Failure of programmed cell death and differentiation as causes of tumors: Some simple mathematical models

(tumorigenesis/apoptosis)

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ABSTRACT Most models of tumorigenesis assume that the tumor grows by increased cell division. In these models, it is generally supposed that daughter cells behave as do their parents, and cell numbers have clear potential for exponential growth. We have constructed simple mathematical models of tumorigenesis through failure of programmed cell death (PCD) or differentiation. These models do not assume that descendant cells behave as their parents do. The models predict that exponential growth in cell numbers does sometimes occur, usually when stem cells fail to die or differentiate. At other times, exponential growth does not occur: instead, the number of cells in the population reaches a new, higher equilibrium. This behavior is predicted when fully differentiated cells fail to undergo PCD. When cells of intermediate differentiation fail to die or to differentiate further, the values of growth parameters determine whether growth is exponential or leads to a new equilibrium. The predictions of the model are sensitive to small differences in growth parameters. Failure of PCD and differentiation, leading to a new equilibrium number of cells, may explain many aspects of tumor behavior—for example, early premalignant lesions such as cervical intraepithelial neoplasia, the fact that some tumors very rarely become malignant, the observation of plateau in the growth of some solid tumors, and, finally, long lag phases of growth until mutations arise that eventually result in exponential growth.

The control of the cell cycle and cell death are frequently deranged in tumors (1). The most frequently mutated loci in tumors, TP53 (2), is centrally involved in pathways leading to programmed cell death (PCD) (3–5). Failure of PCD may be important in causing the development of a tumor and in determining its response to antitumor therapy (6). The precise role of PCD in tumorigenesis is, however, still unclear. There are several ways in which the failure of PCD might lead to or promote tumor growth. One such way may be to give cells the equivalent of a replicative advantage, whereby failure to die is effectively the same as more rapid cell division. Alternatively, the failure of PCD may lead to an increase in the intrinsic mutation rate by permitting cells to live on into senescence and be exposed to mutagens or acquire more spontaneous mutations. Failure of PCD can be regarded as a special case of failure of cell differentiation, which may be of general importance in tumorigenesis.

Basic models of tumorigenesis assume that the tumor grows by increased cell division (7–10). A single mutant cell replicates at a maximum rate of $2^g$ (where $g$ is the number of divisions or “generations” for the mutant). More generally, the rate of replication in the tumor is $(1 + w)^g$ per generation, where $w$ is the selective advantage of the mutant (relative to a mean nonincreasing population of normal cells). All descendants of the original mutant cell simply replicate at that rate. Models of tumorigenesis by failure of PCD or differentiation cannot be assumed to occur via the same straightforward mathematical pathway as tumorigenesis by increased cell division. The reason for this is fundamental to models that incorporate cell death and differentiation: even for the purpose of simplification, it cannot be assumed that descendant cells behave as their parents do. For example, a mutation occurring in a stem cell may have its effects.

The number of cells in any $F_n (n = 0, 1, 2, 3)$ depends on the following variables: (i) the number of cells in $F_{n-1}$ (1 ≤ $n$ ≤ 3); (ii) the rate of division of cells in the $F_{n-1}$; (iii) the probability that cells in $F_{n-1}$ differentiate into $F_n$ cells, rather than remain in $F_{n-1}$ or die; (iv) the rate of division of cells in $F_n$; and (v) the probability that cells differentiate into cells in $F_{n+1}$ or die, rather than remain in $F_n$.

We denote the time for one cell division to occur in population $F_n$ as $t_n$ (where, for convenience, values are normally measured relative to baseline $t_0$). The number of cells after $G$ divisions is denoted by $N_n(G)$. After each cell in $F_0$ divides, it may (i) die, (ii) differentiate to form a $F_{n+1}$ cell, or (iii) renew itself. These proportions are denoted in the $F_0$ by $\alpha_1$, $\alpha_2$, and $\alpha_3$, respectively. $\beta_1$, $\beta_2$, and $\beta_3$ are the respective proportions for $F_1$. $\gamma$ is the probability of a cell in the $F_2$ population dying (that is, passing through to the $F_3$ stage per unit time).

The following restrictions apply to the values of $\alpha$, $\beta$, and $\gamma$:

(i) $\alpha_1 + \alpha_2 + \alpha_3 = 1$; $\beta_1 + \beta_2 + \beta_3 = 1$; (ii) $\alpha_1, \alpha_2, \alpha_3, \beta_1, \beta_2$;

Abbreviation: PCD, programmed cell death.
\( \beta_2, \gamma \leq 0; (iii) \alpha_3 (\text{normally}) = 1/2; (iv) \beta_2 \text{ (normally)} \leq 1/2 \) (owing to differences among \( t \) values); and (v) \( \gamma \leq 1 \).

Results

Model 1: Normal Cell Division. Cell numbers in successive generations in \( F_0 \) are related according to

\[
N_0(G + 1) = 2\alpha_3 N_0(G). \tag{1}
\]

At equilibrium,

\[
N_0(G + 1) = N_0(G),
\]

\[
\Rightarrow 2\alpha_3 N_0(G) = N_0(G),
\]

\[
\Rightarrow \alpha_3 = 0.5. \tag{2}
\]

There is, as expected, a unique point of equilibrium at which the population of stem cells exactly renews itself. If \( \alpha_3 \) rises above or falls below 0.5, \( N_0 \) respectively increases or decreases exponentially.

For \( F_1 \),

\[
N_1(G + 1) = 2\beta_2 N_1(G)t_0/t_1 + 2\alpha_2 N_0(G), \tag{3}
\]

and at equilibrium

\[
N_1(G + 1) = N_1(G),
\]

\[
\Rightarrow N_1(G) = 2\beta_2 N_1(G)t_0/t_1 + 2\alpha_2 N_0(G),
\]

\[
\Rightarrow N_1(G) = \frac{2\alpha_2 N_0(G)}{[1 - (2\beta_2 t_0/t_1)]}. \tag{4}
\]

Unlike \( F_0 \), therefore, there are multiple equilibria depending on \( N_0(G), \alpha_2, \beta_3, t_0, \text{and} \ t_1 \). No equilibrium exists when \( N_0 \) is not at equilibrium or when \( 2\beta_2 t_0/t_1 > 1 \). In the latter case, \( N_1 \) increases exponentially because of self-renewal of \( F_1 \) cells above the number required simply to maintain their steady state.

For \( F_2 \),

\[
N_2(G + 1) = 2\beta_2 N_1(G)t_0/t_1 + N_2[1 - (\gamma t_0/t_2)], \tag{5}
\]

and at equilibrium

\[
N_2(G + 1) = N_2(G),
\]

\[
\Rightarrow N_2(G) = 2\beta_2 N_1(G)t_0/t_1 + N_2[1 - (\gamma t_0/t_2)],
\]

\[
\Rightarrow N_2(G) = \frac{2\beta_2 N_1(G)t_0/t_1}{\gamma}. \tag{6}
\]

As for \( F_1 \), there exist multiple equilibria depending on \( N_1(G), \beta_2, \gamma, t_2, \text{and} \ t_1 \). However, when \( N_1 \) is at equilibrium, so is \( N_2 \).

The simple results illustrate the increased complexity of behavior that accompanies models that consider cell differentiation and PCD. Parameters of replication are constrained within limits for the cell population to be at equilibrium. The limits for stem cells are restrictive but are less so for partially or fully differentiated cells. We now analyze the case in which a mutation has altered the proportions of cells differentiating, dying, or renewing themselves in order to determine the effects on tumorigenesis. The models deliberately do not specify in which cells the mutation occurs or the locus at which it might occur.

Model 2: Change in \( \gamma \), Proportion of Differentiated Cells Undergoing Programmed Death. \( \gamma \) only affects the value of \( N_2 \). It is assumed for the purposes of the model that a mutation causes \( \gamma \) to change by value \( \delta \), where \( 0 \leq \gamma + \delta \leq 1 \). This mutation may have occurred in the \( F_2 \) population itself but would be unlikely to have a large effect, since only one cell and its descendants are affected. It is more likely that the mutation has occurred in the \( F_0 \) or \( F_1 \) population but only has an effect on \( F_2 \) cells.

After the mutation has occurred,

\[
N_2(G + 1) = 2\beta_2 N_1(G)t_0/t_1 + N_2(G)[1 - (\gamma + \delta)t_0/t_2]. \tag{7}
\]

At equilibrium

\[
N_2(G + 1) = N_2(G),
\]

\[
\Rightarrow N_2(G) = 2\beta_2 N_1(G)t_0/t_1 + N_2(G)[1 - (\gamma + \delta)t_0/t_2],
\]

\[
\Rightarrow N_2(G) = \frac{2\beta_2 N_1(G)t_0/t_1}{(\gamma + \delta)}. \tag{8}
\]

Therefore, a change in the proportion of differentiated cells undergoing apoptosis does not lead to exponential tumor growth but rather to a new equilibrium. When \( \delta < 0, N_2(G) \) will be larger than before, and when \( \delta > 0, N_2(G) \) will be smaller than before. Clearly, if \( \gamma + \delta \sim 0, N_2 \) can be very large at equilibrium. \( N_1 \) and \( N_0 \) populations are always unchanged. Fig. 1 shows how \( N_2(G) \) depends on \( \delta \) for representative values of \( \beta_2, N_1, t_2, t_1 \), and \( \gamma \).

Model 3: Change in \( \beta_1 \), the Proportion of Semidifferentiated Cells Undergoing Programmed Death. Here, it is assumed a mutation occurs in an \( F_0 \) or \( F_1 \) cell that causes \( \beta_1 \) to be reduced by an amount \( \delta \) (0 < \( \delta < \beta_1 \)). The cells that fail to die are partitioned between \( \beta_2 \) and \( \beta_3 \) relative to their original values. Here,

\[
N_1(G + 1)
\]

\[
= 2\beta_3[1 + \delta/(\beta_2 + \beta_3)]N_1(G)t_0/t_1 + 2\alpha_2 N_0(G), \tag{9}
\]

and at equilibrium
There is no equilibrium when
\[ 2\beta_3(t_0/t_1)[1 + \delta/(\beta_2 + \beta_3)] > 1, \]
\[ \Rightarrow 2\beta_3(t_0/t_1) + 2\delta \beta_3(t_0/t_1)/(\beta_2 + \beta_3) > 1, \]
\[ \Rightarrow \delta > \left(\frac{1 - 2\beta_3(t_0/t_1)}{2\beta_3(t_0/t_1)}\right)[\beta_2 + \beta_3]. \]  
If this condition is fulfilled, cell numbers in the F1 population undergo exponential growth, but they do not do so otherwise. In the model of normal cell differentiation, the condition \( 2\beta_3(t_0/t_1) > 1 \) must hold for the population not to reach equilibrium. Therefore, the tendency to nonequilibrium is made more likely by the term \( 2\delta \beta_3(t_0/t_1)/(\beta_2 + \beta_3) \) (when \( \delta \) is positive). Again, however, failure of PCD does not necessarily lead to exponential tumor growth. Thus, when \( \delta \) is less than the limit given in Eq. 11, \( N_1(G) \) simply approaches a new, higher equilibrium. At each such stage, however, the probability that a decrease in \( \beta_1 \) caused by a new mutation then leads to exponential growth of F1 cells (and hence of the tumor overall) must increase.

\[ N_0(G + 1) = 2\beta_3[1 + \delta/(\beta_2 + \beta_3)]N_0(G)t_0/t_1 + (1 - \gamma_0/t_2)N_0(G), \]  
and at equilibrium
\[ N_0(G) = 2\beta_3[1 + \delta/(\beta_2 + \beta_3)]N_0(G)t_0/t_1 + (1 - \gamma_0/t_2)N_0(G), \]
\[ \Rightarrow N_0(G)[1 - (1 - \gamma_0/t_2)] = 2\beta_3[1 + \delta/(\beta_2 + \beta_3)]N_0(G)t_0/t_1, \]
\[ \Rightarrow N_0(G) = \frac{2\beta_3[1 + \delta/(\beta_2 + \beta_3)]N_0(G)t_0}{\gamma_1}. \]  
That is, the existence of an equilibrium depends solely on whether \( N_1(G) \) is at equilibrium. \( N_2 \) is always increased, however. It will increase exponentially when \( N_1 \) is also increasing exponentially. Fig. 2 shows both the approach of \( N_1 \) and \( N_2 \) to equilibrium and the exponential growth for \( N_1 \) and \( N_2 \) (with different values of \( \delta \) and appropriate values of \( \beta_2, \beta_3, N_1, t_2, t_1, \) and \( \gamma \)).

**Model 4: Change in \( \alpha_1 \), Proportion of Stem Cells Undergoing Programmed Death.** Here, it is assumed that \( \alpha_1 \) is reduced by an amount \( \delta(0 < \delta < \alpha_1) \) and that the cells that fail to die are partitioned between \( \alpha_2 \) and \( \alpha_3 \). This model, which considers stem cells, provides a comparison with the previous model, which considered semidifferentiated cells. In successive generations
\[ N_0(G + 1) = 2\alpha_3[1 + \delta/(\alpha_2 + \alpha_3)]N_0(G), \]  
and at equilibrium
\[ N_0(G) = 2\alpha_3[1 + \delta/(\alpha_2 + \alpha_3)]N_0(G), \]
\[ \Rightarrow 2\alpha_3[1 + \delta/(\alpha_2 + \alpha_3)] = 1, \]
\[ \Rightarrow \delta = (\alpha_2 + \alpha_3)(1/2\alpha_3 - 1). \]  
There is no equilibrium when these special conditions are not met \((\delta = 0, \alpha_3 = 0.5)\). Otherwise, there is exponential growth in cell numbers in all three. \( N_1 \) and \( N_2 \) rise exponentially according to their normal dependence on \( N_0 \).

**Model 5: Change in \( \alpha_2 \), Proportion of Stem Cells Undergoing Differentiation, Relative to \( \alpha_3 \).** A mutation causes \( \alpha_2 \) to be reduced or increased by an amount \( \delta (-\alpha_2 < \delta < 1 - \alpha_2) \). The cells that fail to die are added to \( \alpha_3 \). Then
\[ N_0(G + 1) = 2(\alpha_3 + \delta)N_0(G), \]  
and at equilibrium
\[ N_0(G) = 2(\alpha_3 + \delta)N_0(G). \]
Clearly, there is no equilibrium (unless $\delta = 0$), and $N_0$ always rises or falls. Simulation shows that, with $\delta < 0$, there is a transient rise in $N_1$ and $N_2$, followed by a decline to zero as the stem cell population is exhausted. In reality, a single mutation may affect only the cell in which it occurs. Hence, $\delta$ may become zero once all of the progeny of that cell are dead. With $\delta > 0$, there is a transient fall in $N_1$ and $N_2$, followed by an exponential rise.

**Model 6: Change in $\beta_2$, Proportion of Semidifferentiated Cells Undergoing Differentiation, Relative to $\beta_3$.** A mutation causes $\beta_2$ to be reduced or increased by an amount $\delta (\beta_2 < \delta < 1 - \beta_2)$. The cells that fail to die are then added to $\beta_3$, and so

$$N_1(G + 1) = 2(\beta_3 + \delta)N_1(G)t_0/t_1 + 2\alpha_2N_0(G),$$

and at equilibrium

$$N_1(G) = \frac{2\alpha_2N_0(G)}{[1 - 2(\beta_3 + \delta)t_0/t_1]}$$

There is no equilibrium and there is exponential growth in cell numbers when $2(\beta_3 + \delta)t_0/t_1 > 1$, again setting lower limits on $\delta$ for exponential growth, as in the case of a decrease in the proportion, $\beta_1$, of cells undergoing PCD (Model 4). $N_0$ does not change. $N_2$ changes with changes in $N_1$ as it would in a normal population. Hence, the model shows that the effects on semidifferentiated cells of changing $\beta_2$ are again to make equilibrium less likely, but not necessarily to abolish it.

**Model 7: Effects of Proliferative Advantage on Cell Differentiation Models.** For the sake of completeness, we shall consider what happens when a proliferative advantage is superimposed on the normal model of cell differentiation and PCD.

Assume first that cells in the $F_1$ population gain a proliferative advantage $w$ and still differentiate into $F_2$ cells. Then

$$N_2(G + 1) = 2\beta_3(1 + w)N_1(G)t_0/t_1 + (1 - \gamma_0/t_2)N_2(G),$$

and at equilibrium

$$N_2(G) = \frac{2\beta_2(1 + w)N_1(G)t_2}{\gamma_1}.$$  

Hence, this proliferative advantage does not overcome apoptosis to cause exponential tumor growth, although it does increase the number of cells at equilibrium by $(1 + w)$, perhaps a small quantity. Similarly, if cells in $F_0$ gained the proliferative advantage $w'$ and differentiated into $F_1$ cells,

$$N_1(G + 1) = 2\beta_3N_1(G)t_0/t_1 + 2\alpha_2N_0(G)(1 + w'),$$

and at equilibrium

$$N_1(G) = \frac{2\alpha_2N_0(G)(1 + w')}{1 - 2\beta_3t_0/t_1}.$$ 

Hence, the conditions for equilibrium are unchanged from the normal population, although the number of cells at any time is increased by $(1 + w')$. Without PCD and differentiation, cell numbers in each generation would have been given by $(1 + w')^G$.

Consider now the situation in which the cells with the proliferative advantage do not differentiate. For the $F_0$ population,

$$N_0(G + 1) = 2\alpha_3(1 + w'\gamma_0G).$$

There is no equilibrium, and the situation is formally very similar to Eq. 16 in Model 5, above with exponential growth. The effects are nearly identical, with differences only in the rate of tumor growth. Similarly, for $F_1$, when an $F_1$ cell with a proliferative advantage does not differentiate,

$$N_1(G + 1) = 2\beta_3(1 + w')N_1(G)t_0/t_1 + 2\alpha_2N_0(G),$$

and at equilibrium

$$N_1(G) = \frac{2\alpha_2N_0(G)}{[1 - 2\beta_3(1 + w')t_0/t_1]}$$

and there is no equilibrium when $2\beta_3(1 + w')t_0/t_1 > 1$.

Hence, the effects on semidifferentiated cells are again to make equilibrium less likely but not necessarily to abolish it. The situation is formally almost identical to Eqs. 18 and 19 in Model 6 above.

The models in this section show that cell proliferation that might otherwise have been considered to lead to tumorigenesis may not do so under a situation where cells differentiate and undergo PCD. In some cases, however, proliferation may substitute for the failure of PCD or differentiation in leading to tumor growth.

**Conclusions**

The failure of PCD or differentiation is sometimes sufficient but is not necessary for tumorigenesis. When stem cells ($F_0$ here) fail to undergo PCD or to differentiate in the models above, exponential growth in cell numbers occurs. This result is intuitive: extra stem cells produce both more differentiated cells and more stem cells, which in turn produce yet more stem cells and so on. This situation is formally very similar to that of tumorigenesis via stem cell proliferation, which also can result in an exponential growth in cell numbers. These are powerful mechanisms for causing tumors to develop.

If, however, a mutation causes semidifferentiated cells ($F_1$ in the models) to fail to undergo PCD to differentiate further, exponential tumor growth does not always result. Sometimes, the cell population reaches an equilibrium at higher numbers than normal; sometimes, exponential growth occurs. As long as the $F_0$ is at equilibrium, whether the $F_1$ population reaches equilibrium or shows exponential growth depends solely on whether a particular function of the parameters of cell replication exceeds some threshold value (see above).

Since the $F_1$ population is potentially self-renewing like the stem cell $F_0$ population, why does exponential growth not always occur in the former when PCD or differentiation fails? The answer is that the semidifferentiated $F_1$ cell population is normally only partly self-renewing: many cells arise not just from the previous generation’s $F_1$ but also from the $F_0$ population. In the $F_0$, any increase in the number of stem cells that subsequently remain as stem cells causes exponential growth because precisely 50% of the $F_0$ daughters self-renew under normal circumstances. When a mutation causes the $F_1$ to become more than self-renewing, exponential growth does occur, just as in the $F_0$. In the $F_1$, however, a mutation must raise the number of $F_1$ cells giving rise to more $F_1$ cells to $>50\%$ from a normal value some way $<50\%$. Therefore, many mutations acting on the $F_1$ population may not have a large enough effect on their own to cause exponential growth of cell numbers.

When PCD of fully differentiated cells (here $F_2$) fails to occur, there is no exponential growth in cell numbers. A decrease in apoptosis in the $F_2$ population leads only to potentially linear growth, since the $F_2$ cells do not themselves proliferate. Although a large decrease in the proportion of cells dying can lead to a significant growth in cell numbers, this always leads to a new equilibrium (at which the linear rate of
increase in cell numbers is balanced by the numbers dying per generation. It has been assumed above that a fixed proportion of cells die per generation, since this is arguably the most realistic scenario. If a constant (or maximum) number of cells dies per generation, failure of PCD in the F₂ could lead to linear growth with no equilibrium.

How might the predicted exponential growth and growth to an equilibrium be reflected in observations of tumors? In both cases, cells gain a selective advantage over normal cells, the essential component of tumorigenesis. Either type of growth may therefore be causal in initiating or promoting tumor growth, no doubt in conjunction with other mutations. Mutations need not occur in, or have their effects on, stem cells for tumors to grow. It is tempting to suggest that growth to equilibrium, which clearly tends to be slower than exponential growth, is more likely to occur in benign lesions or premalignant states such as cervical intraepithelial neoplasia. Malignancies may be more likely to show the rapid, exponential growth. Perhaps the tendency of some tissues to produce benign tumors and others to produce malignant tumors reflects the relative susceptibilities of stem cells in each tissue to mutations affecting PCD and/or the replicative parameters of each tissue. An increase in cell numbers to a new equilibrium is also consistent with the plateaux of growth and lag phases observed in some solid tumors (12). Once equilibrium has been attained, there may be a delay until a new mutation occurs to cause further growth, whether exponential or to another equilibrium; but each such new state reached increases the chance that a further mutation, advantageous to tumorigenesis, will lead to exponential growth and to malignancy.

The interaction between failure of PCD and tumor growth via cell proliferation has been commented on by many workers (13–15). For example, a mutation may cause a cell to proliferate to an excessive degree and thereby initiate tumorigenesis. If, however, all of the daughters of the mutant cell undergo PCD, tumorigenesis will be rapidly aborted. It has been suggested, therefore, that a cell must acquire both a proliferation mutation and a mutation preventing PCD if a tumor is to develop. Two such mutations can undoubtedly give a cell a greater selective advantage than just one mutation—and hence will tend to be observed together in many tumors—but the models suggest that both mutations are not necessary for tumor growth.

There are two reasons for this. First, some models of the failure of PCD and differentiation are very similar to those of proliferation. A single mutation can both overcome PCD and lead to exponential growth in cell numbers. For example, the proportion of cells undergoing PCD in the stem cell (F₀) generation may fall and thereby lead, in effect, to a proliferative advantage for the cells involved. Therefore, failure of PCD may sometimes be sufficient for tumorigenesis: there is no need to invoke a separate proliferative advantage. Second, it is true that if all differentiated cells undergo PCD in each and every generation and the exponential growth of F₀ or F₁ cells does not continue indefinitely, two mutations are necessary. This is, however, an extreme case and unlikely to apply in reality, since apoptosis probably includes some stochastic element and is unlikely to apply uniformly to a population of differentiated cells.

The models have been deliberately imprecise as to the type of mutation that might lead to the differences proposed in the proportion of cells undergoing PCD or differentiation. While the roles of genes such as TP53, MYC, and BCL2 in PCD are being discovered (5, 16–21), the pathways that lead to apoptosis are likely to be complex and varied. There is little virtue in incorporating such complexity into models of tumorigenesis. It is sufficient to ensure that the assumptions made in the models are not known to be unrealistic in any important feature. Further developments of the model will include simulations of stochastic effects and incorporation of successive mutational steps, with mutation rates and selective parameters chosen from appropriate distributions.

In summary, the models presented illustrate the possible roles played in tumorigenesis by the failure of PCD in particular and of differentiation in general. Perhaps most importantly, they show that the incorporation of differentiation and PCD into genetic models of tumor growth can have profound effects. Tumors, for example, grow to some equilibrium rather than the continuing exponential growth that might have been expected. Which path the tumor follows depends on functions of growth parameters, on the values of those parameters and on the stage of differentiation of the cell that fails to apoptose or differentiate. Small changes in these parameters, causing them to exceed or fall below threshold values, can profoundly alter tumor behavior. We believe, therefore, that these models provide effective explanations for the development of benign tumors and premalignant growths as well as the stepwise, gradual growth of many tumors, with sometimes very long apparent lag phases.