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Can Promotion of Initiated Cells be Explained by Excess Replacement of Radiation-Inactivated Neighbor Cells?

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Abstract

Recently, the observed promotion in the clonal expansion of a two-stage cancer model was attributed to a small excess replacement probability for the initiated cells. The proposed mechanism of excess replacement was evaluated for single intermediate cells surrounded by normal cells. This paper investigates this mechanism further using the same biological parameters. If the formation of clones of intermediate cells is taken into account in a quantitative analysis of the proposed mechanism, it turns out that (1) for the initial strong increase of the promotional effect with exposure, a much larger and unlikely excess replacement probability is needed, and (2) the leveling of the promotional effect for high exposures cannot be explained by multiple normal neighbors of an intermediate cell being inactivated within one cell cycle, as it had been suggested. Perhaps these discrepancies could be partly resolved by a reseating of the original parameters, but this should be investigated further.

INTRODUCTION

In two-mutation cancer models, the process of carcinogenesis is modeled by a first mutation followed by a clonal expansion of the intermediate cells and then a second mutation to induce malignancy. For radiation-induced carcinogenesis, the mutational steps are usually taken to be dependent on radiation, but sometimes this is also assumed for the clonal expansion. Recently, a biological mechanism was proposed (1) to explain a radiation-induced increase in clonal expansion (so-called promotion). The mechanism assumes that initiated intermediate cells that are on their way to malignancy have a higher probability than normal cells of replacing cells that are inactivated by radiation. If the excess replacement probability of these intermediate cells is denoted as g and every intermediate cell has N neighboring normal cells, it can be derived (1) that the clonal expansion, γ , amounts to $\gamma \equiv \alpha - \beta = gN\beta$, where α and β denote birth and death rate, respectively.

In ref. (1), the excess replacement probability is calculated for the case of lung cancer in uranium miners due to radon (2). Therefore, in this short paper, the parameters are also taken from this case. If a two-dimensional hexagonal lattice of basal lung cells is assumed ($N = 6$), only a small excess replacement probability [$g = 0.15$ at 10 WLM/month according to Table 1 of ref. (1)] is needed to explain the clonal expansion in the model fit of ref. (2). The leveling of the clonal expansion as observed in ref. (2) may then according to ref. (1) occur when multiple normal cells next to an intermediate cell are being inactivated within

one cell cycle. In that case the intermediate cell would be able to replace only one of the inactivated normal cells per cell cycle.

An important assumption in the proposed mechanism is that intermediate cells are surrounded by normal cells that they can replace upon their inactivation. In reality, clonal expansion leads to clones that may consist of multiple intermediate cells. Many of these intermediate cells will be completely surrounded by other intermediate cells. An excess replacement probability for these cells will not contribute to the desired promotional effect. In the two-stage models, the statistics of clone sizes is known, and thus the effect of the formation of clones on the hypothesis put forward in ref. (1) can be quantified.

This paper contains a quantitative analysis of the heuristic explanation put forward in ref. (1). For this analysis, the same parameters are employed as were used in refs. (1) and (2). A rescaling of these parameters to investigate how the results depend on their choice has not been attempted. The consequences for promotion of an excess replacement probability for intermediate cells are investigated, taking into account a realistic formation and growth of clones.

INCORPORATION OF CLONE SIZE

Without the formation of clones, the number of normal neighbors per intermediate cell in a two-dimensional hexagonal lattice is 6 [as used in ref. (1)]. Since it is the product gN that determines the promotional effect [$\gamma = gN\beta$, and β increases only linearly with dose rate in ref. (1)], a large number of normal neighbors, N , results in a relatively small excess replacement probability, g , to yield the desired effect. Hence we shall first investigate what happens to the number of normal neighbors when clones are considered. Note that all the mathematical machinery used in this section is already available in the literature.

Number of Normal Neighbors

We assume that a clone of intermediate basal lung cells grows in two dimensions to a circle with radius R and that the radius of all individual cells is r . In addition, we use the filling fraction of a hexagonal lattice $\phi = \pi/12$ (3). Then the number of intermediate cells in a clone is $N_I = \phi R^2/r^2$, and the number of normal cells directly surrounding the clone is $N_N = \pi/\sin^{-1}[r(R+r)]$. Note that for the latter equation it was assumed that the clone is surrounded by a ring filled with touching normal cells. By combination of these expressions we find:

$$N_N = \frac{\pi}{\sin^{-1}\left[\left(1 + \sqrt{N_I/\phi}\right)^{-1}\right]}. \quad (1)$$

Size Distribution of Intermediate Clones

For the analytical solution of the two-mutation model, equation 2 in ref. (4) gives the probability $p(t)$ of finding a non-extinct clone consisting of exactly N_I intermediate cells at time t . For time-constant parameters, the integrals for $p(t)$ can be solved analytically to give

$$p(t) = \frac{\left[\frac{\alpha(e^{\alpha t} - e^{\beta t})}{\alpha e^{\alpha t} - \beta e^{\beta t}}\right]^{N_I}}{N_I \{(\alpha - \beta)t - \ln(\alpha - \beta) + \ln[\alpha - \beta e^{(\beta - \alpha)t}]\}}. \quad (2)$$

Normal Neighbors per Intermediate Cell

From Eqs. (1) and (2), the expectation value for the number of normal neighbors per intermediate cell can be calculated as

$$\begin{aligned}\langle N \rangle &= \sum_{N_I=1}^{\infty} p(t) \frac{N_N}{N_I} \\ &= \sum_{N_I=1}^{\infty} \frac{\left[\frac{\alpha(e^{\alpha t} - e^{\beta t})}{\alpha e^{\alpha t} - \beta e^{\beta t}} \right]^{N_I} \pi / \sin^{-1} \left[\left(1 + \sqrt{N_I / \varphi} \right)^{-1} \right]}{N_I^2 \{ (\alpha - \beta)t - \ln(\alpha - \beta) + \ln[\alpha - \beta e^{(\beta - \alpha)t}] \}}.\end{aligned}\quad (3)$$

For a given lifelong exposure rate of 10 WLM/month [as used in ref. (1)], Eq. (3) can be evaluated for different ages. This leads to Fig. 1, which shows that the expected number of normal neighbors per intermediate cell decreases rapidly from approximately $\langle N \rangle = 6$ at very young ages to values below $\langle N \rangle = 1$ at ages above 40 years. This shows that a strong increase in promotion [as is found in ref. (2)] in reality cannot be induced with a small excess replacement probability. In fact, a large excess replacement probability is needed. That, however, is rather unlikely. Thus it is improbable that a steep increase in the clonal expansion with exposure can be explained with this mechanism.

LEVELING OF THE CLONAL EXPANSION

A second feature of the promotion modeled in ref. (2) is the leveling of the clonal expansion at higher radiation dose rates after the initially steep increase. In ref. (1), it is stated that this may be caused by some sort of saturation in the replacement of normal cells. This could take place when multiple normal cells next to an intermediate cell are inactivated so that only one of them could be replaced by an intermediate cell within a cell cycle. We shall now investigate how this turns out in practice when clones are considered.

Number of Contact Cells at the Clone Surface

To investigate the above mechanism for the leveling of the promotion curve (γ), the number of normal cells in contact with an intermediate cell at the clone surface is needed. For small clone sizes, the number of contact cells can be counted easily on a two-dimensional hexagonal lattice. This gives six contact cells per intermediate cell for a clone of one intermediate cell, five per intermediate cell for a clone of two cells, four for a clone of three cells, etc. Furthermore, for perfect circular clones, the number of contact cells will go down to two for clones of infinite size. It is assumed here that due to small deviations from perfect circles this limiting value will be slightly larger in practice (2.2). These numbers can be fitted with a descriptive formula of the form

$$N_c = 6 + a \left(\frac{1 - N_I}{b + N_I} \right). \quad (4)$$

Fitting this formula to the above numbers of contact cells for several clone sizes leads to the values $a = 3.8$ [so that in the limit of very large values of N_I Eq. (4) yields $6 - a = 2.2$] and $b = 0.9$.

Calculation of the Clonal Expansion

For the calculation of the limiting excess replacement, we need to calculate the probability, P_c , that not more than one normal contact cell is inactivated during a cell cycle, because only one normal contact cell can be replaced by an intermediate cell during one cell cycle. That

way, a correct modeling of the limiting biological circumstances is assured. This probability is given by the following binomial distribution:

$$P_c = (1 - p_c)^{N_c} + p_c N_c (1 - p_c)^{N_c - 1}, \quad (5)$$

where p_c is the probability for cell death in one cell cycle; $p_c = (\beta_0 + \beta_r D)/t_c$ [there the usual linear relationship of β with high-LET dose rate D is used (as in ref. 1) and t_c denotes the time for one cell cycle].

Now the limiting value of gN (with not more than one contact cell inactivated) can be calculated as

$$gN \rightarrow \sum_{N_i=1}^{\infty} p(t) \frac{N_N}{N_i} P_c. \quad (6)$$

Equations (1), (2), (4) and (5) can be substituted into Eq. (6), and together with the substitution $\alpha = \beta(1 + gN)$, this yields a limiting value for gN expressed in terms of gN . Input values for gN can be derived from $\gamma \equiv gN\beta$, with both γ and β given in refs. (1, 2). Solutions for gN can be calculated iteratively, starting from the input value for gN , calculating an output value with Eq. (6), averaging input and output values for a new input value, and repeating this process until convergence is reached. The gN calculated in this way, combined with β , gives values for γ that can be compared directly with the clonal expansion given in ref. (2). This comparison is shown in Fig. 2.

Figure 2 shows that the hypothesis that promotion in the two-mutation model is limited by a maximum of one replacement of a normal cell by an intermediate cell in one cell cycle and does lead to a clonal expansion that increases with exposure and that some leveling does occur. However, it does not lead to promotion in the form of almost a step function with a strong initial increase followed by a leveling to a near-constant value as is found in the model fit of ref. (2). The discrepancy between the two clonal expansion rates is considerable, and it should be realized that this has severe consequences for the calculation of the hazard, which depends exponentially on γ .

CONCLUSIONS

In this paper, we have investigated the effect of the formation of clones of initiated cells on the hypothesis put forward by Heidenreich *et al.* (1). For this purpose we considered it appropriate to employ the parameter values used in that paper. The effect of a rescaling of these parameters has not been investigated. Heidenreich *et al.* (1) neglected the formation of clones, but when this is incorporated, it is found that:

1. The expected number of normal neighbors per initiated cell is much lower than estimated in ref. (1). This means that the excess replacement probability needs to be un-realistically high to obtain the desired promotional effect.
2. The leveling of the exposure-dependent clonal expansion as found in refs. (1, 2) cannot be induced by a saturation in the replacement due to multiple normal cells being inactivated next to a single intermediate cell [as suggested in ref. (1)].

In short, the mechanism of excess replacement of normal cells by intermediate cells as proposed in ref. (1) is quantitatively in disagreement with the modeled promotional effect in ref. (2). Perhaps this disagreement could be partly resolved by a rescaling of parameters, but this should be investigated further.

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FIG. 1. Normal neighbors per intermediate cell as a function of age for an exposure rate of 10 WLM/month.

FIG. 2. Comparison of the clonal expansion rate, γ , between the calculations performed in this paper (for which $t = 20$ years) and the model fit of ref. (2) [also used by ref. (1)].