



Emergence of a Subpopulation in a Computational Model of Tumor Growth

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Malignant brain tumors consist of a number of distinct subclonal populations. Each of these subpopulations may be characterized by its own behaviors and properties. These subpopulations arise from the constant genetic and epigenetic alteration of existing cells in the rapidly growing tumor. However, since each single-cell mutation only leads to a small number of offspring initially, very few newly arisen subpopulations survive more than a short time. The present work quantifies “emergence”, i.e. the likelihood of an isolated subpopulation surviving for an extended period of time. Only competition between clones is considered; there are no cooperative effects included. The probability that a subpopulation emerges under these conditions is found to be a sigmoidal function of the degree of change in cell division rates. This function has a non-zero value for mutations which confer no advantage in growth rate, which represents the emergence of a distinct subpopulation with an advantage that has yet to be selected for, such as hypoxia tolerance or treatment resistance. A logarithmic dependence on the size of the mutated population is also observed. A significant probability of emergence is observed for subpopulations with any growth advantage that comprise even 0.1% of the proliferative cells in a tumor. The impact of even two clonal populations within a tumor is shown to be sufficient such that a prognosis based on the assumption of a monoclonal tumor can be markedly inaccurate.

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Introduction

One of the hallmarks of high-grade malignant neuroepithelial tumors, such as glioblastoma multiforme (GBM), is the regional heterogeneity, i.e. the relatively large number of clonal strains or subpopulations present within an individual tumor of monoclonal origin (Berkman *et al.*, 1992; Coons & Johnson, 1993; Paulus & Peiffer, 1989).

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Each of these strains is characterized by specific properties, such as the rate of division or the level of susceptibility to treatment (Yung *et al.*, 1982). Therefore, the growth dynamics of a single tumor are determined by the relative behaviors of each separate subpopulation. For example, the appearance of a rapidly dividing strain can substantially bias tumor growth in that direction. Tumors supposedly harbor cells with an increased mutation rate, which indicates that these tumors are genetically unstable (Nowell, 1976;

Loeb, 1994; Lengauer *et al.*, 1998). Genetic and epigenetic events throughout the tumor may occur randomly or be triggered by environmental and intrinsic stresses. The continuing existence of a subpopulation, however, depends primarily on the subpopulation's ability to compete with the dominant population in its immediate vicinity.

The study of multiple populations in the context of tumor growth entails several interesting features. Most notably, cells within the solid core of a tumor are essentially immobile. This means that clonal subpopulations may be thought of as spatially localized, i.e. as occupying a contiguous region of space. This localization limits the competitive interactions between different subpopulations, allowing very small populations to compete with a dominant population on a somewhat more equal basis than would be the case in a uniform distribution of subpopulations. Another interesting aspect of subpopulation competition within tumors is the relative similarity of the subpopulations. Histological studies do not support the notion of a true polyclonal origin (Berkman *et al.*, 1992). Thus, often only a single behavior of the parent strain, e.g. the cell-doubling time, is altered slightly by the genetic event. Because of this similarity between populations, the chance that a small subpopulation may emerge from a larger population is a largely random process, in which very small fluctuations in short time behavior are of critical importance to long-term growth. The highly localized nature of the competition between cells requires that a model in which the topology of the tumor is specifically tracked be utilized in modeling clonal heterogeneity. In particular, a desirable model would be able to handle small-scale heterogeneities in a three-dimensional setting. These properties were demonstrated in a model developed in our previous work (Kansal *et al.*, 2000), henceforth referred to as Paper I. The importance of topology, and connectivity in particular, to the growth of a subpopulation indicates that a simulation in only one- or two-dimensional space would produce spurious results.

Clonal heterogeneity within a tumor has been shown to have very pronounced effects on treatment efficacy (Schnipper, 1986; Heppner & Miller, 1989). Most dramatically, the existence of a relatively small drug-resistant population

can have an impact upon a tumor's response to treatments (Yung *et al.*, 1982; Panetta, 1998). For example, Tracqui *et al.* (1995) found that the impact of chemotherapy on a brain tumor in one patient could not be adequately fitted to standard growth models without postulating the existence of a second, drug-resistant, population within the tumor. Observations such as this have led a number of authors to study the impact of chemotherapy on populations with differing degrees of sensitivity (Coldman & Goldie, 1985; Birkhead *et al.*, 1987; Panetta, 1998). These models rely on systems of coupled ordinary differential equations to model the size of each clonal population in time. For example, Panetta, (1998) considers two populations—a sensitive cell population x and a resistant population y using the equation set

$$\frac{dx}{dt} = [r_1 - d_1(t)]x, \quad (1)$$

$$\frac{dy}{dt} = b_1 d_1(t)x + [r_2 - d_2(t)]y. \quad (2)$$

In these equations, r_1 and r_2 are growth rates of x and y , respectively, and d_1 and d_2 are measures of drug sensitivity.

A more general consideration of clonal heterogeneity is undertaken by Michelson *et al.* (1987), Gyori *et al.* (1988), and Michelson & Slate (1989), in which a model based on population growth dynamics is used. This model allows the inclusion of competitive effects between different clones and of crowding effects. These models consider the behavior of two uniformly distributed clones that obey logistic-type growth. This type of growth is well-suited to the behavior of populations with a large number of individuals, but does not capture the randomness inherent in very small populations. Further work by Michelson *et al.* (1989) has attempted to capture this small-population behavior by including a "white-noise" term, which allows for small random fluctuations in the population sizes.

In the present work, a computational model is used to study the behavior of a tumor in which two subpopulations are present—a primary, parental strain present from the initiation of the

tumor growth and a secondary, novel strain arising from a mutation within a cell in the proliferative rim of the tumor. The secondary strain is identical to the first, with the exception of an altered cell-doubling time. A small region of this secondary strain (termed the “mutated region”), is introduced and the time evolution of both subpopulations is studied, with particular focus on the issue of the probability of survival of the secondary strain. Note that in this paper we use the term “mutated” in a general sense to indicate a change in either gene sequence (a genetic change) or in gene expression (an epigenetic change). While real heterogeneous tumors have many clonal strains, we will show that the inclusion of even just one secondary strain is sufficient to significantly alter the overall growth dynamics of a brain tumor. In the following section, we present the methods used to assess this probability, including a brief description of the computational algorithm employed. A summary of the emergence probabilities in terms of both the *degree* (i.e. the relative advantage in growth rate) and *size* (i.e. the starting volume of the second strain) of the mutated region is presented in the Results and Discussion section. The significance of our findings as well as reasonable extensions to the work are discussed in the Conclusions.

Methods

The emergence of subpopulations is studied by extending a cellular automaton model of tumor growth developed in Paper I. The description presented here is a brief summary of the key features of this model, the interested reader is referred to the original paper for a more complete description. The underlying lattice for the model is the Delaunay triangulation of space (Okabe *et al.*, 1992). The Delaunay triangulation is the dual lattice of the Voronoi tessellation. In the Voronoi tessellation, a set of random points in space are chosen as lattice sites. The space around these sites is then partitioned into cells. Each cell is associated with a single lattice site and defined as the region of space closer to that site than to any other site. The Delaunay triangulation may then be formed by connecting those sites whose cells share a common face. In

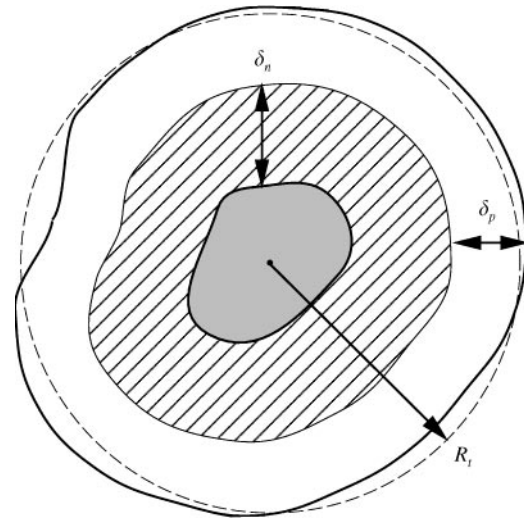


FIG. 1. An idealized tumor. The inner gray region is composed of necrotic tissue. The cross-hatched layer is composed of living, inert cells (non-proliferative). It has a thickness of δ_n . The outer shell, with thickness δ_p , is composed of proliferating cells. The overall radius of the tumor is defined by the average distance of the outer edge from the tumor center and is indicated by the broken circle, of radius R_t .

this construction, sites that are connected are considered to be neighbors of one another. All references to neighbors in this work are those defined in this way.

Once a lattice has been generated, the proliferative growth algorithm that controls each cell’s behavior may be run. This proliferative algorithm assumes that the tumor may be represented as having three distinct regions. The inner region is a mass of apoptotic and necrotic cells. This is surrounded by a layer of living but inert cells termed the non-proliferative, or quiescent, region. Finally, a thin outer layer of rapidly dividing cells, the proliferative rim, surrounds the entire tumor. While an idealized version of this model is a sphere composed of three concentric shells, in general the tumor may take any shape that preserves this layered structure (see Fig. 1).

Figure 2 shows a cut-away view of a simulated tumor in which the mutant subpopulation has emerged. The inner necrotic core is not depicted to bring out the layered structure of the tumor. The yellow shell depicts non-proliferative cells of either clone. The red region is comprised of proliferative cells of the primary strain. The blue region is the mutated subpopulation. This figure

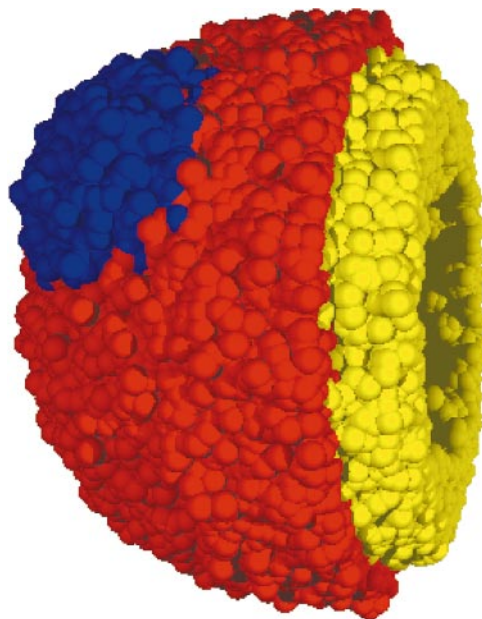


FIG. 2. A cut-away view of a simulated tumor with a mutated population. The inner necrotic core is not depicted in this view. The yellow region is comprised of nonproliferative of either clonal strain. The red region depicts the proliferative cells of the primary strain, while the blue shows the proliferative cells of the secondary strain. Note the layered structure and the small fraction of the secondary strain near the primary strain.

allows the effects of spatial localization to be appreciated. The secondary population must compete with the primary population only in the areas at which the blue and red regions are in contact. The majority of the secondary strain, by contrast, is surrounded only by other cells of the same strain (along with non-proliferative cells). As the secondary population grows to a larger size, this localization has more pronounced effects, due to the reduced fraction of the secondary population in direct competition with the primary population.

The proliferative algorithm depends on four parameters describing a strain's behavior. These parameters reflect the rate at which cells divide, the nutritional needs of actively dividing cells, the nutritional needs of cells in the G_0/G_1 arrested state, and the response of a cell to mechanical pressure. In the present work, only mutations affecting the rate of cellular division are considered. The rate of cellular division is given in terms of the base probability that a cell divides within a single day. For the primary tumor population this probability is denoted as p_0 and for mutated subpopulations as p_1 , p_2 and so on. The probability of division is related to the cell-doubling time, t_d , through the relation

$$t_d = \frac{\ln(2)}{\ln(1 + p_i)}, \quad (3)$$

where p_i is the probability of division for the appropriate strain. The nutritional needs of dividing and arrested cells, and the pressure response parameters are assumed to be the same for all clonal strains and denoted as a , b , and R_{max} , respectively. Future work will relax the assumption that these parameters are constant among strains.

Using these parameters the proliferative growth algorithm may be summarized as follows:

- A small tumor is placed in the center of the lattice.
- At each time step:
 - Apoptotic, necrotic (tumorous) and healthy (non-tumorous) cells are inert.
 - Non-proliferative cells (of any strain) too far away from the proliferative rim are assumed to receive insufficient nutrition and undergo cell death (leading to the necrotic core). The

distance at which this transition occurs, δ_n is defined as

$$\delta_n = aR_t^{2/3}, \quad (4)$$

where R_t is the average overall radius of the tumor and a is a parameter reflecting the nutritional needs of a non-proliferating cell.

—Proliferative cells will attempt to divide with probability p_d .

$$p_d = p_i \left(1 - \frac{r}{R_{max}} \right) \quad (5)$$

with p_i representing the base probability of division for the appropriate clonal strain, r representing the radial position of the proliferative cell, and R_{max} a parameter reflecting the effect of mechanical pressure.

—Given that a cell attempts to divide, it will test whether it has space available to it by searching for a healthy cell within a radius of δ_p . This radius is defined according to the relation

$$\delta_p = bR_t^{2/3}, \quad (6)$$

where b is a parameter of the model reflecting the nutritional needs of a proliferating cell. Those cells that find space divide (taking care to ensure that the division is continuous, i.e. into a neighbor cell), those that cannot become non-proliferative. Note that this process represents the healthy cells as being forced into a surrounding mass of indistinguishable healthy cells (as described in paper I), not as being destroyed by the tumor cells.

In the present work, the initial tumor is composed entirely of cells of the primary clonal population. This tumor is then allowed to grow, in accordance with the algorithm outlined above, until it reaches a pre-determined average overall radius. At this stage, a single automaton cell is changed from the *primary* strain to a *secondary* strain with an altered probability of division. It is important to note that this does not represent a single mutation event. Rather, we only consider

mutations that have reached a size dictated by the limits of our lattice resolution, meaning a mutation event that results in a subpopulation that becomes extinct without ever reaching a sufficiently large size (one automaton cell) are not included in the calculations. If events such as these were included, the effect would be to reduce the probability of emergence for all types of subpopulations. Results are also reported here for simulations in which two or more contiguous cells are mutated. These mutated regions represent very small fractions of the total population of proliferative tumor cells. The position at which the mutation appears is randomly chosen with one constraint—only cells on the outermost edge of the proliferative rim are considered for possible mutation sites. While in theory this restriction is unnecessary, we have found that internal mutations emerge too infrequently to report with sufficient confidence in the present work. In other words, *within the accuracy of our model* mutations that are buried within the proliferative rim almost never emerge.

Once the mutated region has been introduced, the tumor is allowed to continue to grow using the proliferative algorithm. The algorithm is run for a pre-determined number of time steps, after which the behavior of the tumor populations is measured. A subpopulation is considered to have emerged once it comprises 5% of the actively dividing cell population or if it remains in the actively dividing state once the tumor has reached a fully developed size (on the order of 5 cm in diameter, which is the “fatal” volume defined in Paper I). While the first criterion allows for the possibility that the subpopulation may die out (i.e. be overwhelmed by the parental strain) at a later time, many simulations were run for more extended time periods and in each case the subpopulation persisted as a significant fraction of the proliferating cells for a time comparable to the natural life of a tumor. Numerous simulations (roughly 100) were run at each parameter set in order to calculate the expected probabilities of emergence, along with confidence intervals, σ , defined as

$$\sigma = \sqrt{\frac{\bar{p}(1 - \bar{p})}{N}}, \quad (7)$$

where \bar{p} represents the observed probability of emergence in N trials. Each individual simulated run requires an average of 6 min on nine nodes of an IBM SP2, with a total of roughly 3 weeks of CPU time required for the data reported here.

Results and Discussion

The behavior of the secondary strain has been characterized in terms of two properties: the *degree* and the *relative size* of the initial population of mutated cells. The degree of mutation, α , is defined as

$$\alpha = \frac{p_1}{p_0}, \quad (8)$$

which represents the ratio between the base probability of division of the new clone, p_1 , and that of the original clone, p_0 . A degree of mutation $\alpha = 1$ corresponds to a mutation that confers no competitive advantage to the new clone. A positive advantage is conferred for $\alpha > 1$ and a *negative* advantage (i.e. a growth disadvantage) for $\alpha < 1$. The significance of a mutation resulting in a growth disadvantage to the new clone will be discussed below. The relative size of the mutated region, β , is defined as the ratio between the volume occupied by proliferating cells of the new clone and the volume occupied by proliferating cells of the original clone. This initial value of this ratio, $\beta_0 = \beta(t = 0)$, is a parameter of the model reflecting the size of the mutated region introduced. The smallest mutated subpopulations introduced, corresponding to one automaton cell, comprised roughly 0.006% of the proliferative cells (i.e. $\beta_0 \approx 6 \times 10^{-5}$).

The majority of the runs discussed below were run with a parameter set in which

$$p_0 = 0.192, \quad p_1 = \alpha p_0, \quad a = 0.42 \text{ mm}^{1/3},$$

$$b = 0.11 \text{ mm}^{1/3}, \quad R_{max} = 37.5 \text{ mm}$$

for the primary strain, in a simulation in which each time step represents one day. This corresponds to a cell-doubling time of roughly 4 days. In the monoclonal case (i.e. when no mutation occurs), this parameter set has been shown in Paper I to reproduce a test case called from the

medical literature. Thus, the parameters chosen here give growth histories (in the monoclonal case) characteristic of those seen in the literature for patients with GBM tumors. Several additional runs were carried out with a different primary strain whose properties are reflected in the parameter set

$$p_0 = 0.165, \quad p_1 = \alpha p_0, \quad a = 0.42 \text{ mm}^{1/3},$$

$$b = 0.11 \text{ mm}^{1/3}, \quad R_{max} = 37.5 \text{ mm}$$

in order to test if tumor dynamics were properly represented by the parameter α . The range of α values investigated in each case was roughly from 0.8 to 1.6. Runs with each primary strain gave indistinguishable results at the same value of α and, as such, all results reported below are in terms of α only. The mutations were introduced once the average overall radius of the tumor reached $R_t = 3.8$ mm. At this tumor radius a β_0 of 6×10^{-5} corresponds to a second population comprised of roughly 10^4 real cells. Again, this value was varied in several runs and indistinguishable results were obtained for comparable values of α and β_0 .

The emergence of a small mutated subpopulation is shown in Fig. 3, which shows the value of β over time for a single simulated tumor in which $\alpha = 1.29$. As the tumor grows, β increases steadily, eventually approaching 1. This represents the eventual complete dominance (i.e. “outgrowing”) of the secondary strain over the primary strain. Note that for a smaller value of α , such complete dominance likely will not occur, rather a more even mixture of the two clonal strains will be present at large tumor sizes. Figure 3 also depicts the overall volume of the simulated tumor, along with two limiting cases. The lower limiting case, labeled “base p_0 ”, represents a tumor of the same primary strain in which no mutation occurs (a minimally aggressive malignant case). The upper limiting case, labeled “high p_0 ”, represents a tumor which is composed entirely of the secondary strain (a highly aggressive malignant case). The emergent tumor’s growth follows that of the base p_0 case very closely, while the high p_0 case displays markedly different dynamics. At first glance, this suggests that even though the secondary population has come to

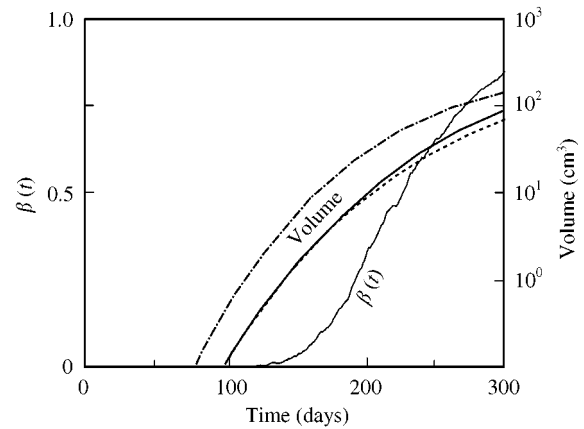


FIG. 3. β and volume for a simulated tumor in which a secondary population emerges. Volumes of tumors composed entirely of the primary strain and the secondary strain are also shown and labeled “base p_0 ” and “high p_0 ”, respectively. Note the emerging tumor follows the volumetric behavior of the base case despite a high β : (---) base p_0 ; (-·-·-) high p_0 ; (—) emergent.

dominate the tumor, the growth dynamics remain largely unaffected.

Careful analysis of the data, however, reveals a more nuanced picture of the tumor dynamics. One of the most important goals of computational modeling is to develop a simulation tool that allow an accurate prognosis to be given to a patient when proposing therapeutic options. In such a situation, the physician is likely to have information regarding the size of the tumor at several closely separated times and therefore some information regarding its dynamics (e.g. the volumetric doubling time). From this information, a good theoretical model could be applied to predict the likely future course of the tumor. In addition, a backward projection in time could provide valuable insight in analysing how the tumor arose. Understanding the dynamics of subpopulations is an essential piece of developing models that can provide this type of predictions.

An example of the importance of subpopulations is depicted in Fig. 4. In this example, a diagnosis has been made (on day t_0) giving information about the macroscopic size and growth rate of the tumor. From this information three possible growth histories of the tumor are plotted. One is the time history of the tumor with an emergent subpopulation discussed above in

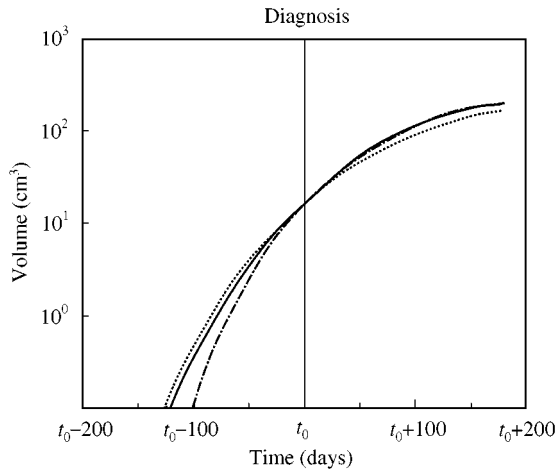


FIG. 4. Volume of a simulated tumor with an emergent subpopulation in time. Volumes of tumors composed entirely of the primary strain and the secondary strain are also shown and labeled “base p_0 ” and “high p_0 ,” respectively. Each tumor is set to have the same volume at some “diagnosis” time t_0 . Note that the emerging tumor’s dynamics initially follow the base case, but later follow the highly aggressive case: (---) base p_0 ; (-·-·-) high p_0 ; (—) emergent.

Fig. 3. The others represent limiting cases, each with a monoclonal tumor of either the primary (“base p_0 ”) or secondary (“high p_0 ”) clonal strain. Note that at the time of diagnosis all three scenarios have very similar dynamics. So any of the three histories is a reasonable prediction given only size and growth rate information. However, if we estimate a fatal tumor volume of 65 cm^3 and define the survival time to be the time required to reach this volume, the base case mispredicts survival times as 90 days, which is 30 days more than the 60 days of the “true” course. It is noteworthy that from this perspective the overall future growth dynamics of the entire tumor closely follow that of the most aggressive case, indicating that the more aggressive clone dominates overall outcome and should therefore also define proper treatment. This finding supports the current medical practice of grading tumors according to the most malignant area (i.e. population) found in any biopsy material. Although of less clinical significance, the high case similarly mispredicts the past history of the tumor. If the diagnosis had been made earlier, the base case would yield still worse future predictions. Similarly, the high case would yield worse past predictions for a

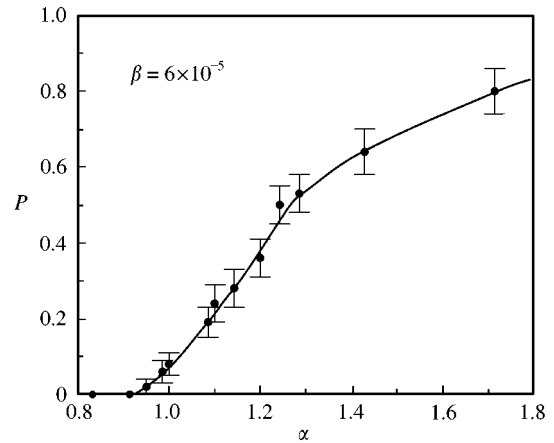


FIG. 5. A plot of probability of emergence, P , vs. the degree of mutation, i.e. growth advantage or disadvantage. The error bar indicate confidence intervals defined by one standard deviation from the mean. Each data point represents the average of roughly 100 simulated tumors. The line is drawn as a guide for the eye.

diagnosis made at a later time. The predictive errors arising from the assumption of a monoclonal tumor indicate how important an accurate estimate of the clonal composition of a tumor is in establishing a complete history and prognosis. Note that the numbers given here are intended to show the scale of the inaccuracy possible, not to reflect any data extracted from actual patients.

Figure 5 depicts the observed probability of emergence, P , for a subpopulation of initial size $\beta_0 = 6 \times 10^{-5}$ as a function of α . The observed probability is measured over a finite number of trials such that

$$P = \frac{\text{number of trials in which emergence occurs}}{\text{total number of trials}} \tag{9}$$

This gives an approximation of the true, asymptotic, probability of emergence. The quantity P is actually a function of the parameters α and β_0 , but for the moment we will suppress indicating this dependence explicitly. Not surprisingly, P is a monotonically increasing function that tends to 0 for $\alpha < 1$ and to 1 as α become significantly greater than 1. Perhaps

the most striking feature of these results is that there is a non-zero probability of emergence for a very small population with no growth rate advantage, or even with a small disadvantage (i.e. $\alpha \approx 0.95$). This suggests that a mutated subpopulation may arise even without any growth advantage. These populations could represent “dormant” clones which confer an advantage not being selected for at the time. An example would be the appearance of hypoxia-tolerant or even treatment-resistant clones. While chemoresistance, for example, confers no advantage to a clone when the drug is not present, this does not exclude the emergence of a specific drug-resistant clone in an as yet untreated tumor. Instead, because of the protection afforded by the localization of each subpopulation, this clone may persist for an extended period and comprise a non-trivial fraction of the tumor at the time of treatment. An example would be the proposed selection of glioma cell clones with high activity of the specific DNA-repair enzyme O⁶-methylguanine-DNA methyltransferase during treatment with chloroethylnitrosoureas such as ACNU (Papavero *et al.*, 1987). As such treatments would select for already existing resistant subclones, rather than inducing novel mutations or epigenetic changes. In reality, both mechanisms are likely to be present.

Figure 6 shows the impact of varying the initial size of the subpopulation on the emergence probability. Again, the general trend is, as expected, a monotonically increasing function in β_0 . The lines in the figure are least-squares fits using a logarithmic function. The mutations of the greatest interest are those that are initially very small. The discrete model used here is limited in resolution by the size of a single automaton cell. As such conclusions regarding mutations at sizes smaller than one automaton cell cannot be drawn from the present work. Work is in progress to allow the resolution of the lattice near a mutation to be increased adaptively thereby allowing smaller mutated populations to be considered. One important conclusion that may be drawn from the present level of resolution, however, is that any mutation that confers even a small advantage in growth rate will emerge with high probability if it can come to comprise as little as 0.1% of the proliferative population.

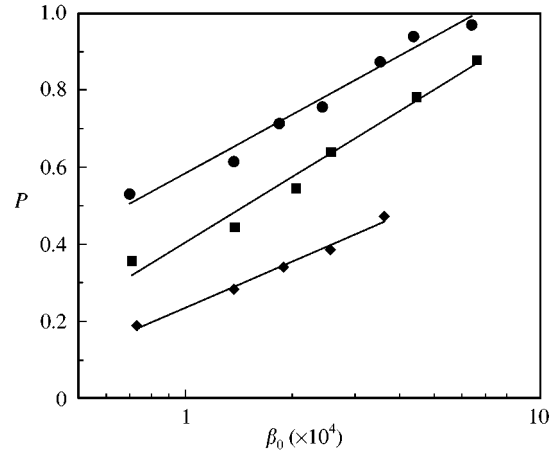


FIG. 6. A plot of the size dependence of the probability of emergence, P . β_0 is the relative size of the initial mutated region. The lines are least-squares fit logarithmic curves. Note that the curves are nearly parallel to one another: \blacklozenge , $\alpha = 1.09$; \blacksquare , $\alpha = 1.20$; \bullet , $\alpha = 1.29$.

The probability of emergence near $\alpha = 1$ (and β_0 small) is of particular importance because it is reasonable to expect that the most likely mutations are those that do not change the behavior of the cell very significantly. As such, even though a subpopulation with a sizable advantage in growth rate (say $\alpha = 1.6$) emerges with a very high probability, such a mutation event may be so rare, that a markedly different population never appears in this manner. Instead, it may be that a series of smaller mutations are responsible for the appearance of the subpopulation.

To compare the relative likelihood of these two pathways, it is necessary to consider the precise physical phenomenon measured here. $P(\alpha; \beta_0)$ actually represents a conditional probability—it is the probability that a subpopulation with a mutation of degree α emerges *given that a region of relative size β_0 has mutated*. The overall probability, P_{ov} , that a mutation of degree α emerges from a tumor of the primary strain, may be expressed as

$$P_{ov} = P(\alpha; \beta_0) P_{mut}(\alpha; \beta_0), \quad (10)$$

in which $P_{mut}(\alpha; \beta_0)$ represents the probability that a region of size β_0 mutates by degree α . If P_{mut} can be estimated or, preferably, measured experimentally, the general probability of emergence, P_{ov} , can be calculated. This then would

allow the importance of two possible pathways—a single large mutation of degree α_1 and a series of smaller mutations within the same population each of degree α_2 —to be compared. Recognizing the number of small mutations required to produce the same change as a single large mutation can be expressed as $n = \ln \alpha_1 / \ln \alpha_2$, the likelihood of the single mutation pathway relative to the multiple mutation pathway can be expressed as

$$\frac{[P_{ov}(\alpha_2)]^n}{P_{ov}(\alpha_1)} = \frac{[P(\alpha_2)]^{(\ln \alpha_1 / \ln \alpha_2)} [P_{mut}(\alpha_2)]^{(\ln \alpha_1 / \ln \alpha_2)}}{P(\alpha_1) P_{mut}(\alpha_1)}. \quad (11)$$

The quantity on the left-hand side of eqn (11) is a measure of the importance of the serial mutation pathway relative to that of the single mutation path for the heterogeneous composition of a macroscopic tumor. A “global” quantity such as this is difficult to measure experimentally, yet it may be found by combining information regarding the way in which individual cells mutate (which may be more readily accessible) with the probabilities of emergence as a function of degree of mutation calculated here. Thus, this approach may also help in studying the origins of GBM tumors for which two potential genetic pathways are proposed: a “progression” pathway emphasizing the gradual increase of malignancy by the accumulation of numerous mutations and the supposedly more rapidly emerging and growing “de novo” tumors, which seem to skip lower malignant pre-steps (Lang *et al.*, 1994). Data from mutational analysis and gene expression profiling from microregions of a tumor may yield the necessary information about mutations within individual cells in the future. Genetic events may cause both differences in gene expression and also epigenetic alterations. Therefore, both pathways potentially lead to differences in the aggressive cellular traits of the emerging clonal strain such as in its tumorigenicity or invasiveness. These characteristics of the harvested cells can be readily measured in standard experimental settings and thus may be used to compare the impact of the specific underlying genetic events on a tumor microregion, giving information on what values of α_1 and α_2 should be considered.

Conclusions and Remarks

In the present work, a quantitative analysis of the growth of a subpopulation within a previously homogeneous tumor has been presented. The growth dynamics of heterogeneous tumors in which the more malignant population is initially a small fraction are shown to mimic the history of the less malignant strain at small tumor sizes (i.e. early stages of the disease). The dynamics at large tumor sizes (such as those of the advanced stage typical at the time of diagnosis), however, become indistinguishable from a tumor composed entirely of the more malignant clone. Important features of heterogeneous tumors, in particular the spatial localization of subpopulations, have pronounced impact on the emergence of a subpopulation. The localization of the subpopulation limits the competitive interactions between the subpopulation and the primary tumor population, thereby allowing even very small populations to emerge with measurable probabilities. In addition, subpopulations with very small advantages in growth rate are observed to have a finite possibility of emergence. This is of particular importance for the situation in which mutations are expected to cause only small behavioral changes. It also has relevance to a situation in which a mutation confers an advantage (such as drug resistance) that takes effect only at some later time. A strong dependence in the probability of emergence is found on the relative size of the initial mutated region. Further work is needed to clarify the behavior of this dependence at length scales beneath the resolution of the current work.

The spatial resolution of the model is also significant in determining the accuracy with which mutated populations of any size (even those above the spatial resolution of the lattice) are simulated. When a single automaton cells divides in the model it represents the division of thousands of biological cells. This may create a larger variance in the local behavior of the model (i.e. at the single automaton cell level) than is expected to be present in the biological system. This effect can be reduced by increasing the spatial resolution of the model and thereby decreasing the number of biological cells represented by each automaton cell. An appropriate choice of

spatial resolution should allow the model to mimic the variance inherent in the biological system.

Aside from work to improve the spatial resolution at which a mutation may be investigated, several directions are available to extend the present work. One simple consideration is the impact of mutations in parameters other than the probability of division. For example, a clone whose R_{max} parameter is increased relative to the primary strain would alter the dynamics of a large tumor, while leaving a small tumor relatively unaffected. This is another case in which a subpopulation can emerge but only affect the growth dynamics at a later stage. Similarly, the emergence of a clone which has two mutations, one beneficial and one disadvantageous, presents an interesting situation. For example, a mutation which allows for a higher rate of cellular division (i.e. a lower cell-doubling time) might also be expected to require a larger amount of nutrition, thereby reducing the thickness of the proliferative rim. These competing mechanisms would have opposite effects on the probability of a mutation emerging and their interaction merits careful investigation.

Because both the probability that a given mutation will occur and the probability that a mutated subpopulation will emerge are dependent on a number of factors that vary with time and position (e.g. the size of the tumor or the surrounding environment at a given time), the combination of these two probabilities gives a complex, continually evolving biosystem. Given an accurate method for predicting the likelihood of any mutation occurring, however, an algorithm such as that presented here, could be used to consider the range of behaviors expected from an evolving tumor. This would allow more quantitative estimates of best-case and worst-case growth scenarios. A simple implementation of such a situation would be to create some fixed number of strains arranged in sequential order, which would be defined as a progression pathway. Then the tumor would be allowed to develop with some probability of each dividing cell progressing to the next step in the pathway. A more realistic model might allow a continuously variable distribution of cell behaviors.

Most importantly, the emergence of clones without an obvious growth advantage suggests clonal coexistence may occur as a result of purely interclonal competition, though the model does not exclude the importance of cooperative effects. Along with cell invasion, the complex interaction of many clonal strains within tumors is likely responsible for many of their most dangerous behaviors, from rapid progression to treatment resistance. Overall, the model already supports the notion that knowledge about the specific clonal composition of the patient's tumor is essential for reliable outcome predictions. This has clear significance for clinical brain tumor management, as it further emphasizes the importance of tissue biopsies followed by genetic and epigenetic profiling of the specimen. Furthermore, while the specific details of the model employed in this study (eg. large central necrosis, gradually thickening proliferative shell, etc.) have only been tested against case studies of GBM tumor growth, some of the broad conclusions are likely to be relevant in other types of tumors. In particular, other types of solid tumors that have topologies qualitatively similar to Fig. 2 (i.e. those that resemble multicellular spheroid) should display the same overall trends in emergence, including the sheltering effect of localization and the emergence of subpopulations with a small (or no) growth advantage.

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