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Tumor Micro-ecology and Competitive Interactions

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Three nested models describing the growth of individual subpopulations in a heterogeneous environment are described. The models represent the dynamics of two populations which compete, to varying degrees, for common resources. The first model describes growth in a totally non-competitive micro-environment, the second model describes an ecology in which competition is proportional to competitor population size, and the third model ecology extends the model described by Jansson & Revesz (1974), which allows one population to emerge from the other. The critical points for each model are defined using the isoclines derived from the Ordinary Differential Equations (ODE's) describing competitive growth. The critical points for each model are characterized by the signs of the eigenvalues of the variational matrix at each point. The theoretical results of the analysis show that a competitive model ecology with Verhulstian logistics allows four critical points: the origin which is a repeller, two competitive exclusion points, and an equilibrium state (Waltman, 1983). The extended model ecology of Jansson & Revesz (1974), allows three critical points: the origin which is a repeller, competitive exclusion of the first population, and an equilibrium point. Data from a human adenocarcinoma of the colon and murine mammary tumors are used as qualitative measures of the dynamics of the three micro-ecologies. Issues such as stochastic extension to model small populations either for clonal extinction or heterogeneous emergence are discussed.

Introduction

This paper analyzes three nested models which describe subpopulation growth in heterogeneous tumors, each constrained by logistic growth. The first model describes a non-competitive environment. The second is a Lotka–Volterra competition Model with Verhulstian logistics (the CV Model), and the third is an extension of a model developed by Jansson & Revesz (1974) that describes cellular mutation in a competitive milieu constrained by Verhulstian logistics (the JRE Model).

Our results show that qualitatively different dynamics can be expected from each of the models. Growth data obtained from a human adenocarcinoma of the colon (Dexter & Leith, 1986; Leith *et al.*, 1987), and a murine mammary tumor (data

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provided by Heppner, Miller, and Miller of the Michigan Cancer Foundation) was used to qualitatively verify growth dynamics in the heterogeneous milieu.

Background

In general, tumor growth *in vivo* has been described by logistic dynamics (e.g., Mayneord, 1932; Steel & Lamerton, 1966; Brunton & Wheldon, 1980; Steel, 1980). From these general observations, mathematical models for logistic growth have been developed into a vast literature for both deterministic and stochastic models (e.g. Laird, 1964; Burton, 1966; Dethlefsen *et al.*, 1968; Fredrickson *et al.*, 1967; Wette *et al.*, 1974a,*b*).

All logistic models assume that growth is bounded by limits in the environment. Damping of the growth rate in the Verhulstian model is due to intraspecific competition for limited nutrients. Even in modified versions, the growth rate of the population is assumed static, and intraspecific competition accounts completely for cell loss and damped overall growth. In one form of the Gompertz model, retardation of growth is attributed to non-specific causes intrinsic to the emerging population. The rate of growth decays exponentially to zero purely as a function of time. Explicit cell loss terms are ignored. All the logistic models assume homogeneous populations, growing at a single rate in a homogeneous environment. In a heterogeneous tumor, emerging subpopulations may invalidate these assumptions.

We assume that cell populations must compete for vital nutrients, growth factors, etc. in the tumor milieu. If two populations compete for a common resource, the generic form for the model ecology is

$$\begin{aligned} dP_1/dt &= [\text{Logistic Growth of } P_1] - [\text{Competition } P_1P_2] \\ dP_2/dt &= [\text{Logistic Growth of } P_2] - [\text{Competition } P_2P_1]. \end{aligned} \tag{1}$$

The simplest biological assumption is that the interspecific competition is proportional to the size of the combined populations. In a tumor, the proportionality constant that represents the interspecific competition between the two populations is a measure of competition for nutrients, growth factors, etc. When defining a simple model such as (1), the two proportionality constants defining interspecific competition need not be the same. In fact, one population may be more efficient in nutrient acquisition or processing than the other.

An extension of the competitive interaction models was derived by Jansson & Revesz (1974). They suggest that cells from one population can be transformed into cells of the other. They observed such transformations in an Ehrlich ascites tumor, and termed them "endomitoses". Diploid cells of the tumor become tetraploid by duplicating their DNA but not dividing. Other tumor systems have been observed that also display mixed populations (e.g. Dexter *et al.*, 1981; Nervi *et al.*, 1982; Jakobsen *et al.*, 1979, 1983; Helio *et al.*, 1985).

The phenomenon of tumor heterogeneity or intraneoplastic diversity has been well described (e.g. Heppner, 1983; Heppner *et al.*, 1984; Spremulli & Dexter, 1983; Dexter & Leith, 1986; Leith & Dexter, 1986). Conceptually, variant subpopulations of tumor cells arise in a neoplasm by the process of mutation and selection during

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tumor evolution and progression (Goldie & Coldman, 1979; Goldie *et al.*, 1982; Foulds, 1954*a,b,c,d*). As a first approximation we will try to describe such heterogeneous tumor growth under three model ecologies: a non-competitive growth model, a Lotka-Volterra competition Model with Verhulstian dynamics (the CV Model), and an extension of the CV model which allows for populational transformation as proposed by Jansson & Revesz (1974), (the JRE Model). In the CV and JRE Models, interspecific competition damps the growth rates of the individual subpopulations, and the measures of gathering and processing efficiencies for nutrients, growth factors, etc. are represented in each model by the interaction and growth parameters. In developing our models, we have assumed that the two cell populations compete for the same resources, and that the "nutrient capacity" of the environment is limited, i.e. what one population gets the other does not.

Definitions

(1) Given a sequence $t_n \to \infty$ and $n \to \infty$, a point P is called an *omega limit point* of a function if there is a sequence t_n such that the function converges to P as $n \to \infty$. The set of all such limit points is the *omega limit set* of the function.

(2) A *critical point* (in 2-space) of the ODE system

$$x' = f(x, y)$$
$$y' = g(x, y)$$

is the solution of $f(x, y) = 0$ and $g(x, y) = 0$.

(3) A *trajectory in phase space* is a parametric (vector) function $(x(s), y(s))$ for s real. If the trajectory is bounded as $s \to \infty$, the omega limit set is not empty and consists of all critical points, trajectories which join them, and closed curves around them (Waltman, 1983).

(4) A critical point is *stable* if for any small number ε, there exists a value, T, such that $(x(t), y(t))$ is within ε of the critical point (x^*, y^*), when $t > T$. It is *asymptotically stable* if it is stable and the limit of the trajectory is (x^*, y^*) as t goes to ∞.

(5) Assuming that f and g are differentiable, the *variational matrix* for the ODE system is given by

$$\mathbf{A} = \begin{bmatrix} \partial f/\partial x & \partial f/\partial y \\ \partial g/\partial x & \partial g/\partial y \end{bmatrix}.$$

(6) The variational matrix $\mathbf{A}$ is evaluated at each critical point. If, for a given critical point, all the eigenvalues of A have negative real parts, then that point is *asymptotically stable.* If they have opposite sign, the point is a *saddle point*, and if both have positive real parts the point is a *repeller.*

Analysis

THE NON-INTERACTIVE MODEL

The first model we analyze describes growth in a non-interactive ecology. In this model, there is no competition for resource and the carrying capacity for each

population determines the total size of the tumor, the dynamics of growth are independent and the model is given by

$$x' = r_1 x(1 - x/K_1)$$
$$y' = r_2 y(1 - y/K_2). \tag{2}$$

If we hypothesize that the carrying capacity of the environment, K, determines the size of the total tumor, i.e. the total population size, $x+y$ approaches K logistically, the model becomes

$$x' = r_1 x(1 - (x+y)/K)$$
$$y' = r_2 y(1 - (y+x)/K). \tag{2'}$$

Equation (2') is just a special case of the CV Model ($r_i xy/K$ is the competitive interaction term). Therefore, if the environment determines the carrying capacity of the tumor system, the resources must be competed for, and a competitive interaction model must be used to describe growth.

Upon simple inspection, the model given by (2) allows four critical points: E_0: (0, 0), E_1: (K_1, 0), E_2: (0, K_2), and E_3: (K_1, K_2). The origin is a repeller, and growth along either axis describes a homogeneous tumor growing logistically in a host. Emergence of one population from the other is ruled out. E_3, the equilibrium point, is globally stable, and any heterogeneous implant must achieve a total tumor size of K_1+K_2 and an end population mix of $K_1/(K_1+K_2)$.

Volumetric and compositional data from the DLD-1 human adenocarcinoma of the colon rule out these dynamics (see Figs 3 and 5 of Leith *et al.*, 1987). Data observed in two murine mammary tumor admixtures also appear to rule out these dynamics (Heppner *et al.*, 1986, personal communication). Rather, there appears to be some form of interaction *in vivo*.

THE CV MODEL

$$x' = r_1 x(1 - x/K_1 - \lambda_1 y)$$
$$y' = r_2 y(1 - y/K_2 - \lambda_2 x). \tag{3}$$

Waltman (1983) analyzes this model in detail. He shows that there are 4 critical points: E_0: (0, 0); E_1: (K_1, 0); E_2: (0, K_2); and E_3: (x_c, y_c), a population equilibrium point. The stability of these points is a function of the choices for (λ_1, λ_2) as follows:

E_0 is *always* a repeller, i.e. if any cells exist in the system at time 0, growth is inevitable, and extinction is impossible.

E_1 is either asymptotically stable, i.e. *competitive exclusion* of the second population occurs (when $\lambda_1 K_1 < 1$ and $\lambda_2 K_2 > 1$), or it is a saddle point, the stable manifold of which is the x-axis. Therefore, depending on the $K_i\lambda_i$, any tumor initially growing in the open quadrant, i.e. as a heterogeneous tumor, either excludes the second population, excludes the first population, or attains an equilibrium. If the implanted tumor is pure, i.e. the initial state lies on the x-axis, then E_1 is asymptotically stable, and the population approaches K_1 asymptotically. Similarly for E_2.

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E_3 is an equilibrium point and is determined by the interaction kinetics ($K_i\lambda_i$) of the system. If both $K_i\lambda_i$ are less than 1, then E_3 is stable. Otherwise, it is a saddle point. If E_3 is asymptotically stable, the populations can co-exist at

$$x + K_1\lambda_1 y = K_1 \qquad (4)$$
$$y + K_2\lambda_2 x = K_2.$$

If E_3 is a saddle point, the system will go to either E_1 or E_2 as a function of the λ's and the initial conditions.

THE JRE MODEL

In this model we assume that transformation from x to y cells occurs. The model is given by

$$x' = r_1 x(1 - x/K_1 - \lambda_1 y) - px \qquad (5)$$
$$y' = r_2 y(1 - y/K_2 - \lambda_2 x) + px.$$

Note that system (5) is identical to system (3) except for the transformation terms $-px$ and $+px$. First we show that Lotka-Volterra Competition type dynamics can actually be extended to the Jansson and Revesz Model. The CV Model is

$$x' = r_1 x(1 - x/K_1 - \lambda_1 y) \qquad (6)$$
$$y' = r_2 y(1 - y/K_2 - \lambda_2 x).$$

Expanding

$$x' = r_1 x - (r_1/K_1)x^2 - r_1\lambda_1 xy \qquad (7)$$
$$y' = r_2 y - (r_2/K_2)y^2 - r_2\lambda_2 xy.$$

For their initial model, Jansson & Revesz (1974) derive

$$D' = aD - D\delta_D F_D(U, D) \qquad (8)$$
$$U' = bU - U\delta_U F_U(U, D)$$

where $D(t)$ is the size of a differentiated population at time t, $U(t)$ is the size of an undifferentiated population at time t, and the damped (i.e. logistic) growth of the populations is described by function F_D and F_U.

They claim that growth is damped due to factors proportional to the number of cells in the total population, i.e. $\delta_D = \delta_U = \delta$ and $F_D(U, D) = F_U(U, D) = U + D$. Their equations then become

$$D' = aD - \delta D^2 - \delta DU \qquad (9)$$
$$U' = bU - \delta U^2 - \delta DU.$$

Their initial model is then a special case of the CV Model, which they extend to include a transformation factor as follows

$$D' = aD - D\delta F_D(U, D) - \mu D \qquad (10)$$
$$U' = bU - U\delta F_U(U, D) + \mu D.$$

Now, considering the general form of the JRE Model (5), one can identify the critical points of this system as the solutions of

$$r_1x(1-x/K_1-\lambda_1y)-px=0$$
$$r_2y(1-y/K_2-\lambda_2x)+px=0 \tag{11}$$

E_0: $(0, 0)$

E_2: $(0, K_2)$

E_3: (x_c, y_c) the solution, if it exists, of

$$(r_1-p)x-(r_1/K_1)x^2-r_1\lambda_1xy=0$$
$$r_2y-(r_2/K_2)y^2-(r_2\lambda_2y-p)x=0. \tag{12}$$

Because, $x\neq 0$ we divide the first equation by x and solve,

$$y=-(1/\lambda_1K_1)x+(r_1-p)/r_1\lambda_1 \tag{13}$$

and,

$$x=(r_2y)(1-y/K_2)/(r_2\lambda_2y-p).$$

For the solution to remain in the positive quadrant, either

$$y>p/r_2\lambda_2 \quad \text{and} \quad y<K_2$$

or

$$y<p/r_2\lambda_2 \quad \text{and} \quad y>K_2.$$

In the admixture experiments we have always assumed that the implants are far smaller than the carrying capacity of the mice. Therefore, $y<K_2$ and we use the first series of conditions as our working hypothesis.

The variational matrix is given by

$$\mathbf{A}=\begin{bmatrix}(r_1-p)-(2r_1/K_1)x-\lambda_1r_1y & -\lambda_1r_1x\\ -\lambda_2r_2y+p & r_2-(2r_2/K_2)y-\lambda_2r_2x\end{bmatrix}.$$

At E_0 the matrix becomes

$$\begin{bmatrix}(r_1-p) & 0\\ p & r_2\end{bmatrix}.$$

If $r_1<p$, then E_0 is a saddle point with the unstable manifold lying along the y-axis. If $r_1>p$ then the origin is a repeller.

At E_2 the matrix is

$$\begin{bmatrix}(r_1-p)-\lambda_1r_1K_2 & 0\\ -\lambda_2r_2K_2+p & -r_2\end{bmatrix}.$$

If $p<r_1-\lambda_1r_1K_2$ then E_2 is a saddle point. Otherwise, it is an attractor.

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We can rule out closed orbits in the closed positive quadrant by using the Dulac Criterion (Waltman, 1983). The Dulac Criterion states that if there exists a function, $q(x, y)$, such that the quantity

$$(q(x, y)f(x, y))_x + (q(x, y), g(x, y))_y$$

does not change sign in a simple closed region about a critical point, then the system has no closed orbits in that region. Let $q(x, y) = 1/xy$. Then, the sum of partial differentials is

$$-(r_1/K_1 y) - (r_2/K_2 x) - p/y^2.$$

Therefore, if E_2 is not asymptotically stable, E_3 must be. Note, that there is no critical point on the x-axis, i.e. competitive exclusion of the y population is impossible as long as the x population exists. In fact, under this model, y cells will emerge from the x population at the given rate p, even when pure tumors are implanted.

Discussion

In the following three subsections we discuss the types of data observed from *in vivo* experiments and how they relate to the theoretical models, the emergence of populations and the types of dynamics which one should expect in a micro-ecology described by the JRE Model, and the dangers of extrapolation of our results to the small population case.

OBSERVED DATA: EQUILIBRIA AND EXCLUSION

The experimental data upon which these analyses were based have previously been published (Leith *et al.*, 1987). In this regard, we note that the heterogeneous DLD-1 (human adenocarcinoma) appears to attain a stable compositional equilibrium at an 88:12 (D:A) composition. A 50:50 admixture always returns to the stable point, and starting at the stable point the tumor remains stable and grows bigger than any other admixture. These dynamics are similar to those observed by Jansson & Revesz (1974) in the Ehrlich ascites tumor.

Mammary tumor admixture data from a 50:50 mix of 410.4 and 66C14 cells exhibits compositional divergence (see Fig. 1(a)(b)) as follows

4 tumors	0–10% type 66C14
2 tumors	10–20% type 66C14
1 tumor	70–80% type 66C14
1 tumor	80–90% type 66C14
2 tumors	90–100% type 66C14.

We term dynamics which exhibit two tracts of competitive exclusion "segregational".

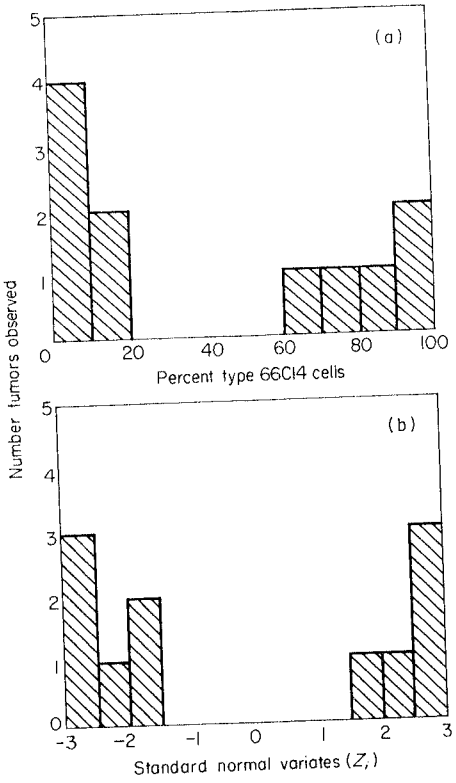


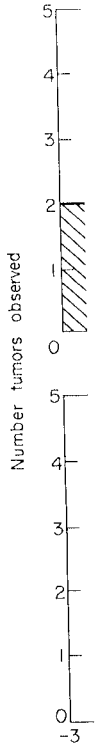
FIG. 1. (a) Raw percentage of type 66C14 mammary adenocarcinoma cells in heterogeneous admixtures. (b) Normalized standard variates of the raw percentages.

Mammary tumor admixtures in a 50:50 mix of 44FT0 and 66 cells exhibit compositional equilibrium as shown in Fig. 2(a) and (b)

2 tumors	0–10% type 66
2 tumors	10–20% type 66
5 tumors	20–30% type 66
0 tumors	30–40% type 66
2 tumors	40–50% type 66.

These equilibrium dynamics are similar to those observed in the Ehrlich ascites data described by Jansson & Revesz (1974) and the DLD-1 data described by Leith *et al.* (1987).

Waltman (1983) described three types of exclusion dynamics in the CV Model. Populations may be excluded when one successfully outcompetes the other for vital nutrients (we term these dynamics CE I and CE II). Or, populations can be excluded when neither is a good competitor, but the initial status of the tumor heavily favors



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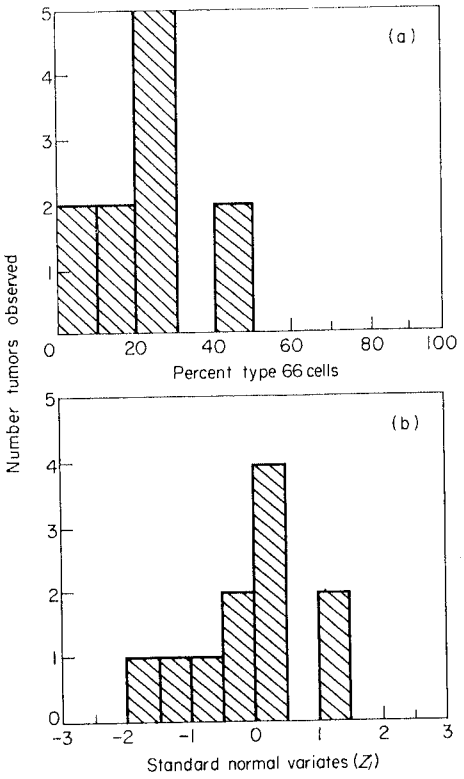

FIG. 2. (a) Raw percentage of type 66 mammary adenocarcinoma cells in heterogeneous admixtures. (b) Normalized standard variates of the raw percentages.

one over the other (we call this CE III). Trajectories following CE III dynamics bifurcate as a function of initial conditions in the ecology (see Fig. 3). Populations can also achieve a co-existent equilibrium as a function of the interactive terms $K_i\lambda_i$ (when both are less than 1).

In the JRE Model, CE I and CE III type trajectories that describe exclusion of the x population are the only allowable exclusion dynamics. However, two new types of dynamics exist. We term them "emergence exclusion" and "emergence equilibrium". We discuss these dynamics in greater detail below.

POPULATION EMERGENCE: EQUILIBRIUM AND EXCLUSION

Figure 4 shows the emergence of y cells from a pure tumor of type x cells which grows according to the JRE Model specifications. The simulated tumor was implanted at time 0. The result is a heterogeneous tumor that converges to a compositional equilibrium after y cells emerge. The parameters used to generate the phase plot were $K_1\lambda_1 = 0{\cdot}071$ and $K_2\lambda_2 = 1{\cdot}34$. These same parameter settings in the CV Model result in competitive exclusion of any y population (CE II dynamics) injected at

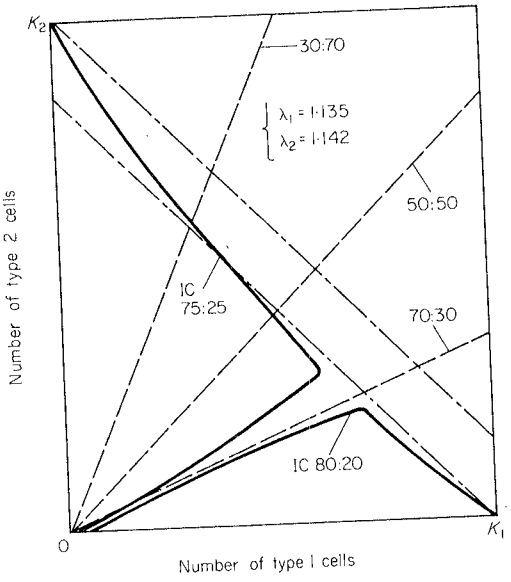

FIG. 3. Trajectories following CE III type dynamics. Trajectory 1 begins with a theoretical 75:25 admixture of competing cells and excludes population 1. Trajectory 2 begins with an initial theoretical admixture of 80:20 and excludes population 2. Competition parameters are 1·135 and 1·142 respectively.

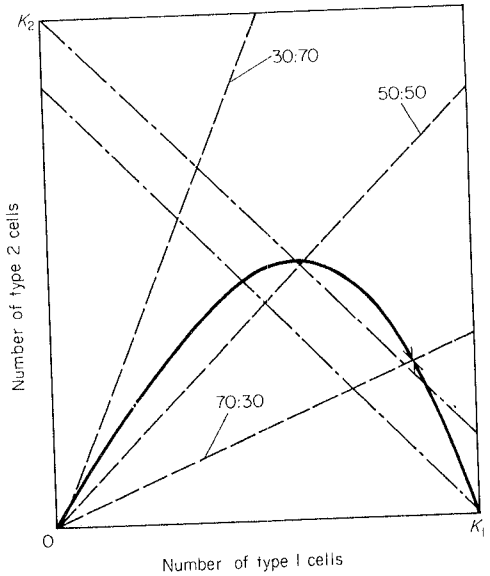

FIG. 4. Tumor equilibrium of an admixed tumor population emerging from a pure type 1 population in the JRE Model. Second trajectory shows equilibrium dynamics for a tumor beginning with a 50:50 mixture.

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EXTRAPOLATIO

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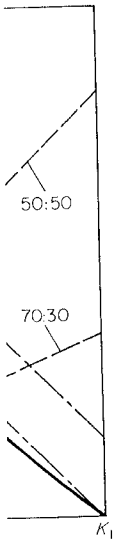

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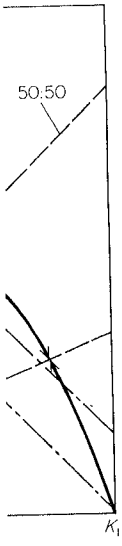

erging from a pure type 1 population
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time 0. However, in the JRE Model, even when p is small (10^{-7}), there still emerges a clone of y cells that grows to an equilibrium point of $6{\cdot}5\times10^3$ cells in a tumor of $1{\cdot}4\times10^9$ cells.

We have observed two types of clonal emergence. In the first, when the transformation rate is less than the reproduction rate of the x cells, the origin is a repeller. The x's replenish faster than they are removed due to competition or transformation. If the λ_i allow it, we call the equilibrium they attain an "emergence equilibrium". When the transformation rate is greater than the rate at which the x cells replenish the population, the origin is a saddle point. This situation may arise in the presence of strong mutagens. In this case, the x cells leave the system (either by dying or transforming into y cells) faster than they enter it. Therefore, the dynamics of this ecology dictate a CE I type of exclusion. We call these dynamics "emergence exclusion".

Other modelers (Goldie & Coldman, 1979; Goldie *et al.*, 1982) suggest that resistance to chemotherapy results from a mutation to the genetic structure of the cell, i.e. x's become y's. They estimate that the rate of transformation is one mutation per 10^6 mitoses. Our initial analyses show that the emerging clone, should it survive an initial growth phase, will either attain an equilibrium mixture, or will, in the face of no back mutations, entirely exclude the original population. Therefore, clonal emergence, even in an established tumor need not result in assigning the emerging clone to a minority status. Furthermore, we have shown that if an equilibrium point is attained, it is globally stable. Therefore, in these models, displacement from equilibrium will result in equilibrium dynamics that return the population to its asymptotic state.

The model proposed by Goldie & Coldman (1979) for the emergence of resistant subpopulations results in a theoretical population which is still highly sensitive to drug therapy. One criticism of the Goldie-Coldman model has been that certain tumors observed *in vivo* (especially those of the GI tract) are predominantly drug resistant (Goldie, personal communication, 1986). Goldie & Coldman have been able to account for this phenomenon by adjusting the growth rates of the emerging population so that by sheer speed the drug resistance of the entire population can be established (Goldie, personal communication, 1986). Our studies with the JRE model suggest a second mechanism, in which two populations, *with the same growth dynamics*, can attain an equilibrium point which corresponds to the drug resistant state.

EXTRAPOLATION TO SMALL POPULATIONS: A DANGER

Deterministic models are limited to describing population behavior by some measure of central tendency, rather than describing the individual behavior of each population member. On the other hand, experimentalists are constrained by the sensitivity of their assays in interpreting their data. So what may appear to be competitive exclusion might, in fact, be an equilibrium point achieved far below the sensitivity threshold of an assaying technique. Furthermore if p is very small, predicted emergence of a clone may, in fact, be thwarted because early random

deaths incurred by the new clone upon emergence result in population extinction before the "average" behavior of the model can take affect.

In the CV and JRE Models, we can adjust the model parameters representing environmental factors so that an equilibrium point is set as near to exclusion limits as we choose. For example, (see system (4)), suppose we wish to exclude the x population in the CV Model. Fix y_c and all model parameters except λ_1. Let m be any small number >0. Then, we can adjust λ_1 so that x_c becomes less than m. We term this phenomenon "asymptotic criticality", because, qualitatively, the x population appears to be excluded in of our assays, when, in fact, an equilibrium point has been achieved with population sizes so small that we will probably never observe them.

On the other hand, predicted equilibria at population levels near either axis may never accrue. As each clonal variant (y cell) emerges, it faces it's own individual competition scenario with the resident population (x cells). When modeling with deterministic models, moderate fluctuations about the mean activity levels in large populations are ignored in that they will probably not result in population extinction. However, in small populations, even minor fluctuations about the mean could result in a measurable probability for population exctinction. Therefore, for dynamics in a small population, one should employ a stochastic model for either emergence or extinction, and not extrapolate these results to the axis. When the population gets "big enough", the deterministic models described above can take over, and if the equilibrium points are globally asymptotically stable, the end dynamics remain as described.

Summary

We have identified three micro-ecologies under which heterogeneous tumors may evolve. The first is the non-interactive growth ecology. The tumors grows to size K_1+K_2 with end population mix K_1/K_1+K_2. Using both compositional and volumetric data observed in the DLD-1 (Leith *et al.*, 1987) and murine mammary tumor systems, we have ruled out these dynamics for these types of tumors.

The second ecology is described by the CV Model. The tumor will never go extinct. Furthermore, either competitive exclusion point can be asymptotically stable. There also exists a compositional equilibrium point which may be asymptotically stable. When a pure tumor of either type is implanted, the resultant tumor will remain pure and grow logistically, approaching the asymptotic carrying capacity of the environment for that cell species.

The third ecology is described by the JRE Model. In this ecology, the origin can be either a repeller or a saddle point with the unstable manifold lying along the y-axis. What this means is that when one starts with a pure tumor of type x and a transformation rate, p. If p is greater than the growth rate of the x population, the trajectory tends toward the origin in the x-direction and toward E_2 in the y-direction. If p is less than the growth rate of the x population, the origin is a repeller, and the trajectory tends away from it in both the x- and y-directions. Competitive exclusion of the x population is an acceptable dynamic, but there is no dynamic

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We would like to thank Dr Ja
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for competitive exclusion of the y population. As long as there are x cells present, y cells will, on average, emerge. Additionally, when the E_2 critical point is a saddle point, a stable equilibrium point must exist.

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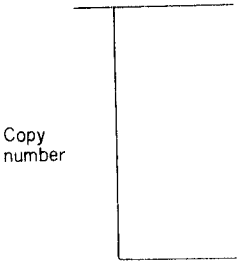

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