Cancer Stem Cells as the Engine of Tumor Progression

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Abstract

Genomic instability is considered by many authors the key engine of tumorigenesis. However, mounting evidence indicates that a small population of drug resistant cancer cells can also be a key component of tumor progression. Such cancer stem cells would be the reservoir of tumor stability while genetically unstable cells would compete with normal cells and invade neighboring host tissue. Here we study the interplay between these two conflicting components of cancer dynamics using two types of tissue architecture. Both mean field and multicompartment models are studied. It is shown that tissue architecture affects the pattern of cancer dynamics and that unstable cancers spontaneously organize into a heterogeneous population of highly unstable cells. This dominant population is in fact separated from the low-mutation compartment by an instability gap, where almost no cancer cells are observed. The possible implications of this prediction are discussed.

Keywords: Cancer, tumor growth, genomic instability, error threshold

I. INTRODUCTION

Cancer is commonly viewed as a micro-evolutionary process (Cairns, 1975; Merlo et al., 2006; Weinberg, 2007; Wodarz and Komarova, 2005). The outcome of such process is strongly tied to different traits of tumor structure, including its heterogeneity (Fearon and Vogelstein, 1990), robustness (Kitano, 2004) and even cooperation (Axelrod et al., 2006). Genomic instability seems to be a common trait in many types of cancer (Cahill et al., 1999) and is a key ingredient in the Darwinian exploratory process required to overcome selection barriers. By displaying high levels of mutation, cancer cells can generate a progeny of diverse phenotypes able to escape from such barriers (Loeb, 2001). Faced with different challenges under the conditions imposed by the given tissue, mutated cells are able to change their pattern of communication, immune markers, migration and adhesion properties.

Genetic instability is present in all solid tumors, particularly under the form of chromosomal instability (CIN). Available evidence shows that CIN is actually an early event in some types of cancer. The presence of a so called *mutator phenotype* (Bielas et al., 2006; Loeb, 2001) has been proposed, suggesting that somatic selection would favor cells having higher mutation rates (Anderson et al., 2001). Genetic instability would then derive from the loss of DNA repair mechanisms and cell cycle checkpoints (Kops et al., 2004, 2005). As Loeb pointed out, a consequence is that tumor progression is genetically irreversible (Loeb, 2001) since genomic instability acts as a rate of change (Lengauer et al., 1998). This leads to cumulative mutations and increased levels of genetic change associated to further failures in genome maintenance mechanisms (Hoeijmakers, 2001). The amount of instability is limited by lethal effects affecting key processes leading to effectively non-viable cells (Kops et al., 2004) thus indicating that thresholds for instability must exist. In fact, many anti-cancer therapies take advantage of increased genomic instability, as is the case of mitotic spindle alteration by taxol or DNA damage by radiation or alkilating agents (DeVita et al., 2005).

The previous observations indicate that instability places cancer cells at some risk: by increasing the number

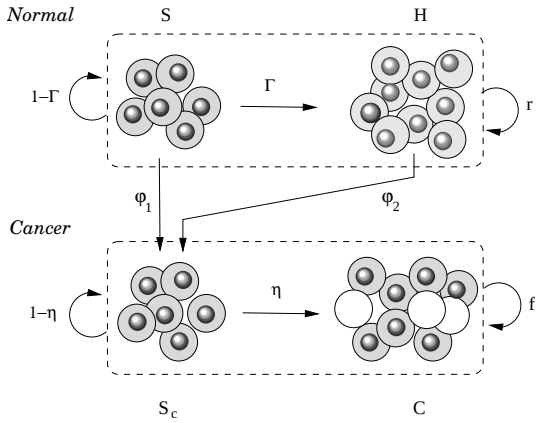

FIG. 1 The architecture of normal and cancer tissue interactions. Four populations are being considered, namely: stem cells (S), host tissue (H), cancer stem cells (S_c) and differentiated cancer cells (C). Cancer stem cells are assumed to emerge either from mutations in normal stem cells or through dedifferentiation, at rates φ_1 and φ_2, respectively. Both normal and cancer differentiated compartments are able to replicate at rates r and f, respectively. If too many mutations occur, new cancer cells might be nonviable. This is indicated here by means of empty circles.

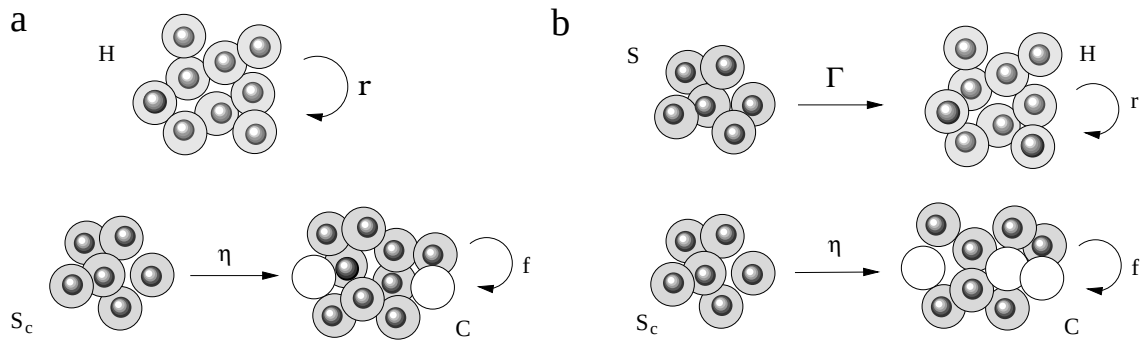

FIG. 2 The two types of tissue architecture considered in this paper. Both are particular cases of the more general scenario given in figure 1. In both cases cancer cells are formed from preexisting cancer stem cells at a rate η. In (a) a so called homogeneous tissue structure is considered, with the normal cell population formed by identical cells replicating at a constant rate r. In (b) a hierarchical tissue is considered, with normal cells being also generated from a stem cell pool.

of errors, cancer cells can also experience a loss in their plasticity or viability due to deleterious mutations. Even for cancer cells, some key genetic components need to be preserved in order to guarantee cell survival. These so-called housekeeping genes are the essential core required to allow reliable self-maintenance and replication to take place. In this context, mutations affecting them would cause cell death (see for example (Jordan and Wilson, 2004; Kops et al., 2004).

A rather different component of cancer success involves a somewhat opposite element dealing with stability: cancer stem cells (Bapat, 2007; Pardal et al., 2003). These cells share the self-renewal character of normal stem cells and have been already found in a number of cancer types. They self-renew to generate additional cancer stem cells and differentiate to generate phenotypically diverse cancer cells with limited proliferative potential. The parallels between somatic and cancer stem cells have long been drawn and are illustrated by many case studies (Pardal et al., 2003; Reya et al., 2001). Their characteristic trait is that self-renew is poorly controlled in cancer, leading to abnormal differentiation. An extreme example of this situation is provided by teratocarcinomas, which give rise to a diverse range of cell types, from respiratory epithelium to cartilage and bone (Sell and Pierce, 1994). In summary, both types of stem cells have organogenic capacity, but somatic stem cells are able to generate normal, well organized tissues whereas cancer stem cells will generate abnormal tissues.

The presence of cancer stem cells is also detected by observing that only a tiny fraction of tumor cells have a high proliferation potential. Although the hypothesis of an emergence of CSC from differentiated cells cannot be ruled out, many evidences point out to their origin from normal stem cells by mutations affecting key pathways (Liu et al., 2005; Wicha and Liu, 2006). These populations have been found in different contexts, including leukemia, brain and breast cancers (Al-Hajj et al., 2003; Bonnet and Dick, 1997; Singh et al., 2004). The self-renewal potential of CSC make them a source of tumor stability. They preserve information and thus define a stable cellular reservoir, whereas the differentiated cell generated from them are not constrained to be stable.

How are both elements reconciled? How does the stable core of a growing tumor, formed by a (presumably) small set of cancer stem cells interact with the much larger, genetically unstable population of differentiated cancer cells? An additional ingredient needs to be also considered: cancer takes place in a well-defined tissue context, where a given cellular environment constraints the tempo and mode of tumor progression. In terms of tissue homeostasis we can find a wide range of tissue architectures among two extremes:

1. Hierarchical tissue organization, where cell homeostasis is supported by a small fraction of proliferative cells (stem cells) able to self-renew themselves and produce nonproliferative cells. This is the case for example of gastric epithelium (Potten, 1998) and skin (Potten and Booth, 2002)

2. Homogeneous tissue organization, such as endothelium (Dejana, 2004) or hepatocytes (Ponder, 1996) in liver where cell homeostasis is maintained by the replication of the very same differentiated cells. In this case stem cells are relegated to tissue regeneration under acute damage (Ponder, 1996).

The dynamics of growth and regeneration resulting from these two basic scenarios will be different and have different consequences to both healthy and neoplasic tissues. In this paper we explore the outcome of the interactions among these components and their consequences using different mathematical models.

II. MEAN FIELD TISSUE-CANCER MODELS

Here we first explore the simplest models involving tumor growth in two alternative types of tissue architecture. In this context, we do not introduce the heterogeneous structure of the cancer population but instead consider it as a population of essentially identical cells.

Both hierarchical and homogeneous tissue architectures are used. The most sophisticated model at this level of description is shown in figure 1. Here four different cell populations are coupled and are assumed to compete for available resources. Here: C are cancer cells, H are host cells, S_c are cancer stem cells and S_H normal stem cells. The associated rates of growth will be indicated as r, f, Γ and η, respectively. Two cell subsets are thus associated to both normal and tumor populations. Each tissue component (healthy tissue and tumor) involves a stem cell and a differentiated compartment. Since cancer stem cells are assumed to result from mutations associated to normal stem cells or matured cell de-differentiation we also indicate their potential origin as two flows φ_1 and φ_2 from S to S_c and from H to S_c, respectively.

The general treatment of this model is not trivial, and here we consider a number of relevant simplifications able to offer insight. In particular, two basic assumptions are made. First, we will decouple CSC from normal cell compartments by setting φ_1 and φ_2 to zero. This assumption is made by considering that the process of CSC production is a slow one and that we start from a given fixed CSC set from which differentiated cancer cells are produced. Additionally, two types of normal tissue structure will be considered, including ($S > 0$) or not ($S = 0$) the presence of normal stem cells.

The two resulting tissue architecture models are shown in figure 2. Our models assume that cancer cell populations are decoupled from the host dynamics, except for the competition introduced by the function Φ. This function will depend on the tissue architecture chosen and the growth functions. CSC and stem cell populations (if present) are considered to be constant. In this way, as shown below, we can easily treat mathematically the two basic scenarios relevant to our discussion.

The two scenarios can be described by a pair of differential equations, namely:

$$\frac{dH}{dt} = G(H) - H\Phi(H, C) \tag{1}$$

$$\frac{dC}{dt} = \eta S_c + fC - C\Phi(H, C) \tag{2}$$

where $G(H)$ introduces the general form of the growth of the normal (host) tissue. Here $\Phi(H, C)$ introduces an outflow term (see below). For the homogeneous tissue architecture model (figure 2a) we have a linear growth term $G(H) = rH$ whereas for the hierarchical model (figure 2b)this is a constant term, namely $G(H) = \Gamma S$.

An additional assumption is that the total cell population is constant. This constant population constraint (CP) is defined by considering the condition

$$\frac{dH}{dt} + \frac{dC}{dt} = 0 \tag{3}$$

which implies that the sum $H + C$ is constant. For simplicity we normalize the total population to one. Using the previous condition we obtain:

$$\Phi = G(H) + fC + \eta S_c \tag{4}$$

Now we can reduce the previous two-equation model to a single-equation model, namely:

$$\frac{dC}{dt} = \eta S_c + fC - C\left(G(H) + fC + \eta S_c\right) \tag{5}$$

and by using the normalization condition $H + C = 1$, the final form of the two tissue architecture models is:

$$\frac{dC}{dt} = \eta S_c + C\left(f(1 - C) - \Gamma S - \eta S_c\right) \tag{6}$$

for the hierarchical model and

$$\frac{dC}{dt} = (1 - C)\left((f - r)C + \eta S_c\right) \tag{7}$$

for the homogeneous tissue[1]

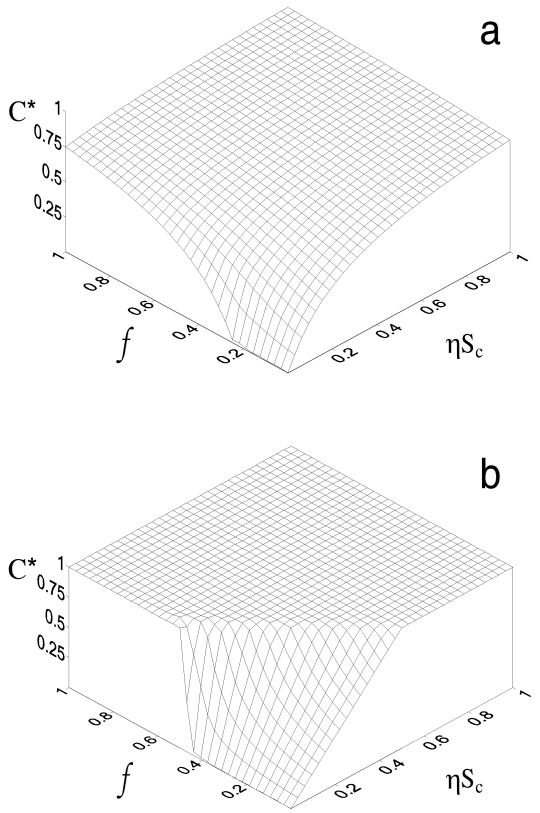

FIG. 3 Stationary populations of cancer cells C^* for the hierarchical (a) and the homogeneous (b) tissue models. The first exhibits a monotonous, single-phase behavior, which is consistent with the presence of a unique fixed point where cancer cells and normal tissue coexist. The second show two phases: a tumor winning phase, where all available space is occupied by cancer cells (the large plateau) and a different phase where both tissues coexist. Here we use: $\eta S_c = 0.25, \Gamma S = 0.25$ and $r = 0.5$.

[1] For both models when $S_c = 0$ we obtain a particular case for a

The two mean field models are now easily analyzed. First, we compute the equilibrium (fixed) points C^*. These points satisfy $dC/dt = 0$. For equation (6) a single fixed point is obtained:

$$C^* = \frac{f - \eta S_c - \Gamma S + \sqrt{(f - \eta S_c - \Gamma S)^2 + 4f\eta S_c}}{2f} \quad (8)$$

(the negative solution has no meaning). This unique point will be always stable provided that $S_c > 0$ i. e. if cancer stem cells are present.

The stability of the equilibrium point is determined following standard methods (Strogatz, 1994). If we indicate as $g(C) = dC/dt$, then the point C^* is stable if the derivative:

$$\frac{dg(C)}{dC} = f - \Gamma S - \eta S_c - 2C \quad (9)$$

is negative for $C = C^*$. If we use $Q = f - \Gamma S - \eta S_c$ and replace $C = C^*$ in equation (9) we obtain

$$\frac{dg(C^*)}{dC} = Q - 2fC^* = -\sqrt{Q^2 + f\eta S_c} \quad (10)$$

Which cannot be positive and thus C^* is always stable. The dependency of the cancer cell population C^* at equilibrium in relation to replication rate f and the production from cancer stem cells ηS_c is shown in figure 3(a). We can see that a continuum of stationary values is obtained, as predicted from the presence of a single fixed point. If cancer stem cells are removed ($S_c = 0$) then the equilibrium point is stable only if $f > \Gamma S$ and otherwise an alternative fixed point $C^* = 0$ is reached with no cancer present. The stability condition just tells us that the rate of cancer growth under the absence of cancer stem cells must be larger than the production rate of normal cells.

For the homogeneous model, we have now two fixed points, namely a tumor-winning state $C_1^* = 1$ and a coexistence point

$$C_2^* = \frac{\eta S_c}{r - f} \quad (11)$$

In this case the stability analysis shows that the tumor winning scenario (C_1^* stable) occurs when the following inequality holds:

$$r < f + \eta S_c \quad (12)$$

and C_2^* will be stable otherwise (i. e. for $r > f + \eta S_c$). The two possible phases are observable in figure 3(b), where a plateau indicates the domain of cancer-winning parameters, whereas the linear decay seen at low parameter values corresponds to the coexistence domain. For $S_c = 0$ we have a classical competition model with two excluding solutions. If $r < f$ then the stable point will be $C_1^* = 1$ and otherwise, $C_2^* = 0$.

The previous approach can be generalized by considering other types of functional dependencies among cell types. For example, we could use a dynamical model where the Φ function is a different one, including other types of biologically sensible limitations. In appendix I we consider a general class of model that includes the previous one as a particular case. As shown there, our previous results are robust and do not change by using other types of functional responses.

III. THE ROLE OF GENETIC INSTABILITY

As mentioned at the introduction, cancer stem cells are the reservoir of stability in a tumor. They are able to maintain their cellular organization and simultaneously generate further cancer cells that are free from such constraint. What is the impact of an unstable cancer cell population on the final outcome of tumor progression? A first approximation to this problem can be obtained by considering an extension of the previous two models that incorporates instability. Since we consider all cells within one compartment as equal, all cancer cells will share a common instability level. This is of course a rough approximation, which we will relax in the next section by considering a hierarchy of instability levels and thus population heterogeneity.

In order to choose an appropriate form of both growth and instability constraints, we will use the following functional form for the replication rate of cancer cells:

$$f(\mu) = r(1 + g(\mu))d(\mu) \quad (13)$$

where the functions $g(\mu)$ and $d(\mu)$ will introduce both the selective advantage and the deleterious effects on replication, associated to each instability level μ, respectively.

As discussed above, $g(\mu)$ will be an increasing function, since it indicates that higher replicating strains are more easily found as instability increases. This can be understood in terms of the potential number of oncogenes and tumor suppressor genes that, if mutated, can favor increased proliferation. Moreover, the function $d(\mu)$ must introduce the deleterious effects of instability and thus needs to be a decreasing function. Assuming that instability causes changes in r we consider that for $\mu = 0$ cancer populations will have the same replicative power than healthy cells, i. e. $f(0) = r$.

Many possible choices for $g(\mu)$ and $d(\mu)$ can be made. Here we show our results for a linear dependency in the growth term, $g(\mu) = \alpha\mu$: the higher the instability, the more likely is to hit a proliferation-related gene. For the second term, we need to consider the probability of affecting housekeeping genes. Here we can make a rough estimation using available data on housekeeping (HK) genes and therefore leading to a nonviable cell. The probability $P_h(\mu)$ of hitting a HK gene for a given instability rate

cancer without any stable reservoir.

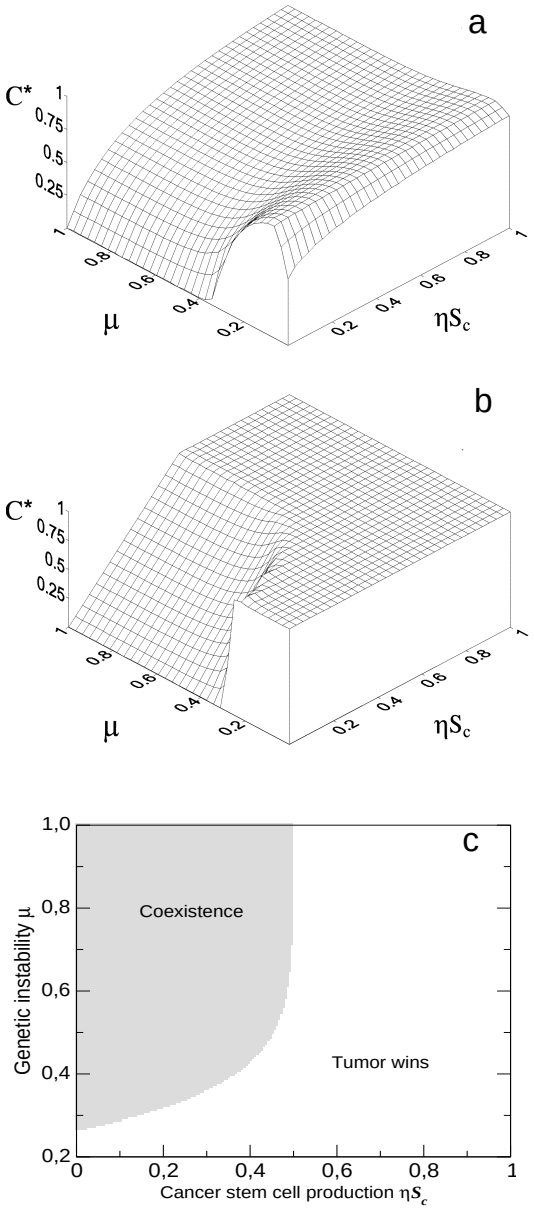

FIG. 4 The role of genetic instability in the two previous models is summarized here by plotting the cancer cell population against both instability level μ and CSC production ηS_c for: (a) the hierarchical model and (b) the homogeneous model. In (c) we represent the two domains of behavior shown in (b) by means of a two-dimensional parameter space. We can see that in order to have a successful expansion of the unstable tumor, a given amount of cell proliferation from the CSC compartment is required. All parameters as in figure 3, with $\alpha = 100$ and $\mu_c = 0.08$.

will be

$$P_h(\mu) = 1 - (1 - \mu\rho_h)^{n_h} \tag{14}$$

where ρ_h is the relative frequency of HK genes and n_h their absolute number. Current estimates (Eisenberg and Levanon, 2003) give $n_h \approx 500 - 600$ to be compared with the total number of genes $N_g \approx 3 \times 10^4$. This gives $\rho_h = n_h/N_g \approx 0.016 - 0.02$. Assuming $\mu\rho_h$ small and $n_h = 600$, we can write

$$P_h(\mu) = 1 - e^{-\mu\rho_h n_h} \tag{15}$$

(using the Taylor expansion $e^{-z} \approx 1 - z$) with $\rho_h n_h \approx 12$. The probability of generating a viable cell will be $1 - P_h(\mu)$ and thus we can use an exponential form for the effect of deleterious mutations, namely $d(\mu) = \exp(-\mu/\mu_c)$, with $\mu_c \approx 0.08$. Note that $\mu = 0$ leads to $d(0) = 1$, i. e. no deleterous effect by instability and by contrast $g(0) = 0$, no selective advantage. Here α will be a given constant (not estimated from real data). The resulting function will have a maximum at some given μ^* value, i. e.

$$\left(\frac{\partial f(\mu)}{\partial \mu}\right)_{\mu=\mu^*} = 0 \tag{16}$$

and also

$$\left(\frac{\partial^2 f(\mu)}{\partial \mu^2}\right)_{\mu=\mu^*} < 0 \tag{17}$$

which in our case gives a maximum at

$$\mu^* = \mu_c - \frac{1}{\alpha} \tag{18}$$

Such value will be positive provided that $\mu_c > 1/\alpha$ and this inequality actually defines a necessary condition for a successful unstable tumor to propagate.

In figure 4 we summarize our results for the mean field model incorporating genetic instability. Once again, the hierarchical tissue displays a continuous, although non-linear relation between the stationary cancer population and instability levels. In particular, if the production term is small, a large cancer cell population can be sustained only if the instability level is small enough. Once it keeps increasing, a rapid decay occurs. The homogeneous model shows again two well-defined phases. These two phases can be obtained from the generalized condition for stability:

$$r < f(\mu) + \eta S_c \tag{19}$$

which leads to

$$\left(1 - \frac{\eta S_c}{r}\right) e^{\mu/\mu_c} < 1 + \alpha\mu \tag{20}$$

The two phases are clearly indicated in figure 4(c). Here we can appreciate the effects of instability and cancer stem cells in terms of a threshold phenomenon. In order for the tumor to grow and outcompete the host tissue, we need either low levels of instability if the production term is small or large production rates able to overcome the deleterious effects of instability. It can be easily shown that the limit value of ηS_c for high instability levels is $\eta S_c = r$: the rate of cancer cell production must (at least) equal the normal tissue growth rate.

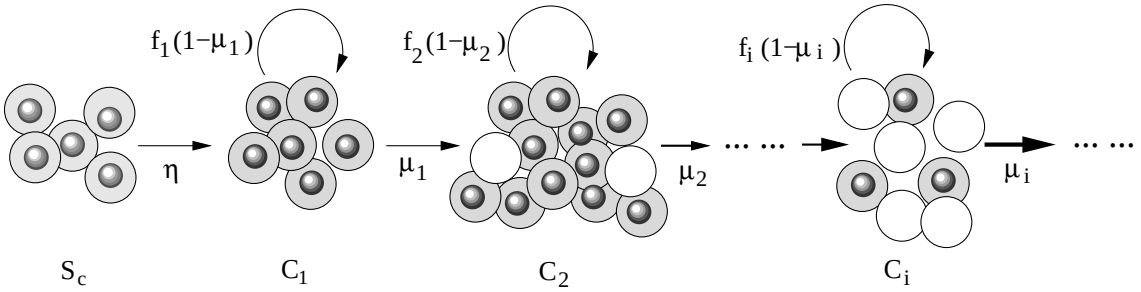

FIG. 5 Sequential unstable cancer model. Here only the cancer cell populations are shown, starting at left from a compartment of cancer stem cells of fixed size S_c. At a rate η, differentiated, unstable proliferating cancer cells C_1 are generated, with an increased instability level μ_1. New cells are generated at a rate $f_1(1-\mu_1)$ whereas mutated cells C_2 originate at a rate $f_1\mu_1$. The new population has an average instability $\mu_2 = \mu_1 + \Delta\mu$. The process continues and as we move to the right increasing instability levels are involved.

IV. MULTISTEP MODEL OF GENETIC INSTABILITY

The previous models considered a homogeneous cancer cell population, being all cells equal in terms of their dynamics. A tumor is far from a homogeneous system and mounting evidence indicates that they might actually display high levels of genetic heterogeneity in space and time (González-García et al., 2002). Due to genetic instability, the possible spectrum of replication and death rates (as well as many other aspects of cell function) will typically present a large variance. How is the introduction of such heterogeneity changing our previous picture? If a spectrum of instability levels is reached, how are mutation rates distributed over the population structure? How is this heterogeneous structure affecting tumor dynamics?

Each time a cancer cell replicates, new mutations can arise. In such scenario, genes controlling genome integrity will fail to do so and further mutations will arise. Eventually, the increasing mutation rate will affect other repair and stability genes. Each time new mutations occur, new opportunities will appear for finding cell phenotypes that replicate faster. In parallel, increasing mutation rates will also jeopardize cell replication due to deleterious effects. The two conflicting constraints can be introduced in a general model of unstable tumor progression where a range of possible instability levels is introduced explicitly.

Instead of lumping together all cancer cells in a single phenotype, we will describe the cancer population as a set of compartments $\mathbf{C} = \{C_1, C_2, ..., C_M\}$ where M is the maximum number of cancer cell types. This linear chain model allows defining a multistep model of unstable tumor progression. Each compartment C_i is characterized by a given replication rate f_i and a given instability level μ_i. Increasing instability allows a one-directional flow $C_{i-1} \to C_i \to C_{i+1}$.

As we move to higher instability levels, the likelihood to generate nonviable cancer strains increases. The basic scheme of this model is outlined in figure 5. A linear chain of events connects cancer cells through increasing levels of mutation. Of course this is again an oversimplification of reality, since each compartment actually includes a diverse zoo of cells sharing common instabilities but having different replication rates. Once again, we collapse all this diversity in a single number.

The new model (following figure 5) is described by a system of $M+1$ coupled differential equations:

$$\frac{dH}{dt} = G(H) - H\Phi(H, \mathbf{C}) \tag{21}$$

$$\frac{dC_1}{dt} = \eta S_c + f_1(1-\mu_1)C_1 - C_1\Phi \tag{22}$$

$$\frac{dC_2}{dt} = f_1\mu_1 C_1 + f_2(1-\mu_2)C_2 - C_2\Phi \tag{23}$$

$$\ddots \tag{24}$$

$$\frac{dC_i}{dt} = f_{i-1}\mu_{i-1}C_{i-1} + f_i(1-\mu_i)C_i - C_i\Phi \tag{25}$$

$$\ddots \tag{26}$$

$$\frac{dC_M}{dt} = f_{M-1}\mu_{M-1}C_{M-1} + f_M C_M - C_M\Phi \tag{27}$$

For this system, we have now:

$$\Phi(H, \mathbf{C}) = G(H, \mathbf{C}) + \sum_{j=1}^{M} f_j C_j + \eta S_c \tag{28}$$

which generalizes our previous expression. As we move to higher instability levels, we should expect to reach some critical level where cells are nonviable. In that sense, we will assume that the maximum number of cell compartments M is large enough so that $f_M \approx 0$. The role of instability can be introduced as already defined for the mean field (one-dimensional) models, but now we can consider different levels for each compartment and thus different replication rates:

$$f_i = r(1 + g(\mu_i))d(\mu_i) \tag{29}$$

Following our previous discussion, we have $g(\mu_i) = \alpha\mu_i$ and $d(\mu_i) = \exp(-\mu_i/\mu_c)$, respectively.

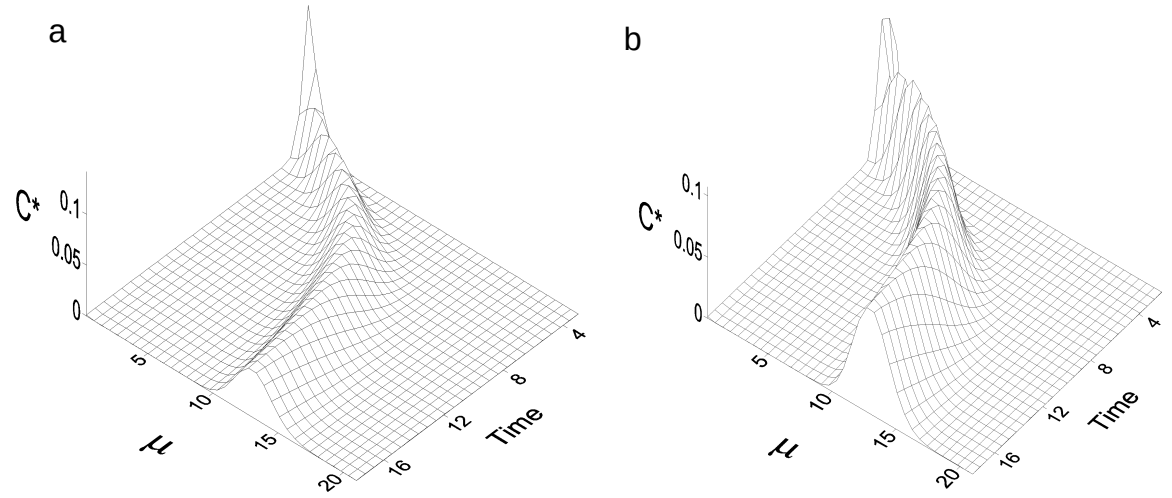

FIG. 6 Time evolution of the multistep model for (a) hierarchical tissue ($\eta S_c = 0.0001, \mu_c = 0.08, \Gamma S = 0.5$ and $\alpha = 20$) and (b) homogeneous tissue ($\eta S_c = 25 \times 10^{-3}, \alpha = 20, \mu_c = 0.08$ and $r = 0.25$). The change in mutation rate from compartment to compartment is $\Delta\mu = 0.001$ and the compartment number needs to be rescaled by $\mu \times 10^{-3}/3$ in order to obtain the exact genetic instability level. In both cases, the tumor diffuses through instability space as a wave, reaching a steady distribution near the optimal mutation rate μ^* (but typically moving beyond this value). In the first case, a stable tumor of finite size is formed, whereas in the second all the invaded tissue becomes tumor. In both cases, a gap is formed between the CSC compartment and the unstable tumor population.

Starting from an initial condition including just healthy and cancer stem cells, new cancer cells are generated and the population structure starts moving through the instability space. The dynamics of the population structure is shown in figure 6 for both homogeneous and hierarchical models. Here we show the frequency of cancer cell types along time and mutation space. We can see that there is a steady state where most cancer cells become organized close to a maximal level of instability. This is actually the result of the spontaneous tendency of moving towards higher mutation rates and the brakes associated to increasing deleterious effects. A small peak is observable at small mutation rates, indicating the presence of cancer stem cells.

A remarkable outcome of our models is the presence of a gap in instability space. Such *stability gap* implies that the largest part of the tumor will be displaced towards high instability and high replication, leading to a highly heterogeneous population as it is occurs in real tumors (particularly when CIN is present). Under our continuous approximation, evolves towards a high instability level and the population distribution eventually reaches a steady state. The distribution has a peak close to the optimal instability level μ^* but typically moves beyond this value. Such result would suggest that tumors growing under the mutator phenotype might become too unstable and thus more fragile than expected. It also seems consistent with the observation that cells taken from samples obtained from tumors seldom develop colonies except for special cell types that correspond to CSC. If no instability gap were present, we would expect having a continuum of colony-forming capacities associated to cancer cells having more or less stability levels. The all-or-none pattern observed from experimental systems indicates that non-CSC are highly unlikely to develop colonies. which will be the case for the unstable population. This pattern is a prediction of our model.

V. DISCUSSION

Cancer dynamics display most features common to other biological systems experiencing Darwinian selection (Merlo et al., 2006). The lack of cooperation and inhibition among cancer cells leads to the survival of the fittest: the most efficient replicators are the winners. But the whole picture is more complicated and the study of complexity in cancer development can benefit from modelling approaches (Dingli and Nowak, 2006; Spencer et al., 2006; Wodarz and Komarova, 2005). Spatial heterogeneity and genetic instability introduce several relevant components that can modify the standard predictions of a purely Darwinian dynamics. Previous theoretical works (Solé, 2002; Solé and Deisboeck, 2003) (see also (Poyatos and Carnero, 2004)) suggest that genetically unstable cancer population exhibit an error threshold of instability beyond which population drift occurs. As a consequence, increasing mutation rate we would force tumor regression. However, mounting evidence reveals that tumors benefit from a highly stable component: cancer stem cells. Such a small, but robust ingredient seems to play the role of a reservoir of stability. In this context, it has been postulated that such stem cells are likely to be very resistant against the action of drugs since they

present different cell cycle kinetics, more active mechanism for drug exclusion than cancer cells (Dean and Bates, 2005) as well as DNA repair mechanisms (Dean and Bates, 2005; Wicha and Liu, 2006). In addition, stem cells avoid mutation accumulation by keeping the same parental DNA strand into stem cell by a selective segregation process (Merok et al., 2002; Potten et al., 2002). According to this cancer stem cell surveillance even if tumor ressection is successful, the preservation of these CSC allows a new tumor to be formed. Cancer stem cells and unstable cancer cells thus define a complex system, where information is preserved in the stable compartment while exploration and adaptation takes place thanks to the intrinsic lack of reliable genome replication of unstable cancer cells.

In this paper we have considered the problem of the interplay between cancer stem cells and genetic instability within the context of tissue architecture. A previous model (Komarova, 2005) suggested that hierarchical tissues appear as a solution to prevent cancer and cell aging thus reinforcing the relevance of tissue structure in understanding oncogenesis. In our paper we have shown that appropriate simplifications allow treating the all these components in a theoretically meaningful way. We have seen that (under the assumptions made here) the presence of cancer stem cells acts as the engine of tumorigenesis and presents a number of tradeoffs with genetc instability. Beyond the mean field models (reduced to a single equation by using the constant population constraint) the use of a multistep model of instability reveals that we should expect most cells in the tumor to be highly unstable and distribute close to the optimal instability level. Therapies detecting CIN cells could exploit this feature and take advantage of the tumor fragility. This is actually consistent with the observation that tumors displaying high CIN have better prognosis. On the other hand, tumor resection affecting only unstable cells will not prevent the emergence of a new tumor mass, since the instability wave easily reappears (results not shown). Future work should consider several generalizations of our theoretical models using stochastic implementations (such as branching processes, see (Kimmel and Axelrod, 2002)) spatially-explicit models and more accurate representations of cell genomes and the cell cycle.

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VI. APPENDIX: GENERALIZED MODEL OF CANCER-NORMAL TISSUE INTERACTIONS

In this appendix we consider the effect of changing our previous model description of the cancer-normal tissue interactions. Starting with our initial set of equations (1,2) let us assume that $\Phi(H, C)$ is a continuous differentiable function in both arguments $H \geq 0$ and $C \geq 0$, is such that the following set of conditions is fulfilled:

$$\Phi(0, 0) = \Phi_0 \geq 0 \tag{30}$$

$$\frac{\partial \Phi}{\partial H} > 0 \tag{31}$$

$$\frac{\partial \Phi}{\partial C} > 0 \tag{32}$$

(and thus $dC/dh < 0$). We will show that the basic results presented in section II hold, provided the previous conditions are met.

A. Homogeneous tissue

For the homogeneous tissue, we would have

$$\frac{dH}{dt} = H(r - \Phi(H, C)) \tag{33}$$

$$\frac{dC}{dt} = C(f - \Phi(H, C)) + \eta S_c \tag{34}$$

The nullclines of this system for $H > 0$ are thus

$$\Phi(H, C) = r \tag{35}$$

$$\Phi(H, C) = \frac{\eta S_c}{C} + f \tag{36}$$

Under the previous assumptions on $\Phi(H, C)$, Eq. (36) implicitly defines the function $H = \hat{H}(C)$. Moreover, from the total derivative of the previous expression, we have that $\hat{H}(C)$ is a decreasing function with

$$\frac{d\hat{H}}{dC} = -\left(\frac{\eta S_c}{C^2} + \frac{\partial \Phi}{\partial C}\right)\left(\frac{\partial \Phi}{\partial H}\right)^{-1} \tag{37}$$

which implies that $\hat{H}(C) \to \infty$ as $C \to 0$. Therefore, if C^* is the value of C such that $\hat{H}(C^*) = 0$, the system has $P_1^* = (0, C^*)$ as an equilibrium point. Now, comparing (35) with (36), it follows that, in order to find a condition for a coexistence equilibrium P_2^*, we have to consider two possible cases. The first one corresponds with the inequality

$$\frac{\eta S_c}{C^*} + f < r \tag{38}$$

For this situation, it is not difficult to show that P_1^* is a saddle point whereas P_2^* is globally stable (for $H_0 > 0$). When the opposite inequality is at work, namely

$$\frac{\eta S_c}{C^*} + f \geq r \tag{39}$$

P_1^* is the only equilibrium point which is globally stable for $H \geq 0$ and $C \geq 0$.

B. Hierarchical tissue

For the second type of tissue structure, the equations now read

$$\frac{dH}{dt} = \Gamma S - H\Phi(H, C) \tag{40}$$

$$\frac{dC}{dt} = C(f - \Phi(H, C)) + \eta S_c \tag{41}$$

The new nullclines are now

$$\Phi(H, C) = \frac{\Gamma S}{H} \tag{42}$$

$$\Phi(H, C) = \frac{\eta S_c}{C} + f \tag{43}$$

respectively. We need to characterize the relative position of the nullclines in the (H, C)-plane in order to determine the existence and stability of a positive equilibrium point P^*.

From the first nullcline, we have:

$$\frac{\partial \Phi}{\partial C} + \frac{\partial \Phi}{\partial H}\left(\frac{d\hat{H}}{dC}\right) = -\frac{\Gamma S}{H^2}\left(\frac{d\hat{H}}{dC}\right) \tag{44}$$

which can be written as:

$$\left(\frac{\partial \Phi}{\partial H} + \frac{\Gamma S}{H^2}\right)\frac{d\hat{H}}{dC} = -\frac{\partial \Phi}{\partial C} < 0 \tag{45}$$

Similarly, from the expression of the second nullcline we obtain:

$$\frac{\partial \Phi}{\partial H}\left(\frac{d\hat{H}}{dC}\right) = -\frac{\eta S_c}{C^2} - \frac{\partial \Phi}{\partial C} < 0 \tag{46}$$

Therefore, the existence of a globally stable equilibrium-point P^* follows since

$$\left(\frac{d\hat{H}}{dC}\right)_{\dot{C}=0} < \left(\frac{d\hat{H}}{dC}\right)_{\dot{H}=0} < 0 \tag{47}$$

the nullcline $H' = 0$ is tangent to the C-axis and the nullcline $C' = 0$ is tangent to the H-axis and thus they cross each other.

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