in the life span of a genetically altered cell could increase the possibility that cell undergoing malignant transformation. In the skin, chemically induced sustained hyperplasia correlates well with tumor-promoting activity; however, there is no evidence that cell proliferation, without initiation by a DNA-altering agent, produces skin tumors (8).

The suggestion has been made that increased cell proliferation increases the probability of mutations arising from endogenous oxidative DNA damage, and thus agents given at doses that increase cell proliferation will likely be carcinogenic (9). In contrast to this view, an examination of site-specific histopathologic correspondence between carcinogenicity and chemically induced target organ toxicity for approximately 500 long-term experiments in rats and mice did not demonstrate a correlation between these two morphologic endpoints (10). Thus cytotoxicity does not necessarily predispose a tissue to cancer; organ toxicity occurs without evidence of carcinogenesis and carcinogenesis occurs without evidence of toxicity. Furthermore, in some experimental models, mitogen-induced liver growth, unlike compensatory cell proliferation, was ineffective in supporting the initiation or promotion of liver carcinogenesis (11). It is also hypothesized that increased cell proliferation may result in DNA hypomethylation, leading to increased expression of proto-oncogenes involved in carcinogenesis (12).

**Critical Evaluation of Cell Proliferation Methodologies**

Chemically induced cell proliferation is generally assessed after the administration of DNA precursor labels, 'H-thymidine or bromodeoxyuridine, or by analysis of endogenous cell replication markers, such as proliferating cell nuclear antigen (13). Measurements of overall cell replication in an organ do not distinguish between cell division in normal differentiated cells from that in stem cells or in preneoplastic cells. To better understand potential relationships between
cell proliferation and chemical carcinogenesis, a considerably larger database is needed of sustained cell replication induced by mitogenic and cytotoxic chemicals in target cell populations and in preneoplastic lesions. Subtle treatment-related differences in labeling index can be detected with properly designed experiments, by analyzing a sufficient number of tissue samples to minimize variance in estimated values, and by employing appropriate statistical evaluations (14). In addition, quantification of cell proliferation in the rodent liver using DNA precursor labels or by measuring mitotic activity should be considered in conjunction with the variables of hepatocyte ploidy and nuclearity in rats and mice (15); this can be partly facilitated by using flow cytometry in conjunction with DNA labeling techniques.

**Cell Proliferation and Modeling Organ-Specific Carcinogenesis**

Studies of proliferative responses in specific organ systems and mathematical models of chemical carcinogenesis were presented to further explore relationships between these processes. Although an empirical association between cell proliferation and cancer in certain tissues of rodents and humans has been proposed, an increasing number of examples have been found in which cell proliferation or chronic toxicity and epithelial cell degeneration/regeneration was not associated with carcinogenesis (16). Chemically induced cell proliferation frequently does not correlate, qualitatively or quantitatively, with the development of tumors. The data currently used for estimating parameters in mathematical models of carcinogenesis are generally not sufficient to differentiate between chemical effects on initiation, promotion, progression, or completion stages (17). These data also fail to allow multistage models of carcinogenesis that incorporate growth kinetic data to be distinguished from those that do not (e.g., Armitage-Doll models). Furthermore, measuring mitotic rates without considering relative changes in DNA repair may not necessarily provide meaningful estimates of changes in mutation rates. Mathematical models that reduce complex processes to single parameters may lead to erroneous results.

The growth of normal tissue and of cancer tissue is determined by the difference between rates of cell replication and cell death. Studies on the regulation of apoptosis (programmed cell death) may be helpful for understanding mechanisms of initiation and promotion of liver cancer (18) and of tumor development in other organs. Transforming growth factor, TGF-β, appears to be involved in the initiation of apoptosis in hepatocytes. Experimental studies of liver carcinogenesis indicate that initiated cells may die without giving rise to intermediate populations of preneoplastic foci; this occurrence has also been predicted in mathematical models of carcinogenesis that include rates of cell division and rates of cell death as separate parameters (19).

In experimental studies of skin carcinogenesis, stimulation of epidermal cell proliferation alone is not a reliable predictor of tumor promotion; hyperplastic transformation with keratinocyte activation and the release of growth factors are critical for tumor development (20). A stochastic-based mathematical model of skin tumor promotion predicts that initiated cells may not exhibit a growth advantage over normal cells (21).

Induced cell proliferation was proposed to influence dose-response relationships for urinary bladder carcinogenesis induced by genotoxic chemicals and to be an important factor in the induction of urinary bladder cancers by certain nongenotoxic chemicals (e.g., sodium saccharin (22)). Formation of microcrystals containing sodium saccharin in alkaline urine of rodents may be responsible for the enhanced urothelial proliferation that is associated with the induction of bladder tumors by sodium saccharin. α2u-Globulin nephropathy and sustained increases in cell proliferation have been correlated with kidney neoplasia in male rats exposed to unleaded gasoline and to d-limonene; additional correlative studies and a better understanding of mechanisms of chemically induced renal cell proliferation are necessary before cause-and-effect relationships can be established (23). In studies of nasal lesions induced by inhaled chemicals, correlations between site specificity of carcinogenesis and increases in cell proliferation are important in understanding relationships between these two occurrences and in assessing human risk. Although sustained increases in cell proliferation appear to be relevant to nasal carcinogenesis induced by formaldehyde, it was also noted that some inhaled irritant gases do not induce nasal tumors after chronic exposure (24). Various nonmutagenic phenolic antioxidants (e.g., BHA: butylated hydroxyanisole, caffeic acid, sesamol, 4-methoxyphenol) cause dysplastic lesions with accompanying increases in DNA synthesis in the forestomach of the rat; these lesions persist after cessation of chemical treatment and presumably play a role in the development of forestomach carcinomas (25).

**Cell Proliferation and Human Carcinogenesis**

Mutations in the p53 tumor-suppressor gene may alter cell-cycle control, differentiation, and neoplastic potential and are often present in diverse types of human cancer. Inactivation of p53 may occur by genetic or epigenetic (e.g., DNA hypomethylation) alterations (26). Human cancers associated with steroid hormones, drugs, infectious agents, chemicals, or chronic irritation were suggested to be due to an increase in the rate of genetic errors resulting from enhanced cell division (27).

Different views were expressed by representatives of regulatory and international cancer research agencies. For nonmutagenic compounds that produce toxic lesions or regenerative hyperplasia before the forma-
tion of tumors, it may be possible to establish a safe level of exposure by determining the threshold level of response and applying a suitable safety factor (28). Risk assessments should factor in cell proliferation data for chemicals that are believed to cause cancer secondary to enhanced cell replication (29). All factors that are known to influence the carcinogenic response should be incorporated into quantitative estimates of human cancer risk; however, the use of cell proliferation data to modify tumor dose-response data has not been justified (30). A greater understanding of carcinogenic mechanisms is needed before data on chemically induced cell proliferation can become incorporated into an overall evaluation of carcinogenicity (31).

Summation and Future Directions

Although cell proliferation is involved in the development of cancer, chemically enhanced cell division does not reliably predict carcinogenicity. To clarify the role of cell proliferation in chemical carcinogenesis, a larger database is needed on possible dose-response correlations between sustained cell proliferation and carcinogenicity. Further work is also needed to increase our understanding of factors influencing sex, species, or tissue specificity and to enable comparisons between the mutational spectra for background tumors and those associated with chemically induced cell proliferation in normal tissue (32). Many animal organ systems can tolerate high levels of cell proliferation without developing cancers. Molecular biomarkers are needed that will identify and distinguish clinical and subclinical changes occurring in normal cells undergoing neoplastic transformation (33). A greater understanding of the cellular and molecular events in chemical carcinogenesis should improve all aspects of the risk assessment process, including extrapolations based on dose, species, and interindividual differences.

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