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From mechanisms to risk estimation – bridging the chasm

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Abstract

We have a considerable amount of work ahead of us to determine the importance of the wealth of new information emerging in the fields of sub-cellular, cellular and tissue biology in order to improve the estimation of radiation risk at low dose and protracted dose-rate. In this paper, we suggest that there is a need to develop models of the specific health effects of interest (e.g., carcinogenesis in specific tissues), which embody as much of the mechanistic (i.e., biological) information as is deemed necessary. Although it is not realistic to expect that every radiation-induced process should or could be included, we can hope that the major factors that shape the time dependence of evolution of damage can be identified and quantified to the point where reasonable estimations of risk can be made. Regarding carcinogenesis in particular, the structure of the model itself plays a role in determining the relative importance of various processes. We use a specific form of a multi-stage carcinogenic model to illustrate this point. We show in a review of the application of this model to lung cancer incidence and mortality in two exposed populations that for both high- and low-LET radiation, there is evidence of an "inverse dose-rate" or protraction effect. This result could be of some considerable importance, because it would imply that risk from protracted exposure even to low-LET radiation might be greater than from acute exposure, an opinion not currently held in the radiation protection community. This model also allows prediction of the evolution of the risk over the lifetimes of the exposed individuals. One inference is that radiation-induced initiation (i.e., the first cellular carcinogenic event(s) occurring in normal tissue after the passage of the radiation) may not be the driving factor in the risk, but more important may be the effects of the radiation on already-initiated cells in the tissue. Although present throughout the length of the exposure, radiation-induced initiation appears to play a dominating role only very late in life, and only for those individuals who began their exposure early in life. These conclusions are very dependent, of course, on the hypotheses embodied in the initiation–promotion–conversion paradigm of carcinogenesis. We suggest that recently identified processes, such as the "bystander effect", might affect initiation, promotion, and malignant conversion in different ways. Finally, the manner in which the quality of radiation affects these processes must be understood in the context of the mixed high- and low-LET radiations that are found in the space environment. Important directions in critical experiment definition are suggested, including a renewed emphasis on well-designed animal experiments over extended periods of time.

Keywords: Radiation risk; Cellular biology; Tissue biology; Radiation carcinogenesis models

1. Introduction

A tremendous amount of new information on radiation molecular biology has emerged in the last several years. It is important to acknowledge, however, that problems may exist as to the relevance of in vitro data to in vivo processes, animal data to human data and, when considering cancer-induction, non-carcinogenic end points to the very complex end point of carcinogenesis itself. In this paper, we confine ourselves to human cancer as end point, as it is a recognized risk factor that should be evaluated for extended space missions. An enormous task remains to tie the new information coming from the laboratory into a coherent picture of radiation carcinogenesis so that reliable predictions of risk from protracted radiation exposures in space can become possible. First, we believe it is important to develop specific models for each of the various types of cancer inducible by radiation. For instance, leukemogenesis probably involves different mechanisms than

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those operating in solid tumorigenesis, simply because the time course for induction of each is so different. Even for the group of tumors labeled "solid tumors", different processes (certainly different mutational events) play prominent roles. The present paper discusses a well-known carcinogenesis model (the two-stage clonal expansion model) and the implications arising from using it to analyze lung cancer incidence/mortality in populations exposed to protracted doses of high-LET radiation in the case of the Colorado Plateau miners (Luebeck et al., 1999) and low-LET radiation in the case of the Canadian radiation workers (unpublished).

2. The two-stage clonal expansion (TSCE) model

The main hypotheses of the model are:

1. Two (or more) events must occur within a cell to produce a malignant cell. The events, presumably mutational in nature, may be caused either spontaneously or by specific carcinogenic agents.
2. At some point between the first and last event, a cell emerges that has altered net cell proliferation kinetics: the net average rate of growth of this population of cells is greater than that of the surrounding cells. The clone of these intermediate (or initiated) cells has a proliferative advantage over other cells in the tissue. The process of enhancing the growth of such clones is called "promotion". The cell kinetic parameters of this population have been altered from those of the normal cell population.
3. An initiated cell can sustain further spontaneous or induced genomic events that may lead to the appearance of a malignant cell, and ultimately to a malignant tumor.

Specifically, we assume that the number of initiated cells arising from normal stem cells can be described by a non-homogeneous Poisson process with intensity $\nu(t)N(t)$, where $N(t)$ is the number of normal susceptible cells in the tissue of interest at time (age) t and $\nu(t)$ is the rate per cell of the process (which may involve multiple mutations) that results in an initiated cell with altered proliferation kinetics. These cells divide at a rate $\alpha(t)$ per cell and die or differentiate at a rate $\beta(t)$ per cell. They divide into one intermediate cell and one malignant cell (malignant conversion) at a rate $\mu(t)$ per cell. A pictorial representation of the model is shown in Fig. 1. Further details of the model can be found in Moolgavkar and Luebeck (1990) and Heidenreich et al. (1997).

2.1. Lung cancer risk in the TSCE model

In this paper we concentrate on lung cancer as an example of a radiogenic cancer. In an analysis of lung cancer mortality in Colorado Plateau miners, application of the hypotheses of the TSCE model has shown

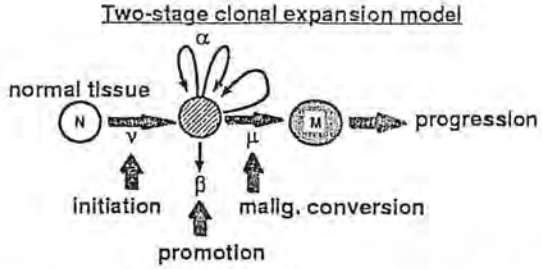

Fig. 1. A pictorial representation of the two-stage clonal expansion (TSCE) model.

that radiation-induced promotion dominates radiation-induced initiation (Luebeck et al., 1999), and it has been suggested that this could be due to a bystander effect (Curtis et al., 2002a). This domination of promotion over initiation is shown in Fig. 2 where the lifetime excess absolute risk at age 70 is shown for 1- and 12-year exposures as a function of total exposure with initiation "turned off" and with the initiation coefficient equal to 10 times the value obtained by the maximum likelihood analysis. We see that there is little change in the risk for either the 1- or 12- year exposures. The best functional dependence of the promotion term as a function of exposure rate was an initial increase followed by saturation at higher rates. Such saturation can contribute to an "inverse dose-rate effect", i.e., for the same total exposure, more risk occurs for longer exposures than for shorter ones. This phenomenon has been reported in

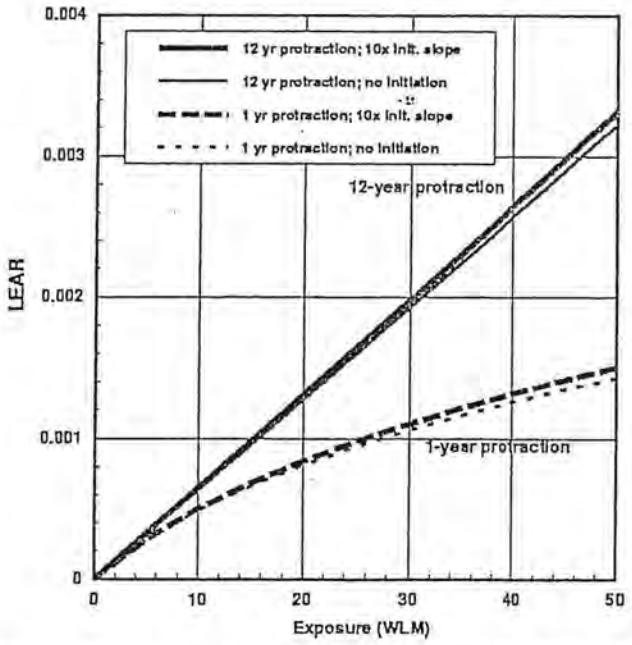

Fig. 2. Lifetime excess absolute risk, LEAR (at age 70) as a function of exposure for 1- and 12-year protraction intervals. The radiation-induced initiation rate was set to zero and to 10 times the value obtained by the maximum likelihood method. Each exposure was centered on age 42. (from Curtis et al., 2001).

earlier studies using the TSCE model (e.g., Moolgavkar et al., 1993) and has been detected in the miner cohort studies using conventional statistical techniques (e.g., Lubin et al., 1995). We call it a protraction effect (Curtis et al., 2001) to distinguish it from the "inverse dose rate effect" seen in cell culture experiments, which arises from an entirely different mechanism and is seen at much shorter protraction times. This protraction effect is clearly seen in Fig. 2, where the 12-year exposure risk is significantly greater than the 1-year exposure risk at any given total exposure except at very low exposures where they converge.

Another study has used the TSCE model to analyze 95,000 Canadian male radiation workers (unpublished). Here lung cancer incidence from the Canadian Cancer Data Base was linked to the Canadian National Dose Registry. The exposures were to whole body gamma radiation with a small contribution of tritium to a few workers. The mean cumulative dose was 18.2 mSv. Thirty-three out of 322 cancers were attributable to radiation. The analysis indicated that a protraction effect was present in these data as well. Along with promotion, malignant conversion was seen to play a role in certain situations. This is seen in Fig. 3, where the excess absolute risk (here defined as an excess in the hazard function) is shown as a function of attained age for two accumulated doses (10 and 100 mSv) for two protraction intervals (1 and 10 years). The ages at which the three different carcinogenic processes (initiation, promotion and malignant conversion) dominate are indicated. It is seen that for younger ages, malignant conversion (of already initiated cells) dominates, for middle to early old age, promotion (again, of already initiated cells) dominates, and only at older ages does initiation dominate. We note that the amount of the initiation contribution is highly uncertain, and even its presence is not statistically significant. We also can see that a protraction effect exists from all three processes, i.e., more contribution to the risk for the 10-year than for the 1-year protraction. Although no smoking data are available on this cohort, it is generally recognized that smoking enters many populations in a birth cohort-wise fashion. Therefore, introducing such an effect is expected to account for much of the effect of smoking. The assumption has been made that there is no correlation between radiation duration and smoking frequency.

In both these studies, promotion plays a dominant role in shaping the carcinogenic response. In addition, the conclusion is inescapable that already initiated cells, i.e., those already along the carcinogenic pathway, play a crucial role in determining the ultimate probability of radiation-induced cancer for protracted exposures. The suggestion has been made that bystander effects may be important in the radiation-induced promotion of such intermediate cells in the high-LET radiation case (Curtis et al., 2002a,b). Signals sent out by radiation-damaged cells may be interpreted, as being mitogenic by neighboring intermediate cells (or clones) which are not as controlled by homeostatic pressure as are normal tissue cells. Malignant conversion of intermediate cells may be important in those individuals exposed late in life, when background intermediate cells become more prevalent. The time inversion of the importance of the processes (i.e., malignant conversion, promotion, and initiation) is clearly evident in Fig. 3, when compared to the original time sequence of the stages shown in Fig. 1.

Assuming in the human that there is radiation-induced promotion that can affect already initiated cells, these analyses suggest that: (1) this phenomenon dominates the rate of tumor induction (the hazard function) over the other processes (initiation and malignant conversion) at least for individuals exposed at young to middle age, (2) greater risk is generated for protracted than for shorter (i.e., acute) exposures; that is, there may be a protraction effect even for low-LET radiation. If this is true, the value for the dose and dose rate effectiveness factor (DDREF) may have to be revised downward, and, this evaluation suggests, might even be set at a value less than unity. Much has to be done, however, to substantiate these results. In particular, we suggest that the animal data on which the DDREF was based should be revisited. These data have suggested that a value between two and ten be used (see, for instance, NCRP, 1980; NCRP, 2001). ICRP and NCRP have chosen the value two as a conservative choice. The question arises as to why the risk in animals decreases with increased protraction of low-LET radiation. Clearly in the human case, it cannot be due to the same repair processes that are acting for the end point of cell killing, since the time scale for repair of sub-cellular damage relative to cell killing (several hours up to a day) is much less than the time scale for the increases in risk seen in the above epidemiological analyses (1–10 years or longer). The answer might be that human cells are intrinsically less prone to initiation than rodent or other animal cells. Perhaps they need to accumulate more (or more complex) damage than do animal cells to become initiated. This would explain why the presence of initiation is so hard to detect in the human epidemiological data, although we acknowledge that some epidemiological studies have shown initiation to be significant (e.g., Heidenreich et al., 2003).

Another point is that much of the animal data were obtained with experiments designed such that the low and high dose rate groups began their exposures at the same age. It has been shown both for the Colorado miner (high-LET) data and the Canadian worker (low-LET) data that cohorts starting exposures at the same age could show a "direct dose rate effect". Fig. 4 shows this for these two data sets. It is clear that either "direct" or "inverse" effects can be measured, depending on the timing of the start dates of the experiments.

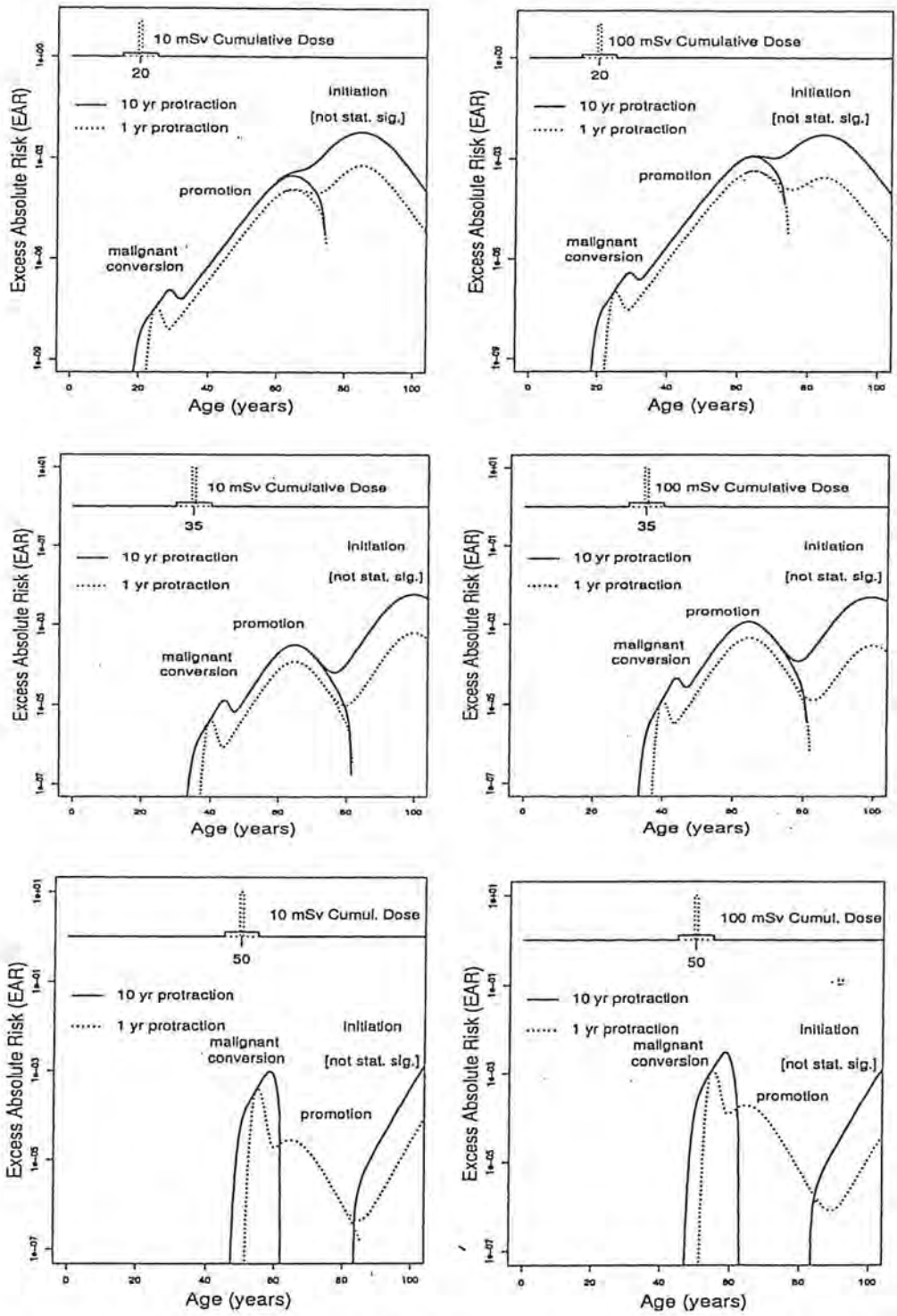

Fig. 3. The excess absolute risk (hazard function) for the Canadian (low-LET) workers as a function of attained age for 10 mSv (left panels) and 100 mSv (right panels) for exposures centered at 20, 35 and 50 years (top, middle and bottom panels) and for 1- and 10-year exposures (dotted and solid lines, respectively).

In any case, we suggest that the animal data leading to the large values of DDREF should be reevaluated with multi-stage models to see if a coherent picture (for both animals and humans) can be gained of the pattern of tumor incidence after protracted low-LET irradiation. For instance, if initiation dominates in animal radiation carcinogenesis, perhaps long term repair of and/or recovery from initial lesions plays a role, and the "direct dose rate effect" will become apparent, while in situations where promotion dominates (in the

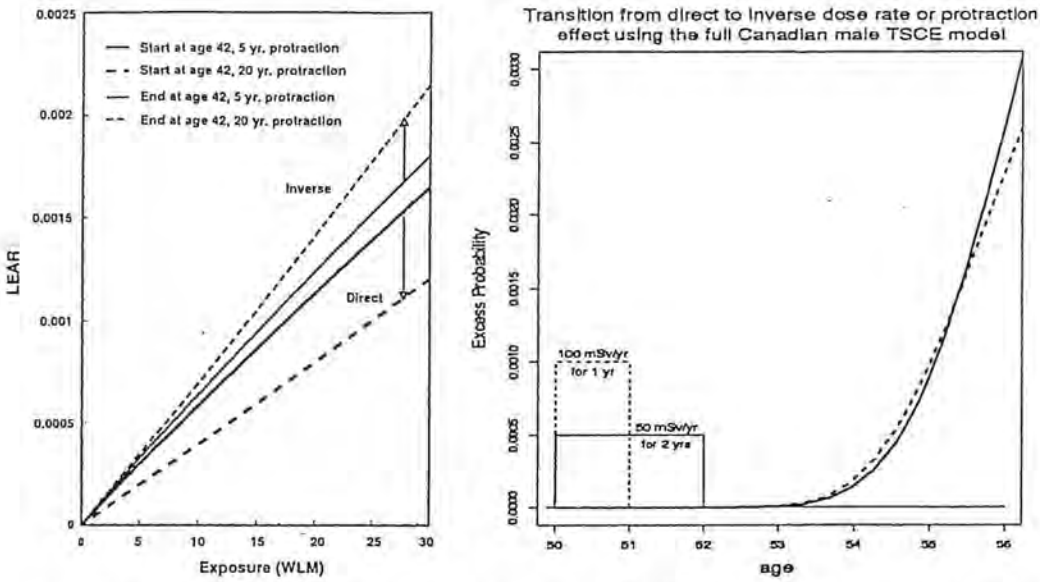

Fig. 4. Lifetime excess absolute risk (LEAR) at age 70 is plotted against exposure for the Colorado plateau miners on the left, with exposure started (bottom two curves) and ended (top two curves) at age 42 (from Curtis et al., 2001). On the right, excess probability is plotted vs. age for the Canadian radiation workers data. Here a transition is seen from a direct to an inverse dose rate effect when an individual ages. The exposures both start at age 50.

human case), protraction effects may become more important.

The foregoing suggests that careful new animal experiments should be designed to test this hypothesis. In particular, experiments should be designed such that two exposure regimes producing the same dose are given to animals in which the exposures are *centered* on the same age. For example, animals exposed for 20 weeks and 6 weeks might be irradiated from 15 to 35 weeks of age in the first case and from 22 to 28 weeks of age in the second case, i.e., both centered at 25 weeks of age. Other "centered" ages, of course, should be chosen as well. Animal model systems having a significant background of cancer over their lifetimes should be selected to mimic the human case. This would ensure that already-initiated cells would be present in the animals.

3. Conclusions

If we accept the initiation–promotion–malignant conversion paradigm, the use of this relatively simple TSCE model in analyzing the two epidemiological data sets just discussed leads to several interesting conclusions.

1. The processes leading to radiation-induced initiation, e.g., mutations in normal lung tissue, may not be as important as the other two processes, promotion and malignant conversion, in the radiogenesis of human lung cancer from protracted exposure. The dominance of initiation appears late in life, and may well play a significant role only if the radiation exposure begins at an early age. We note that neither the low-LET nor the high-LET radiation cohort produced a statistically significant value for the initiation coefficient.
2. A protraction effect, i.e., increased risk with increased exposure duration for the same total dose, occurs not only in a population exposed to high-LET radiation (the Colorado Plateau miners inhaling radon) but also appears to occur in a population exposed to low-LET radiation (the Canadian radiation workers exposed to gamma rays and tritium). This effect in these cohorts is caused primarily by the promotion process.
3. The model calculations show that the importance of the three processes appear in time in reverse order to that in which they occur. The reason is the two later processes, promotion and malignant conversion, act on already initiated cells or clones in the tissue. We suggest that bystander effects, in which mitogenic signals are received from neighboring irradiated cells, promote initiated cells or clones. Such initiated cells or clones may be less influenced than normal cells by homeostatic processes and might not be able to ignore the mitogenic signals. This mechanism would then lend these clones to gain proliferative advantage.
4. The protraction effect seen in the low-LET radiation cohort would imply that the DDREF may be less than, not greater than, unity for humans, at least for radiogenic lung cancer. This in turn implies that the animal data, which have consistently given values greater than unity, may not be relevant in all cases to the human situation. It is suggested that this might be

because radio-oncogenic initiation is more prevalent in animal cells than in human cells. There may be less complex or fewer mutational events necessary to produce a cancer in animals; therefore, radiation-induced initiation may be less important in human than in animal tumorigenesis. This implies that the animal data should be revisited and analyzed with the model to see if a coherent picture of carcinogenesis can emerge for both humans and animals. New protracted irradiation experiments are suggested, which are centered at the same age rather than begun at the same age, since in some cases, even in the human case, a direct dose rate effect can be shown to occur briefly if the exposures begin at the same time.

We emphasize that these conclusions result from the analysis of a very few data sets with the model. Although a birth-cohort effect accounts for some of the smoking effect, we acknowledge that since smoking is the overriding risk factor for lung cancer, our assumption that there is no correlation between smoking frequency and radiation exposure duration is crucial in arriving at the above conclusions.

Finally, it is clear that we are a long way from understanding the carcinogenic process either in animals or in humans. The above conclusions imply that there is indeed a wide chasm that separates our emerging knowledge of what radiation does at the tissue, cellular and sub-cellular levels from our experience with the emergence of radiation-induced cancer in humans. Carcinogenic models can play a crucial role in suggesting new experiments to test hypotheses. As more is learned about which processes are important and when in the life of the individual they play an important role, models may eventually emerge that can be used to predict the probability of cancer induction after an arbitrary radiation exposure. Because it appears that at least three processes can be important at different times in an individual's life, there may be at least three different dependencies on LET that are important, each playing a role at different ages. Therefore, it would not be surprising that risk would have a different dependence on LET depending on such parameters as age at exposure and exposure duration. We have a considerable way to go to bridge the gap between identifying the critical events occurring at a local (cellular/subcellular) level in the tissue during and after an exposure and arriving at the probability of the emergence of a tumor many years later.

Acknowledgements

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