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Genetic instability and clonal expansion

Martin A. Nowak[a,*], Franziska Michor[a,b], Yoh Iwasa[c]

[a]*Program for Evolutionary Dynamics, Department of Organismic and Evolutionary Biology, Department of Mathematics, Harvard University, Cambridge, MA 02138, USA*
[b]*Society of Fellows, Harvard University, Cambridge, MA 02138, USA*
[c]*Department of Biology, Faculty of Sciences, Kyushu University, Fukuoka 812-8581, Japan*

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Abstract

Inactivation of tumor suppressor genes can lead to clonal expansion. We study the evolutionary dynamics of this process and calculate the probability that inactivation of a tumor suppressor gene is preceded by mutations in genes that confer genetic instability. Unstable cells might have a slower rate of clonal expansion than stable cells because of an increased probability of generating lethal mutations or inducing apoptosis. We show that the different growth rates of genetically stable and unstable cells during clonal expansion represent, in general, only a small disadvantage for genetic instability. The intuitive reason for this conclusion is that robust clonal expansion, where cellular birth rates are significantly greater than death rates, occurs on a much faster time scale than waiting for those mutations that allow clonal expansion. Moreover, in special cases where clonal expansion is very slow, genetically unstable cells have a higher probability to accumulate additional mutations during clonal expansion that confer a selective advantage. Clonal expansion represents a major disadvantage for genetic instability only when inactivation of the tumor suppressor gene leads to a very small increase of the cellular reproductive rate that is cancelled by the increased mortality of unstable cells.

Keywords: Mathematical biology; Population genetics; Stochastic dynamics; Tumor suppressor genes; Chromosomal instability

1. Introduction

Cancer progression is an evolutionary process. Cells of somatic tissues receive mutations that can alter their phenotype leading to increased reproductive rates and increased mutation rates. Mutations in oncogenes and tumor suppressor genes (TSGs) enhance the net reproductive rates of a cell, while mutations in genetic instability genes increase the rate of mutational processes such as point mutations, insertions, deletions, gene amplification, chromosome rearrangements and gain or loss of whole chromosomes (Vogelstein and Kinzler, 1998). There is much discussion at present whether genetic instability is an early event and therefore a driving force of cancer progression or a late stage consequence of the somatic evolution that leads to cancer (Rajagopalan et al., 2003; Sieber et al., 2003).

The idea that cancer progression is accelerated by an enhanced mutation rate (a so-called 'mutator phenotype') was first introduced by Loeb (1991). So far, two main types of genetic instability have been observed in human cancers. Mutations in mismatch repair genes lead to microsatellite instability (MIN) while mutations in genes that maintain the integrity of the chromosomes during cell division can lead to chromosomal instability (CIN) (Lengauer et al., 1997, 1998; Cahill et al., 1998; Loeb, 2001; Duval and Hamelin, 2002; Rajagopalan et al., 2004; Rajagopalan and Lengauer, 2004). Whereas MIN is rarely found in cancers other than colon cancer, CIN appears to be a common genetic instability which is widespread among solid tumors (Vogelstein and Kinzler, 1998).

Genetic instability can increase the mutation rate of precancer cells, but it will also cause many deleterious mutations that might slow down the growth rate of a population of cells. This is called the 'cost' of genetic instability. The main question is: under which circumstances will the benefits of an increased mutation rate

*Corresponding author. Tel.: +1 617 496 4737; fax: +1 617 496 4629.
E-mail address: martin_nowak@harvard.edu (M.A. Nowak).

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outpace the cost of genetic instability? In previous papers, we have investigated whether tumorigenesis could be initiated by mutations in genes that confer CIN or MIN (Nowak et al., 2002; Komarova et al., 2002). We have concluded that it is very likely that CIN mutations induce the first phenotypic change in cancer pathways where inactivation of a TSG represents the first step towards cancer. In particular, we have calculated the probability that inactivation of the APC TSG in colon cancer is preceded by mutations in CIN genes (Michor et al., 2004a, 2005a).

In this paper, we analyze the following question. Suppose genetically unstable cells have a reduced rate of successful cell division and/or an increased rate of cell death. This cost of genetic instability could lead to a slower clonal expansion of pre-cancer cells. What is the effect of this slower clonal expansion on the overall probability that cancers are initiated by mutations that confer genetic instability?

This paper is part of the rapidly growing effort to understand the evolutionary dynamics of cancer progression with the help of mathematical models (Moolgavkar and Knudson, 1981; Sheratt and Nowak, 1992; Wodarz and Krakauer, 2001; Luebeck and Moolgavkar, 2002; Gatenby and Maini, 2003; Gatenby and Vincent, 2003; Little and Wright, 2003; Iwasa et al., 2004a, b; Michor et al., 2004a, b, 2005a, b; Nowak et al., 2004; Frank, 2005; Breivik, 2005; Wodarz and Komarova, 2005; Iwasa et al., 2005).

2. Cancer initiation by tumor suppressor gene inactivation

Let us design the simplest possible model that allows us to study the effect of clonal expansion on CIN. Consider a somatic tissue that is subdivided into compartments. Each compartment is fed by a small number of stem cells. For simplicity, let us assume that there is only one stem cell per compartment. Suppose the inactivation of a TSG, A, in a stem cell leads to clonal expansion. A wild type stem cell has two unmutated alleles of A, $A^{+/+}$. A mutation in one allele leads to a cell with genotype $A^{+/-}$. A mutation in the second allele or loss of heterozygosity (LOH) can lead to a cell with genotype $A^{-/-}$. Let us denote these three cell types by X_0, X_1 and X_2. In addition, there can be mutations in CIN genes which lead to the three cell types $A^{+/+}CIN$, $A^{+/-}CIN$, and $A^{-/-}CIN$. These three cell types are, respectively, denoted by Y_0, Y_1 and Y_2.

The mutational diagram is shown in Fig. 1. The first allele of the TSG is inactivated by a point mutation or a small genetic modification such as an insertion or a deletion; this mutation rate is not affected by CIN. Therefore, the rate for inactivating the first allele of the TSG is the same in CIN and non-CIN cells and is given by u_1. The rate for inactivating the second allele of the TSG (including LOH events) is given by u_2 for non-CIN cells and by u_3 for CIN cells. The rate of acquiring CIN mutations is given by u_c. All mutation rates are given as probabilities per cell division. Time is also measured in units of cell divisions. For example, if the stem cell divides once a week, then the unit of time is one week.

The probability that a compartment has generated a stem cell of type X_2 by time t is given by

$$P_x(t) \approx u_1 u_2 t^2/2. \tag{1}$$

The mutation from cell type X_0 to X_1 occurs at rate u_1. The mutation from cell type X_1 to X_2 occurs at rate u_2. The probability that the stem cell has mutated to type X_2 by time t is given by the convolution $P_x(t) = \int_0^t u_1 e^{-u_1\tau}(1 - e^{-u_2(t-\tau)})\,d\tau$. For $u_1 u_2 t^2 \ll 1$ this expression leads to Eq. (1). Similarly, the probability that a compartment has generated a stem cell of type Y_2 by time t is given by

$$P_y(t) \approx u_1 u_c t^2. \tag{2}$$

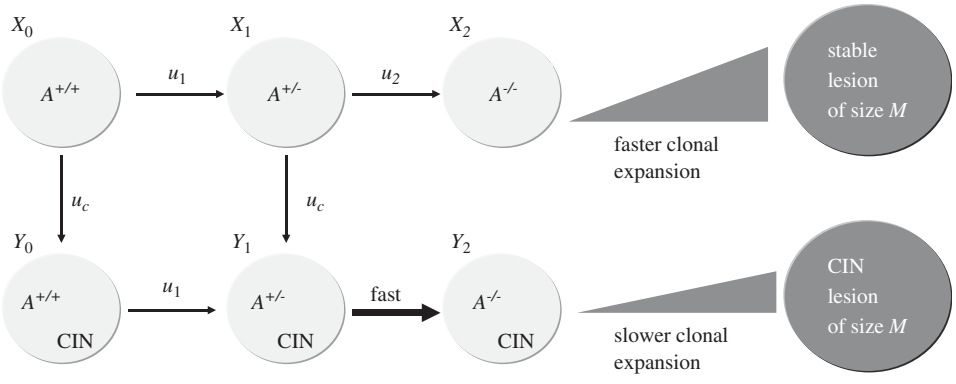

Fig. 1. The mechanism of cancer initiation that is analyzed in this paper assumes that inactivation of a tumor suppressor gene, A, leads to clonal expansion. The mutation rates for inactivating the first and second alleles are given by u_1 and u_2, respectively. There can also occur mutations, at rate u_c, which lead to chromosomal instability (CIN). CIN causes very fast inactivation of the second allele of the tumor suppressor gene (TSG) by loss of heterozygosity (LOH); this step occurs on a much faster time scale than in stable cells and is usually not rate limiting. Thus, it requires two rate limiting hits to inactivate a TSG with or without CIN (Eqs. 1 and 2). We assume that the subsequent clonal expansion occurs at a faster rate in stable cells than in CIN cells, because the latter might suffer from a reduced rate of successful cell division and/or an increased death rate. We calculate the probability that a lesion consisting of M cells with or without CIN has arisen by a certain time (see Eqs. 4 and 5).

These equations hold for $u_3 \gg 1/t \gg u_1, u_2, u_c$ which is certainly the case for reasonable values of the mutation rates and the time scale of human life. The probability to inactivate one allele of a TSG with a point mutation is estimated to be about 10^{-7} per cell division. Therefore, $u_1 \approx 2 \times 10^{-7}$ because there are two alleles that represent targets for the first hit. The second allele of a TSG can be inactivated by another point mutation or by LOH. The rate of LOH in normal cells is not well-known, but estimates range from 10^{-7} to 10^{-6}. In CIN cells the rate of LOH was determined to be 10^{-2} (Lengauer et al., 1997). The rate of acquiring CIN depends on the number and type of CIN genes (Michor et al., 2004b); a reasonable range is $u_c = 10^{-7}$ to 10^{-5}. If the relevant cells divide once per day then $t = 30\,000$ cell generations occur in about 82 years. For detailed derivations of Eqs. (1) and (2) including more general cases see Nowak et al. (2002), Komarova et al. (2003) and Iwasa et al. (2004a).

If $P_y(t) > P_x(t)$, then the majority of cancer lesions will start from cells where the inactivation of the second allele of the TSG was preceded by a CIN mutation. This inequality leads to

$$u_c > \frac{u_2}{2}. \tag{3}$$

If the mutation rate that leads to CIN is greater than half of the mutation rate for the second hit in stable cells, then the majority of cancers will start with CIN. The factor $\frac{1}{2}$ comes from the fact that the CIN mutation could occur before or after the inactivation of the first TSG allele; in Fig. 1, there are two evolutionary pathways from X_0 to Y_1. If, for some reason, the CIN mutation could only occur after inactivating the first allele, then the relevant condition is $u_c > u_2$. In general, if the mutation rate conferring CIN is of the same order of magnitude as the inactivation rate of the second allele, then a significant fraction of cancers will start with CIN.

The mutation rate conferring CIN depends on the number of CIN genes in the human genome. In yeast, more than hundred genes have been identified, each of which is sufficient to trigger CIN when genetically altered (Shonn et al., 2000; Kolodner et al., 2002; Nasmyth, 2002). By analogy, we expect hundreds of human CIN genes, but only few have been identified so far (see Michor et al., 2004b). Therefore, the mutation rate triggering CIN could be hundred times larger than the mutation rate inactivating the first TSG allele, and CIN will almost certainly arise very early in tumorigenesis.

3. Clonal expansion

Let us now add clonal expansion. Suppose clonal expansion starts once both alleles of the TSG have been inactivated. This means clonal expansion is initiated by cells of type X_2 or Y_2. We model clonal expansion as a probabilistic branching process describing the birth and death of cells. In a branching process, there are two possibilities: either the cell population becomes extinct after some time or continues to grow. We interpret extinction as follows: the mutated stem cell initiates a clonal expansion, but the stochastic fluctuations in cell birth and death lead to the extinction of this clone. In this case, we assume that also the mutated stem cell that has initiated the wave of clonal expansion has died. This assumption describes a scenario where the mutated stem cell is just one of the cells of the clonally expanding population. If this population dies out, then naturally the mutated stem cell has also disappeared. Another plausible scenario is that the mutated stem cell sits in a protected niche, will not disappear and might generate several waves of clonal expansions. We do not discuss this case in the present paper, but note that both the analysis and the conclusions are very similar.

For X_2 cells, the rate of cell division is given by a and the rate of cell death by b. For Y_2 cells, the rate of cell division is given by c and the rate of cell death by d. The probability that clonal expansion starting from a single X_2 cell results in extinction is given by b/a. The probability that such clonal expansion leads to a lesion is given by $1 - b/a$. Similarly, the probability that clonal expansion starting from a single Y_2 cell results in extinction is given by d/c. The probability that such clonal expansion leads to a lesion is given by $1 - d/c$.

In the Appendix, we derive the following results. The probability that a compartment has given rise to a lesion of size M without CIN by time t is approximately given by

$$Q_x(t) = \frac{1}{2} u_1 u_2 \left(1 - \frac{b}{a}\right)(t - T_x)^2. \tag{4}$$

The probability that a compartment has given rise to a lesion of size M with CIN by time t is approximately given by

$$Q_y(t) = u_1 u_c \left(1 - \frac{d}{c}\right)(t - T_y)^2. \tag{5}$$

Here T_x and T_y indicate the time scale of clonal expansion for stable and unstable lesions. We have

$$T_x = \frac{1}{a - b}\left[\ln M + \ln\left(1 - \frac{b}{a}\right) + \frac{1}{2}\right] \tag{6}$$

and

$$T_y = \frac{1}{c - d}\left[\ln M + \ln\left(1 - \frac{d}{c}\right) + \frac{1}{2}\right]. \tag{7}$$

Figs. 2 and 3 show the excellent agreement between these equations and exact numerical simulations.

If $Q_y(t) > Q_x(t)$, then the majority of cancer lesions (by time t) have been initiated with a CIN mutation. In the reasonable parameter regime where the time scale for inactivating the TSG is much larger than the time scale for the clonal expansion, $t \gg T_x$ and $t \gg T_y$, the inequality

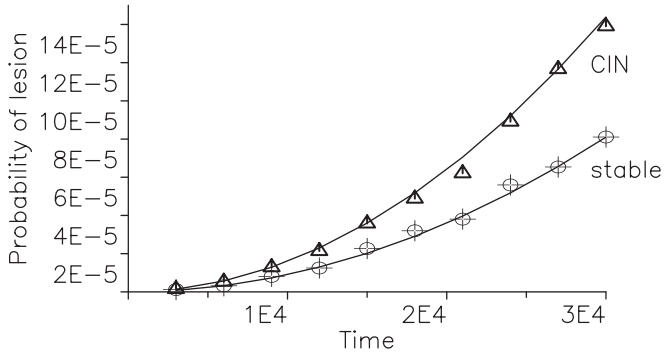

Fig. 2. Perfect agreement between the analytical approximations given by Eqs. 4 and 5 (lines) and exact numerical simulation (points). We perform a stochastic simulation for the cancer initiation process described in the main text and illustrated in Fig. 1. In each time step, a random event is chosen proportionally to its rate. Before clonal expansion, there is only one (stem) cell. Initially, this cell is of type X_0. Mutation to X_1 or Y_1 occurs with probabilities u_1 and u_c, respectively. A Y_0 cell can mutate to a Y_1 cell with probability u_1. An X_1 cell can mutate to an X_2 cell with probability u_2 or to a Y_1 cell with probability u_c. A Y_1 cell can mutate to a Y_2 cell with probability u_3. An X_2 cell initiates clonal expansion; X_2 cells divide with rate a and die with rate b. Similarly, a Y_2 cell initiates clonal expansion; Y_2 cells divide with rate c and die with rate d. The simulation is stopped if the clone has died out or has reached M cells. The figure shows the probability that a single (stem) cell has given rise to a stable lesion or a CIN lesion. Parameter values are $u_1 = 2 \times 10^{-7}$, $u_2 = 10^{-6}$, $u_3 = 10^{-2}$, $u_c = 5u_1$, $a = 1$, $b = 0.1$, $c = 0.5$, $d = 0.1$, and $M = 10^6$. The probability is evaluated over 10^7 runs.

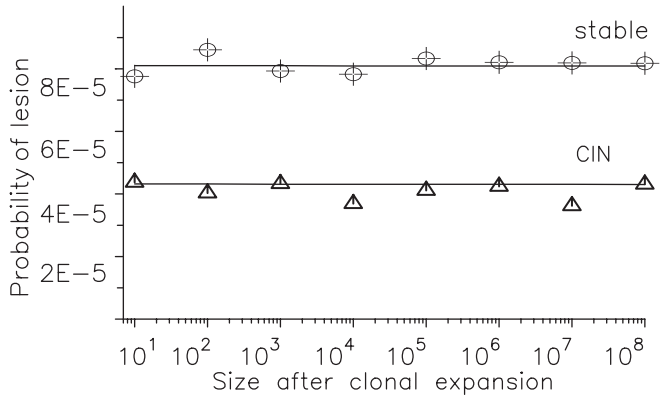

Fig. 3. Comparison between the analytical approximations given by Eqs. 4 and 5 (lines) and exact numerical simulation (points) for a different choice of parameter values. The same process is simulated as described in the legend of Fig. 2. The figure shows the probability that lesions of sizes 10 to 10^8 cells have arisen after $t = 30\,000$ time units (days). For the chosen parameter values, the magnitude of the clonal expansion has no effect. This underlines our conclusion that robust clonal expansion has no effect for evaluating the cost of CIN. The time for clonal expansion is short compared to the waiting time of inactivating the tumor suppressor gene. Parameter values are $u_1 = 2 \times 10^{-7}$, $u_2 = 10^{-6}$, $u_3 = 10^{-2}$, $u_c = 2u_1$, $a = 1$, $b = 0.1$, $c = 0.5$, $d = 0.2$, and $t = 30\,000$. The probability is evaluated over 10^7 runs.

$Q_y(t) > Q_x(t)$ leads to

$$u_c > \frac{u_2}{2}\frac{1 - b/a}{1 - d/c}. \quad (8)$$

Let us define robust clonal expansion as the birth rate of cells being much larger than their corresponding death rate. For stable cells, this means $a \gg b$, and for unstable cells, $c \gg d$. Either by inspecting the simple inequality (8) or by comparing the more accurate Eqs. (4) and (5) we conclude that robust clonal expansion has only a very small effect on the relative importance of genetic instability for initiating tumor progression. For example, if $a = 4b$ and $c = 2d$, then the non-CIN clone has an exponential growth rate which is twice as fast as the CIN clone. This corresponds to a 50% fitness disadvantage of CIN. In this case inequality (8) leads to $u_c > (\frac{3}{4})u_2$. This has to be compared with inequality (3), $u_c > (\frac{1}{2})u_2$, which neglects the effect of clonal expansion. Therefore, robust clonal expansion has almost no consequence for evaluating the contribution of early genetic instability.

4. Discussion

In this paper, we have analyzed the effect of clonal expansion on genetic instability. Robust clonal expansion is defined by the property that cell division is significantly faster than cell death. In this case, clonal expansion has little effect for evaluating the probability that CIN precedes inactivation of the first TSG on the way to cancer. Our conclusions differ from those reached by Komarova and Wodarz (2004) who argue that CIN is always slower than non-CIN when clonal expansion is taken into account. Their conclusion is based on the time at which the average number of clonally expanding cells reaches a certain size. In their approach, however, the average number of cells includes evolutionary trajectories where early mutations have given rise to a clonal expansion that leads to unreasonably large population sizes (more than the total number of all cells in the body, for example). Such trajectories could never occur and therefore must be excluded from the calculation. This effect is stronger for faster clonal expansion, and therefore, Komarova and Wodarz (2004) have concluded that the faster growing non-CIN cells always outperform the slower growing CIN cells.

The problem with using the average number of cells can be illustrated by studying the numerical example of Fig. 2. Using the calculation of Komarova and Wodarz (see Appendix B), the average number of X_2 cells reaches M at time $\bar{t}_x = 47.72$ while the average number of Y_2 cells reaches M at time $\bar{t}_y = 101.88$. From these results we cannot conclude that non-CIN outperforms CIN, because CIN lesions (of size M) are always more abundant than non-CIN lesions—as shown in Fig. 2. Therefore, in this example, the probability that a cancer is initiated with an early CIN mutation is higher than the probability that a cancer is initiated without a CIN mutation.

If clonal expansion is not robust, then the situation can turn against CIN. Suppose that inactivation of the TSG leads to a weak clonal expansion where the birth rate a is only marginally larger than the death rate b in the stable

cell type. The cost of CIN could then imply that the birth rate c is smaller than the death rate d in the unstable cell type. In this special case, CIN lesions cannot form.

Whenever the possible relevance of early genetic instability is evaluated, there is the concern that the increased mutation rate may not compensate for the reduced fitness of unstable cells. But it is important to keep in mind that genetic instability could also be associated with an increased rate of cell division, if for example certain cell cycle checkpoints are ignored. Thus, in some cases, unstable cells could have a fitness advantage (Nowak et al., 2002). It has also been proposed that certain carcinogens could select for genetic instability (Bardelli et al., 2001; Breivik, 2001; Blagosklonny, 2001). Moreover, if there are factors that select for genetic instability before clonal expansion, they could also do so during clonal expansion.

For most cancers, inactivation of the first TSG may not lead immediately to an enormous clonal expansion, but instead the first wave of clonal expansion comes to a halt when a certain size is reached. At this point, additional mutations in TSGs or oncogenes may be necessary for further clonal expansion. In pathways of tumorigenesis where two or more TSGs must be inactivated in rate limiting situations (when the number of replicating cancer cells is not too large), CIN will almost certainly occur before the inactivation of the first TSG (Michor et al., 2005a).

In summary, we conclude that robust clonal expansion even to very large cell numbers has only a small effect when evaluating the probability that tumorigenesis is initiated via genetic instability. The intuitive reason is that the time scale for clonal expansion is much shorter than the time scale of waiting for those mutations that cause clonal expansion.

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Appendix A

Eq. (1) gives the probability that a compartment has generated a stem cell of type 2 before time t. The clonal expansion of type 2 cells can either reach size M or die out again which results in the elimination of the whole compartment. We are interested in the probability that the clone reaches size M before time t. We consider the extinction probability and the time delay required for the cell population to increase from 1 to M. Let s denote the time delay required for the clonal expansion. Consider a continuous-time branching process starting from a single individual, $N(0) = 1$, that reaches population size M after time s. We have $N(s) = M$. Note that the population grows exponentially with rate $a - b$ when it is sufficiently large. For a very large population size, the stochasticity caused by demographic process becomes less important, and the population can be approximated by deterministic exponential growth. Trajectories of different simulation runs differ in s and the timing of reaching M due to the stochasticity while the population size is small. Hence we have

$$N(t) \approx M\mathrm{e}^{(a-b)(t-s)}. \tag{A.1}$$

According to the branching process calculation, the arithmetic average of the population size is $E[N(t)] = \exp[(a-b)t]$. This average is calculated by considering both trajectories of clonal expansion and of extinction. In contrast, the amount of delay for the clonal expansion should be averaged over only those runs that show exponential growth. Let $\langle\bullet\rangle$ denote the average with respect to the trajectories that do not go extinct. Using this average, we have $E[N(t)] = (1 - b/a)\langle N(t)\rangle + (b/a)\cdot 0$, leading to $\langle N(t)\rangle = \exp[(a-b)t]/(1-b/a)$. If $\langle N(t)\rangle$ is equivalent to $N(t)$ in Eq. (A.1), the time required for clonal expansion is given by

$$s \approx \frac{1}{a-b}\ln[M(1-b/a)]. \tag{A.2}$$

In the following, we estimate the mean time delay more carefully by considering the variance of trajectories.

A.1. Mean and variance of the cell population size

Let $g(\xi, t)$ be the generating function of the cell number, defined as $g(\xi, t) = E[\xi^{N(t)}|N(0) = 1]$, where ξ is a positive parameter ($0 < \xi \leqslant 1$). Within a short time interval of length Δt, a cell dies with probability $b\Delta t$, divides (becomes two cells) with probability $a\Delta t$, and remains unchanged otherwise. We obtain

$$g(\xi, t+\Delta t) = \Delta t + [1-(a+b)\Delta t]g(\xi, t) + a\Delta t g(\xi, t)^2.$$

Here we have used $E[\xi^{N(t)}|N(0) = 2] = E[\xi^{N(t)}|N(0) = 1]^2 = g(\xi, t)^2$, because two lineages starting from different cells at a given time behave independently. In the limit of infinitesimal Δt, we obtain $\partial g/\partial t = (ag - b)(g - 1)$ (see Iwasa et al., 2004a, b). With the initial condition $g(\xi, 0) = \xi$ we have

$$(\xi - b/a)(g-1) = (\xi - 1)(g - b/a)\mathrm{e}^{(a-b)t}. \tag{A.3}$$

When $\xi \to 0$, we have the extinction probability, which becomes $g(0, \infty) = b/a$ when $t \to \infty$. By taking the derivative of Eq. (A.3) and letting $\xi \to 1$, we have

$$\partial g/\partial \xi(1, t) = \mathrm{e}^{(a-b)t}.$$

This is the unconditional mean number of cells, where the average is calculated including paths that go extinct. Also, by the second derivative of Eq. (A.1) and by letting $\xi \to 1$, we have

$$\partial^2 g/\partial \xi^2(1, t) = \frac{2}{1-b/a}\mathrm{e}^{(a-b)t}(\mathrm{e}^{(a-b)t} - 1).$$

Note that $\partial^2 g/\partial\xi^2(1,t) = E[N(N-1)]$. This too is the unconditional mean. Let us now calculate the conditional mean and conditional variance, i.e. the mean and variance calculated only for the paths that grow exponentially. These are given by

$$\langle N\rangle = \partial g/\partial\xi(1,t)/(1-g(0,t)) \approx \mathrm{e}^{(a-b)t}/(1-b/a), \quad \text{(A.4a)}$$

$$\begin{aligned} Var(N) &= \langle N^2\rangle - \langle N\rangle^2 \\ &= \frac{\partial^2 g/\partial\xi^2(1,t) + \partial g/\partial\xi(1,t)}{1-g(0,t)} - \left(\frac{\partial g/\partial\xi(1,t)}{1-g(0,t)}\right)^2 \\ &\approx \mathrm{e}^{2(a-b)t}/(1-b/a)^2 \end{aligned} \quad \text{(A.4b)}$$

Here we pick up the leading order terms only. We have $Var(N)/\langle N\rangle^2 \approx 1$. Now consider the Taylor expansion of $\ln N$ around $\langle N\rangle$,

$$\langle \ln N\rangle \approx \ln\langle N\rangle - var(N)/2\langle N\rangle^2 + \cdots.$$

Hence we have

$$\ln\langle N\rangle - \langle \ln N\rangle = \tfrac{1}{2}. \quad \text{(A.5)}$$

Here only leading order terms are shown. They are accurate if M is sufficiently large.

A.2. Delay caused by clonal expansion

Due to the stochasticity when the cell number is small, a different realization of the cell population growth has a different time at which the population reaches size M. This time, denoted by s, differs between simulation runs (i.e. different clones). Let $\langle\bullet\rangle$ be the average with respect to the different runs that end up with exponential growth. Then we have from Eq. (A.1)

$$\langle \ln N\rangle = \ln M + (a-b)(t - \langle s\rangle). \quad \text{(A.6)}$$

Hence, from Eqs. (A.6), (A.4a), and (A.5), we have

$$\langle s\rangle = \frac{1}{a-b}\left[\ln M\left(1-\frac{b}{a}\right) + \frac{1}{2}\right], \quad \text{(A.7)}$$

which is denoted by T_x and is given by Eq. (6) in the text. Using this estimate of the mean delay, we can obtain Eq. (4) by multiplying the probability of non-extinction, $(1-b/a)$, and by replacing t by $t - T_x$.

Similarly, the time delay for the clonal expansion of type 2 cells with CIN is given by Eq. (7), which is Eq. (A.7) where a and b are replaced by c and d, respectively.

Appendix B

B.1. The time until the average cell number reaches M is much shorter than the average time until the cell number reaches M

To explain the reason why Komarova and Wodarz (2004) reach a different conclusion than we do, consider the average number of type X_2 cells. When the first X_2 cell arises at time t', the number of type X_2 cells increases exponentially following $\mathrm{e}^{(a-b)(t-t')}$. The rate at which new type X_2 cells arise is given by $\mathrm{d}P_x/\mathrm{d}t = u_1u_2t$. The probability that the clone does not go extinct is $1-b/a$. Hence the mean number of type X_2 cells averaged over different patients is given by

$$Z_x(t) = \int_0^t u_1u_2t'\left(1-\frac{b}{a}\right)\mathrm{e}^{(a-b)(t-t')}\,\mathrm{d}t'. \quad \text{(B.1)}$$

In a similar way, the mean number of Y_2 cells averaged over different patients is given by

$$Z_y(t) = \int_0^t 2u_1u_ct'\left(1-\frac{d}{c}\right)\mathrm{e}^{(c-d)(t-t')}\,\mathrm{d}t'. \quad \text{(B.2)}$$

By integration by part, we have $Z_x(t) = (u_1u_2/a(a-b))\mathrm{e}^{(a-b)t}$. Here we neglect small terms. Now consider the time at the mean number of X_2 cells reaches size M, and denote it by $\bar{t}_x$. From $Z_x(\bar{t}_x) = M$ we have

$$\bar{t}_x = \frac{1}{a-b}\ln\left(\frac{Ma(a-b)}{u_1u_2}\right).$$

Similarly, we calculate the time at which the mean number of Y_2 cells reaches M as $Z_y(\bar{t}_y) = M$. This leads to

$$\bar{t}_y = \frac{1}{c-d}\ln\left(\frac{Mc(c-d)}{2u_1u_c}\right).$$

With the parameter values used in Fig. 2, we have $\bar{t}_x = 47.72$ and $\bar{t}_y = 101.88$. Hence, using this criterion, the mean number of type X_2 cells reaches size M earlier than the mean number of type Y_2 cells. This might suggests that tumorigenesis without CIN is more important than tumorigenesis with CIN, if clonal expansion is taken into account. This conclusion, however, is opposite to the conclusion that is reached by considering the mean time until the clone becomes size M (Fig. 2).

Note that the estimates of $\bar{t}_x = 47.72$ and $\bar{t}_y = 101.88$ are much shorter than the mean waiting times shown in Fig. 2. There is a large variation between patients concerning the timing at which the first X_2 cell (or Y_2 cell) arises. In rare cases, in which the first mutant cell arises much earlier than in most cases, the cells quickly increase exponentially to unrealistically high numbers. When calculating the average cell number over different patients, those rare cases cause a large contribution to the average number and decreases the time at which the mean cell number reaches M. Since this tendency is stronger when the exponential growth rate is faster, X_2 cells (with exponential growth rate $a-b$) show an apparently earlier increase than Y_2 cells (with exponential growth rate $c-d<a-b$). Komarova and Wodarz (2004) carried out essentially the same calculation as above, although they used a convolution integral of two exponential functions rather than a linear function for the rate of producing mutants.

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