Dynamics and Pattern Formation in Invasive Tumor Growth

Evgeniy Khain and Leonard M. Sander

Department of Physics and Michigan Center for Theoretical Physics, The University of Michigan, Ann Arbor, Michigan 48109, USA

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We study the in vitro dynamics of the malignant brain tumor glioblastoma multiforme. The growing tumor consists of a dense proliferating zone and an outer less dense invasive region. Experiments with different types of cells show qualitatively different behavior: one cell line invades in a spherically symmetric manner, but another gives rise to branches. We formulate a model for this sort of growth using two coupled reaction-diffusion equations for the cell and nutrient concentrations. When the ratio of the nutrient and cell diffusion coefficients exceeds some critical value, the plane propagating front becomes unstable with respect to transversal perturbations. The instability threshold and the full phase-plane diagram in the parameter space are determined. The results are in a qualitative agreement with experimental findings for the two types of cells.

Glioblastoma multiforme (GBM) is an aggressive form of primary brain tumor. The prognosis for victims of this disease is very poor [1]. One of the main reasons for such high mortality is the fact that GBMs are highly invasive [2]. The growing tumor sheds invasive cells which run through the brain, see Fig. 1; secondary tumors are produced by the invasive cells even if the primary is removed. In this Letter we introduce a reaction-diffusion model for invasion. By comparing the behavior for two different cell lines we hope to get insight into invasion dynamics which needs to be better understood.

This work is inspired by recent in vitro experiments [3–5] where microscopic tumor spheroids (radius about 250μ) were placed in collagen-I gel and allowed to grow. The cell lines used were U87 and U87-ΔEGFR. The first type is called “wild-type” in what follows. The second is a mutant line [6] in which there is an amplification of the epidermal growth factor receptor (EGFR) gene. This amplification occurs in approximately 40% of cases of GBM [6] and is associated with enhanced malignancy.

If we compare the growth of the two cell lines in vitro we see two main differences; cf. Fig. 1. The invasive region for the wild-type cells grows faster than for the mutant cells, and produces a diffuse spherically symmetric pattern, while mutant cells produce a branching pattern [5]. In the present work, we formulate a simple reaction-diffusion model which shows an instability for certain sets of parameters (which we identify with mutant cells) and stability for other sets (which could be the wild-type). The parameters could be measured to confirm the identification. Our model may give insight into the functional significance of the mutation.

Analogous instabilities were studied in the theory of combustion [7], and in studies of the self-organization of microorganisms [8,9]. In the present context, one possible mechanism for instability is attraction between cells [10] due to the production of growth factors, see also [11]. In this Letter we propose another possible mechanism based on the biology of GBM.

We give a continuum description which treats the density of cells \( u(\mathbf{r}, t) \) and the density of some growth factor or nutrient (whichever controls the growth), \( c(\mathbf{r}, t) \). We assume that \( c \) diffuses to the tumor from far away. Each cancer cell is able to proliferate as well as to perform random motion. The key feature of our model is to build in the experimental fact that within the inner region there is rapid proliferation whereas in the invasive region, the cells have high motility, but slow proliferation [12]. We model this dynamical switch of phenotype by introducing a density-dependent proliferation term where the proliferation rate increases with cell density. The simplest form for such a term is \( \partial u/\partial t \sim u^2 c \) rather than the usual \( uc \). A rough derivation of this might go as follows: suppose that the growth rate depends on the local cell density, perhaps through the number of gap junctions [12]. Then the proliferation rate per cell, \( (1/u)\partial u/\partial t \) increases with, and in the

FIG. 1. Growing tumor spheroids from in vitro experiments [5] in collagen gel for the wild-type (a) and mutant (b) cells. These in vitro cell clusters consist of an inner proliferation zone with a very high density of cells and an outer invasive zone, where the cell density is smaller. The structure in vivo is believed to be similar. The radius of the inner zone here is about 250μ. (a) For wild-type cells a spherically symmetric pattern is observed. (b) Mutant cells are organized in branches. Note also that the invasive region for the mutant-type cells grows slower than for wild-type cells.
simplest model, is proportional to \( u \). This term drives the instability and leads to branching.

We assume that in order to proliferate a cell needs to consume some amount of \( c \). The density \( c \) obeys a diffusion equation with a sink at the tumor cells. Encoding these assumptions, we have

\[
\begin{align*}
\frac{\partial u}{\partial t} &= \nabla \cdot (D_u \nabla u) + \alpha u^2 c, \\
\frac{\partial c}{\partial t} &= \nabla \cdot (D_c \nabla c) - \beta u^2 c.
\end{align*}
\]

(1)

Here \( D_u \) and \( D_c \) are the diffusion coefficients of \( u \) and \( c \), \( \alpha \) is a proliferation coefficient, and \( \beta \) is the coefficient of nutrient consumption. The nutrient concentration is assumed to be constant far from the tumor: \( \lim_{r \to \infty} c(r) = c_\infty \). Now, we take into account cell-matrix interaction. It is known that the experimental medium, collagen-I, is very soft (much softer than brain tissue) and glioma cells remodel its structure \([4]\). When an initial tumor is introduced into the gel, the fibers near the tumor rearrange and become pulled in a radial direction. Therefore, in the vicinity of the tumor, cells move mostly in a radial direction. We incorporate this idea assuming that the radial diffusion is larger than the azimuthal diffusion, by introducing an anisotropy parameter \( p < 1 \).

We will measure cell density in the units of some characteristic density \( u_0 \), nutrient density in units of \( c_\infty \), distance in units of \([D_c/(\beta u_0)]^{1/2}\), and time in units of \((\beta u_0)^{-1}\). This gives

\[
\frac{\partial u}{\partial t} = \frac{1}{\delta} \nabla^2 u + \frac{1}{m} u^2 c \quad \text{and} \quad \frac{\partial c}{\partial t} = \nabla^2 c - u^2 c,
\]

(2)

where \( \delta = D_c/D_u \) is the ratio of the nutrient and cell diffusion coefficients and \( m = \beta u_0/(ac_\infty) \) is the ratio of consumption and proliferation rates.

The nutrients or growth factors represented by \( c \) are small molecules which diffuse much faster than cells. For example, the diffusion coefficient of glucose in the brain is of the order of \( 10^{-7} \text{ cm}^2/\text{s} \), while the effective cell diffusion coefficient is of the order of \( 10^{-9} \text{ cm}^2/\text{s} \) \([10]\), so that \( \delta \sim 100 \). A typical nutrient consumption is \( 10^{-12} \text{ g/cell)/ min} \) \([13]\), and a typical glucose concentration is of the order of 1 g/l. Assuming that typical cell density within the invasive region is of the order of \( 10^3 \text{ cell/cm}^3 \), we estimate the consumption rate as \( 1.7 \times 10^{-6} \text{ s}^{-1} \). The typical proliferation rate in experiments \([5]\) is of the order of 1/day, so that \( m \) turns out to be of the order of 0.1.

To analyze the instability we work in a two-dimensional channel geometry. Let \( x \) be the direction of tumor growth, and \( y \) the perpendicular direction. In the \( y \) direction we use periodic boundary conditions with a finite channel width. Far ahead of the tumor the cell concentration is zero, \( u(x = \infty) = 0 \), and the scaled nutrient concentration is unity, \( c(x = \infty) = 1 \). At \( x = -\infty \) we put \( c = 0 \). There is a conservation law in Eq. (2): the volume integral of \((mu + c)\) is a conserved quantity; that is in our model a cell needs some amount of food to divide. Therefore, at \( x = -\infty \), \( u = 1/m \) if there is a steady state. Our initial conditions are \( u = 1/m \) for \( x \leq 0 \); \( u = 0 \) for \( x > 0 \), and \( c = 0 \), for \( x \leq 0 \); \( c = c_\infty \) for \( x > 0 \). We assume that a steady propagating state has been established.

First, we consider the solutions of Eq. (2) in the form of plane propagating fronts: \( u = u_0(\xi), c = c_0(\xi) \), \( \xi = x - vt \). Substituting into Eq. (2) we arrive at

\[
\frac{u_0'/\delta + v u_0' + u_0^2 c_0/m = 0, \quad c_0'/v + u_0 c_0 - u_0^2 c_0 = 0.}
\]

(3)

To obtain the profiles and the velocity of front propagation, we performed a numerical shooting procedure. First, we write down Eq. (3) as four coupled first-order differential equations in the form \((\tilde{a})' = M(\tilde{a})\), where \( \tilde{a} \) is the column of solutions with elements \( u_0, u_0', c_0, c_0' \). Then we find the eigenvalues and eigenvectors of \( M \) at \( \xi = \pm \infty \). Starting with the solution \( \tilde{a} \) that is proportional to the eigenvector belonging to the positive eigenvalue at \( \xi = -\infty \) (using the linearity of the problem, we choose the constant of proportionality to be unity), we perform shooting by the velocity of front propagation \( v \). We find the profiles by demanding that the solution \( \tilde{a} \) at \( \xi = +\infty \) is a linear combination of the two eigenvectors \( \tilde{y}_1 \) and \( \tilde{y}_2 \) belonging to negative eigenvalues \( \lambda_1 \) and \( \lambda_2 \) at \( \xi = +\infty \): \( \tilde{a} = b_1 \tilde{y}_1 \exp(\lambda_1 \xi) + b_2 \tilde{y}_2 \exp(\lambda_2 \xi) \). Figure 2 shows a typical solution of Eq. (3).

We now perform a linear stability analysis. Consider perturbations in the transverse direction \( u = u_0(\xi) + u_1(\xi) \exp(\gamma t + iky), c = c_0(\xi) + c_1(\xi) \exp(\gamma t + iky) \) and substitute into Eq. (2). We have

\[
\begin{align*}
\frac{1}{\delta} u_1'' + vu_1' + \left(\frac{2}{m} c_0 u_0 - \frac{p k^2}{\delta} - \gamma\right) u_1 + \frac{1}{m} u_0^2 c_1 &= 0, \\
c_1'' + vc_1' - (u_0^2 + k^2 + \gamma) c_1 - 2c_0 u_0 u_1 &= 0,
\end{align*}
\]

(4)

where \( p < 1 \) is the anisotropy parameter. For a fixed value

FIG. 2. Density profiles of cells and nutrient from Eq. (3). Cell density \( u = u(\xi) \), solid curve. Inset: nutrient concentration, \( c = c(\xi) \), dashed curve. Parameters are \( m = 1, \delta = 100 \).
of $k$, we find the perturbations $u_1(\xi)$ and $c_1(\xi)$, and the growth rate $\gamma$ by a numerical shooting. Changing $k$ for fixed $\delta$ and $m$ gives the dispersion curve $\gamma(k)$.

As was found previously in the context of chemical reactions for $m = 1$ [14], plane fronts can become transversally unstable if the ratio of diffusion coefficients $\delta$ exceeds a certain critical value. Indeed, for $\delta > \delta_{cr}$, the growth rate $\gamma$ is positive for small $k$, while for larger $k$, cell diffusion in the transverse direction stabilizes the instability. That is why the anisotropic case (with smaller lateral diffusion) is more unstable. We checked that $\delta_{cr} = 2.300$, in agreement with previous results [14,15]. Figure 3 shows an example of the dispersion curve for $\delta > \delta_{cr}$ both for isotropic and anisotropic diffusion. An inset shows the dependence of the largest unstable wave number $k_*$ on $\epsilon = (\delta - \delta_{cr})/\delta_{cr}$. For a system of finite width we need to form a discrete set of modes from the modes of the infinite-width system by imposing $kL = 2n\pi$, where $n = 1, 2, \ldots$ and $L$ is the width of the system. For a small enough width we can “freeze out” the instability if $k_0 < 2\pi/L$, even for $\delta > \delta_{cr}$ (see Fig. 4, the left panel.) For a spherical tumor $L$ is of the order of the diameter; Fig. 1(a) could correspond to small $k_*$.

We also performed numerical simulations of Eq. (2), using the third order Runge-Kutta method. We did 2D simulations both in channel and radial geometries both for isotropic and anisotropic diffusion. Simulations in a channel geometry support the results of linear stability analysis. To be closer to the experimental observations, we present here the radial results for cell density, both in the stable (the left panel) and unstable (the center and right panels) cases, see Fig. 4. We start from initial conditions for the cell density in the form of radial step function (with initial radius $r = 7$, which corresponds to $280\mu$) with random azimuthal perturbations [16]. The snapshots in Fig. 4 are presented at time $t = 1.2$, which corresponds to 7 days of experiment. One can see that the anisotropic case (the central panel, $t = 1.2$) is more unstable and the developing branches are much thinner than for isotropic case (the right panel, $t = 1.95$).

For a very wide system the instability threshold $\delta_{cr}$ does not depend on $m$. To show this we introduce new dimensionless variables $r = (m/\delta^{1/2}) R$, $t = m^2 T$, and $u = U/m$. In this case, $m$ drops out of the problem. One can easily find the dependence of the velocity and of the wave number on $m$: $v = m^{-1} \delta^{-1/2} V$, $k = m^{-1} \delta^{1/2} K$. Since the scaled front velocity, $V$, and the scaled wave number, $K$, must be independent of $m$, $v$ and $k$ are proportional to $m^{-1}$. However, we find it more convenient to retain $m$ as a parameter because different types of cells have different diffusion coefficients $D_u$ and different proliferation rates $\alpha$. It is convenient that the dependence of the physical quantities $k_{ph} = k[D_c/(\beta u_0^2)]^{-1/2}$ and $v_{ph} = \nu(D_c \beta u_0^2)^{1/2}$ on $D_u$ and $\alpha$ enters only via the dimensionless variables $v$ and $k$.

We now consider a phase plane of parameters $(\delta, m)$, see Fig. 5. The main differences between the experiments with wild-type and mutant cells are in the velocity of the front propagation and possible symmetry breaking. Thus the region of larger $v$ and smaller $k_*$ in this phase plane should correspond to wild-type cells, while the region of smaller $v$ and larger $k_*$ should correspond to mutant cells. We consider two families of curves: $v = const$ and $k_* = const$. It was shown previously that for $m = 1$ and large $\delta$, $\nu = 1.219 \delta^{-1}$ [17]. Combining this with the $m$ dependence, one can see that in the $(\delta, m)$ phase plane the curves of constant velocity for large $\delta$ are given by $\delta = const/m$. Now consider $k_* = const$. Our numerical calculations indicate that for large $\delta$, $k_*$ tends to a constant independent of $\delta$; for $m = 1$, $k_* \approx 0.5$. Therefore, for large $\delta$, $k_* = 0.5/m$, and the curves of constant $k_*$ are given by

![FIG. 3. Dispersion curve $\gamma(k)$. The instability occurs if the ratio of diffusion coefficients $\delta > \delta_{cr} \approx 2.300$. The growth rate $\gamma$ is positive for small $k$; for larger $k$, cell diffusion in the transverse direction stabilizes the instability. Parameters are: $m = 0.1$, $\delta = 20$, $p = 1$ (the dashed line), and $p = 0.3$ (the solid line). Inset: dependence of the largest unstable wave number $k_*$ on $\epsilon = (\delta - \delta_{cr})/\delta_{cr}$ for $p = 1$. Numerical simulations are the asterisks, the asymptote $k_* = (0.36/m)\epsilon^{1/2}$ is the dotted line.](image)
FIG. 5. Phase plane (δ, m) with two families of curves: ν = const and k_s = const, calculated from Eqs. (3) and (4) for an isotropic case p = 1. The two curves marked by ○’s are ν = const; ν = 0.1 (left), and ν = 0.02 (right). The two curves marked by *’s are k_s = const. The left-hand curve corresponds to a stronger instability, k_s = 10, while the right one corresponds to a weaker instability, k_s = 1. Also shown are the large δ asymptotes: δ = const/m for ν = const curves, and m = const for k_s = const curves, see text. The instability threshold for an infinite stripe, δ ≈ 2.300, is the dotted line. The large-δ and small-m region corresponds to wild-type cells, while mutant cells are in the region of smaller δ and larger m. This suggests that wild-type cells have a larger D_u and a smaller α compared to mutant cells.

m = const. Figure 5 shows also these large δ asymptotes. A typical length scale is [D_u/(βu_0^2)]^{1/2} ≈ 0.2 cm and a typical velocity scale (D_uβu_0) ≈ 4 × 10^{-7} cm/s. Comparing this with experimental data [5], one can see that the dimensionless wave number k_s and front velocity ν should be of the order of 0.5 and 0.1, correspondingly.

In Figs. 4 and 5, large δ and small m give behavior like that of mutant-type cells, while wild cells act as if they had small δ and large m. Thus we predict that wild-type cells have a larger diffusion constant but a smaller proliferation rate than mutant cells in the invasive zone. Measuring these quantities would be very interesting.

An additional point should be taken into account when we compare theoretical predictions with experiments. The basic solutions of our model are plane fronts which propagate with constant velocity. However, in experiments, the more dense inner proliferative region grows more slowly than the invasive region [5]. Probably, the system is in a transient regime, and the steady-state behavior will set in later [18]. Nevertheless, our qualitative predictions should hold in for the experiment.

In summary, we have considered the growth of GBM tumors. In vitro experiments [5] showed that the dynamics of growth and resulting patterns are quite different for wild-type and mutant cells. For the wild-type the invasive region grows faster, and tumor remains spherically symmetric. On the other hand, the invasive region grows slower for the mutant cells, and there are indications of symmetry breaking of spherically symmetric growth. We formulated a simple reaction-diffusion model that captures these experimental findings. Based on our model, we predict different diffusion constants and proliferation rates of wild-type and mutant cells: wild-type cells diffuse faster, but have a lower proliferation rate in the invasive zone. We think that an attempt should be made to test these predictions and relate them to the microscopic biology of the two cell lines.

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[16] We use a circular grid with radial mesh size δr = 0.02, azimuthal mesh size δθ = 2π/180, and a time step δt = 3 × 10^{-7}.