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COMMENTARY

Mechanisms, Chemical Carcinogenesis, and Risk Assessment: Cell Proliferation and Cancer

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Mechanisms of carcinogenesis — and in particular chemically associated carcinogenicity-have attracted considerable scientific and public attention in the last decade. Much insight has been gained that will lead to more reasoned and better prevention, intervention, and treatment for the reduction of environmentally caused cancers. However, there seems to be an exaggerated tendency to embrace "mechanisms" not yet fully characterized, completely tested, unequivocally proven, and consensus accepted. More than 100 agents and exposure circumstances have been identified as causally or strongly associated with human cancers; for many the evidence was discovered first in experimental animals. More chemicals have been uncovered as carcinogenic in experimental animals, with as yet no or little available information in exposed human populations. Additional and expanded mechanistic and epidemiological studies should further elucidate the relevance of these agents to adverse human health effects, including cancers. Claims are being posed that certain chemical-specific "mechanisms" in experimental systems are irrelevant to humans, and thus chemicals thought to be aberrantly carcinogenic in animals would present no cancer hazard to exposed humans. Nonetheless before undeniable proof becomes available, we must continue to proceed with sensitive and responsible caution. This commentary offers a central and personal view of one such mechanism: cell proliferation and cancer. © 1995 Wiley-Liss, Inc.*

Key words: carcinogenesis; cell proliferation; risk assessment; experimental carcinogenesis; bioassays; animal carcinogens; human carcinogens

INTRODUCTION

Strategies for accomplishing risk assessments for protecting workers and the general public from exposures to harmful circumstances are being revamped to include "mechanistic information." A major difficulty confronting researchers and regulators centers on what is actually meant by the oft-used term "mechanism": to many this term connotes simply pharmacokinetics, whereas to others the word means molecular bases of action. Some have additional notions with respect to genotoxicity,

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metabolism, toxicity, and so forth. In chemical carcinogenesis, for example, discussion and research center on the role that exogenously enhanced cell proliferation may have on the carcinogenesis process. No consensus exists on this particular issue; for some, confusion persists because without cell proliferation cancer could not occur, and thus individuals might conclude that if a chemical induces cell proliferation this must then be mechanistically influential in the carcinogenic process. Not true.

CELL PROLIFERATION

Cell proliferation is typically estimated (assumed) by quantifying S-phase nuclei. Most reported cell proliferation data reflect replicative DNA synthesis, without demonstrating that the S-phase nuclei proceed to divide and accumulate. Thus, the term cell proliferation and other synonyms should be replaced by more specific terms such as what was specifically measured: replicative DNA synthesis, S-phase labeling index, BrDU labeling index, and so on. Clearly, reporting mitotic indexes allows more confidence in cell division taking place; yet even in this instance, without cell death rate measurements, there may be no net increase in cells. Several views expressed at a recent National Institute of Environmental Health Sciences (NIEHS) international symposium on cell proliferation and carcinogenesis placed this important issue in better perspective: while cellular replication must take place for cancer development, chemical-induced cell proliferation per se may **or** may not influence chemical carcinogenesis; enhanced cell division is not reliably predictive of carcinogenicity; and no generic postulate can be formulated.'

Nonetheless, some scientists and administrators continue to insist that chemicals causing increases in cell replication would also lead to cancer, and studies of cell proliferation would divulge the mechanism of tumor formation. This notion has been used to "explain" why certain chemicals induced cancer in laboratory animals; but a testable or biologically based "mechanistic hypothesis" has yet to be systematically formulated and evaluated. At times we develop and use certain terminology for communicative purposes that tends to lack precision: e.g., "tumor promoters'' and "nongenotoxic carcinogens.'' Unfortunately, these terms often become "mechanistic-based,'' rather than only operational, and we begin to "believe" our own hypothesis without having the necessary supporting experimental information (re: satisfaction of Koch's postulates). This seems to be where we are at the moment regarding the impact of enhanced cell division on chemical carcinogenesis. Much work is being accomplished; yet considerably more needs to be done, however, to enable us to make better and more scientific-seated mechanistic decisions.

EXPERIMENTAL STUDIES

Few studies have been designed and conducted to answer the question: "What influence does chemically enhanced cell proliferation have on chemical carcinogenesis?" In the aggregate, these have been relatively short-term experiments of comparatively limited duration: **1–13** weeks. However, in most reports the connection between chemically augmented cell proliferation and cancer has not been proven; and in most, has been shown to play no obvious role in the carcinogenic process. Better

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and more experiments are needed (e.g., using the same long-term exposure conditions of carcinogenesis bioassays) before we will be able to reliably conclude whether the proposed mechanistic hypothesis is or is not correct. And these will likely be chemical and organ specific.

Thus, cell replication (proliferation, turnover, mitogenesis, division) is an important and essential factor in chemical carcinogenesis, yet available scientific data do not sustain the hypothesis that enhanced cellular proliferation in a particular organ associates consistently with or mechanistically causes an increased induction of neoplasia. Increased cellular proliferation is but one of a number of hypotheses that might explain or contribute to the resultant neoplastic transformation.

Cell proliferation per se may influence the carcinogenic processes but there are arguments for and against this hypothesis. As mentioned, one of the major confusing factors that leads some to the notion that agent-enhanced cell proliferation persuades towards cancer comes from the knowledge that without cell proliferation there would be no cancer. That is, in the absence of cell division, any "damaged" DNA would be repaired or remain without consequence. Because cell proliferation is necessary for carcinogenesis, then the implication is frequently made that enhanced cell turnover would be mechanistically associated with the resultant cancer. However, the available experimental data do not support the view that simply increasing division of a "normal" cell population is or would be sufficient for carcinogenesis.

CHEMICALS AND CELL TURNOVER

Chemicals that only enhance cell proliferation may have no or little influence on the multistep and multimechanistic carcinogenesis processes. However, if a carcinogenic substance also induces sustainable cell proliferation, it might influence one or more or a combination of steps in the overall process by potentially involving one or both of two possible effects: 1) reduce the latency period for tumor development by obviously increasing populations of both transformed and nontransformed cells or/and 2) promote an increase in incidence of detectable tumors induced by alternative mechanisms by simply stimulating more potential stem cells. Still, chemicals having no inherent carcinogenic potential would not be expected to "cause" cancer simply by increasing cell proliferation. Chemical combination experiments including "noncarcinogenic cell proliferators" could lead to better understanding of the role this biological effect has in carcinogenesis processes.

Available studies of cellular proliferation of known carcinogens have been examined and the unambiguous conclusion is that short time-point measurements of cell replication do not predict long-term effects, and that mitogenesis or cell proliferation is merely a part of the carcinogenic process. In many instances, the observational data actually contradict the hypothesis: i.e., tumors occur in organs exhibiting no "increases" in cell division, cell proliferation is significantly enhanced without any tumors being discovered, or combinations of these two findings. In some instances, DNA synthesis is substantially increased in the same organ of both sexes or in the same sex in two species—yet tumors develop in only one of the sexes or in one of the species. Experimental findings such as these need to be fittingly pursued, deciphered, and resolved.

RESEARCH QUESTIONS AND CELL REPLICATION

Answers to the following (and other) questions may allow a better foundation to formulate a mechanistic-based hypothesis about cell proliferation and cancer:

1. whether chemically induced cell proliferation in normal tissue can be taken to indicate that a greater incidence of ''spontaneous" mutations (initiating events??) would occur
2. whether measuring cell turnover in normal tissue (vs. preneoplastic or neoplastic tissue) has any relevance to the carcinogenesis process
3. whether an "adaptive" short-term (e.g., a few to several days) increase in cellular proliferation would influence the carcinogenic process (especially and typically observed for nongenotoxic chemical carcinogens thought to need "continuous" and long-term exposures for carcinogenesis)
4. whether enhanced cell replication must take place in stem cells vs. those terminally differentiated (and unable to clonally expand)
5. whether "pure" mitogenic (and nontoxic) chemicals are carcinogenic by the simple mechanism of cell replication (and hence increase the probability of "mutations" or "genetic errors")
6. whether the impact of chemically induced cell proliferation always, never, or only sometimes leads to or accelerates cancer
7. whether cell replication influences each (or one) step of the multistage and multimechanistic processes of carcinogenesis
8. whether this mechanism would likewise increase the incidence of cancers in humans exposed to drugs, alcohol, tobacco, industrial chemicals, environmental. estrogens, "promoters," viruses, irritants, heat, and any number of other potential agents known or capable of inducing cellular damage or death and subsequent regenerative physiological adaptive processes, including cellular replacement.

MECHANISMS AND RISK ASSESSMENT

As with pharmacokinetics, metabolism, toxicity, genotoxicity, other mechanisms, and auxiliary experimental data (apoptosis; proto-oncogene activation; tumor suppressor gene inactivation) and carcinogenesis hypotheses, one would be ill–advised to seriously consider using cell proliferation or other mechanistic findings to significantly influence the risk assessment and risk management processes necessary to protect public health. Available data are incomplete, hypotheses being promoted are under test, and the scientific debates rightly continue. Meanwhile, we need to honestly communicate not only what we do (or think we) know, but also we must disclose our lack of conclusive knowledge to the public and to the public health administrators who must deal with these frequently contentious and subjective issues.

Interestingly enough, as we learn more and more about the intricacies and multiplicities of "mechanisms of carcinogenesis" and carcinogenesis processes, we have not been able to conclusively and unequivocally describe—after only four or five decades of intense searching—the complete mechanistic process for a single chemical carcinogen. Undoubtedly significant gains have been and are being made, but we still await a solitary and inarguable mechanism of action of carcinogenicity for even one chemical. For a few we are close. Some believe, including myself, that a

mechanism or "unified theory" of carcinogenesis may not be congruent with available information; perhaps more logically, we must continue to approach each chemcial (or class of chemicals) as if it exhibits a distinct mechanism or mechanisms of cancer induction (e.g., individual benzene metabolites; dioxin and receptor-mediated effects; diethylstilbestrol (DES) and hormonal perturbations), and may indeed function differently among different species or genders. Of course this hypothesis may be disproven as well, as we gather further information and more knowledge.

α-2μ-GLOBULIN

Meanwhile regulators and administrators should continue to be considerably cautious before embracing a purported mechanism—even a reasonable proposed hypothetical mechanism of carcinogenesis—as being pivotal to public health decisions. One example still garnering considerable debate centers on whether chemicals that induce cancers of the tubular cell epithelium of the kidney in male rats, concomitant with an increase in cell proliferation and testosterone-mediated α-2μ-globulin protein, are relevant or useful to overall hazard identification for humans. The U.S. Environmental Protection Agency (for example) has embraced the concept that these experimental carcinogenic responses are not important to the human situation, whereas others (e.g., the International Agency for Research on Cancer) appear properly cautious, or have proposed equally reasonable alternative hypotheses. For example, and yet to be clarified, there are several chemicals known to induce this specific nephropathy syndrome that do not cause kidney cancers, and may or may not produce cancers in other organs; this lack of consistency must be explained. Importantly, gasoline is one complex mixture that induces these "rodent-unique" cancers with the opine of having no real or potential risk to humans; yet accumulating epidemiological evidence shows an association with increases in cancers of the kidney in cohorts exposed occupationally to aviation fuels. Continuing experimental and epidemiological efforts should lead to more definitive mechanistic and public health answers.

MECHANISM, LOGIC AND PUBLIC HEALTH

Thus, practicality and prudence commands us to be convincingly certain before using experimental data to promote or formulate mechanistic hypotheses in an attempt to permit increased (or to promulgate decreased) levels of exposures to chemicals known to cause cancers in laboratory animals. Conversely (and meanwhile), one should continue to use in vivo data to protect public health and to reduce the incidence, morbidity, and mortality of cancers from exposures to chemicals known to cause these diseases in animals or in humans. On the other hand, we should be willing to believe, for example, that another benzidine-based dye or nitrosoamine or anthraquinone or aniline or phenylenediamine would behave toxicologically like all the other chemicals belonging to those groups already shown convincingly and consistently to be carcinogenic. Likewise, one should be reasonbly confident that the next classical antihistamine or penicillin/tetracycline antibiotic would not be carcinogenic in experimental animals. Obviously, empirical relationships or predictions such as these need mechanistic or bioassay substantiation.

Ergo, mechanisms of action are not considered to be understood well enough to become significant factors in the evaluations of chemically induced carcinogenesis, but certainly all relevant and available biological data and other pertinent information including available mechanistic hypotheses information should be considered when deciding on the overall evidence of the carcinogenic responses. And, thus, for extrapolating or interpolating among mammalian species in risk assessment and risk management approaches. The cascade of events collectively termed carcinogenesis may eventually be deciphered fully enough to allow better predictions of carcinogenic risks to humans. After all, enriched human health and prolongation of life remain the ultimate collective goals of biomedical research efforts.

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