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Author(s): W. F. Heidenreich, H. M. Cullings, S. Funamoto, and H. G. Paretzke
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Promoting Action of Radiation in the Atomic Bomb Survivor Carcinogenesis Data?

W. F. Heidenreich,[a,1] H. M. Cullings,[b] S. Funamoto[b] and H. G. Paretzke[a]

[a] *GSF—National Research Center for Environment and Health, Institute for Radiation Protection, 85764 Neuherberg, Germany; and*
[b] *Department of Statistics, Radiation Effects Research Foundation, Hiroshima, Japan*

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The age–time patterns of risk in the atomic bomb survivor data on incidence of solid cancers suggest an action of low-LET radiation not only on the initiating event but also on promotion in a biologically motivated model that allows for both actions. The favored model indicates a decrease of radiation risks with age at exposure due to the initiating effect and with time since exposure due to the promoting effect. These result in a relative risk that depends mostly on attained age for ages at exposure above 20 years. According to the model, a dose of 100 mGy is inducing about the same number of initiating events that occur spontaneously in 1 year. Assuming that several mutations are needed to obtain intermediate cells with growth advantage does not improve the quality of fit. The estimated promoting effect could be explained if the number of intermediate cells increases by 80% at 1 Gy, e.g. due to stimulated cell repopulation.

INTRODUCTION

The development of a solid cancer in humans is a multistep process (*1*), the details of which are not known. Essential are several changes that can be inherited (on a cellular level) and clonal expansion of intermediate (premalignant) cells. The two-stage clonal expansion (TSCE) model provides a stochastic mathematical framework that incorporates these features in a minimal way (*2, 3*). It can be used as a basis for testing hypotheses on mechanisms of radiation action and other modeling assumptions quantitatively. While it was originally developed with Knudson's two-mutation hypothesis in mind (*4*), the stem cell hypothesis of cancer (*5*) now gives new momentum to the notion that a small number of events (rate-limiting events) are necessary for the development of cancer, while other changes occur regularly and comparatively fast. The mathematical structure of the model readily allows for genes to act as caretakers or gatekeepers (*6, 7*). From a systems biology point of view (*8*), the model allows one to predict features of hazard functions from hypotheses about the mechanisms that can be compared with experimental and epidemiological data.

The simplest and often used hypothesis is that radiation action affects only the initiating event. This assumption was used e.g. in previous analyses of the atomic bomb survivor data (*9–11*). If the initiation event is a mutation, radiation-induced mutations occur in addition to those that have accumulated due to background mutations and clonal expansion. The model allows one to calculate the fraction of radiation-induced mutations over the background mutation rate from cancer incidence data.

Intermediate (i.e. initiated) cells in the model are defined as cells with a small growth advantage. Radiation exposure may modify this clonal expansion rate during the exposure. Such a promoting effect cannot be caused by the mutagenic action of radiation. Indications of promoting actions were found for protracted high-LET radiation in several studies (*12, 13*). In this paper, the promoting potential of acute irradiation is estimated in addition to initiation effects in the large data set on the atomic bomb survivors. This is possible because initiating and promoting radiation actions give different age and time trends of risk later in life for an acute exposure (*14*). For reasons given below, initiating action gives a relative risk (RR) at a given time since exposure that decreases with age at exposure, while a promoting action leaves it basically constant. For constant age at exposure, the RR in time since exposure is constant or increasing for some time before eventually decreasing to 1 for an initiating action, while it can soon decrease for a promoting action.

Several elementary events (e.g. mutations) may be needed before a cell develops a clonal advantage. This possibility is also tested in this paper by an extended version of the TSCE model.

MATERIALS AND METHODS

The Data Set

Data on 113,251 people [the set of persons with known DS02 doses in the Life-Span Study (LSS) cohort] were available at the Radiation

[1] Address for correspondence: GSF—National Research Center for Environment and Health, Institute for Radiation Protection, 85764 Neuherberg, Germany; e-mail: heidenreich@gsf.de.

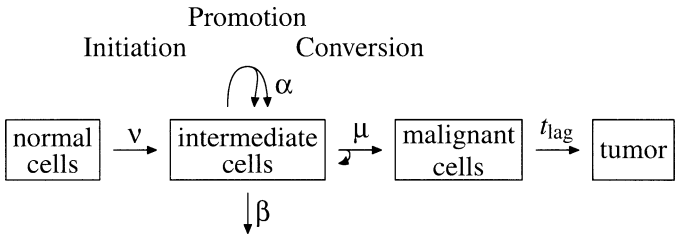

FIG. 1. Sketch of the TSCE model.

Effects Research Foundation (RERF) in Hiroshima. Cancer incidence is used here, because the cancer mortality data are influenced by the quality of cancer treatment. Analyses were based on a subset of the full cohort including persons who have an estimated kerma in air of less than 4 Gy, had not died before 1958, and were within 3 km of the epicenter at the time of the bombing. Although most RERF analyses make use of LSS cohort members who were within 10 km of the hypocenter at the time of exposure, our restriction to the 3-km zone was motivated by earlier analyses at RERF (*15*). This leaves data on 22,489 males and 33,943 females. A grouped data set for Poisson regression, stratified by city, gender, age at exposure, calendar time (through the end of 1997), and radiation dose, was produced from these individual data for the incidence of tumors at the sites stomach, colon, liver, lung and all solid tumors and for a collection of nine tumor sites (esophagus, stomach, colon, rectum, liver, gallbladder, pancreas, lung, and bladder). These latter nine sites are fairly homogeneous with respect to their age dependences according to earlier analyses (*16*). The values for the person-years in the cells of the grouped data are corrected for migration, and the average doses in the cells are adjusted for estimated dose uncertainties (*17, 18*). As usual for analysis of this data set, the neutron doses are added with a weighting factor of 10 to the respective γ-ray doses.

The TSCE Model

The two-stage clonal expansion (TSCE) model is outlined in Fig. 1; an earlier version has been described in detail previously (*9*). The baseline hazard of the first appearance of a malignant cancer-causing cell as a function of age depends on three combinations of the biological parameters shown in Fig. 1: (1) The product X_0 of the baseline initiation rate ν_0 and the baseline rate μ_0 of malignant conversion, (2) the effective clonal expansion rate $\gamma_0 = \alpha_0 - \beta_0 - \mu_0$, which is close to the difference between the cell division rate α_0 and the rate for cell death or differentiation β_0 of the intermediate cells, and (3) the parameter $q = \frac{1}{2}(-\gamma_0 + \sqrt{\gamma_0^2 + 4\alpha_0\mu_0})$, which determines the hazard at high age, $h(t = \infty) = X_0/q$. A lag time of 5 years is used to approximate the processes from the appearance of the first malignant cancer-causing cell to an observable tumor. In these parameters, as derived in ref. (*9*), the explicit expression for the baseline hazard at age t is

$$h(t) = \frac{X_0\{\exp[(\gamma_0 + 2q)(t - t_{lag})] - 1\}}{q\{\exp[(\gamma_0 + 2q)(t - t_{lag})] + 1\} + \gamma_0}. \quad (1)$$

In contrast to the earlier analysis (*9*), here we do not treat the childhood period in a special way. This is motivated by the following reasoning: The nature of the target cells for cancer development is not certain. This number may be constant in the growing organ. Alternatively, their number could be increasing over time during childhood. Then an intermediate cell initiated during childhood has two growth modes, one from the average growth of the organ and the other from the additional growth advantage of being an intermediate cell. Estimates suggest that the expected number of intermediate cells in a young adult is about the same if a constant number of sensitive cells from birth is assumed, without the growth mode due to the growing organ.

The atomic bomb survivors were exposed to radiation for a short time. This is modeled here by changing some parameters for a period Δt. Tests showed that the precise value of this period is of little importance for the form of the resulting hazard functions. Below, parameters are chosen such that they are also insensitive to the value of Δt, as long as it is small relative to $1/\gamma_0$. We use 1 week. In principle, radiation can act on each of the four biological parameters of the model. Next we describe the dependences on dose rate that we use and the risk pattern that they can explain (*14*).

An action of radiation on the initiation parameter ν is modeled with the parameter x_r as dependence on dose rate $D/\Delta t$ during the period Δt,

$$X(D) \equiv (D)\mu_0 = X_0(1 + x_r D/\Delta t). \quad (2)$$

Due to the exposure, $(X_0/\mu_0)x_r D$ cells are newly initiated, while in a time span ΔT, $(X_0/\mu_0)\Delta T$ initiated cells occur spontaneously. Therefore, the number of cells initiated by an acute exposure to dose D is equal to the number of cells that would be initiated in the period $x_r D$, or equivalently, $1/x_r$ is the dose that gives the same number of initiated cells that would occur in 1 year in the absence of exposure.

For an initiating action of radiation, after the lag time, the relative risk associated with an exposure is approximately equal to the ratio of the number of cells initiated by the exposure to the total number of intermediate cells at the time of exposure. Since the number of intermediate cells increases with increasing age, the relative risk associated with initiation decreases with increasing age at exposure (*9, 14*).

An effect of radiation on the rate μ of malignant conversion would be observable as a peak in the hazard rate function after the lag time. For plausible values of the lag time, including the 5-year lag assumed in these analyses, this peak would occur before the beginning of the follow-up for these incidence data since they have no information on risks prior to 13 years after exposure. Therefore, it is not modeled in this context.

A promoting action of radiation on the number of initiated cells is modeled with the parameter g_r by a dose dependence of the effective clonal expansion rate during Δt. A logarithmic dependence of promotion on dose is assumed in the form

$$\gamma(D) = \gamma_0 + \ln(1 + g_r D)/\Delta t. \quad (3)$$

At low doses this function has a linear dependence on D, but the slope decreases at higher doses. It is chosen so that $\Delta\gamma\Delta t = \ln(1 + g_r D)$: The number of available initiated cells is increased by roughly a factor of $\exp(\Delta\gamma\Delta t)$ for short periods relative to $1/\gamma_0$. Therefore, the initial ERR from promotion is roughly $g_r D$, independent of age at exposure. Depending on the parameter q, it can decrease with time since exposure.

Radiation could also modify the parameter q. Tests showed that this would influence the risk only very long after exposure and is not estimable from the present data set. Therefore, it was not used in this study.

We call the TSCE model with both an initiating and a promoting action of radiation the "IP" model. If the promoting action is not used ($g_r = 0$), we call it the "I" model; if the initiating action is not used ($x_r = 0$), we call it the "P" model.

Birth-year effects are allowed for by multiplying the overall hazard function by a factor $e^{\delta b}$, where b is the difference between the birth year and the year 1900. This is equivalent to making X_0 dependent on birth year. In this way, behavioral changes are taken into account. Preliminary tests had shown that other possible ways to deal with the birth-year effects did not give improvements over this customary term (*11, 16*). Since all persons were exposed on the same date and the baseline TSCE model gives the hazard as a function of age, further calendar-year effects cannot be separated.

For an infinitesimally short exposure acting on the initiation effect only, a simple formula for the hazard function can be given, which was used in earlier work (*9, 11*). The work here with a promoting action requires use of the iterative formulas given in ref. (*3*).

A Model with Several Steps Leading to Intermediate Cells

A possible generalization of the TSCE model is to assume that it takes several events (e.g. mutations) before a cell acquires a growth advantage (*19*). When $n - 1$ mutations are necessary, the model assumes a total of

TABLE 1
Deviances of the Fits of the Different Models to the Data

Site	Cases	IP model deviance	I model Δ deviance	P model Δ deviance	Baseline Δ deviance
Male					
Stomach	1269	3396.1	1.8	1.9	7.3
Colon	363	1543.8	15.9	0.5	24.2
Liver	448	1891.7	15.3	0.0	17.2
Lung	520	1941.7	3.7	1.2	7.2
Nine sites	3302	4941.3	16.8	6.7	45.7
Females					
Stomach	1221	3445.9	3.1	10.8	31.4
Colon	422	1626.8	1.3	0.4	3.1
Liver	301	1436.9	1.6	1.0	4.1
Lung	427	1959.8	20.8	1.5	45.8
Nine sites	3083	5057.4	25.8	8.0	78.0

Notes. For each cancer site, or group of sites, and each gender, the deviance for the IP model is given. For the I and P models (one parameter less), and the baseline model without dose–response parameters (another parameter less), the increase in deviance is given relative to the IP model. For comparisons such as those among the I, P and IP models, we suggest the usual likelihood ratio tests used in Poisson regression; i.e., the difference in deviance between two models differing by the addition of one or two parameters is distributed approximately χ^2 with one or two degrees of freedom, respectively, if the model with fewer parameters is correct, so that a χ^2 statistic exceeding the critical value suggests that the model with more parameters is a better model (*33*).

n mutations, the last one leading to the malignant conversion. A mathematical approach is to calculate the probability $p_{n-1}(t)$ that a particular target cell becomes an intermediate cell as a function of age. The initiation rate ν from N target cells of an organ is then proportional to $Ndp_{n-1}(t)/dt$. As is frequently done, the rates λ for the $n-1$ mutations are all assumed to be equal. The radiation-induced mutation fractions are also assumed to be equal for each mutation. These assumptions are similar to those frequently made in the Armitage-Doll and the Nordling models, except that here the rate $\nu(t)$ for initiated cells is calculated, not the rate for the appearance of malignant cells. Unfortunately, the assumed simplifications are needed, because all the parameters cannot be estimated from the data. A similar model with all parameters slightly different is used in ref. (*20*). The dose response is described here by x_r, which is chosen such that x_rD is the fraction of cells mutated by dose D, divided by the spontaneous mutation rate λ. Thus it is defined in the same way as in Eq. (2). With the usual approximation for $\lambda t \ll 1$ and $x_rD \ll 1$, we get up to linear terms in x_r

$$\begin{aligned} \nu(t) &= ct^{n-2} \\ &\quad \text{before exposure,} \\ \nu(t) &= ct^{n-2}(1 + x_rD/\Delta t) \\ &\quad \text{during the short exposure,} \\ \nu(t) &= ct^{n-2}(1 + (n-2)x_rD/t) \\ &\quad \text{after exposure.} \end{aligned} \tag{4}$$

The first and last formula correspond to Eqs. (A9) and (A10) in ref. (*11*), and the second is calculated from the difference in $p_{n-1}(t)$ before and after the exposure. The number of sensitive cells N and the mutation rate λ enter in the parameter c, which is fitted to the data. We used the above relationships in an alternative TSCE model in which the age-dependent initiation rates were described using piecewise constant $\nu(t)$ in time steps of 1 year before and after exposure and a time step of Δt during exposure. No attempt was made to implement the stochastic versions of the Nordling or Armitage-Doll model for the initiating events since they may give unrealistic parameter estimates for N and λ (*11*).

TABLE 2
Estimated Parameter Values for the Nine Sites in the IP Model and Their Standard Errors

Parameter	Males	Females
X_0 (10^{-6} year^{-2})	0.36 ± 0.08	1.1 ± 0.2
x_r (year/Gy)	7.2 (4.0, 10.9)	11.6 (6.8, 17.2)
x_r in I model	18 ± 4	37 ± 7
γ_0 (year^{-1})	0.147 ± 0.005	0.100 ± 0.004
g_r (Gy^{-1})	0.7 (0.5, 0.9)	0.9 (0.7, 1.1)
q (10^{-4} year^{-1})	0.15 ± 0.03	0.64 ± 0.10
δ (10^{-2} year^{-1})	1.5 ± 0.2	0.75 ± 0.19

Note. Also given is the dose–response parameter x_r for the I model.

Method of Parameter Estimation

The parameter values and their uncertainties for all the models described above were calculated by Poisson regression, using the nonlinear function minimization program "Minuit" of the CERN computer program library (*21*).

RESULTS

We fitted the full model and sub-models to the data for the chosen tumor sites separately for men and women. The deviances of the various fits are presented in Table 1. When the nine sites are considered, for both males and females, omitting radiation effects on either initiation or promotion led to a statistically significant increase in deviance. For the individual cancers, the 95% significance level is reached for some of these comparisons, but not for all. In the majority of the cases considered, the statistical evidence for a promoting action of radiation is larger than it is for an initiating action.

The fitted optimal parameter values and their standard errors are given in Table 2 for the IP model and the nine sites. Also given are the dose–response parameters of the I model. The four estimated background parameters differ significantly between the two genders. The estimated dose–response parameters are larger for females than for males, but for the IP model, one of them could be equal for the two genders.

For each of the fitted models, the observed and expected number of cases were compared for various groupings of the data, and a good agreement was found.

In Fig. 2, the estimated hazard functions after an exposure of 1 Gy at ages 20, 40 and 60 years are shown. In Fig. 3, the predicted relative risks (RR) for several age-at-exposure groups are plotted for 15 to 50 years after exposure (roughly the observation period). The IP model gives both a decrease with age at exposure (from I) and a decrease with time since exposure (from P). These two effects together give an RR that depends mostly on attained age if age at exposure is above 20 years. For a given attained age, the RR is increased somewhat for the youngest age-at-exposure group but is similar for other ages at exposure.

It should be stressed that this detailed dependence of rel-

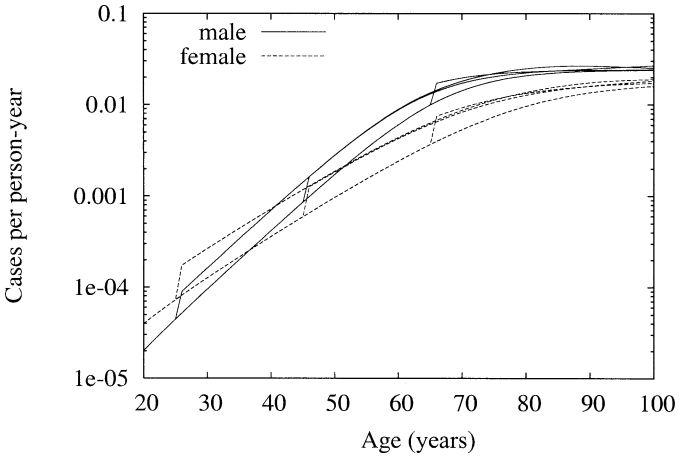

FIG. 2. Estimated hazard functions in the IP model for the nine sites, spontaneously, and for exposure to 1 Gy at an age of 20, 40 and 60 years. The step-like behavior of the hazard function 5 years after exposure plays no role in the estimates, because the data begin 12 years after the exposure.

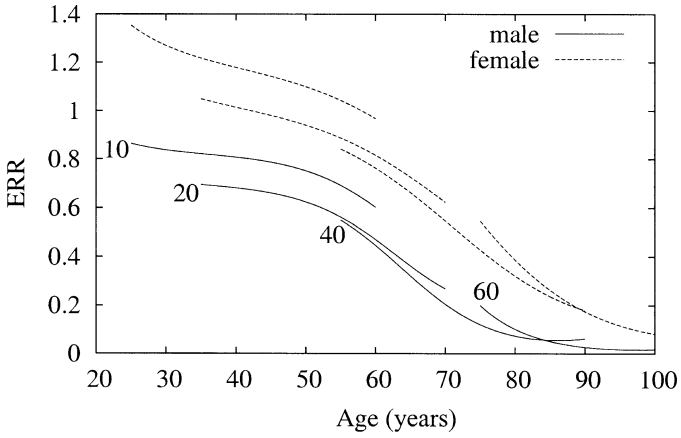

FIG. 3. Estimated ERR functions in the IP model for the nine sites for age at exposure of 10, 20, 40 and 60 years, each from 15 to 50 years after exposure to 1 Gy.

ative risk on two of the three quantities age at exposure, time since exposure, and age is a result of the IP model. To find out the extent to which it can be obtained directly from the data, we estimated the relative risk for combinations of ranges of age at exposure and observation periods. The technique of ref. (*22*) was used. In Fig. 4, the result is shown together with the prediction of the IP model applied to the full data set. We conclude that age and time trends are not easily seen in the data directly due to a lack of statistical power. It is necessary to combine data from a sufficient range in age and calendar time, for example by estimating simple parametric functions of age and time.

The shape of the dose–response curves is fixed in the IP model by Eqs. (2) and (3). The quality of these choices can be tested by assuming different shapes. For this purpose we used piecewise linear functions, with nodes at 0.1, 1 and 3 Gy. We either used one shape (I or P) from Eqs. (2) and (3) and piecewise linear functions for the other one, or we used piecewise linear functions for both shapes. Figure 5 shows the results for the end point nine sites. The standard error bars give the estimate of the nodes for the piecewise linear curves and their uncertainties. The dose–response curves appear to describe the data well. There is a slight tendency, more pronounced in the females, for a quadratic component in the dose dependence for initiation, and possibly a more substantial leveling of the dose dependence describing the promoting effect.

The model with several initiating events described in the Materials and Methods section was applied to the statistically most powerful subset for females and the end point nine sites. Table 3 shows the resulting deviance values and some of the estimated parameters. The largest tested value for n is the value obtained with a model without clonal expansion. It is similar to the approximated Armitage-Doll or Nordling model (*11*). The best fit is obtained with the IP model. More initial events do give a poorer agreement with the data. This may have to do with a feature of the model used such that at high age the hazard is not leveling to a constant but to age to the power $n - 2$. The estimated dose response of mutations x_r depends only weakly on the

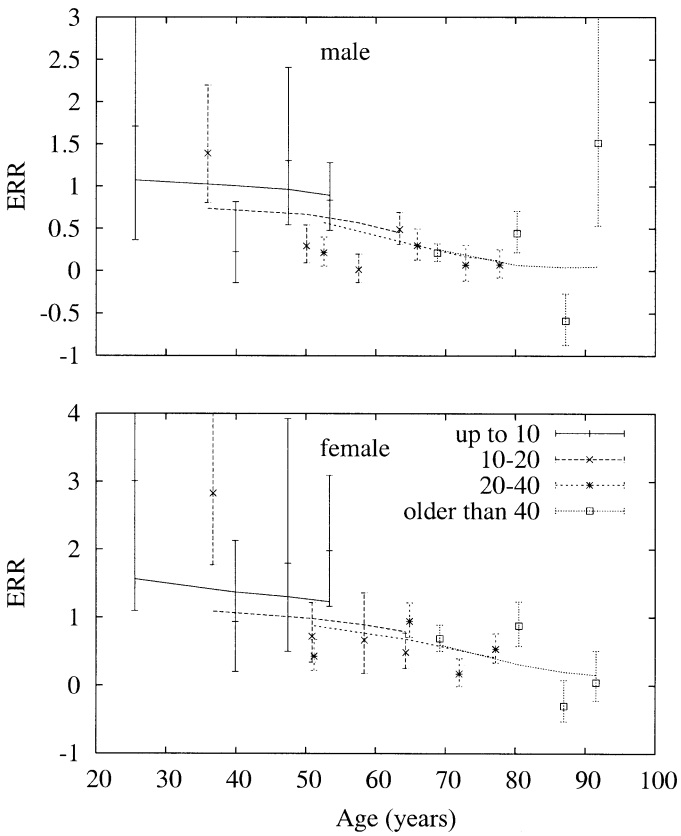

FIG. 4. Comparison of the estimated ERR functions in the IP model for the nine sites (lines) with estimates based on subsets of the data (points with error bar). The subsets used contain all cells with age at exposure up to 10, 10–20 and 20–40 years and older, for the periods 1958–1975, 1976–1985, 1976–1990 and 1990–1997.

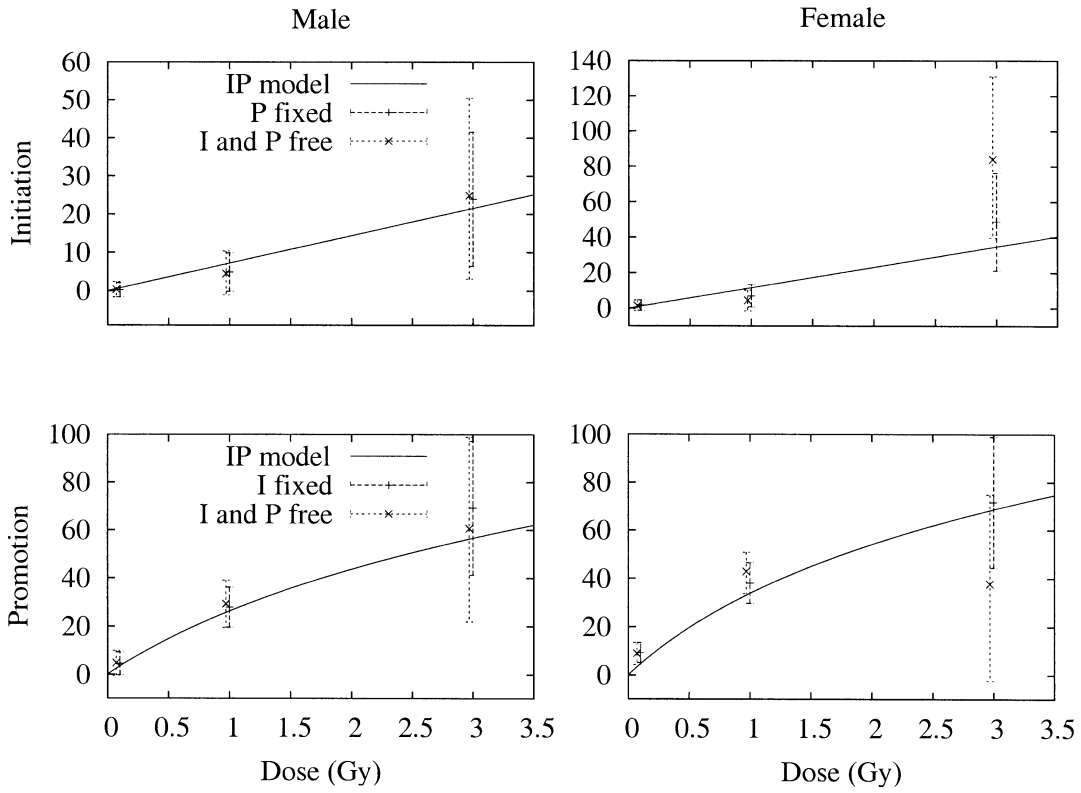

FIG. 5. Estimated dose dependence of the initiation function $X(D)\Delta t/X_0$ and the promotion function $\gamma(D)$ for the end point nine sites. The error bars represent the nodes of piecewise linear models.

number of initiation events. The clonal expansion rate decreases very slowly with increasing number of initiating events, until the number of the events of the Armitage-Doll model is approached (*19*).

DISCUSSION

Intermediate cells can be created from normal cells (Fig. 1) by two mechanisms in the present model: spontaneously and by the short-term action of radiation. For males and the nine sites, the parameter x_r in Table 2 is about 18 (years/Gy) for the I model, while it is 7.2 (years/Gy) for the IP model. Alternatively, 55 mGy would produce the same number of intermediate cells that occur in 1 year spontaneously according to the I model and 140 mGy according to the IP model. The relative importance of initiation is decreased to less than half when a promoting effect is also allowed. It would be most desirable if the value of x_r could be measured directly in some experimental setup. This could help to connect experimental work and epidemiological results and to understand in greater detail the mechanisms of radiation-induced carcinogenesis.

TABLE 3
Deviance, Effective Clonal Expansion Rate and Dose–Response Parameter of Multistage Models with Various Numbers of Stages

Model	Deviance	n	x_r (year/Gy)	$\gamma(0)$ (year^{-1})
IP model	5057.4	2	11.6	0.10
$n = 3$ fixed	5060.0	3	9.5	0.10
$n = 4$ fixed	5067.8	4	8.8	0.10
$n = 5$ fixed	5073.3	5	9.2	0.08
$\gamma = 0$	5078.9	5.2	9.3	0

Notes. The fit is to data for females and the end point nine sites. The number of stages is denoted as n, x_r is the period during which the same number of baseline mutations occur as after 1 Gy of radiation, and $\gamma(0)$ is the baseline effective clonal expansion rate. Since the number of parameters being estimated does not depend on n, we suggest simply comparing the deviances; i.e.,the model with the smallest deviance is the best-fitting model.

As an example, we compare the values reported above with the rate of stable translocations found in human lymphocytes. The background level increases roughly linearly to about 1.5×10^{-2} translocations per cell at an age above 80 years (*23*). The slope is about 1.7×10^{-4} per cell per year. The linear term of the radiation response for stable translocations is estimated from various sources as about 1.5×10^{-2} per cell per Gy for ^{60}Co γ rays (*24*). Thus about 11 mGy gives the same number of stable translocations as occur in 1 year spontaneously. This indicates a much larger effect of radiation than is needed for the solid cancer models above. In addition, at doses above 0.5 Gy, where the statistical power of the cancer data lies, a quadratic term has to be included for the dose response of the stable trans-

locations, giving an even larger discrepancy. If this discrepancy also holds for stable translocations in the precursor cells of solid cancers, this would suggest that stable translocations are not the dominating effect in the initiation of solid cancers.

For another comparison, work on *HPRT* mutations in Chinese hamster cells *in vitro* can be used (*25*). The authors found that there are 5.4×10^{-7} mutations per cell per generation spontaneously, while 1 Gy of γ rays induces 5×10^{-6} mutations. Thus, in about nine cell cycles, the same number of *HPRT* mutations in Chinese hamster cells occur spontaneously as are induced by 1 Gy of γ rays. The magnitude of the spontaneous occurrence was confirmed in another hamster cell line (*26*); the same magnitude of radiation-induced *HPRT* mutations was also found in human leukemia cells (*27*). Basal cells are one possible target cell type in the human lung. Measurements (*28*) suggest that these cells divide about once a month, so there are about 12 cell divisions per year. If the cited ratio for *HPRT* mutations in Chinese hamster cells is also typical for cancer-inducing genes in human lung cells, 1 Gy would induce fewer initiating mutations than occur spontaneously in 1 year. This would be far fewer radiation-induced mutations than are needed for the I model and less than the best value for the IP model. The IP model for lung cancer could be adapted within the statistical uncertainties to such a small mutation induction by radiation by increasing the relative importance of promotion.

Promotion effects of radiation were suggested from the protraction effects found in various epidemiological studies and from analyses of animal experiments for high-LET radiation (*12, 13*). The present work also suggests that γ radiation might act not only on initiation but also on promotion. This is in line with an analysis of lung cancer in mice after acute and fractionated exposure, where a promoting effect of γ rays was also found (*29*).

The mechanism for the estimated promoting effect of radiation is still unknown. Cell inactivation due to radiation has been suggested (*9*) and applied to promotion of radon-induced lung cancer (*30*). We may tentatively test this mechanism for numerical consistency: It may be assumed that 1 Gy of acute γ radiation inactivates about 30% of cells in an organ. If initiated cells have twice the replacement probability of a normal cell, then their number increases by 30%, inducing an initial ERR after 1 Gy of 0.3. For most cancer sites, the estimated initial ERR from promotion after 1 Gy (g_r) is larger, but it is of the correct order of magnitude.

The patterns of the time-since-exposure dependence of lung cancer risk for miners (*31*) and of the atomic bomb survivors (*32*) become more similar. In both cases, a promoting effect of radiation is also suggested. Whether the actual mechanisms of radiation action are also similar requires further studies.

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