Cell Proliferation and Carcinogenesis Models: General Principles with Illustrations from the Rodent Liver System

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Rates of cell proliferation, cell death, and cell differentiation affect the risk of cancer profoundly. An increase in cell proliferation rates leads to an increase in mutation rates per unit of time, which, in turn, leads to an increase in the risk of cancer. An increase in cell division rates relative to death or differentiation rates may lead to an increase in the population of critical target cells, which, again, leads to an increase in cancer risk. These fundamental principles are well illustrated by the rodent liver model for carcinogenesis. In this paper I briefly discuss some of the consequences of incorporating cell proliferation kinetics into quantitative models of cancer risk assessment. Consideration of cell kinetics can shed light on apparently paradoxical observations, such as the observation that the administration of two different promoters may lead to the same volume fraction in the rodent liver, with one promoter giving rise to a large number of small foci and the other to a small number of large foci. Another observation that can be illuminated by a consideration of cellular proliferation kinetics is the phenomenon of the inverse dose-rate effect. It has been observed with exposure to high LET radiation and to certain chemicals that fractionation of a given total dose of the agent leads to an increased lifetime probability of tumor. A biological explanation of this finding can be given in terms of the effect of the agent on cell proliferation kinetics.

Introduction

There is now considerable evidence that the somatic mutation theory of carcinogenesis is essentially correct. At the same time, there is increasing appreciation that cell proliferation kinetics play a critical role in malignant transformation (1). Cell proliferation may affect the risk of cancer in one or more of at least two ways. First, an increase in the rate of cell proliferation may lead to an increase in the rate of mutation per unit time and thus to an increase in the risk of cancer. Second, an increase in cell division relative to cell differentiation or cell death may lead to an increase in the population of cells susceptible to malignant transformation, which, in turn, has the most profound effect on cancer risk. In this paper, I present some of the general principles that arise as a natural consequence of consideration of cell kinetics in quantitative models of carcinogenesis.

There is now clear-cut evidence that programmed cell death, or apoptosis, is just as important as cell division in determining cancer risk. Some promoters may inhibit apoptosis (2) and some oncogenes may do the same (3). Thus, it is important to explicitly consider apoptosis in mathematical models that incorporate cell proliferation kinetics. There is a mistaken impression (1) that, in mathematical models with cell kinetics, it is sufficient to consider only the net rate of growth of cells, i.e., it is sufficient to consider the difference between the rate of cell division and the rate of cell death. This is false, as I will try to illustrate by means of examples. Similarly, some so-called simulation models (1) consider only the mean number of cells in each of the stages on the pathway to malignancy. This again leads to erroneous results: stochastic considerations cannot be ignored.

The Model

Most of my qualitative conclusions are general and do not depend on a specific model of carcinogenesis. Quantitative conclusions are based on a simple model for chemical carcinogenesis (Fig. 1).
Initiation and Conversion

The model assumes that normal target cells are transformed into cancer cells via an intermediate stage in two rate-limiting, irreversible, hereditary (at the level of the cell) steps. Intermediate cells are assumed to be generated from normal cells as a nonhomogeneous Poisson process with intensity $\nu(s)X(s)$, where $\nu(s)$ can be thought of as the rate of initiation per cell per unit time and $X(s)$ is the number of normal cells at risk at time $s$. The second step is the conversion of intermediate cells into malignant cells with rate $\mu(s)$ per cell and unit time. Precisely, an intermediate cell divides into one intermediate cell and one malignant cell with rate $\mu(s)$.

Promotion

The salient feature of promotion is assumed to be growth (clonal expansion) of intermediate cells as a stochastic birth-death process with cell division rate $\alpha(s)$ and death (differentiation) rate $\beta(s)$. Details of the model can be found elsewhere (4,5).

Some Consequences of Explicitly Considering Cell Division and Cell Death

If the rate of apoptosis is greater than zero, then there is a non-zero probability that an initiated cell will die without giving rise to a detectable lesion such as a papilloma on the skin or an altered focus in the liver. To some biologists, this conclusion may come as a surprise, because the irreversibility of initiation is current dogma. I am not suggesting that individual initiated cells revert to the normal phenotype, but because some initiated cells die, initiation is partially reversible on the level of the organ. Because normal cells that are initiated (altered) may die without giving rise to foci, it is impossible to determine from the number of observable foci alone how strong the initiating action of an agent is (6,7).

For the sake of simplicity, let us assume that the rates of cell division and apoptosis are constant. Then, the probability that an initiated cell and all its progeny die is given (asymptotically) by the ratio of the apoptosis rate and the cell division rate. If the rate of apoptosis is larger than the cell division rate, then the (asymptotic) probability of extinction is 1. However, some foci may still be visible because of the stochastic nature of the process. In recent analysis of altered foci in the rat liver, we conclude that the vast majority (approximately 90%) of initiated cells die without giving rise to foci (8). Some support for this conclusion is provided by the observations of Satoh and colleagues (9) and more recently by those of Schulte-Hermann (10) on the time-course of the number of GST-P-positive single cells following initiation. From his data, Schulte-Hermann estimates that approximately 80% of GST-P-positive cells become extinct without giving rise to foci. This figure is in remarkably good agreement with the estimate above, which is derived from theoretical considerations.

The mean number of initiated cells at any time depends on the net rate of cell division $\alpha - \beta$. However, there is considerable stochastic variation around this mean number, and this variation depends on $\alpha$ and $\beta$ individually, not just upon their difference. Further, the distribution of altered cells in foci also depends on $\alpha$ and $\beta$ individually. Thus, for a given value of $\alpha - \beta$, large values of $\alpha$ and $\beta$ lead to small numbers of large foci, and small values of $\alpha$ and $\beta$ lead to large numbers of small foci. Consider a hypothetical example. Suppose $\alpha - \beta = 0.01$ per cell per day, and consider the following two combinations of parameters: $\alpha = 0.5$, $\beta = 0.49$ and $\alpha = 0.1$, $\beta = 0.09$. Both these combinations of parameters lead to $\alpha - \beta = 0.01$ and thus to the same mean number of initiated cells (assuming, of course, that the rate of initiation is identical). However, the first set of parameters will lead to a small number of large foci, whereas the second set will lead to a large number of small foci. All other things being equal, the first combination of parameters carries a higher risk of malignant transformation than the second. This is because, in the absence of the compensatory increase in the rate of repair, a high cell division rate implies a high mutation rate. Examples of the phenomenon described here are provided by promoters such as 4-dimethylaminoazobenzene and the peroxisome proliferators, which lead to a small number of large foci, and others such as N-nitrosodimethylamine (NDEOL) and phenobarbital, which lead to a large number of small foci. By measuring labeling indexes, it should be possible to confirm that division rates in foci associated with the former compounds are higher than the division rates in foci associated with the latter compounds.

The incidence of malignant tumors depends on both $\alpha$ and $\beta$, not on just their difference. One reason for this was pointed out above: a large cell division rate implies a large mutation rate. However, even if the mutation rates are assumed to be independent of cell division rates, the incidence function depends on both...
α and β individually. This is a simple mathematical consequence of the model. Thus simulations that take into account only the mean behavior of cells in the intermediate compartment (1) lead to erroneous results for the incidence of malignant tumors.

**Inverse Dose-Rate Effect**

The inverse dose-rate effect is a curious phenomenon that has been described with respect to exposure to high LET radiation and certain chemicals. It involves fractionation of a given total exposure leading to an increase in the lifetime risk of tumor. It turns out that agents that affect cell proliferation kinetics are predicted to have such an effect. I discuss this in greater detail below.

**An Example.** We have used the model presented above for the analysis of data on altered hepatic foci in rodent hepatocarcinogenesis experiments. It is well known that the rodent hepatocarcinogenesis model is characterized by the appearance of foci that exhibit alterations of enzyme expression. These foci are clonal, and at least some of them are believed to be premalignant lesions. It seems reasonable to believe that by studying the temporal evolution of these foci as functions of the doses of the agents under investigation, one should be able to derive estimates of the parameters relevant to initiation and promotion. I will illustrate by means of a simple example without going into any technical details, which are given in recent papers (8,11). In this example, rats were exposed to various concentrations (0, 0.1, 1, 5, 10, 20, 40 ppm) of N-nitrosomorpholine (NNM) in their drinking water. The following information was available on each animal: the concentration of NNM in the drinking water, the age of the animal when it was sacrificed, the number of ATPase-deficient foci observed in a two-dimensional section of the liver, and the area of the examined section, the radii in microns of each of the observed foci. The objective of the analysis was to study the effect of NNM on the parameters of the model and thus to study the initiation and promotion potencies of the compound. The relevant expressions required for the analysis can be found in a recent paper (8). Briefly, the parameters of the model were estimated by maximizing the appropriate likelihood function. The results of the analysis are presented in Figure 2. It can be seen from the figure that, although there are outliers, the rates of initiation and net proliferation of initiated cells are both linear functions of the dose of NNM. When the dose response functions are linear, we (8) proposed the following definitions of initiation and promotion potencies. Consider the appropriate linear regressions through the dose-response curves and define the potency as the quotient of the slope and the intercept. Thus, potency measures the proportionate increase over background of initiation or promotion per unit of dose. Potency, as defined here, depends on the units in which dose is measured; however, once the potency is computed in a given system of units, it is an easy matter to express it in any other system of units. For the NNM data, the regression lines are given by $IR(d) = 5.9 + 0.93 d$, where $IR(d)$ is the number of cells initiated per day per milliliter of liver as a function of dose, and $NP(d) = 0.009 + 0.0003 d$, where $NP(d)$ is the net proliferation rate ($α - β$) expressed as a function of dose. It follows that initiating potency is 10.93/5.9 = 1.85 per ppm, and promoting potency is 0.0003/0.009 = 0.034 per ppm. Thus, by these definitions of initiating and promoting potencies, NNM is a strong initiator and a weak promoter. These definitions of initiating and promoting potencies make sense only when the dose-response functions are linear, and the rates of initiation and promotion are constant over time.

The number of normal cells per milliliter of liver that becomes initiated ranges from about 10 per day in the control group to about 380 per day in the 40-ppm group. That is, approximately 3,600 cells are initiated per milliliter per year in the control group and 140,000 in the 40-ppm group. In contrast, the model predicts about 100 nonextinct foci in the control group and about 1,700 nonextinct foci in the 40-ppm group at the end of 1 year of treatment. Thus the model suggests that the vast majority of initiated cells dies without giving rise to foci. These numbers must be taken as rough estimates derived from the model. Due to technical considerations beyond the scope of this paper, these estimates could
be made more precise if information were directly available on cell division rates in the foci.

**Explanations for Inverse Dose-Rate Effect.** As stated earlier, an interesting phenomenon observed with exposure to high LET radiation and to some chemicals is that fractionation of a given total exposure results in an increased lifetime probability of tumor (12). There are some obvious biological explanations for this phenomenon. For example, if a chemical needs to be metabolically activated and if high exposure rates lead to saturation of the relevant enzyme pathway, then one would expect to see an inverse exposure-rate effect. Similarly, with radiation, one would expect to see an inverse exposure-rate effect if a high exposure rate leads to cell killing. With agents that affect cell proliferation kinetics, however, an inverse exposure-rate effect can be predicted even if neither saturation nor cell killing is taking place. Consider an agent that increases $\alpha - \beta$, and suppose that $\alpha - \beta$ is a linear function of the exposure of the agent. In particular, suppose that neither threshold nor saturation phenomena are operating. Then, mathematical calculations based on the model in Figure 1 show that the lifetime probability of tumor is higher with fractionation of a given total exposure. Another way of stating this fact is that a given total dose of a promoter is more effective in bringing about malignant transformation with prolonged application. Figure 3 illustrates this situation. Figure 3 is a plot of probability of occurrence of malignant tumor as function of time after application of promoter is begun. The curves in Figure 3 represent tumor probability for identical total promoter dose but at different dose rates. Exposure to promoter is assumed to begin at age 10. The total administered dose is 500 units, and five different dose rates are shown. Thus, for example, with dose rate 10, exposure begins at age 10 and continues until age 60. The parameters assumed for this example are as follows: $\nu X = 0.1$, $\beta/\alpha = 0.9$, $\alpha - \beta = 0.04 + 0.01d$ where $d$ is the dose rate, and $\mu = 10^{-4}$. It is quite clear from the figure that, eventually, the probability of tumor is highest at the lowest dose rate.

**Concluding Remarks**

The consideration of cell kinetics in models of carcinogenesis leads to some surprising and unexpected results. Perhaps the most important consequence of considering both cell division and cell death explicitly is the conclusion that some initiated cells must die without giving rise to foci. This conclusion also underscores the importance of considering not just the net cell division rate, but cell division and cell death separately. Another important consequence is that agents that increase the net proliferation rate exhibit the inverse dose-rate effect.

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