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The Two-Stage Clonal Expansion Model as an Example of a Biologically Based Model of Radiation-Induced Cancer

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A model with two stages and clonal expansion (TSCE) is reviewed as a prototype for biologically based models of cancer development. Applications of the TSCE model to data sets for animals and humans for particle radiation (α particles) are presented. The results suggest that the radiation not only influences the initiating mutation, but may also act as a promoter. A possible mechanism for the promoting action is described. The consequences of these results for the shapes of the radiation dose–response curves at low doses and dose rates are discussed.

INTRODUCTION

Cancer cells differ from normal healthy cells as a result of several mutations. Over the years, detailed information has been collected about these mutations in many tumor types (*1*), and more information is to be expected, in part because of the human genome project. Mathematical models that describe a multistep process of carcinogenesis have been proposed since the 1950s (*2, 3*). It was determined that n mutations would give a cancer incidence which increases roughly with age raised to the power $n - 1$. Fitting to the data for the age dependence of incidence (or mortality) gave $n \approx 6$ for several solid tumors. It is widely believed that multiple mutations may be required for the development of a malignancy (noting that inactivation of a tumor suppressor gene requires two recessive mutations). However, there is then some tension to be resolved between the predictions of cancer incidence based on spontaneous mutation rates and the observed cancer rates. Naive application of the Armitage-Doll model might suggest that the mutation rates required to explain observed cancer rates must be much greater than the spontaneous mutation rates usually considered (see e.g. ref. *4*). Possibilities for reducing the tension include (a) clonal expansion, (b) allowing that some mutations may greatly increase the rate of subsequent mutations by impairing repair mechanisms, and (c) other types of genomic instability. Some discussion of these issues, although not in terms of the Armitage-Doll model, is given in refs. (*5, 6*). When the clonal expansion of some intermediate cells plays an important role, the observed age dependence of the cancer incidence can be fitted with fewer steps. When one of the early steps increases mutation rates, the subsequent steps happen faster and therefore may not be rate-limiting.

Such considerations make a two-stage model with clonal expansion (TSCE) an attractive possibility (*7*). Consideration of familial predispositions for tumors, specifically retinoblastoma, suggested that the inactivation of the two copies of a tumor suppressor gene may be the two rate-limiting steps for the development of solid tumors (*8, 9*). This suggestion encouraged the mathematical development of this particular cancer model (*10, 11*). Mathematically rigorous formalisms were obtained for piecewise constant parameters that can be implemented in fast computer code (*12*). Although two mutational events are insufficient for most solid tumors, two rate-limiting steps and clonal expansion may contain some of the important lessons from molecular biology (*13*).

In spite of the progress in understanding the development of cancer, a precise model based on proven biological steps cannot yet be formulated, due to a lack of knowledge of the details of the mechanisms. Nevertheless, a mathematical cancer model can satisfy several purposes:

1. Collecting biological knowledge about the essential steps in cancer development.
2. Extracting insights from cancer incidence data about the process of cancer development and the effects of external exposures.
3. Providing a framework for the creation of hypotheses about the process of cancer development and the effects of external exposures.
4. Suggesting qualitative features of hazard functions.
5. Providing a framework for combining radiation risk estimates and toxicological risk estimates.

The use of the TSCE model for these purposes is dis-

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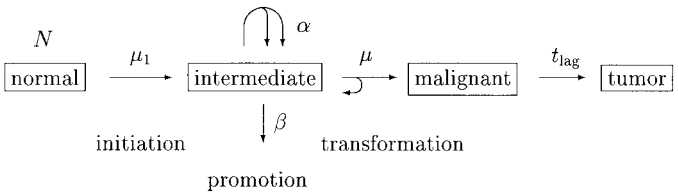

FIG. 1. Schematic of the TSCE model.

cussed here. This particular model is well developed, and it may serve as a guide for biologically more refined approaches.

THE STANDARD TSCE MODEL

A schematic of the two-step clonal expansion model is given in Fig. 1. Normal healthy cells (whose number N is usually not known) mutate at a rate μ_1 to intermediate cells. Thus the intermediate cells are created at a rate $\nu = N\mu_1$. An intermediate cell can divide into two intermediate cells at rate α, die or differentiate at rate β, or divide into one intermediate cell and one malignant cell at rate μ. The progression from a malignant cell to an observable tumor is usually described with a lag time. Although the two rates μ, ν can be mutation rates, the mathematics of the model only requires that they represent rate-limiting events on the way to a cancer cell. Thus initiation is considered to be a Poisson process, and not necessarily a single specific mutational event. The lag time may also include late, but not rate-limiting, events in the development of a tumor cell. This reasoning may help to bridge the gap between ''two-stage'' and ''multistage'' modeling, but more work with simulations or concrete data needs to be done to examine the limits of the applicability of the approximations which are involved in such extensions of the model. Most tumor end points can be reached by several different pathways; the model simplifies this, with some effective parameters.

The hazard function of the model for constant parameters

$$h(t) = \frac{X(e^{(\gamma+2q)t} - 1)}{q(e^{(\gamma+2q)t} + 1) + \gamma}, \tag{1}$$

can be described qualitatively in three phases (*12, 14*):

1. For young age t, the hazard is well approximated by

$$h(t) \approx Xt; \; X \equiv \nu\mu. \tag{2}$$

In this early phase, the majority of intermediate cells are created by initiation from normal cells.

2. Later, the majority of new intermediate cells are created by the division of intermediate cells, giving rise to the exponential growth of the hazard. During this period, the formula

$$h(t) \approx \frac{X}{\gamma}, (e^{\gamma t} - 1), \qquad \gamma \equiv \alpha - \beta - \mu \tag{3}$$

describes the hazard well.

3. Eventually, when stochastic effects become important, the hazard levels off to a constant asymptotic value

$$h(t) \approx \frac{X}{q}, \qquad q \equiv \frac{1}{2}(-\gamma + \sqrt{\gamma^2 + 4\alpha\mu}). \tag{4}$$

The three parameters X, γ and q describe distinct features of the hazard function and therefore can be estimated well from incidence data. Surprisingly, these three parameters determine the hazard function completely (*14*). Notably, only the product $\nu\mu$ enters into the hazard function, not ν and μ separately. Similar problems of ''identifiability'' are to be expected in all multistep tumor models.

Radiation or chemical substances can modify the rate of initiation (change from a normal cell to an intermediate cell) or of transformation (change from an intermediate cell to a malignant cell). In principle, such exposures can also modify the effective clonal expansion rate γ. Such an effect would correspond to the promotion step in tumor development. It has been shown (*12*) that the quotients $\nu(d)/\nu(0)$ and $\mu(d)/\mu(0)$ are identifiable from incidence data [while for example $\mu(0)$ itself is not identifiable, as pointed out above]. Thus it is possible to separate the initiating, transforming and promoting effects of external exposures using data on cancer incidence. Models with age-dependent initiation could mimic some of the features of models requiring multiple mutations before clonal expansion, thus narrowing the gap to multistep models further. The characteristic feature of the TSCE model is clonal expansion, described as a birth–death process.

APPLICATION TO CANCER INCIDENCE DATA

In several applications of the model to data on radiation-induced tumors shown in Table 1, an initiating action of radiation was found. For protracted exposure to radon in

TABLE 1
Action of Radiation and Cigarette Smoke in the TSCE Model

Data set	Initiation	Transformation	Promotion
Atomic bomb survivors (*21, 22*)	+	−	−
Radon-exposed rats (*15, 16*)	+	−	+
Colorado miners (*17*)	+	−	+
Smoking[a]	−	+	+
	mutational event		clonal expansion

[a] Heidenreich *et al.*, unpublished results.

TABLE 2
Action of Radiation on Mutational Events Observed in Some Published Studies

Study	Doubling dose
Rats: fatal lung tumors (*16*)	3 WL ≈ 800 mSv/year
Colorado miners (*17*)	2.5 WL ≈ 700 mSv/year
	Acute exposure
Atomic bomb survivors (*21*)	30 mSv ≈ 1 year spontaneous
Kai *et al.* (*22*)	1.25–125 mSv ≈ 1 year spontaneous
	Experimental mutation rates
Kai *et al.* (*22*)	
HPRT	23 mSv ≈ 1 year spontaneous
Glycophorin A	16 mSv ≈ 1 year spontaneous

Note. A conversion of 1 WLM ≈ 5 mSv is used.

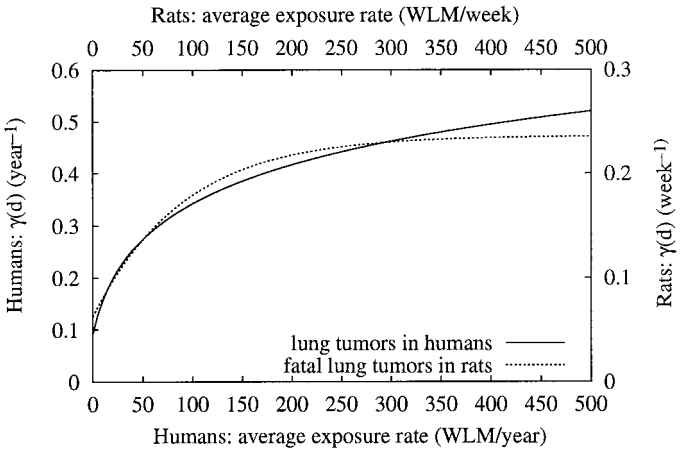

FIG. 2. Dependence of the effective clonal expansion rate $\gamma(d)$ as estimated in refs. (*16, 17*). The labels on the top and right axes refer to the curve for rats, the others to the curve for humans.

both humans and rats, the assumption of a promoting action of radiation was necessary to explain the protraction effects in the data (*15–17*). The atomic bomb survivor data contain no information about protraction, because the exposure was acute. Radiation was not found to produce transformation. This may be an indication that the second rate-limiting event is of a different nature from the initiating events. For comparison, the action of cigarette smoke found in the analysis of a large German case–control study is given in the last line of Table 1, where these actions are summarized.

For high-LET radiation, the observed mutation rates depend roughly linearly on exposure. Therefore, a doubling dose can be defined at which the rate of radiation-induced mutations is equal in magnitude to the spontaneous mutation rate. For low-LET radiation, to which the atomic bomb survivors were exposed, a linear dependence of initiation on the acute dose was found to describe the data adequately. An identifiable quantity is the acute dose that induces as many mutations as occur spontaneously in 1 year. These parameters are summarized in Table 2 for several studies.

In Fig. 2, the fitted dependence of the effective clonal expansion rate $\gamma(d)$ is given for humans and rats. It starts out linearly and levels off at high exposure rates.

A HYPOTHESIS ON THE PROMOTING ACTION OF RADIATION

These observations suggest that a part of the radiation risk for radon-induced lung tumors is not due to the mutagenic action of the α-particle radiation, but rather is due to an effect of radiation on the effective clonal expansion rate. A possible mechanism for this effect could be the inactivation of cells by radiation (*18*). According to this model, inactivated stem cells of the lung epithelium [basal cells or secretory cells (*19*)] might be replaced by the division of neighboring stem cells. Intermediate cells (which have a growth advantage when compared to normal cells) may fill the deficit faster than normal stem cells. Less than a doubling of the normal replacement probability would be sufficient to explain the promoting action of radiation, as found in the initial increase for the Colorado miners shown in Fig. 2. A crucial input in this calculation is the number of stem cell nuclei hit by α particles (*19*).

The mechanism proposed above would have interesting consequences:

1. Tumors induced by the increased promotion would show no ''fingerprints'' of radiation, because the mutations that lead to tumor formation occur spontaneously. The cells that are inactivated by radiation die and disappear from the developing tumor.
2. The transfer of relative risks (but not of absolute risks) between populations and between animal species is plausible: The organ-specific spontaneous mutation rates are promoted proportionally by cell inactivation.
3. Dose–response curves for cell inactivation by low-LET radiation have wide shoulders. A promoting action of these radiations could be greater than linear ($n > 1$) at low dose rates.

These considerations suggest that physiological processes occurring in individual cells may not be sufficient for quantitative risk assessment. The communication between the cells within an organ may play a more important role than has been assumed in most models of radiation carcinogenesis (*20*).

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