

# Non-neutral role of replicative senescence in tissue homeostasis and tumorigenesis

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## Abstract

Normal somatic cells divide only a limited number of times reaching a state known as replicative senescence. This restraint in reproductive potential has been proposed as a mechanism evolved in higher eukaryotes to protect the organism from developing cancer. However, despite this protection there is a positive correlation between tumor incidence and organism aging when cells are potentially closer to their replication limit. We use simple mathematical models derived from quasispecies theory to analyse the role of senescence in various scenarios with different cell types according to their replicative capacity. The models predict that a situation with cells launching more often the senescence response plays against tissue homeostasis favoring tumor initiation. It is also shown that cancer cells arising early in organism life are more sensitive to genetic instabilities progressing less often toward tissue invasion. The passage of cells through crisis emerges as a mechanism to maintain tissue homeostasis that is weakened in aged individuals. The models introduced, though simple, help to integrate experimental information relating tumorigenesis with cellular and organism aging phenomena.

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## 1. Introduction

Normal human somatic cells display limited proliferative potential (Hayflick, 1965). This limited replicative capability, called replicative senescence, is genetically controlled (Vojta and Barrett, 1995; Guarente and Kenyon, 2000; Clark et al., 2003) and characterized by terminal growth arrest, insensitivity to growth factors and apoptotic signals and flat, enlarged, usually polynucleated morphology (Mathon and Lloyd, 2001). Moreover, numerous biochemical and molecular changes can be followed as markers during the process of cellular senescence (Dice, 1993). Telomere shortening is considered to be the most probable molecular mechanism explaining the existence of such mitotic

clock controlling replicative senescence (Wright and Shay, 2002; Sherr and DePinho, 2000). Eukaryotic cells cannot replicate the very end of their chromosomes, the telomere, shortening its length every cell division until it reaches a critical threshold at which cells stop replicating (Collins, 2000). Other mechanisms may also exist (Guarente and Kenyon, 2000; Dice, 1993; Finkel and Holbrook, 2000; Howard, 1996; Rattan et al., 1992; Collins, 1999; Vijg, 2000; Knight, 1995). Cells displaying characteristic senescent features can be observed in response to oncogenic stress, DNA damage or oxidative stress (Reddel, 1998). More recently, the senescent phenotype of some cell types unresponsive to telomere enlargement have been considered a consequence of inappropriate culture conditions (Clark et al., 2003), a situation that has been speculated to mimic some tissue micro-environments. It is however important to remark that regardless the exact mechanisms of replicative senescence, the bypass of this checkpoint does not directly imply cellular immortalization (Wright and

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Shay, 1992, 2002; McEachern et al., 2000; Kim et al., 2002) as we will discuss in the following sections.

Cellular entry into the senescent state limits the replicative potential of somatic cells and therefore has been proposed to be an important anti-tumor mechanism (Campisi, 2001; Wright and Shay, 2001). In fact, most tumor cells present unlimited replicative potential and signals controlling cellular senescence are usually altered in tumors (Hanahan and Weinberg, 2000). Essentially, a tumor is the breakage of tissue homeostasis where one specific cellular population proliferates overcoming its innate genetic control and invading the space and resources of other coexisting populations. This breakage gradually leads to tissue dysfunction. During this process, cells can diversify into different “species” in terms of replication potential ranging from limited to boundless replication. These different stages of replication are linked to different genetic instability conditions.<sup>1</sup> Genetic instability is a property commonly found in tumors (Lengauer et al., 1998). It seems to arise in different steps of tumor progression as a mean to escape from diverse host mechanisms to maintain tissue homeostasis. However, instability might play against such progression when uncontrolled, since it reduces the growth rate of the tumor cell population. This ultimately leads to the activation of stabilizing mechanisms to allow the progression toward malignancy. In this paper, we introduce simple mathematical models to explore the evolutionary dynamics of tissue homeostasis and tumorigenesis generated by populations with different proliferative capabilities and genetic instability ratios. We can then identify the range of conditions necessary to cause the proliferation of each cellular population.

## 2. Models and results

We consider a given tissue in which different cell types, in terms of replicative potential, arise during proliferation. Such diversity is a consequence of normal replicative transitions or is due to mutations and chromosomal instability which change the proliferative potential of cells. We thus introduce four different cell populations describing experimentally well-documented observations. These cell types have been widely characterized in vitro and signatures of their presence have been also confirmed with various experiments in vivo (Mathon and Lloyd, 2001): (i) normal somatic cells with limited replicative potential,  $y_1$ . Even though tissues could be composed of various types of somatic cells, we consider here that  $y_1$  uniquely describes a single cell type

as the main constituent of the tissue, (ii) senescent cells,  $y_2$ , (iii) at a certain point, due to genetic alterations a third population arises which is characterized by an extension of lifespan bypassing the senescent limit,  $y_3$ . This population experiences an increasing cell death while replicating, reaching the so-called *crisis* state (Wright and Shay, 2002; Kim et al., 2002), and, (iv) a population characterized by new acquired capabilities for telomere length stabilization,  $y_4$ . This population will not have genetically controlled replicative limits, i.e., it becomes immortal (McEachern et al., 2000; Wright and Shay, 1992). The presence of cell species with different replication and mutation rates competing for limited resources inside the tissue allows natural selection to act. A process analogous to Darwinian selection has been widely argued to drive the progressive transformation of normal human cells into cancer ones (Nowel, 1976; Cahill et al., 1999; Tomlinson and Bodmer, 1999). To describe mathematically the competition and transition among species we make use of the quasispecies framework. Quasispecies theory was initially introduced to study how a collection of RNA molecules would evolve under the prebiotic conditions of the early Earth (Eigen, 1971), and then further applied to more general molecular evolution scenarios (Nowak and May, 2000). It thus seems appropriate to apply this mathematical framework to the study of the evolutionary dynamics of the different coexisting populations inside the tissue. In the following, we analyse several situations which are likely to be found in the process of tumorigenesis. We study several scenarios in which different cell types are considered. The more complex scenario is that describing a situation where immortal cells could arise (Fig. 1a). Some aspects of these models could be reminiscent of the earlier commitment theory of cellular ageing (Holliday et al., 1977). However, while this theory was aimed to clarify the emergence of apparent immortality in culturing procedures with unlimited resources, here we analyse the role of senescence in scenarios with limited resources in vivo by introducing a different mathematical framework.

### 2.1. Senescent vs. non-senescent cells

We introduce some of the mathematics of quasispecies theory in a simple model of two populations. We consider two cell types: normal replicative cells ( $y_1$ ) and senescent cells ( $y_2$ ). Replicative cells constitute a heterogeneous mixture of cells with different limited lifespans due to the inherent stochastic behavior of the senescent mechanisms (Blackburn, 2000). However, we lump all cells together in a single population type. Replicative cells could become senescent, a transition mainly associated to telomere shortening, although telomere-independent cellular damage or cellular stress

<sup>1</sup> We use here the term genetic instability in its most general sense, i.e., it refers to both increased rate of mutation and chromosomal instability.

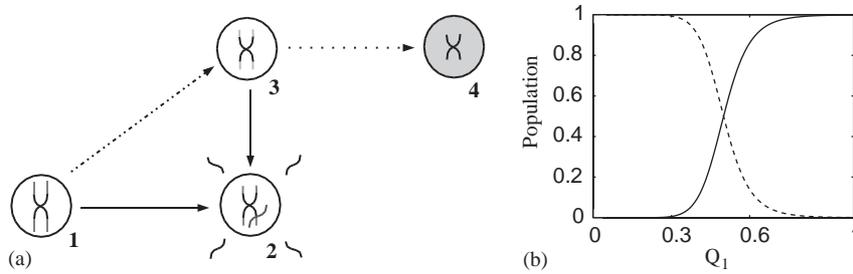


Fig. 1. (a) Cell replicative states: (1) divide with an upper limitation, (2) reach senescence, (3) experience a state of elongated lifespan (EL), (4) achieve immortalization. Telomeres (gray) are shorter in senescent cells and much shorter in EL cells (light gray). Senescence can be driven also by cellular damage and stress signals (wavy lines). (b) Equilibrium population of replicative,  $y_1$  (solid line), and senescent,  $y_2$  (dashed line), cells as a function of  $Q_1$ . Threshold behavior is due to the proximity of the distribution of telomere length of the initial replicative population to the Hayflick limit. Parameters as follows:  $Q_H = 1/2$  and  $n = 10$ .

signals can also contribute to this phenotype (see introduction). All these factors contributing to replicative senescence are incorporated. A simple model describing the transition toward senescence would then read,

$$\begin{aligned} \dot{y}_1 &= (r_1 Q_1 - \delta_1) y_1, \\ \dot{y}_2 &= (r_2 - \delta_2) y_2 + r_1 (1 - Q_1) y_1. \end{aligned} \tag{1}$$

Here,  $r$  and  $\delta$  are the average growing and death rate of populations  $y_1$  and  $y_2$ . The parameter  $Q_1$ ,  $0 \leq Q_1 \leq 1$ , known as the *quality factor* in standard quasispecies theory (Eigen, 1971; Nowak and May, 2000), specifies the fraction of replicative cells that keeps a replicative potential. A fraction  $r_1(1 - Q_1)y_1$  of replicative cells become senescent. In a general case with two different populations without mutation, i.e.,  $Q_1 = 1$ , the cell type which replicates fastest would be selected and, as the model stands, growth without limit. We introduce a constraint of constant population size to reflect a maximum size that cell populations can achieve. This implies a competition of both populations in such limited environment. The equations now read

$$\begin{aligned} \dot{y}_1 &= (r_1 Q_1 - \delta_1 - \bar{E}) y_1, \\ \dot{y}_2 &= (r_2 - \delta_2 - \bar{E}) y_2 + r_1 (1 - Q_1) y_1, \end{aligned} \tag{2}$$

where  $E_i = r_i - \delta_i$  is the excess productivity of species  $i$ , i.e., the excess of growing rate over the death rate and  $\bar{E} = \sum_i E_i y_i$  is the net rate at which the population size would change. Without loss of generality, we set  $\sum_i y_i = 1$ . These are the standard nonlinear quasispecies equations for two species. We further specify these equations by considering that senescent cells neither grow nor die, i.e.,  $r_2 = \delta_2 = 0$ , and that for replicative cells  $\delta_1 \approx 0$ . Then we have

$$\begin{aligned} \dot{y}_1 &= (r_1 Q_1 - r_1 y_1) y_1, \\ \dot{y}_2 &= -r_1 y_1 y_2 + r_1 (1 - Q_1) y_1. \end{aligned} \tag{3}$$

Thus, growth of  $y_2$  is only due to the fraction of replicative cells becoming senescent. The population converges to the state  $(y_1^*, y_2^*) = (Q_1, 1 - Q_1)$ . When  $Q_1 \ll 1$  almost the whole population becomes senescent.

Otherwise, since the senescent population reduces the available resources, e.g., space, it reduces the capacity of  $y_1$  to keep growing. This can be easily understood by rewriting the equation for the replicative population as:  $\dot{y}_1 = r_1 Q_1 y_1 (1 - \frac{y_1}{Q_1})$ , which is the familiar logistic equation in which  $Q_1$  acts as the carrying capacity or maximum number of cells this population can achieve (Murray, 1993).

We could consider telomere shortening as the major inductor of the senescent phenotype. A population  $y_1$  whose cell replicative lifespans are mostly distributed near the limit of population doublings, the so-called Hayflick limit (Hayflick, 1965), would experience a drastic increase in the probability of becoming senescent, i.e.,  $1 - Q_1$ . This could be for instance related to organism aging. We can take this into account by expressing  $Q_1$  as a threshold function around such Hayflick limit:  $Q_1 \rightarrow \frac{Q_1^n}{Q_1^n + Q_H^n}$ . Here,  $Q_H$  is the quality factor denoting a population  $y_1$  with approximately half of their telomere lengths close to the Hayflick limit and  $n > 0$  is a measure of how switch-like this transition is. In this case the population converges to  $(y_1^*, y_2^*) = (\frac{Q_1^n}{Q_1^n + Q_H^n}, \frac{Q_H^n}{Q_1^n + Q_H^n})$  (Fig. 1b). The main conclusion of this simple model is that in a limited environment the presence of senescent cells reduces the expansion of the non-senescent ones. The parameter  $Q_1$  acts as a measure of how often the replicative senescence response is launched, i.e., low  $Q_1 \rightarrow$  high senescence. A straightforward biological consequence of this mathematical conclusion is that all cells in a tissue will never reach complete senescence, since an increase in the number of senescent cells will lead to a reduction in the transition of new cells to senescence. This allows tissues to remain functional even though their activity can decrease as a consequence of an increase in the number of senescent cells.

### 2.2. Extension of lifespan

Cells can bypass senescence entering a state of temporal extension of their lifespan. This can be a

consequence, for instance, of the inactivation of pRb and p53 tumor suppressor proteins (Shay et al., 1991). Such extension of lifespan is only temporal leading to a state with high rates of cell death as a result of increasing chromosomal abnormalities termed *crisis*. To analyse this situation, we introduce a third population type,  $y_3$ , describing cells which are in such extension of lifespan state (denoted as EL cells from now on). The model now reads

$$\begin{aligned} \dot{y}_1 &= (r_1 Q_1 - \bar{E})y_1, \\ \dot{y}_2 &= -\bar{E}y_2 + w_{21}y_1 + r_3(1 - Q_3)y_3, \\ \dot{y}_3 &= (r_3 Q_3 - \delta_3 - \bar{E})y_3 + w_{31}y_1, \end{aligned} \quad (4)$$

and  $\bar{E} = r_1 y_1 + (r_3 - \delta_3)y_3$ . Here,  $Q_1$  and  $Q_3$  represent the probability of replicative and EL species to preserve their proliferative potential, respectively. Replicative cells could become senescent with rate  $w_{21}$  or bypass the senescence arrest with rate  $w_{31}$ , such that  $r_1(1 - Q_1) = w_{21} + w_{31}$  and, normally,  $w_{31} \ll w_{21}$ . EL cells are considered to have a very similar average growing rate to that of replicative cells  $r_3 \approx r_1$ . Further, since EL cells experience a very low senescent response, by its very definition, one has  $Q_3 \approx 1$  (we keep explicitly  $Q_3$  and  $r_3 \neq r_1$  in the following discussion to better understand the different contributing factors to the dynamics).

The previous equations describe a competition for the available resources among three types of cellular species, replicative, senescent and pre-immortal EL cells. This could be considered a likely scenario in initial stages of tumorigenesis. We are interested to analyse under which conditions EL cells can or cannot invade the micro-environment. This is manifested in the existence of two stable equilibrium states of the dynamical system. By studying the stability properties of these states, we will understand the factors contributing to the expansion of pre-immortal cells. We could have

(e.1) All three populations different from zero. This state is given by

$$\begin{aligned} y_1^* &= \frac{r_1 Q_1 [r_1 Q_1 - r_3 Q_3 + \delta_3]}{r_1 [r_1 Q_1 - r_3 Q_3 + \delta_3] + w_{31} (r_3 - \delta_3)}, \\ y_2^* &= \frac{w_{21} [r_1 Q_1 - r_3 Q_3 + \delta_3] + w_{31} r_3 (1 - Q_3)}{r_1 [r_1 Q_1 - r_3 Q_3 + \delta_3] + w_{31} (r_3 - \delta_3)}, \\ y_3^* &= \frac{w_{31} r_1 Q_1}{r_1 [r_1 Q_1 - r_3 Q_3 + \delta_3] + w_{31} (r_3 - \delta_3)}. \end{aligned} \quad (5)$$

Note that the populations of both senescent and EL cells are directly proportional to the mutation rates from replicative cells. The stability of this equilibrium state is determined mathematically by the eigenvalues of the Jacobian matrix of first order partial derivatives. These eigenvalues are  $\{r_3 Q_3 - r_1 Q_1 - \delta_3, -r_1 Q_1, -r_1 Q_1\}$ . The equilibrium is stable whenever these eigenvalues are negative, i.e.,  $r_1 Q_1 > 0$  (always fulfilled when  $Q_1 \neq 0$ ) and  $r_1 Q_1 > r_3 Q_3 - \delta_3$ . This last condition implies that the

effective growth rate of the EL population is smaller than that of the replicative population.

(e.2) Only senescent and EL cells, which is described by the equilibrium

$$y_1^* = 0, \quad y_2^* = \frac{r_3(1 - Q_3)}{r_3 - \delta_3}, \quad y_3^* = \frac{r_3 Q_3 - \delta_3}{r_3 - \delta_3}. \quad (6)$$

In this case the eigenvalues of the Jacobian matrix are  $\{-r_3 Q_3 + r_1 Q_1 + \delta_3, -r_3 Q_3 + \delta_3, -r_3 Q_3 + \delta_3\}$ . This equilibrium is stable whenever  $r_1 Q_1 < r_3 Q_3 - \delta_3$  and  $r_3 Q_3 > \delta_3$  (trivially fulfilled when the other condition is satisfied). This parameter regime is the opposite to the previous one. In this case, the effective growth of the replicative population is smaller than that of the EL population. This equilibrium remains a theoretical limit. It will never be achieved in realistic scenarios due to crisis (see discussion below).

We want to analyse how both the senescence response of replicative cells and the instability properties of EL cells influence the transition between the previous dynamical regimes. We can easily derive threshold conditions relating these features from the previous stability inequalities. First, we want to focus on the role played by the senescence response. The following threshold condition can be introduced:  $r_1 Q_1^{\text{th}} = r_3 Q_3 - \delta_3$ . This can be rewritten as  $Q_1^{\text{th}} = Q_3 - \frac{\delta_3}{r_1}$ , when the value  $r_3 \approx r_1$  is explicitly considered. This reflects the existence of a kind of *error-threshold* of replication, a key concept in quasispecies theory. In the standard quasispecies scenario the error-threshold quantifies the minimum replication accuracy maintaining the wild-type sequence (Nowak and May, 2000). In our case, it implies a condition relating the amount of senescent response experienced by  $y_1$  cells and the instability of the EL population. Error-thresholds and cancer have been recently discussed in a more general setting (Solé and Deisboeck, 2003).

We have plotted how the equilibrium populations vary with respect to the quality factor threshold in Fig. 2 for two different instability values of  $y_3$  cells. Instability in this population is considered to be due to telomere dysfunction as a consequence of telomere erosion which causes chromosomal abnormalities increasing the cell death rate  $\delta_3$ . Fig. 2(a) corresponds to the case in which EL cells are still very stable,  $\delta_3 \ll 1$ . This could describe an scenario of early elongated lifespan. In this regime, the only difference between  $y_1$  and  $y_3$  cells is that the last ones hardly launch the senescence response, i.e., they experience a low slow-down in growth caused by senescence. EL cells will always invade unless the senescence response suffered by  $y_1$  is very small. Senescence then promotes EL invasion. Fig. 2(b) corresponds to a case with more unstable EL cells,  $\delta_3 = .7r_1$ , describing a later period of EL, i.e., crisis. EL cells invade much less the population, both in relative size and in  $Q_1$  range. Thus, equilibrium (e.2), in which

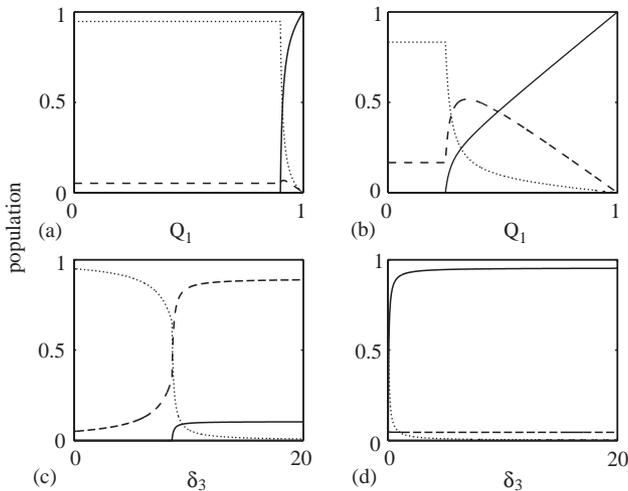


Fig. 2. Thresholds in equilibrium populations when three cell species are present: replicative cells  $y_1$  (solid line), senescent cells  $y_2$  (dashed line), and elongated lifespan (EL) cells  $y_3$  (dotted line). Threshold in quality factor  $Q_1^{\text{th}} = Q_3 - \frac{\delta_3}{r_1}$  with (a) low instability ( $\delta_3 = 0.05r_1$ ), (b) high instability ( $\delta_3 = 0.7r_1$ ). Threshold in genetic instability  $\delta_3^{\text{th}} = r_1Q_3(1 - \xi)$ , and  $Q_1 = \xi Q_3$  with (c) high senescence response ( $\xi = 0.1$ ), (d) low senescence response ( $\xi = 0.8$ ). Common parameters to all figures as follows:  $r_1 = 10$ ,  $Q_3 = 0.95$ ,  $w_{31} = r_1(1 - Q_1)/10$ , and  $w_{21} = r_1(1 - Q_1) - w_{31}$ .

homeostasis is lost, is ultimately never achieved due to crisis. The increase in instability diminishes the role played by senescence as a mechanism of EL progression.

A complementary threshold condition could be introduced to analyse the influence of the genetic instability suffered by EL cells in the dynamics. This is derived by rewriting the previous condition in an alternative way as  $\delta_3^{\text{th}} = Q_3r_1(1 - \xi)$ . Here  $Q_1 = \xi Q_3$  with  $\xi < 1$  since EL cells hardly launch senescence and thus  $Q_1 < Q_3$  normally. We have studied this threshold in Fig. 2 for two different  $Q_1$  regimes. For low quality factor (Fig. 2c), e.g., population close to Hayflick limit, when most replicative cells become senescent, there exists a wide instability regime where the EL population still invades. For high quality factor (Fig. 2d), i.e., low senescence response, the EL population disappears at very low levels of instability. In this case tissue homeostasis is favored, i.e., replicative cells invade. These conclusions seem to support a picture in which both the senescence response and genetic instability act as key factors in the progression of pre-immortal cells.

### 2.3. Immortalization

EL is not the final replicative stage experienced by cells. More than 90% of cancers reflect an ultimate replicative status: tumor cells are immortal. Immortalization per se is not sufficient to generate tumors and other additional “acquired capabilities” are needed (Hanahan and Weinberg, 2000). We consider immortalization as a first step in an hypothetical model of

multistep tumorigenesis. Therefore, we include a fourth cell type in our model,  $y_4$ , to describe the appearance of immortal cells. The model now reads

$$\begin{aligned} \dot{y}_1 &= (r_1Q_1 - \bar{E})y_1, \\ \dot{y}_2 &= -\bar{E}y_2 + w_{21}y_1 + w_{23}y_3, \\ \dot{y}_3 &= (r_3Q_3 - \delta_3 - \bar{E})y_3 + w_{31}y_1, \\ \dot{y}_4 &= (r_4 - \delta_4 - \bar{E})y_4 + w_{43}y_3, \end{aligned} \tag{7}$$

and  $\bar{E} = r_1y_1 + (r_3 - \delta_3)y_3 + (r_4 - \delta_4)y_4$ . Here  $r_3(1 - Q_3) = w_{23} + w_{43}$ , and  $r_1(1 - Q_1) = w_{21} + w_{31}$ .  $w_{43}$  and  $w_{23}$  denote the transition rate of EL cells to become immortal and senescent, respectively. We take  $w_{43} \ll w_{23}$  and  $w_{31} \ll w_{21}$ , where we have considered that shortening of telomeres continues during the elongated lifespan period therefore launching senescence. All other rates represent similar quantities as in previous cases. In this final scenario, there exists a competition among four different cell populations. The final outcome of this competition is again manifested in several stable equilibria of the dynamical system. As before, the effective growth factors of the populations, i.e.,  $r_1Q_1$ ,  $r_3Q_3 - \delta_3$ , and  $r_4 - \delta_4$ , will determine the different distribution of populations in these equilibria. We have:

(e.1) All four populations coexist in equilibrium, i.e.,  $y_1^* \neq 0$ ,  $y_2^* \neq 0$ ,  $y_3^* \neq 0$ ,  $y_4^* \neq 0$ . The eigenvalues of the Jacobian matrix are  $\{-r_1Q_1 + r_4 - \delta_4, -r_1Q_1 + r_3Q_3 - \delta_3, -r_1Q_1, -r_1Q_1\}$ . Stable when  $r_1Q_1 > 0$  (always fulfilled when  $Q_1 \neq 0$ ) and

$$r_1Q_1 > r_3Q_3 - \delta_3, \quad r_1Q_1 > r_4 - \delta_4. \tag{8}$$

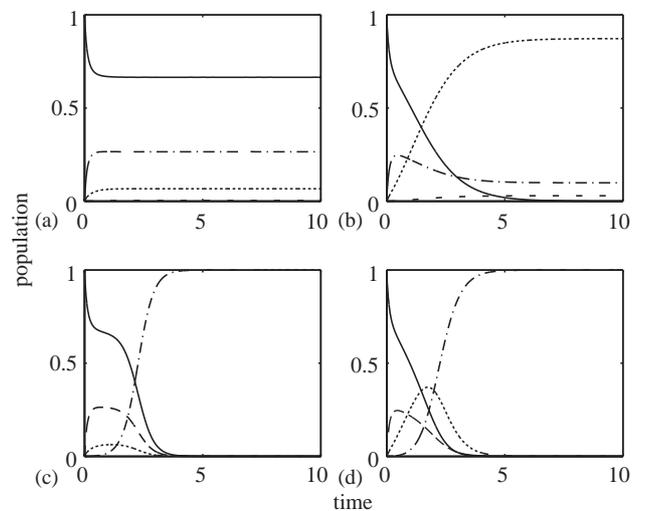


Fig. 3. Dynamical behavior of all four cell types for different parameter regimes. In (a) and (b) all types coexist in equilibrium as a *quasispecies*, while in (c) and (d) the immortal species dominates the population. Parameters: (a)  $\delta_3 = r_1/2, \delta_4 = r_1/2$ , (b)  $\delta_3 = r_1/10, \delta_4 = r_1/2$ , (c)  $\delta_3 = r_1/2, \delta_4 = 0$ , and (d)  $\delta_3 = r_1/10, \delta_4 = 0$ . Common parameters to all figures as follows:  $r_1 = 10$ ,  $r_3 = r_1$ ,  $r_4 = r_1$ ,  $Q_1 = 0.7$ ,  $Q_3 = 0.9$ ,  $w_{31} = r_1(1 - Q_1)/10$ ,  $w_{21} = r_1(1 - Q_1) - w_{31}$ ,  $w_{43} = r_3(1 - Q_3)/10$ ,  $w_{23} = r_3(1 - Q_3) - w_{43}$ . Line codes:  $y_1$ : solid,  $y_2$  dashed,  $y_3$ : dotted,  $y_4$ : dash-dotted.

This equilibrium would imply that all four cell types coexist (Fig. 3a).

(e.2) Replicative cells disappear, i.e.,  $y_1^* = 0$ ,  $y_2^* \neq 0$ ,  $y_3^* \neq 0$ ,  $y_4^* \neq 0$ , with the eigenvalues  $\{r_1 Q_1 - r_3 Q_3 + \delta_3, -r_3 Q_3 + \delta_3 + r_4 - \delta_4, -r_3 Q_3 + \delta_3, -r_3 Q_3 + \delta_3\}$ .

Stable when  $r_3 Q_3 > \delta_3$  and

$$r_3 Q_3 - \delta_3 > r_1 Q_1, \quad r_3 Q_3 - \delta_3 > r_4 - \delta_4. \tag{9}$$

EL cells would invade in this case (Fig. 3b). Note that, as equilibrium (e.2) in the previous section, this represents an hypothetical situation since this equilibrium is never achieved due to the increasing instability suffered by EL cells toward crisis ( $y_1$  will never completely disappear in a realistic scenario).

(e.3) Immortal cells invade all available space, i.e.,  $y_1^* = 0$ ,  $y_2^* = 0$ ,  $y_3^* = 0$ ,  $y_4^* = 1$ , with the eigenvalues  $\{r_1 Q_1 - r_4 + \delta_4, \delta_4 - r_4, \delta_4 - r_4, r_3 Q_3 - \delta_3 - r_4 + \delta_4\}$ .

Stable when  $r_4 > \delta_4$  and

$$r_4 - \delta_4 > r_1 Q_1, \quad r_4 - \delta_4 > r_3 Q_3 - \delta_3. \tag{10}$$

This implies invasion of immortal cells, a potential initiation of a malignant tumor.

In Fig. 3, we have plotted the dynamical behavior of all four population types for the cases just discussed. Figs. 3(a) and (b) display an equilibrium where a combination of species in fixed proportions is achieved. This is what is normally called a *quasispecie* (Nowak and May, 2000). This would reflect a situation of homeostasis even when EL and immortal cells are present, though in very low levels. Figs. 3(c) and (d) relate to the same equilibrium (e.3), but with different progression to it due to the different ratio between  $r_1 Q_1$  and  $r_3 Q_3 - \delta_3$ .

Let us study now the influence of different parameter regimes on the dynamics. We are particularly interested in analyzing the dual role played by the genetic instability associated with crisis in tumor progression. Such trade-off between genetic instability and tumor progression is one of the fundamental open questions about tumor invasion (Cahill et al., 1999). At least three alternative theories are currently under debate: (1) emergence of a mutator phenotype which increases the basal mutation rate (Loeb et al., 2003), (2) existence of “master” genes whose alteration gives rise to chromosomal instabilities (Lengauer et al., 1998; Nowak et al., 2003) and (3) existence of aneuploidy conditions from the very beginning (Duesberg and Li, 2003). In any case, it is clear that too much genomic instability would be fatal to the cell. On the other hand, increased genetic instability is likely to speed up tumor progression. In this context, telomere dysfunction may appear as an alternative source of genetic instability. For instance, chromosomal abnormalities induced by short telomeres lead to an increase in the mutation rate in yeast (Hackett et al., 2001), mouse (Blasco, 2003), and humans (Vulliamy et al., 2001). We include such competing forces in the model as follows. We consider that the

progression of EL cells toward crisis implies, beyond a given threshold, a considerable decrease in growth rate of the emerging immortal population. This threshold is marked by the minimum size of telomeres beyond which cells suffer chromosomal reorganization minimizing survival potential (McEachern et al., 2000). Thus, we redefine the growth rate of immortal cells as a threshold function of  $Q_3$ , such that  $r_4 \rightarrow r_4 \frac{Q_3^n}{Q_{th}^n + Q_3^n}$  (Solé and Deisboeck, 2003), with  $Q_{th}^n$  denoting the  $Q_3$ -threshold value and  $n > 0$  denoting how sharp this threshold is. Recall that  $Q_3$  is the probability that  $y_3$  cells stay as EL cells after replication. Thus, high  $Q_3$ 's imply a situation of early EL with low instability and low probability of emerging immortal cells which, on the other hand, would have a high growth rate. On the contrary, low  $Q_3$ 's imply late EL, i.e., crisis, with higher instability and probability of emerging immortal cells but lower growth rate. A complementary stabilizing force can also emerge. This is due mostly to the activation of telomerase (Mathon and Lloyd, 2001; Wright and Shay, 1992; Kim et al., 2002), although alternative mechanisms are also known (Reddel, 2003). We then introduce the following decay function  $\delta_4 = \Delta \delta_3$ . Here,  $\Delta$  denotes the magnitude of the stabilization mechanism with  $0 \leq \Delta \leq 1$ . All together we write  $r_4 - \delta_4 \rightarrow r_4 \frac{Q_3^n}{Q_{th}^n + Q_3^n} - \Delta \delta_3$ . Further, we select a range of parameters fulfilling the conditions  $r_1 Q_1 > r_3 Q_3 - \delta_3$  (with  $r_3 Q_3 > \delta_3$ ). This condition reflects the fact that immortal cells arise in the period of late elongated lifespan, i.e., crisis.

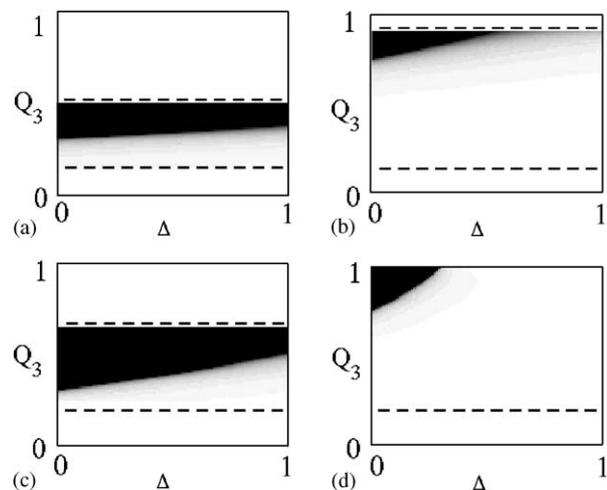


Fig. 4.  $(Q_3, \Delta)$  parameter space for immortal cells invasion. Black areas between dashed lines show the parameter regime in which immortal cells invade the whole population. Dashed lines denote the range of parameters where the crisis conditions  $r_1 Q_1 > r_3 Q_3 - \delta_3$  (upper line) and  $r_3 Q_3 > \delta_3$  (lower line) are fulfilled. Parameters were chosen as follows: (a)  $Q_1 = 0.4$ ,  $\delta_3 = r_1/10$ , (b)  $Q_1 = 0.8$ ,  $\delta_3 = r_1/10$ , (c)  $Q_1 = 0.4$ ,  $\delta_3 = r_1/4$ , and (d)  $Q_1 = 0.8$ ,  $\delta_3 = r_1/4$ . Common parameters to all figures as follows:  $Q_{th} = 3/8$ ,  $n = 2$ . All other parameters as Fig. 3.

In Fig. 4, we have plotted the parameter space ( $Q_3, \Delta$ ) in which immortal cells would invade the whole population (black areas). This figure shows one of the key conclusions of the model. By comparing Figs. 4(a) and (c) and 4(b) and (d), one sees that a bigger parameter regime in which immortal cells invade is experienced in a situation of high senescence, i.e., low  $Q_1$ . In other words, a situation with cells launching more often the senescence response favors tumor invasion. Figs. 4(a) and (c) show a situation with high senescence,  $Q_1 = 0.4$ , and increasing instability,  $\delta_3 = r_1/10$  in (a), and  $\delta_3 = r_1/4$  in (c). Note that both figures present a parameter regime where immortal cells invade even without any stabilization activity ( $\Delta = 1$ ). This is a consequence of the threshold of instability considered, i.e.,  $Q_{th}$ . We have considered a situation with relatively low  $Q_3$  value for the decrease in growth rate to arise, i.e.,  $Q_{th} = 3/8$ , to help us in the discussion. With a slightly higher threshold such parameter regime disappears and stabilization is always needed for invasion of immortal cells (data not shown). Figs. 4(b) and (d) show the same instability situations but with lower senescence response,  $Q_1 = 0.8$ . In this case the high potential growth rate of the  $y_1$  population,  $r_1 Q_1$ , protect from invasion by immortal cells. This is the case even when immortal cells suffer only a small decrease in growth rate. Immortal cells would invade in a very restricted parameter regime with low decrease in growth rate, i.e., cells derived from a EL population in early crisis, and high stabilization.

*Non-neutral senescent cells:* We have discussed how the sheer presence of senescent cells influences tumorigenesis. Additionally, it has been noted recently an *active* influence of senescent cells in tissue homeostasis by means of releasing growth inhibitors or stimulators to the medium (Krtolica and Campisi, 2002). This active influence can be taken into account easily in our models by including a “non-neutrality” coefficient,  $\kappa$ , measuring such effect, i.e.,  $r_1 \rightarrow \kappa r_1$ . Note that this would assume that such non-neutral senescent cells influence both replicative and EL cells. This factor influences the threshold conditions shifting the balance toward or against tumor progression whenever  $\kappa > 1$  or  $\kappa < 1$ , respectively.

### 3. Discussion

Replicative senescence has long been thought to be a tumor suppressor mechanism. We have introduced simple mathematical models to examine this issue in a more formal setting. Our objective was to understand the role played by replicative senescence in the complex dynamics contributing to tissue homeostasis and tumor development. We studied the evolutionary dynamics of three scenarios with the presence of different cell types: (i)

replicative and senescent cells, (ii) replicative, senescent and elongated lifespan cells, and (iii) replicative, senescent, EL and immortal cells. The first case describes tissues without any mutation altering the normal replicative behavior of cells. The main conclusion is that the presence of senescent cells is not innocuous. The presence of these cells contribute to the inhibition of the transition to senescence. A straightforward biological conclusion is that tissues never reach full senescence. This situation might explain what is observed in vivo. Given the correlation between replicative senescence and organism aging (Rubin, 1997; Dimri et al., 1995; Martin, 1977; Kirkwood and Austad, 2000; Imai and Kitano, 1998) we would expect a very large presence of senescent cells in aged organisms. The percentage of tissue showing senescence is however moderated (Rubin, 1997; Dimri et al., 1995; Martin, 1977).

In the second case, our model predicts different equilibrium populations of EL cells depending on  $Q_1$  and the instability of EL cells. Recall that  $Q_1$  could reflect how close to the Hayflick limit (and, more or less directly, to organism aging) the lifespans of the replicative population are. If EL cells arise early in organism life (high  $Q_1$ ) when cells are not too close to the Hayflick limit, low instability rates induce an equilibrium with these cells kept at a minimum and normal somatic cells being the most common ones (Fig. 2d). However, if the EL population arises when the replicative cells have reduced proliferative capabilities, i.e., low  $Q_1$  (late in organism life), there is a wide instability range in which EL cells are still the most common ones (Fig. 2c). This population can form a considerably non-tumoral mass that can increase the risk of cancer depending only on telomerase activation. Thus, the model predicts an increased presence of EL cells in those individuals whose tissue cells have a reduced replicative potential, e.g., aged individuals.

Alternatively, we can examine this situation in terms of tissue homeostasis, i.e., predominance of non-EL cells. We can observe two main biological conclusions: (1) Very stable EL cells ( $\delta_3 \ll 1$ ) as a consequence of long telomere length (EL cells in early period of elongated lifespan). In this regime tissue homeostasis is only achieved under situations when senescence has been hardly launched. This would be consistent with the low rate of development of tumors in early organism life even in regimes with very stable EL populations. Thus, this population could never progress toward immortality (Fig. 2a). (2) EL cells are very unstable due to telomere shortage (EL cells in late period of elongated lifespan, i.e., crisis). In this regime replicative cells are the most common ones in the tissue except in situations of very high senescence (Fig. 2b). Thus, crisis emerges as a mechanism to maintain tissue homeostasis. However this role of crisis seems to be diminished in aged individuals. When senescence is high (low  $Q_1$ ) there is a wide instability range in which

senescence acts as a mechanism that favors the presence of EL cells. However, beyond the instability threshold, i.e., *crisis*, the EL cell population diminishes recovering the previous case.

In the last scenario, our model incorporates a fourth cell type: Immortal cells. This population might not yet be a tumoral one since it is not tumorigenic, i.e., it has not altered genes conferring transforming properties. We can consider it nevertheless as an initial state toward tumor formation. This is a theoretical assumption and has nothing to do with the order of achieving the acquired capabilities characterizing cancer cells (Hanahan and Weinberg, 2000). The main conclusion of our model is extracted from Fig. 4. By comparing Figs. 4(a) and (c) and 4(b) and (d) we observe how immortal cells invade the tissue for a larger parameter regime in a situation of high senescence (low  $Q_1$ ). In other words, in situations where there is a high transition toward senescence (aging, progeria syndromes) the presence of immortal cells is favored increasing consequently the probability of cancer. This is an important conclusion since it matches with the observation that age is the largest single risk factor for the development of cancer in mammals. The incidence of cancer in humans rises exponentially with age beginning at about the middle age (Krtolica and Campisi, 2000; Balducci and Beghe, 2001; DePinho, 2000; Balducci and Lyman, 1997). In general, the rate at which cancer develops is proportional to the rate of aging (Miller, 1991; Martin, 1996). This has been also found in animals models. Practically all models of accelerated aging showed increased incidence and shorter latency of tumors (Anisimov, 2001). A complementary observation can also be extracted from our mathematical model. In low senescence regimes (high  $Q_1$ ), the replicative potential of normal cells contributes to tissue homeostasis. In this case, immortal cells only invade in a parameter regime of low instability, i.e., telomerase activated. This situation is similar to that observed in many childhood tumors that seems to originate in stem cells (with telomerase constitutively activated) failing to differentiate, rather than from somatic mutations. The most common cancers in children and young adults are hematological malignancies and sarcomas. It is commonly admitted that they are the consequence of genetic alterations that inhibit normal differentiation of progenitor stem cells (Mackall et al., 2002; LeBien, 2000; Greaves, 1999), with each lineage giving rise to specific malignancies. Progenitor cells have activated telomerase therefore decreasing the range of instability needed by immortal cells to become the most common in the population.

#### 4. Summary

In conclusion, we have introduced simple mathematical models to study the role of replicative senescence in

tissue homeostasis and tumorigenesis. We have shown how the sheer launching of replicative senescence, more often in aged organisms, favors tumor progression. The genetic instability associated to the crisis stage emerges as a mechanism to keep tissue homeostasis. We hope that the introduction of this and similar mathematical models (Tomlinson and Bodmer, 1999; Solé and Deisboeck, 2003; Nowak et al., 2003) will help to integrate experimental information leading to a better knowledge of cancer dynamics (Gatenby and Maini, 2002) and the development of more effective therapies.

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