

Mathematical model for the cancer stem cell hypothesis

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Abstract. Recent research on the origin of brain cancer has implicated a subpopulation of self-renewing brain cancer stem cells for malignant tumour growth. Various genes that regulate self-renewal in normal stem cells are also found in cancer stem cells. This implies that cancers can occur because of mutations in normal stem cells and early progenitor cells. A predictive mathematical model based on the cell compartment method is presented here to pose and validate non-intuitive scenarios proposed through the neural cancer stem cell hypothesis. The growths of abnormal (stem and early progenitor) cells from their normal counterparts are ascribed with separate mutation probabilities. Stem cell mutations are found to be more significant for the development of cancer than a similar mutation in the early progenitor cells. The model also predicts that, as previously hypothesized, repeated insult to mature cells increases the formation of abnormal progeny, and hence the risk of cancer.

INTRODUCTION

Self-renewal is an intrinsic property of some cancer cells. Various genes that regulate self-renewal in normal cells are also found in cancer cells (Al-Hajj & Clarke 2004; Clarke 2004). This implies that cancers can occur because of mutations in normal stem and early progenitor cells or from abnormal self-renewing cancer cells. The cancer stem cell hypothesis states that just as mature cells are maintained by self-renewing stem cells, cancerous tumours are the abnormal progeny of abnormal stem cells or of progenitor cells whose DNA carries mutation(s). There is recent evidence that brain cancer tumours are driven by a very small subpopulation of self-renewing brain cancer stem cells (Reya *et al.* 2001; Pardal *et al.* 2003; Singh *et al.* 2003, 2004; Clarke 2004). Brain tumour growth bears similarities to other cancer pathways for blood or epithelial cells (Al-Hajj & Clarke 2004). It is likely that cancerous stem cells may often be responsible for recurrences that occur after treatment.

Transformation of stem cells into malignant cells is important because they already have an activated self-renewal mechanism. These cells therefore require fewer mutations to become abnormal cells than are required to ectopically activate a more differentiated cell. Because stem cells are self-renewing, they often persist for longer periods and are thus more likely to accumulate mutations than a short-lived progenitor or differentiated cell. A progenitor cell could inherit mutations from a stem cell and itself could undergo continuing mutation to cause

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transformation. When the signalling pathways that normally regulate stem cell self-renewal are disrupted, newly arising cancer cells appropriate the machinery for self-renewal that is normally only expressed in stem cells. Many pathways associated with normal stem cells are also be involved in regulating the development of cancer cells, for example, Bmi-1 (Lessard & Sauvageau 2003; Molofsky *et al.* 2003), sonic hedgehog (Taipale & Beachy 2001), and Wnt signalling (Polakis 2000; Taipale & Beachy 2001), which are all implicated in both oncogenesis and stem cell renewal.

Predictive models of stem cell systems provide the means to pose and validate non-intuitive hypotheses. Hence, they serve as important tools to distinguish the underlying regulatory mechanisms that govern stem cell fate decisions. A model can also provide a basis for estimating the potential risk of malignant tumour growth in the event of a detectable oncogenic event (for example, formation of a cancer cluster), or physiological perturbation to the tissue (for example, radiation injury).

The literature cites several techniques for mathematical modelling of haematopoietic, neural and embryonic stem cells (Viswanathan & Zandstra 2003). However, none of these models can quantitatively describe the recently proposed neural cancer stem cell hypothesis (Reya *et al.* 2001; Al-Hajj & Clarke 2004; Clarke 2004; Singh *et al.* 2004). Here we describe a mathematical model for the hypothesis that is based on the cell compartments method (Wichmann & Loeffler 1984). The role of internal cell signalling during cell proliferation is discussed. Growths of abnormal (stem and early progeny) cells from their normal counterparts are ascribed with separate mutation probabilities. The relative importance of mutation probabilities of stem and early progenitor cells on abnormal progeny's growth rates are discussed. It is also demonstrated how repeated insult to mature cells can lead to enhanced population growth rate of the abnormal cells.

THE MODEL

The mathematical model describes formation of cancer cells from stem cells. We have used it to analyse the influence of a stimulus, such as depletion of mature cells because of injury, on overall cancer growth. The model is deterministic (that is, not stochastic) so that for specified input conditions, it always provides the same prediction. However, this is not restrictive, since randomness is readily modelled by changing various parameters (such as those reported in Tables 1 and 2).

Table 1. Parameters used in the model

Parameter	Symbols	Values
Cell cycle time for SC, EP and LP compartments	$\tau_{SC}, \tau_{EP}, \tau_{LP}$	8 h
Cell maturation time for LP compartment	τ_m	40 h
Self-renewal probability upper and lower limits for SC	$P_{max,SC}, P_{min,SC}$	0.6, 0.4
Self-renewal probability upper and lower limits for EP	$P_{max,EP}, P_{min,EP}$	0.5, 0.5
Upper and lower limits of mitotic fractions for stem cells	$\alpha_{max,SC}, \alpha_{min,SC}$	1, 0.01
Upper and lower limits of mitotic fractions for EP cells	$\alpha_{max,EP}, \alpha_{min,EP}$	1, 0.3
Number of EP cell self-renewals	k	5
Upper and lower limits of the number of mitotic cycles	$n_{LP,max}, n_{LP,min}$	3, 9
Death rate (apoptosis) of MC	$\dot{\omega}_{0,MC}$	0.01
Death rate (apoptosis) of AP	$\dot{\omega}_{0,AP}$	0.01

Table 2. Mathematical representations of the regulatory signals (Wichmann & Loeffler 1984)

Controlled parameter	Controlling parameter	Feedback loops involved (cf. Fig. 1)	Functional relationship
P_{SC}	$N_{SC}, N_{EP}, N_{LP}, N_{MC}$	①, ②, ③, ④	$P_{SC} = P_0 \tanh(-Y) + 0.5, \text{ where}$ $Y = \left\{ (N_{SC} - 1) \cdot \max \left(c_1, \frac{c_1}{N_{SC}^{0.6}} \right), \right. \\ \left. + \frac{1}{3} c_2 (N_{EP} + N_{LP} + N_{MC} - 3) \right\}$ <p>and P_0, c_1 and c_2 are constants.</p>
α_{SC}, α_{EP}	$N_{SC}, N_{EP}, N_{LP}, N_{MC}$	①, ②, ③, ④	$\alpha = \frac{a_1 \exp(-X') + a_2 \exp(X')}{\exp(-X') + \exp(X')},$ <p>where $X' = a_3(XS + XE) + a_4$; $XS = b_S \begin{cases} \ln(N_{SC}) & \text{for } N_{SC} \leq 1 \\ (N_{SC} - 1) & \text{for } N_{SC} > 1 \end{cases}$ and $XE = b_E \ln \left\{ \frac{1}{3} (N_{EP} + N_{LP} + N_{MC}) \right\}$. a_1, a_2, a_3 and a_4 are separate constants for SC and EP cells (i.e. for α_{SC} and α_{EP}); b_S and b_E are constants.</p>
n_{LP}	N_{MC}	⑤	$n_{LP} = \frac{1}{\ln 2} \ln \left\{ Z_{\min} + (Z_{\max} - Z_{\min}) \times \right. \\ \left. \exp \left[-\ln \left(\frac{Z_{\max} - Z_{\min}}{Z_{\text{norm}} - Z_{\min}} \right) N_{MC}^d \right] \right\},$ <p>where $Z_{\max} = 2^{n_{LP, \max}}$, $Z_{\min} = 2^{n_{LP, \min}}$ and $Z_{\text{norm}} = 2^{n_{LP, \text{norm}}}$, and d is a constant.</p>

Figure 1 is a schematic representation of how normal stem cells and early progenitor cells undergo mutation to produce abnormal cells that give rise to abnormal progeny which ultimately form tumours (Clarke 2004). It is an evolutionary perspective that assumes that cells regularly undergo mutation in their DNA (the vast majority of which are detrimental to their survival). However, some cells, particularly those that are capable of self-renewal, infrequently survive these mutations and subsequently drive the formation of pathological tumours.

The incorporation of cell biological mechanistic information into stem cell models requires that signalling thresholds be mathematically represented in the context of cell fate choices. It has been suggested that the diffusion of a soluble signal can establish a concentration gradient and that cells within the gradient can adopt alternate, 'all-or-none' fates at critical threshold levels of the signal (Jessel & Lumsden 1997; O'Neill & Schaffer 2004). Our model is consistent with this principle, but we assume that this concentration gradient becomes negligible when the mature cell population reaches a desired sustainable level (that is, there must be an upper signalling threshold that should be modelled).

Consistent with previous findings (Oliver & Wechsler-Reya 2004), the growth mechanisms and factors influencing stem cell self-renewal are considered to be the same for both normal and abnormal stem cells. Consequently, in our model, the self-renewal probabilities for normal and

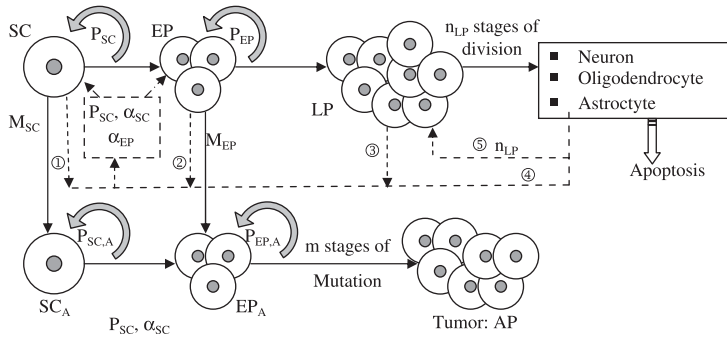


Figure 1. Schematic representation of the model. SC, stem cells; EP, early progenitor cells; LP, late progenitor cells; SC_A , abnormal stem cells; EP_A , abnormal early progenitor cells; AP, abnormal progeny (tumour); P, self-renewal probability.

abnormal (DNA mutated) stem cells are identical. The same assumption applies for normal and abnormal early progenitor cells. Stem cells are assumed to self-renew an unlimited number of times (Al-Hajj & Clarke 2004) with a self-renewal probability P_{SC} . Cells that do not self-renew differentiate to form early progenitor cells. Depending on the stem cell and early and late progenitor cell populations, the self-renewal probability varies between a maximum $P_{max,SC}$ and a minimum $P_{min,SC}$.

Unlike stem cells, early progenitor cells can self-renew only a limited number of times (Al-Hajj & Clarke 2004), assumed to be k_{EP} in our analysis. Depending on early and late progenitor cell populations, the early progenitor cells' self-renewal probability P_{EP} lies between $P_{max,EP}$ and $P_{min,EP}$. Late progenitor cells are assumed to undergo n_{LP} stages of cell division. Here, depending on the mature cell population, cell division regulatory feedback signalling controls the number of cell division stages (between $n_{LP,min}$ and $n_{LP,max}$). This regulatory signal is responsible for maintaining a steady population of mature cells. The mature cell population also provides feedback to the stem cells and thus influences their mitotic fraction and self-renewal rate. In Fig. 1, the regulatory feedback signals are represented by dotted lines and numbered ① through ⑤. Table 2 summarizes the mathematical representations for these signals.

We have considered cancer-initiating mutations both in normal stem cells and in early progenitor cells. Abnormal progeny are produced either through mutation of stem cells into abnormal stem cells and their subsequent self-renewal and differentiation, or from differentiated early progenitor cells. Occurrences of mutation are probabilistic events. Each cell division event for stem cells or for early progenitor cells has the associated probabilities, M_{SC} and M_{EP} , that represent the occurrence of oncogenic mutation during DNA transcription. Hence, following cell division, one daughter cell acquires the mutated gene while the other retains the original DNA. As the self-renewal process has a probability P_{SC} , the probability that the mutated gene is inherited in the abnormal stem cell lineage is $M_{SC} \times P_{SC}$. The same rationale applies to mutated early progenitor cells. Cancer arises if a cell undergoes a critical number of consecutive mutations and acquires the ability to proliferate indefinitely. Thereafter, the associated cell cluster produces a malignant tumour through its uncontrolled proliferation (Reya *et al.* 2001).

Each cell type is considered as a separate compartment. Cell population growth is modelled by considering individual rate expressions for each compartment.

(1) Stem cell compartment. During each cell division, there is a probability, P_{SC} , that the two daughter stem cells retain their original features. Thus, the stem cell population increases by an amount $(2P_{SC}-1)$. These self-renewed cells add to the stem cell lineage, whereas the differentiated

cells contribute to the early progenitor cell pool. When a mutation occurs during the DNA replication process (which is assumed to have a probability M_{SC}), one of the two daughter cells inherits the mutated gene. Thus, for each cell division event, the number of cells lost from the normal stem cell pool is M_{SC} . Consequently, the net rate of change of the stem cell population is

$$\frac{dN_{SC}}{dt} = \dot{\omega}_{SC}(2P_{SC} - 1)N_{SC} - \dot{\omega}_{SC}M_{SC}N_{SC}, \quad (1)$$

where $\dot{\omega}_{SC} = (\alpha_{SC}/\tau_{SC})\ln 2$ denotes the cell division rate, α_{SC} the mitotic fraction and τ_{SC} the cell cycle time (that is, the time required to complete a full cycle of cell division). The self-renewal probability $P_{SC} = P_{SC}(N_{SC}, N_{EP}, N_{LP}, N_{MC}) \in [P_{\max,SC}, P_{\min,SC}]$. The mitotic fraction is a bounded function of the stem and early progenitor cell populations (Wichmann & Loeffler 1984), that is, $\alpha_{SC} = \alpha_{SC}(N_{SC}, N_{EP}) \in [\alpha_{\max,SC}, \alpha_{\min,SC}]$.

(2) Early progenitor (EP) cell compartment. The model for this compartment considers that EP cells undergo a limited number (k) of self-renewal steps. Thus, the early progenitor cells are split into k subcompartments that contain identical EP cells, which differ only with respect to the number of times they have undergone self-renewal (and, hence, the number of times they can further self-renew). We refer to these subgroups as $EP_1, EP_2 \dots EP_k$.

When a subgroup EP_i undergoes cell division, it self-renews (with a probability P_{EP} that is assumed identical for $i = 1$ through $k - 1$) into a subgroup EP_{i+1} and also differentiates into LP. The first subgroup receives a flux of differentiated stem cells from the stem cell compartment, whereas the last one completely differentiates into LP (late progenitor) (i.e. $P_{EP} = 0$ for the k^{th} compartment). Each subgroup has a mutation probability M_{EP} (assumed identical for $i = 1$ through k). The relations describing the population changes in each subgroup are

$$\frac{dN_{EP_i}}{dt} = 2\dot{\omega}_{SC}(1 - P_{SC})N_{SC} - \dot{\omega}_{EP}N_{EP_i}, \quad \text{and} \quad (2a)$$

$$\frac{dN_{EP_i}}{dt} = \dot{\omega}_{EP}(2P_{EP_{i-1}} - M_{EP})N_{EP_{i-1}} - \dot{\omega}_{EP}N_{EP_i} \quad \text{for } i = 2 \dots k, \quad (2b)$$

where $\dot{\omega}_{EP} = (\alpha_{EP}/\tau_{EPC})\ln 2$. This is a set of state equations for the state variables N_{EP_i} .

The output equation $N_{EP} = \sum_{i=1}^k N_{EP_i}$ can be used to determine the EP cell population. Because EP cells do not differ in cell characteristics (other than their ability to further self-renew), the same values of $\dot{\omega}_{EP}$, P_{EP} and M_{EP} are considered for all subgroups 1 through k_{EP} (except that the value of P_{EP} for the k^{th} subgroup is zero). The total efflux of differentiated EP cells that enters the LP compartment is

$$\dot{N}_{EP}^{\text{out}} = \sum_{i=1}^{k-1} 2\dot{\omega}_{EP}(1 - P_{EP})N_{EP_i} + 2\dot{\omega}_{EP}N_{EP_k}. \quad (3)$$

Thus, the resulting efflux of mutated EP cells is $\sum_{i=1}^{k-1} \dot{\omega}_{EP}M_{EP}N_{EP_i}$.

(3) Late progenitor cell compartment. The LP compartment receives an influx $\dot{N}_{EP}^{\text{out}}$ as described previously. The cells contained in it undergo n_{LP} successive stages of cell division before transforming into mature cells, where $n_{LP} = n_{LP}(N_{MC}) \in [n_{LP,\max}, n_{LP,\min}]$. Although a state-space model for the n stages of cell division can be explicitly written (as for the EP compartment), its solution would be needlessly complicated, as the dimension of the state-space matrix is itself a variable in this case. Instead, we follow an approach (Wichmann & Loeffler 1984) that assumes that the efflux from the EP compartment is amplified by a factor Z^n as soon as the cells enter

the LP compartment. Immediately before leaving the LP compartment, they are further amplified by a factor Z^{out} . These two factors are selected such that $Z^{\text{in}} \times Z^{\text{out}} = 2^{n_{\text{LP}}}$.

The total time τ_{LP} spent by a cell in the LP compartment is the sum of the cell generation time ($\tau_g = \alpha_{\text{LP}} \ln 2 / \tau_{\text{LP}}$) and the cell maturation time τ_m . The factor $Z^{\text{in}} = (2^n - 1) \tau_g / \tau_{\text{LP}} + 2^n \tau_m / \tau_{\text{LP}}$, and the LP population change is

$$\frac{dN_{\text{LP}}}{dt} = Z^{\text{in}} \left\{ \sum_{i=1}^{k-1} 2\dot{\omega}_{\text{EP}}(1 - P_{\text{EP}})N_{\text{EP}_i} + 2\dot{\omega}_{\text{EP}}N_{\text{EP}_k} \right\} - \dot{\omega}_{\text{LP}}N_{\text{LP}}, \quad (4)$$

and the efflux from the LP compartment (that is, the generation rate of mature cells) $\dot{N}_{\text{LP}}^{\text{out}} = Z^{\text{out}}\dot{\omega}_{\text{LP}}N_{\text{LP}}$. As before, $\dot{\omega}_{\text{LP}} = (\alpha_{\text{LP}}/\tau_{\text{LP}})\ln 2$.

(4) Mature cell compartment. The mature cell compartment receives an efflux of $\dot{N}_{\text{LP}}^{\text{out}}$ from the LP compartment. Considering a cell death (or apoptotic) rate of $\dot{\omega}_{0,\text{MC}}$, the rate of change in the mature cell population

$$\frac{dN_{\text{MC}}}{dt} = \dot{N}_{\text{LP}}^{\text{out}} - \dot{\omega}_{0,\text{MC}}N_{\text{MC}}. \quad (5)$$

(5) Abnormal stem cell compartment. As mutated stem cells behave in the same manner as normal cells, the relation representing the growth rate of the abnormal stem cell population is analogous to (1) except that the mutating fraction must be added to the cell population in this case. Thus,

$$\frac{dN_{\text{SC,A}}}{dt} = \dot{\omega}_{\text{SC}}(2P_{\text{SC}} - 1)N_{\text{SC,A}} + \dot{\omega}_{\text{SC}}M_{\text{SC}}N_{\text{SC}}. \quad (6)$$

(6) Abnormal early progenitor cell compartment. The equations for the abnormal EP cell population also resemble Eq. (2a) and (2b), except that here too the mutating cells add to the population. Hence,

$$\frac{dN_{\text{EP,A}_i}}{dt} = 2\dot{\omega}_{\text{SC}}(1 - P_{\text{SC}})N_{\text{SC,A}} - \dot{\omega}_{\text{EP}}N_{\text{EP,A}_i}, \quad \text{and} \quad (7a)$$

$$\frac{dN_{\text{EP,A}_i}}{dt} = 2\dot{\omega}_{\text{EP}}P_{\text{EP}}N_{\text{EP,A}_{i-1}} - \dot{\omega}_{\text{EP}}N_{\text{EP,A}_i} + \dot{\omega}_{\text{EP}}N_{\text{EP}_{i-1}}M_{\text{EP}} \quad \text{for } i = 2 \dots k. \quad (7b)$$

The overall population of abnormal EP cells can be found by solving the above state equations and substituting the state variables into the output equation $N_{\text{EP,A}} = \sum_{i=1}^k N_{\text{EP,A}_i}$. The outflux of abnormal EP cells

$$\dot{N}_{\text{EP,A}}^{\text{out}} = \sum_{i=1}^{k-1} 2\dot{\omega}_{\text{EP}}(1 - P_{\text{EP}})N_{\text{EP,A}_i} + 2\dot{\omega}_{\text{EP,A}}N_{\text{EP}_k}. \quad (8)$$

(7) Abnormal progeny compartment. If they originate from cancerous abnormal stem cells or from abnormal early progenitor cells, the abnormal progeny undergo uncontrolled cell division leading to a malignant tumour. The growth of the abnormal progeny

$$\frac{dN_{\text{AP}}}{dt} = \dot{N}_{\text{EP,A}}^{\text{out}} - \dot{\omega}_{0,\text{AP}}N_{\text{AP}}, \quad (9)$$

where $\dot{\omega}_{0,\text{AP}}$ denotes the abnormal progeny cell death rate (or apoptosis).

RESULTS AND DISCUSSION

Equations 1–9 are numerically solved through time marching using the parameters specified in Table 1 (Wichmann & Loeffler 1984). The model is first tested for its stability and steady state response by selecting an initial normalized condition $N_{sc} = 1.0$, with all other normalized cell populations set to zero. The response of the SC, EP, LP and MC populations is then examined for a mature cell death rate of 0.01/h. For this base case, the mutation probabilities are assumed to be zero.

Figure 2a presents the growth of the EP, LP and MC populations from stem cells in response to these conditions. Initially, when these populations are very small, feedback signals ②, ③ and ④ from the EP, LP and MC compartments increase the values of α_{sc} and α_{ep} so as to quickly replenish the respective cell populations (Fig. 2b). Because $P_{sc} = P_{sc,min}$ (that is, 0.4) initially, rapid cell division leads to an early depletion in the SC pool. Subsequently, as the value of P_{sc} slowly increases, so does the SC population stabilizes at $N_{sc} = 0.77$. The values of α_{sc} and α_{ep} concurrently decrease to steady values of 0.28 and 0.34, and the EP, LP and MC cell populations likewise stabilize at various steady values (3.19, 96.85 and 678.1, respectively) within the first 400 h. Figure 2 confirms that the modelled process is self-regulating, that is, proper cell signalling limits cell proliferation to a steady value that is sufficient to replenish the steady death (apoptosis) of mature cells.

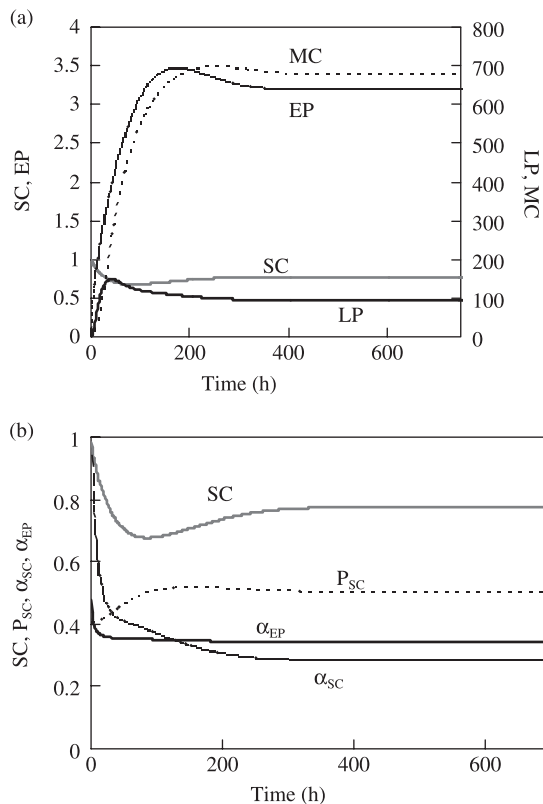


Figure 2. Transient response of the model beginning with $N_{sc} = 1.0$ and with all other cell populations set to zero. (a) The SC, EP, LP and MC populations and (b) the internal signalling described by the model in terms of the transient values of α_{sc} , α_{ep} and P_{sc} .

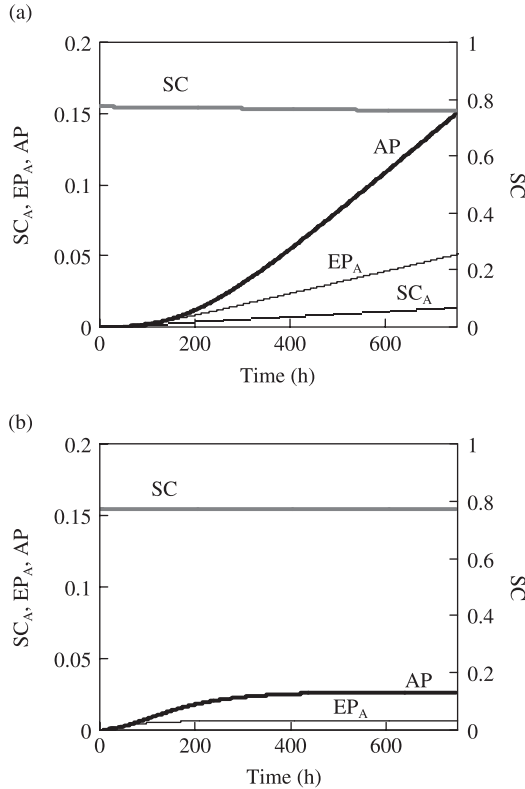


Figure 3. Growth rate of populations of abnormal stem cells, EP cells and abnormal progeny for a specific mutation probability in the SC or EP cell compartments. Model response for (a) $M_{SC} = 10^{-3}$, $M_{EP} = 0$, and (b) $M_{SC} = 0$, $M_{EP} = 10^{-3}$.

Next, we consider the occurrence of an oncogenic event in either of the SC or of the EP populations. First, we assume that there is a 10^{-3} probability that a mutation occurs during stem cell self-renewal with $M_{SC} \gg M_{EP}$ (or $M_{EP} \approx 0$). Next, assuming that the abnormal progeny have an apoptotic rate (1%/h) equals the mature cell death rate, the growth rates of the SC_A, EP_A and AP populations are determined as shown in Fig. 3a. Since the mutation rate is very small, it has a negligible effect on the SC population. The abnormal cell populations are much smaller than those of healthy cells. Following an initial rapid growth period, the AP population grows steadily and nearly linearly to a normalized value of 0.15 at 700 h. This indicates that loss of self-regulation (that is otherwise observed in healthy cells) is successfully modelled. When EP cells undergo mutation (with $M_{EP} = 10^{-3}$, and $M_{EP} \gg M_{SC}$), the AP population growth rate shown in Fig. 3b is far smaller than when the SC population is allowed to mutate. This is to be expected, since while stem cells can self-renew indefinitely, EP cells have limited capability to do so. Hence, SC mutations have a much larger proliferation potential than mutations in EP cells. The model confirms that mutations leading to cancer are more significant when they occur in stem cells than when they occur in early progenitor cells.

Regulation of healthy cells occurs through feedback signals that alter the values of P_{SC} , α_{SC} and α_{EP} (Fig. 1). (This mechanism is also responsible for tissue healing; Craig *et al.* 1996; Calza *et al.* 2004.) In order to investigate the influence of these feedback loops on the onset of cancer,

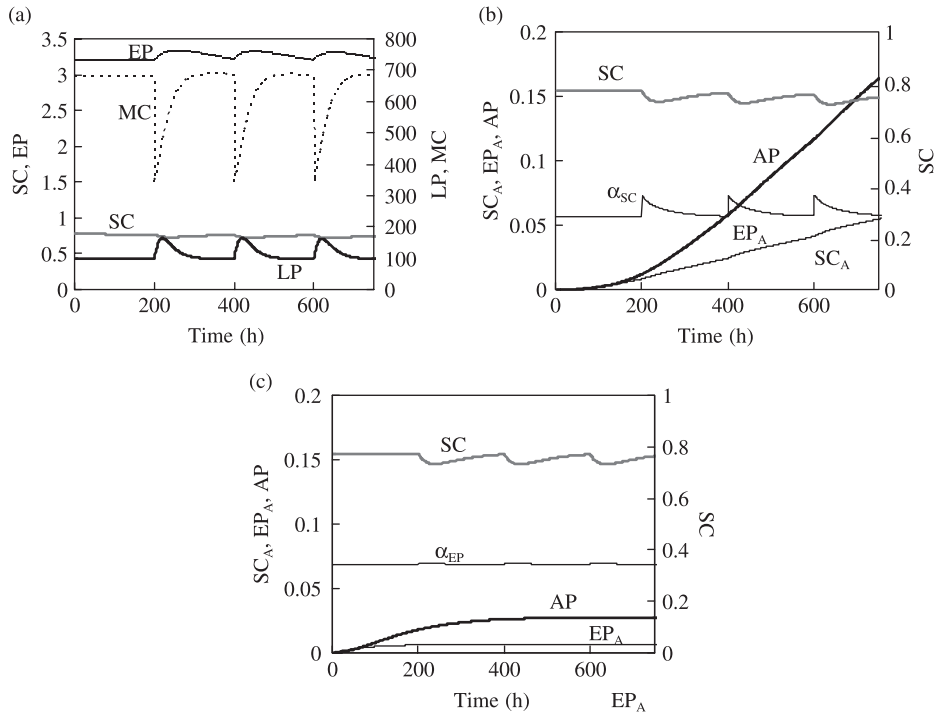


Figure 4. (a) Transient response of the SC, EP, LP and MC populations to periodic insult that depletes 50% of the MC population every 200 h. The corresponding response in the SC_A, EP_A and AP populations is shown for (b) $M_{SC} = 10^{-3}$, $M_{EP} = 0$, and (c) $M_{SC} = 0$, $M_{EP} = 10^{-3}$.

we simulated repeated insult to the mature cell population by periodically depleting its population by a specified amount. Figure 4a shows the response of the SC, EP, LP and MC populations when 50% of mature cells are removed at 200 h intervals. (Repetitive radiation therapy used to treat cancer could lead to a similar condition, that is, when some mature cells also periodically die because of treatment; Fujimaki 2005.)

Upon loss of mature cells, the feedback loop ⑤ immediately increases proliferation of LP cells, and feedback signal ④ contributes to an immediate rise in the proliferative fraction of stem cells. This is reflected in Fig. 4b, which shows that the value of α_{SC} temporarily increases to 0.34 from a steady value of 0.28. Therefore, repeated insult increases the proportion of the stem cell population that participates in cell division. Even assuming that the probability of cell mutation remains unchanged, the growth rate of the abnormal progeny is consequently enhanced.

A comparison of Figs 3b and 4b indicates that the abnormal progeny population becomes larger with repeated injury (when $M_{SC} = 10^{-3}$ and $M_{EP} = 0$, the AP population increases to 0.16 in 700 h for repeated insult, whereas it would have been 0.15 in the absence of any insult). This is in conformity with the earlier findings (Beachy *et al.* 2004), which showed that repeated tissue invasion leads to an increased cancer risk. In comparison, the increase in the value of α_{EP} following insult is negligible (Fig. 4c). Hence, when only EP cells are assumed to undergo mutation (for $M_{EP} = 10^{-3}$ and $M_{SC} = 0$), the AP cell population is relatively unaffected by repeated insult to the MC population. The model indicates that repeated insult increases the cancer risk by influencing the mitotic fraction in the stem cell population.

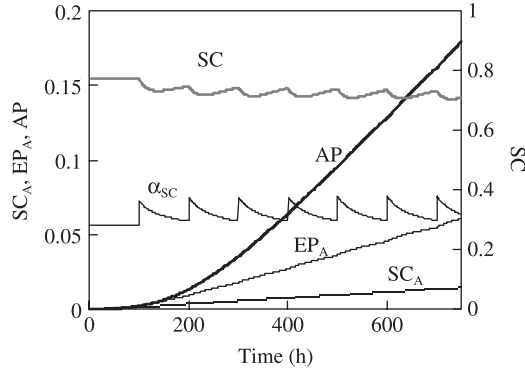


Figure 5. Response of the in SC_A , EP_A and AP populations when 50% of the MC population is depleted every 100 h.

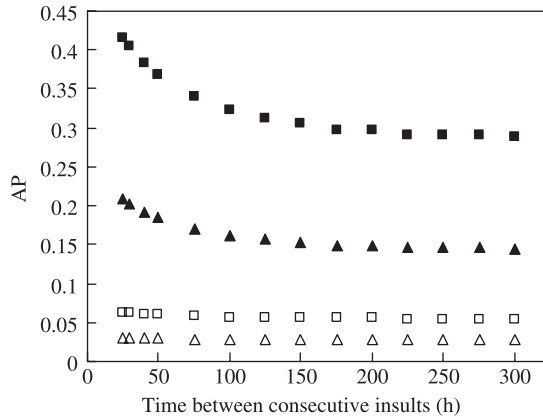


Figure 6. AP cell growth as a function of the time between consecutive insults. The AP cell population (after 700 h) increases with increasing frequency of insult. The plots correspond to the conditions: $M_{EP} = 0$ (solid symbols) with $M_{SC} = 0.002$ (■) and 0.001 (▲); and $M_{SC} = 0$ (empty symbols) with $M_{EP} = 0.002$ (□) and 0.001 (△).

When repeated MC insult occurs every 200 h as shown in Fig. 4b, the value of α_{SC} first rapidly increases to a maximum but then decreases to its initial value within 200 h. However, when the frequency of insult is increased to once every 10 h, α_{SC} does not decrease to that initial value at the end of each cycle. Here, Fig. 5 shows that the actively proliferating stem cell pool keeps increasing with time and consequently the AP population grows to an even larger value (N_{AP} increases from 0.16 to 0.18 as the time between two consecutive insults is decreased from 200 to 100 h). This indicates that the cancer risk increases with increasing frequency of injury to the mature cell population.

The trend becomes obvious when the AP populations after 700 h are plotted as a function of the mean time between two consecutive insults (Fig. 6). Here, four different combinations of the SC and EP cell mutation probabilities are considered ($M_{SC} = 0.002$ and 0.001 with $M_{EP} = 0$; and $M_{EP} = 0.002$ and 0.001 with $M_{SC} = 0$) while all the other parameters were kept unchanged. As before, 50% insult of the MC population is considered. It is evident from Fig. 6 that the AP population increases as the time between two subsequent (regularly imposed) insults is reduced.

The effect of changing the frequency of repeated insult is more profound when the mutations occur in SC rather than in EP cells. Also, the effect of changing the insult frequency is more pronounced at higher insult frequencies (that is, at smaller mean time between the insults – less than 100 h for the case shown here). With smaller time between two insults, MC population fails to rise to its normal value – leading to a high level of actively proliferating SC and EP cells at the end of each cycle. This means that the SC and EP cells undergo more frequent cell division. For a given probability of mutation, this essentially means a higher growth rate of the AP cells. When the mean time between two insults is very large (e.g. larger than 250 h for the cases presented here), nearly the steady normal MC population is re-established after each insult. Each consecutive insult therefore can be treated as nearly isolated events, and the AP growth rate becomes almost insensitive to the insult frequency.

CONCLUSION

A predictive model based on a cell compartments method is presented to pose and validate non-intuitive scenarios arising from the cancer stem cell hypothesis.

- 1 Under normal circumstances, the model converges to a steady population of the stem, early and late progenitor cells that maintain a constant mature cell population for a specified cell apoptotic rate.
- 2 An oncogenic event in stem cells or EP cells produces abnormal stem or EP cells and eventually the abnormal progeny. For the same occurrence probability, mutations in stem cells (rather than in EP cells) lead to faster growth of the abnormal progeny.
- 3 When the mature cell population undergoes a sudden depletion because of an insult (for example, radiation damage to the mature tissue), regulatory feedback signals (that are responsible for tissue healing) increase the stem and EP cell proliferative fractions. This in turn leads to an increased growth rate of the abnormal progeny cells.
- 4 The abnormal progeny cell population growth rate increases as the mean time between two consecutive insults is reduced. This trend is more pronounced when the insult frequency is higher. However, when this frequency is small enough that the stable cell populations are restored between two consecutive insults, the AP growth rate is relatively unaffected.

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