Three-Dimensional Diffuse-Interface Simulation of Multispecies Tumor Growth–II: Investigation of Tumor Invasion

H.B. Frieboes\textsuperscript{a}, S.M. Wise\textsuperscript{b}, J.S. Lowengrub\textsuperscript{b}, V. Cristini\textsuperscript{a,b,1}

\textsuperscript{a}Biomedical Engineering Department, University of California, Irvine, CA 92697-2715, USA
\textsuperscript{b}Mathematics Department, University of California, Irvine, CA 92697-3875, USA

Abstract

Using the diffuse interface model presented in Part I, we investigate non-symmetric tumor growth and invasion in two and three dimensions using biological input parameters calculated from glioma cell culture experiments. Here we simulate non-linear, multispecies tumor growth in three spatial dimensions in order to study in detail disease progression and treatment response. Results obtained using the computational method described in Part I, are compared to recent experimental findings \textit{in vitro} and \textit{in vivo}. This work supports the hypothesis that heterogeneity in the tumor micro-environment, caused by non-uniform distribution of nutrient or genotype mutations, can be a mechanism of tumor invasion via tumor morphological instability that leads to infiltration of surrounding healthy tissue. Using patient-specific parameter values, tumor simulators such as this one may in the future help to elucidate the role of heterogeneity in the tumor micro-environment and lead to improved treatment modalities by providing a quantitative means to link \textit{in vivo} tumor morphology and clinical response.

\textit{Key words:} cancer, necrosis, invasion, metastasis, computer simulation, three dimensional model

1 Introduction

Most tumors become life-threatening because of their invasive phenotype. Tumor morphology is believed to be a function of several biological factors, including cell population genetic diversity, interactions between cells and extra-

\textsuperscript{1} Corresponding author: cristini@math.uci.edu
cellular matrix, and ability by the cancer to access oxygen and life-supporting nutrients (Alberts et al., 2002). Although extensive research has been performed to investigate tumor invasion, especially at the genetic and cellular scales, our understanding is still lacking regarding the mechanisms driving spread of cancer. A quantitative analysis of morphological stability at the tumor scale may be of value to therapeutic strategies that aim to control disease extension into surrounding healthy tissue.

Cancer drug therapy, which generally can be classified as either chemotherapeutic (targeting tumor cells) or antiangiogenic (targeting vascular endothelial cells), can help control the growth of tumor lesions by triggering apoptosis in tumor or endothelial cells, respectively. However, there is recent evidence that such therapies may also unwittingly aid tumor morphological instability in at least two ways. One is by selecting for cells genetically resistant to subsequent treatment by eliminating cell species that are sensitive to the drug. The remaining tumor cell population can then aggressively expand without competition for nutrient and oxygen from drug-sensitive cells (Tannock, 2001). Another way is by creating inhomogeneous conditions, such as hypoxia, in the tumor micro-environment. It is known that hypoxia selects for cells that are more resistant to apoptosis and necrosis (Graeber et al., 1996). For example, Pennacchietti et al. (2003) have found that antiangiogenic therapy, which deprives a tumor of nutrient by blocking the formation of neovascuclature, can exacerbate hypoxic conditions and lead to increased cell motility. Tumor cohesion is thus decreased and invasiveness is increased. Furthermore, it has been observed that treatment of glioblastoma in mice with an antiangiogenic drug led to tumors becoming consistently more invasive, with satellite clusters breaking-off and migrating towards surrounding vessels (Kunkel et al., 2001; Lamszus et al., 2003), as shown in Fig. 1. Similar results were observed in a rat model (Rubenstein et al., 2000). Drug therapy may also stimulate tumor morphological instability by non-uniform drug application, exposing some tumor cells to sub-optimal drug concentrations and thus introducing variability in their therapeutic response (Durand, 2001). Drug transport to tumors may be compromised by the irregular tumor-generated vasculature and by high interstitial pressure (Jain, 2001a). However, using two-dimensional simulations, Sinek et al. (2004) recently predicted that even spatially and temporally uniform application of chemotherapeutic drugs via nanoparticles may lead to tumor fragmentation.

Novel treatment concepts have been proposed to alleviate the shortcomings of traditional drug therapy. In order to minimize induction of hypoxia during anti-angiogenic treatment and allow for uniform delivery of chemotherapy, it has been proposed to “normalize” the irregular tumor vasculature (Jain, 2001b, 2005). Normalization of blood flow to a tumor means that hypoxic regions would be reduced in number and size, decreasing the likelihood of tumor invasion. In addition, tumor cells would be exposed to higher, more
optimal drug concentrations. Recently, using the cancer simulator of Zheng et al. (2005), Cristini et al. (2005) showed that tumor morphological stability may be further enhanced by therapies that promote cellular adhesiveness in addition to tumor vascular normalization. This approach would increase tumor mass cohesion and prevent fragmentation by reducing cell motility.

This paper is the second part of a two-part series. In Part I (Wise et al., 2006) a tumor-level diffuse interface model of cancer growth, in avascular or vascular stage growth, was presented. Owing to an efficient nonlinear multigrid/finite-difference algorithm, the tumor simulator based on this model provides for the first time the capability to simulate fully three-dimensional, non-symmetric multispecies tumor growth. This model can describe biological events such as mutations that select for more malignant cell species exhibiting invasive phenotypes, development of necrotic regions, and tumor response to drug therapy. The model can also describe effects of tumor growth on gradients of nutrient and tissue pressure in the micro-environment. The model represents an improved growth module that can be plugged into the cancer simulator of Zheng et al. (2005), and therefore can be dynamically coupled to current modules for angiogenesis and chemotherapy (Sinek et al., 2004), and future components such as anti-angiogenic therapy.

In this paper we use this multispecies cancer growth simulator to predict tumor morphology in two and three dimensions. Specifically, we investigate: (i) effects of the nutrient profile in the micro-environment, and (ii) differential proliferation caused by cells consuming nutrient and undergoing apoptosis at different rates due to varying genotypes and application of drug therapy. In agreement with the conclusions of Cristini et al. (2005), our investigation in two and three dimensions indicates that even in an environment where nutrient is spatially nearly uniform (e.g., in vitro), morphological instability may occur if tumor cell adhesiveness is low. This instability triggers tumor invasion by generation of hypoxic regions (Young et al., 1988; Cairns et al., 2001; Rofstad et al., 2002) and by gradients of nutrient in the micro-environment that favor subsets of tumor cells for proliferation (Frieboes et al., 2006). Tumor invasion may thus occur without recourse to angiogenesis. The in vitro instability is characterized by sub-tumors growing off the main tumor mass, as shown in Fig. 7 for two different types of cancers. This is further evidence that the employment of adhesive-promoting therapy is vital to maintain compact, non-invasive tumors. For the study of in vivo tumors, we take parameters consistent with modeling glioma growth (Zheng et al., 2005; Frieboes et al., 2006), which is known to be highly invasive. The micro-environment of a pre-vascular, peri-necrotic tumor may be characterized by large gradients of nutrient. Our results suggest that under these conditions a tumor may undergo a more dramatic instability than that encountered in vitro, where nutrient is spatially homogeneous in the micro-environment. The in vivo instability can be characterized by elongation and growth of invasive fingers of tumor cells.
infiltrating the nutrient-rich areas of the host tissue. In both scenarios, cell adhesion has a key role in the wavelength and magnitude of the instability.

We study in more detail how tumor morphology may be affected when the tumor cell population contains varying genotypes. Main gene categories that can affect tumor growth and survival include oncogenes and tumor suppressor genes. An oncogene may enhance nutrient uptake and cell proliferation while a tumor suppressor gene affects cell death rates (Benjamin et al., 2003). Nutrient heterogeneity can be introduced when a more aggressive subset of cells, embedded randomly among other tumor cells, expresses oncogenes that increase nutrient uptake or expresses tumor suppressor genes that affect rates of cell death. Such mutations are known to occur in tumors over long periods of time, since cells with these phenotypes are favored for survival (Benjamin et al., 2003). Proliferation of cells expressing a more aggressive phenotype can also be unwittingly facilitated in a shorter amount of time by therapeutic strategies employing drugs that are more lethal to less malignant cell species, thus furthering heterogeneity in the micro-environment through non-uniform cell death. Our model predicts that mutations in oncogenes and tumor suppressor genes, as well as drug therapy applied to genetically diverse tumor cell populations, can introduce sufficient nutrient heterogeneity in the micro-environment to dramatically increase morphological instability, thus driving the cancer to aggressively invade healthy tissue. An example of an aggressive genotype is shown for human breast cancer in vitro in Fig. 7, where ERBB2 oncogene activation leads to a multiacinar phenotype (Debnath et al., 2002; Debnath and Brugge, 2005).

This paper is organized as follows. In §2 we summarize the non-dimensional model of Wise et al. (2006) in a two-species representation. In §3 we present simulations of tumor growth with a cell population of one genotype containing both viable and necrotic cell species. In §3.1 we show radially symmetric, steady-state solutions to the model. In §3.2 are two-dimensional simulations of in vitro tumor growth. In §3.3 are non-symmetric three-dimensional simulations of in vitro and in vivo tumor growth. Finally, in §4 we investigate the effect that a cell population containing two genotypes expressing varying degrees of nutrient uptake and cell death has on nutrient heterogeneity, tumor morphology, and response to therapy.

2 Diffuse Interface Model

Here we summarize the non-dimensional model from Wise et al. (2006). The dependent variables in the model are

- the mass fraction of tumor cell species 1 $\rho_1$, 

• the mass fraction of tumor cell species 2 $\rho_2$,
• the total mass fraction of both tumor cell species $\rho_T$,
• the mass fraction of the host (healthy) cells $\rho_H$,
• the concentration of vital nutrient $n$,
• the pressure in the tumor $p$, and
• the cellular velocity $u$.

The equations governing the evolution of these variables are

\[
\frac{\partial \rho_T}{\partial t} = M_{1,2} \nabla^2 \mu + S_1 + S_2 - \nabla \cdot (u\rho_T),
\]
(1)

\[
\mu = f'(\rho_T) - \varepsilon^2 \nabla^2 \rho_T,
\]
(2)

\[
\frac{\partial \rho_1}{\partial t} = M_{1,2} \nabla \cdot \left( \frac{\rho_1}{\rho_T} \nabla \mu \right) + S_1 - \nabla \cdot (u\rho_1),
\]
(3)

where $M_{1,2}$ and $\varepsilon$ are constants, and the $S_i$ are source terms for the respective cell species. The $S_i$ terms contain all the information about how the tumor mass grows and shrinks, and how mass is transferred during genetic mutation of one cell species into another. According to model approximations, the equations describing mass transport of interstitial water decouples from those for the motion of cells. The mass needed for cell mitosis is assumed to come directly from interstitial water, and, conversely, the mass lost due to cell lysing is assumed to go directly into water. As a simplifying assumption the transport of growth factors, proteins et cetera, which are required during cell proliferation, and debris, which is created during necrosis, is neglected.

The function $f$ has the form $f(\rho_T) = \frac{1}{4} \rho_T^2 (1 - \rho_T)^2$. This energy function, together with the diffuse interface mobility $M_{1,2}$ and the diffusion term $\varepsilon^2 \nabla^2 \rho_T$, control the “adhesiveness” of the tumor. In this model the interface between tumor and host tissue is diffuse, rather than sharp. The more adhesion there is, the thinner the diffuse interfacial region, i.e., the closer the diffuse interface is to being sharp. It is generally assumed that $0 \leq \rho_1 \leq \rho_T$, so that the mobility in Eq. (3) satisfies $0 \leq \rho_1/\rho_T \leq 1$. The inequality is generally enforced via the source terms.

The mass fraction of host and mass fraction of the second viable tumor cell species are found using the following relations:

\[
\rho_H = 1 - \rho_T,
\]
(4)

\[
\rho_2 = \rho_T - \rho_1.
\]
(5)

These relations come from the assumption that the total cellular density remains constant in time and space, and by the definition of the total tumor cell density: $\rho_T = \rho_1 + \rho_2$. All the density fields are approximately bounded
above by 1, the scaled uniform cell density, and bounded below by 0. Inside the tumor \( \rho_T \approx 1 \), and outside the tumor, in the host medium, \( \rho_T \approx 0 \). When we speak of a diffuse interface, it is with respect to the total tumor cell density \( \rho_T \). The tumor interface is assigned as the set \( \Sigma_T = \{ x | \rho_T(x) = 0.5 \} \).

Cells are treated as a very viscous fluid, flowing through a porous medium (e.g. the extra cellular matrix). Volume change is accounted for in the continuity equation

\[
\nabla \cdot \mathbf{u} = S_1 + S_2,
\]

and cellular motion in response to pressure is modeled by Darcy’s Law:

\[
-\delta_\varepsilon \kappa_\varepsilon \zeta_\varepsilon = \mathbf{u} + \nabla p,
\]

where the surface tension term \( \delta_\varepsilon \kappa_\varepsilon \zeta_\varepsilon \) and interpolation function \( Q \) are defined by

\[
\delta_\varepsilon = \varepsilon |\nabla \rho_T|^2 6\sqrt{2}, \quad \kappa_\varepsilon = \nabla \cdot \zeta_\varepsilon, \quad \zeta_\varepsilon = -\frac{\nabla \rho_T}{|\nabla \rho_T|},
\]

\[
Q(\rho_T) = 3\rho_T^2 - 2\rho_T^3.
\]

We model the transport of a single vital nutrient; its concentration obeys

\[
0 = \nabla \cdot (D(\rho_T) \nabla n) + T_C(\rho_T, n) - U(\rho_1, \rho_2) \frac{Q(\rho_T)}{\rho_T},
\]

\[
T_C(\rho_T, n) = \left( \nu^H_P (1 - Q(\rho_T)) + \nu^T_P Q(\rho_T) \right) (n_C - n),
\]

\[
D(\rho_T) = D_H (1 - Q(\rho_T)) + Q(\rho_T).
\]

\( D \) is the interpolated nutrient diffusivity, and \( T_C \) is a nutrient source from a pre-existing uniform vasculature. Note that the nutrient diffusivity \( D \) is constructed so that it has the constant value 1 inside the tumor (\( \rho_T = 1 \)) and the constant value \( D_H \) in the host medium (\( \rho_T = 0 \)).

The function \( U(\rho_1, \rho_2) \) models uptake of nutrients by tumor cells. As a first approximation, we model the host tissue at equilibrium, where net nutrient consumption therein is negligible compared to that of the tumor. In particular, we assume that whatever nutrient is uptaken by the host tissue is replaced by supply from the normal vasculature. This may not be the case in the tumor, where not only the uptake in general greatly exceeds the supply, but also can be much higher than that of the host tissue (Ramanathan et al.,...
2005; Esteban and Maxwell, 2005). See Tab. 1 for a brief description of the parameters introduced above, and their relation to physical parameters.

To solve for the pressure and velocity, we combine Eqs. (6) and (7) to obtain

\[ \nabla^2 p = -S_1 - S_2 - \gamma \nabla \cdot (\delta \kappa e \zeta e) . \]  

(13)

Knowing the pressure, we calculate \( u \) using Eq. (7).

These equations hold over a computational domain \( \Omega \), with boundary \( \Sigma_\infty \). The boundary conditions are taken as

\[ \mu = 0, \quad \zeta_\infty \cdot \nabla \rho_T = \zeta_\infty \cdot \nabla \rho_1 = 0, \quad n = 1, \quad p = 0, \]  

(14)

where \( \zeta_\infty \) is the outward-pointing unit normal on the outer boundary \( \Sigma_\infty \). Note that in the diffuse interface framework there are no boundary conditions at the tumor/host tissue interface \( \Sigma_T \).

2.1 Necrotic and Viable Tumor Cells

To describe a tumor comprised of dead and viable cells, we identify \( \rho_1 \) as the mass fraction of dead cells, say \( \rho_D \), and identify \( \rho_2 \) as the mass fraction of viable cells, say \( \rho_V \). For this case the source terms have the form

\[ S_1 = \frac{1}{\rho_T} (\lambda_A \rho_2 + \lambda_N H(n_N - n)\rho_2 - \lambda_L \rho_1) Q(\rho_T), \]  

(15)

\[ S_2 = \frac{1}{\rho_T} (n\rho_2 - \lambda_A \rho_2 - \lambda_N H(n_N - n)\rho_2) Q(\rho_T). \]  

(16)

The dead cell population includes the viable cells that have undergone apoptosis or necrosis. Dead cells are assumed not to consume nutrients and the nutrient uptake function is taken as

\[ U(\rho_1, \rho_2) = n\rho_2. \]  

(17)

See Tab. 2 for a brief description of the parameters used in the source terms.

2.2 Two Viable Tumor Cell Species

To describe a tumor comprised of two viable cell species, each with its own genotype, and neglecting for simplicity the build-up of necrotic cells, we use...
the following mass source terms

\[ S_1 = \left( \lambda_M^{(1)} n - \lambda_A^{(1)} - \lambda_N^{(1)} \mathcal{H} (n_N^{(1)} - n) \right) \rho_1 - \mathcal{M}^{1-2}(\mathbf{x}, t, \rho_1, \rho_2), \]  
\[ S_2 = \left( n - \lambda_A^{(2)} - \lambda_N^{(2)} \mathcal{H} (n_N^{(2)} - n) \right) \rho_2 + \mathcal{M}^{1-2}(\mathbf{x}, t, \rho_1, \rho_2), \]

where \( \mathcal{M}^{1-2} \) is a function modeling the mutation of cells of species 1 to cells of species 2. The mutation function is assumed to have the simple form

\[ \mathcal{M}^{1-2}(\mathbf{x}, t, \rho_1, \rho_2) = g(\mathbf{x}, \mathbf{x}_i) a(t, t_i) \rho_1(\mathbf{x}, t), \]

where \( g \) is a Gaussian spatially located at \( \mathbf{x}_i \), and \( a \) is a time activation function located at time \( t_i \). Precisely,

\[ g(\mathbf{x}, \mathbf{x}_i) = C \exp(-((x - x_i)^2 + (y - y_i)^2)/r^2) \]

\[ a(t, t_i) = \frac{1}{t_a} \begin{cases} 
0 & \text{if } |t - t_i| > t_a \\
(t - (t_i - t_a)) & \text{if } t_i - t_a < t \leq t_i , \\
-(t - (t_i + t_a)) & \text{if } t_i < t < t_i + t_a
\end{cases} \]

where \( C > 0 \) is a rate constant, \( r \) is a measure of the spatial extent of the mutation event, and \( t_a \) is a measure of its temporal extent. The choice of the mutation event \( (\mathbf{x}_i, t_i) \) has a random component, and the probability of spatial occurrence is assumed to be proportional to the nutrient concentration. This reflects the assumption that the number of mutated clones is greater the higher the effective rate of mitosis in the population.

The source terms \( S_1 \) and \( S_2 \), which are non-dimensionalized by the mitosis rate \( \lambda_M^{(2)} \) of Species 2 (see Tab. 3), quantitatively specify tumor volume change due to mitosis, apoptosis, and necrosis at the cellular scale. The original tumor tissue (cell species 1) is assumed to have a volume that is increased by nutrient consumption \( n \) and cell proliferation \( \lambda_M^{(1)} \), and decreased by apoptosis \( \lambda_A^{(1)} \), necrosis \( \lambda_N^{(1)} \), and mutation function \( \mathcal{M}^{1-2} \). The mutated tumor tissue (cell species 2) has volume that is likewise increased by nutrient consumption and cell proliferation, as well as mutations from Species 1, and decreased by apoptosis \( \lambda_A^{(2)} \) and necrosis \( \lambda_N^{(2)} \). The effects of the mutations on phenotype are coupled nonlinearly to the tumor scale through the numerical calculation of cellular pressure and nutrient gradients in the micro-environment and their effect on cell proliferation and apoptosis (Wise et al., 2006).

Finally, the nutrient uptake function is

\[ U(\rho_1, \rho_2) = \nu_1^{(1)} n \rho_1 + n \rho_2. \]

8
See Tab. 3 for a brief description of the parameters used in the source terms and the uptake function.

3 Tumor Invasion: Development of Diffusional Shape Instability

In this section we investigate in one, two and three dimensions the effects that the nutrient profile in a tumor micro-environment may have on tumor morphology and invasion, and present solutions to the diffuse interface model for solid tumor growth obtained using the numerical method in Wise et al. (2006).

We simulate two cases, in vitro tumor growth and in vivo tumor growth. The model tacitly assumes that tumors grow surrounded by a material, e.g. a gel or a scaffold in vitro or healthy tissue in vivo, that provides resistance to growth by sustaining a pressure field. In vitro we simulate a tumor spheroid growing under standard culture conditions, where nutrient levels are spatially nearly uniform in the extra-tumoral environment. This is simulated via a high nutrient diffusivity $D_H$ (Tabs. 4 and 5, Figs. 3 and 5). Note that the same nutrient profiles can be simulated using the nutrient diffusivity for in vivo—$D_H \approx 1$, i.e. the diffusivity is about the same in the host and the tumor—with a uniform vasculature in the extra-tumoral environment represented by nutrient production terms $\nu_H \approx 1, \nu_T = 0$. For the in vivo case simulated in this section we set the nutrient diffusivity in the host equal to that of the tumor, $D_H = 1$, and assume that there are no sources of nutrient in the tumor, $\nu_T = 0$, and the nearest capillaries are at the computational boundary, $\nu_P = 0$ and $n = 1$ on $\Sigma_\infty$. See Tab. 6 and Fig. 6.

3.1 Radially Symmetric Solutions

Radially symmetric solutions are easily computed using a sharp interface model. For the single species models used by Byrne and Chaplain (1995), Byrne and Chaplain (1996), and Cristini et al. (2003), analytic, radially symmetric solutions can be found that show the existence of steady states. That a model admits steady state solutions is crucial because stable, compact tumors are observed in vivo and in vitro. Steady states can be achieved by cell proliferation in viable tumor areas that is balanced by cell death so that the size of the tumor does not change in time. In both the diffuse and sharp interface models presented in Wise et al. (2006), the proliferation is a positive mass divergence, and death is a negative divergence, which implies that even at steady states there is nonzero flow within the tumor. Here we show that steady state solutions of the diffuse interface model can be obtained numerically.
We model the case of a tumor growing \textit{in vitro}, supposing the diffusivity of the nutrient in the medium to be 1000 times larger than that in the tumor interstitium. In all the simulations for which this is true we replace Eq. (12) with

\[ D(\rho_T) = (D_H - D_T)(1 - \rho_T)^8 + D_T, \]

(24)

where the exponent 8 numerically enforces a ratio of diffusivities approximately equal to 1000. Otherwise with Eq. (12), which is appropriate for a ratio of the diffusion coefficients less than 10, the model greatly over-predicts the diffusivity of nutrient in the diffuse tumor interface. The parameters for the test are given in Tab. 4. In Fig. 2 we calculate the steady state solutions of two two-dimensional, radially symmetric tumors with different cell viability limits \( n_N \). In Fig. 2a the necrotic limit is \( n_N = 0.6 \), and the tumor reaches a steady radius of about 3.75. For an intrinsic length scale based on diffusion and uptake of oxygen, \( \mathcal{L} = 200 \, \mu\text{m} \) (according to Zheng et al. (2005)), this represents a tumor of radius 750\( \mu\text{m} \), which is consistent with our observations \textit{in vitro} (Frieboes et al., 2006). In Fig. 2b the necrotic limit is smaller, \( n_N = 0.2 \), meaning cells remain viable at a smaller nutrient concentration. Consequently, the tumor reaches the larger radius of 6.0, or 1200 \( \mu\text{m} \). The steady states are (effectively) reached by about time \( t = 100 \); we show the solutions at time \( t = 150 \), at which time the mass and the interfacial position have negligibly increased from those values at \( t = 100 \). For a mitosis time scale \( \bar{\lambda}_M^{-1} = 1 \text{ day} \) (Zheng et al., 2005; Frieboes et al., 2006), the nondimensional time \( t = 100 \) corresponds to 100 days.

Notice in these simulations that at the steady state the velocity at the tumor interface—\( x_T \), such that \( \rho_T(x_T) = 0.5 \)—is practically zero. At steady state the cellular advection velocity \( \mathbf{u} \) need not be precisely zero at the interface, since there is an effective diffusion velocity associated with adhesion that acts against it. We also point out that there is a small amount of mass lost due to shedding at the interface. In addition, there can be a small amount lost due to numerical error. The shedding effect models some cells separating from the tumor because the adhesive force is not strong enough to hold all of the proliferating cells together. However, the degree of shedding can be controlled with introduction of a more sophisticated proliferation term and a sharper diffuse interface corresponding to higher adhesion. Inside the tumor the cellular velocity is negative, reflecting the flow of cells toward the center of the spheroid as mass is lost due to degradation of necrotic cells. There is still proliferation, but only enough to exactly balance the loss due to degradation. The interface between the necrotic core (where \( \rho_D \approx 1 \)) and the proliferating rim (where \( \rho_D \approx 0 \)) is well-delineated for this set of parameters. However, because viable cells do not become necrotic instantly, but at a certain rate (controlled by \( \lambda_N = 3.0 \)), the necrotic interface is not sharp. In fact there are some viable
cells in the region where \( n < n_N \). This situation represents what is observed in tumors grown \textit{in vitro} and \textit{in vivo}, where there can be peri-necrotic regions. In the limit as \( \lambda_N \to \infty \) the necrotic interface becomes sharp, and the nutrient concentration in the necrotic core approaches the value \( n_N \).

### 3.2 Two-Dimensional Solutions

Here we present a fully two-dimensional simulation of a tumor growing \textit{in vitro}. The parameters are the same as for the radially symmetric case, Tab. 4, with \( n_N = 0.6 \). According to the results presented above, a radially symmetric tumor with these parameters would reach a steady size with radius 3.75. The initial condition for the simulation shown as snap-shots in Fig. 3a is a slightly elliptical tumor, with its major axis along the x-direction. In particular, \( \Sigma_T = \{(x,y)|x^2/1.1 + y^2 = 2^2\} \). Initially there are no necrotic cells in the tumor, but they quickly begin to accrue since the nutrient concentration falls below 0.6 in the center of the tumor from the initial time. The step-sizes for the simulation are \( \Delta x = 20.0/256 \), \( t = 5.0 \times 10^{-3} \), and the computational domain is \( \Omega = [0, 20] \times [0, 20] \).

As in the radially-symmetric case of §3.1, we are modeling a tumor spheroid growing \textit{in vitro}, taking the nutrient diffusivity to be 1000 times larger in the medium than inside the tumor. We use the form of the nutrient diffusion coefficient in Eq. (24). This makes the nutrient level surrounding the tumor very nearly one throughout the simulation, as suggested in the radially symmetric cases, provided the tumor boundary is almost circular. However, when the tumor boundary develops finger-like protrusions, creating invaginations, there may be areas along the boundary where the nutrient is significantly lower than one (see Fig. 3b and Fig. 3c). This effect occurs because nutrient molecules must follow a more specific path to reach cells in the recesses of the invaginations. Recall that, as there is no vasculature, there is no supply of nutrients inside the outer boundary \( \Sigma_\infty \); this is modeled by setting \( \nu^* = 0 \). The adhesion energy gives rise to a relatively small body force which tends to hold the tumor together. However, we assume for this simulation that there is no excess surface tension; \textit{i.e.}, we set \( \gamma = 0 \). This is an important parameter in two- and three-dimensional simulations. It models cell adhesion and the presence of a membrane, or capsule, formed of extra-cellular matrix macromolecules that may encapsulate tumors \textit{in vitro} and \textit{in vivo}. For \( \gamma \) large, we would expect the tumor to stay compact, and nearly circular. For small (or negligible) \( \gamma \) we expect that the tumor boundary \( \Sigma_T \) can evolve to a very complicated shape, as is the case for the following simulation. A linear stability analysis of a similar model of tumor growth with surface tension was fully developed by Cristini et al. (2003) to study adhesion effects on tumor morphological stability.
It is important to point out that in this simulation the material outside the tumor is assumed to have the same hydraulic conductivity (cell mobility) as the tumor ($k_H = 1$), and thus may sustain pressure gradients as inside the tumor. The extra-tumoral material could represent a gel or porous scaffold, interpenetrated by a nutrient-rich fluid. This is different from many \textit{in vitro} experiments where tumor spheroids are grown surrounded only by nutrient in cytophobic plates. The latter case can be more complicated because the dynamics of the nutrient fluid might be important. This would require $k_H$ to be large so that only very small pressure gradients are sustained. Both cases can be simulated with the model.

As seen in Fig. 3a, at time $t = 5$ (left), the tumor has a fully developed necrotic core, and the perturbed radius of the tumor boundary is about 3.5. According to the radially symmetric results of the last section, the tumor has almost reached its diffusion-limited size, provided it remains nearly circular. However, the mode of the small perturbation (mode 2), present from the initial condition, is preserved. One can slightly discern the symmetric bulge oriented along the $x$ direction. The pressure field is highest just outside the tumor boundary, indicating that the tumor is still growing, while the nutrient is nearly one outside the almost circular tumor boundary.

The tumor is shown at $t = 20$ in Fig. 3a (right). In agreement with the linear analysis (Cristini et al., 2003), the perturbation grows and the tumor elongates along the $x$-direction. This diffusional instability enables the tumor to increase its exposure to nutrient as its surface area increases relative to its volume. Notice that the pressure has decreased to almost zero along the top and bottom of the tumor, and is largest at its right and left edges indicating continued proliferative growth of the 2-mode perturbation. At the center of the tumor, the pressure is negative indicating the presence of a mass sink in the necrotic core. We point out again that, for large enough $\gamma$, the perturbation would be completely damped out and the tumor would tend to the radially-symmetric steady state as calculated in the last section (Cristini et al., 2003). In other words, the degree of the diffusional instability strongly depends on the surface tension. Incidentally, it also depends upon the hydraulic conductivity of the extra-tumoral scaffold, which is equal to that of the tumor tissue here ($k_H = 1$).

In Fig. 3b (left), we show the snap-shot at time $t = 30$. From the pressure we see the continued (and even accelerated) growth of the perturbation, since the pressure is largest at its left and right edges. Along the mid-line at $x = 10$ the tumor is being pinched, as indicated by the drop in pressure in the areas surrounding the points $(10, 7)$ and $(10, 13)$. Notice that the inferred shape of the necrotic interface corresponds well to the shape of the contour $n = 0.6$. In Fig. 3b (right) the tumor is shown at $t = 40$, and we see that the right and left bulbs are growing rapidly. Moreover, inspecting the pressure field, it is clear
that the right and left will form two sub-bulbs each. In particular, notice that
the pressure has dropped in the regions surrounding the points (4, 10) and
(16, 10). Thus, the diffusional instability will repeat itself on the sub-bulbs.

In Fig. 3c (left) we show a close-up view of the pressure field at \( t = 45 \) for
the same simulation. Superimposed to the pressure are the three tumor cell
mass fraction contours and the cell velocity field. One can see by the cell
fluxes that the tumor is pinching in the middle, and growing at its bulbs. In
particular around the points (11, 12) and (16, 10) the velocity at the tumor
boundary points inward towards the center of the tumor, where the pressure is
lowest due to necrotic cell degradation. On the other hand, around the point
(11, 16), at the tip of the bulb where there is considerably more proliferation,
the velocity field points outward from the tumor. This phenomenon provides
a quantitative explanation for the circular growth arrangement of glioma cells
as seen in our previous \textit{in vitro} experiments (Frieboes et al., 2006).

The snap-shot at \( t = 47.5 \) is shown in Fig. 3c (right). Four bulbs are now
clearly visible. The tumor has evolved into a complicated structure in order
to maximize its surface area relative to its sustainable volume. Notice that
the shape of \( \Sigma_T \) has very near perfect reflectional symmetry of about \( x = 10 \) and \( y = 10 \), forming from an initially perturbed shape having the same
symmetries. Notice also that the nutrient is noticeably lower than one in the
regions surrounding the points (10, 7) and (10, 13), due to the reasons detailed
earlier.

Recent experiments indicate that this diffusional instability may occur \textit{in vivo}.
The tumor shown in Fig. 4 (Gatenby et al., 2006) is growing subcutaneously
in a mouse model and visible through a dorsal window. The boundary of
the mass, as visible through the window, has been highlighted to show three
major bulbs growing outward (corresponding to a low-wave-number instability,
mode 3 (Cristini et al., 2003)) towards regions of higher nutrient as the shape
instability develops. This boundary also contains a fractal component, mainly
noise, at a smaller, cellular scale. Values of pH were measured inside and
outside the tumor (Gatenby et al., 2006) and are shown in Fig. 4. Variations
in pH suggest corresponding variations in nutrient, \textit{e.g.}, glucose, since pH can
be a measure of metabolic activity as a result of nutrient consumption (Alberts
et al., 2002). Oxygen tension levels may also follow pH trends (Carlsson and
Acker, 1988), although there is evidence that local pH and pO\(_2\) levels may in
some cases not correlate (Helmlinger et al., 1997). The experimental data show
that whereas pH levels are fairly uniform outside the tumor, there exist wide
variations inside the tumor mass. Thus, variations of nutrient inside the tumor
are expected as well, leading to tumor regions proliferating faster than others.
This differential proliferation may cause the formation of tumor bulbs that
grow to become unstable, as predicted by the model. Variations of nutrient
predicted \textit{in vivo} are shown in Fig. 3 in Part I.
The evolution depicted in this simulation would have been very different had the diffusivity outside the tumor been the same as inside. In two-dimensional simulations of the latter case (results not shown), we observed that the tumor tends to elongate towards the boundary due to the large nutrient gradients that form outside. In many cases, the tumor tears apart in the middle as the tips nearest to the outer boundary $\Sigma_\infty$ grow rapidly. The general trend can be summed up by the following: if the nutrient is approximately constant outside, the tumor tends to recursively branch into bulbs; if the nutrient has large gradients outside, the tumor tends to elongate and possibly break apart. In either case we see that low wave number shape perturbations lead to unstable configurations. The two cases are documented in the following three-dimensional simulations. Finally, we note that the effects of variable diffusivity and nutrient gradients were also recently investigated by Macklin and Lowengrub (2006).

3.3 Three-Dimensional Solutions

We now present the results of non-symmetric three-dimensional simulations. The first simulation is analogous to the two-dimensional simulation shown in Fig. 3a. The parameters are given in Tab. 5, and results are shown in Figs. 5a–5d. In particular, the nutrient diffusivity outside the tumor is 1000 times larger than that inside. In the sequel we refer to this as the in vitro simulation. The parameters for the second simulation are given in Tab. 6, and a single snapshot of the evolution is shown in Fig. 6. For the second simulation, the nutrient diffusivity outside the tumor is the same as that inside, and we refer to this as the in vivo simulation. As the nutrient is fixed with $n = 1$ at the outer boundary, and there is no other source of nutrient inside the computational domain ($\nu^*_P = 0, * = H, T$), large gradients in the nutrient develop outside the tumor and contribute to a very different evolution than the in vitro case. This case models a tumor growing under nearly hypoxic conditions in vivo, with a surrounding vasculature located at the outer boundary $\Sigma_\infty$.

For both three-dimensional simulations the computational domain is $\Omega = [0, 20] \times [0, 20] \times [0, 20]$, and the spatial step size is $\Delta x = 20/128$. The time step is $\Delta t = 5.0 \times 10^{-3}$ for the in vitro simulation and $\Delta t = 1.0 \times 10^{-2}$ for the in vivo simulation. (With these parameters, the three-dimensional, radially-symmetric, steady-state tumors have final radii of approximately 3.8 (760$\mu$m) for the in vitro, and 4.3 (860$\mu$m) for the in vivo tumors.) The initial shape of the tumor interface $\Sigma_T$, composed mainly of a four- and a two-mode spherical harmonics, is the same for both simulations, and can be viewed in Fig. 5a. In Figs. 5a–6, we show the tumor interface $\Sigma_T$ and contour plots of the nutrient and pressure fields on the slice $x = 10$. On top of the pressure plot we superimpose the iso-contours $\rho_T = 0.5 \ (\Sigma_T)$, and $\rho_D = 0.5$ (the 50% necrotic contour). Note that there are no dead cells initially, and thus the
necrotic contours are not present on the pressure plot in Fig. 5a.

The initial in vitro tumor interface, shown in Fig. 5a, has a four-bulbed shape, corresponding to a low-wave-number perturbation of mode four (Cristini et al., 2003). As there are initially no necrotic cells the amount of cell growth in the tumor is relatively high, reflected by the high pressure region surrounding the tumor. Later at time $t = 12.5$, Fig. 5b, there is a fully developed necrotic core; cell proliferation has slowed and is highest at the outer tips of the bulbs. By time $t = 22.5$, Fig. 5c, the tumor is growing very slowly, and is beginning to collapse in the center as it changes shape to maximize access to the limited nutrient supply. The nature of the diffusional instability is much more complicated in three dimensions than in two dimensions, as can be seen from the snapshot at time $t = 32.5$, Fig. 5d. By this time each of the four outer bulbs have divided into two sub-bulbs, and the center of the tumor has further collapsed. While the shape of $\Sigma_T$ is complicated, it is not unexpected. The more folded the surface becomes, the more nutrient can be accessed by proliferating cells (Cristini et al., 2005). A similar type of growth has been observed in vitro. Glioblastoma spheroids grown in culture grew by generating sub-spheroids on their surface, as shown in Figure 7 (Frieboes et al., 2006). These sub-spheroids could recursively divide into sub-spheroids as predicted by both the two- and three-dimensional simulations for which $D_H = 1000$. A similar effect is seen with transformed mammary epithelial cells, also in Figure 7 (Debnath et al., 2002), where tumor growth leads to a complicated morphology. In this case, excess proliferation and luminal filling lead to a morphology composed of multiple acinar structures (Debnath and Brugge, 2005).

The simulated in vivo tumor is shown at the simulation time $t = 45$ in Fig. 6. The evolution of the tumor boundary in this case is different from that of the simulated in vitro tumor. Since there are large nutrient gradients outside, the tumor elongates as the four bulb tips grow increasingly faster towards the outer, nutrient rich boundary $\Sigma_\infty$, where the capillaries are. In this case there is no great advantage for the tumor interface $\Sigma_T$ to become folded in a complicated way because tumor mass growth is favored by its proximity to the boundary. In fact, the tumor has split into two halves to allow it better access to the boundary. This is a common characteristic of a tumor growing in a high-nutrient-gradient environment, according to the model, suggesting one possible mechanism for tumor invasion in vivo. Note that if there is excess surface tension, $\gamma > 0$, as in Fig. 4 of Part I (Wise et al., 2006), this splitting may be delayed in time, Fig. 4a ($\gamma = 0.1$), or suppressed entirely, Fig. 4b ($\gamma = 1.0$).
4 Tumor Invasion: Effect of Nutrient Heterogeneity

In §3 we investigated how perturbations in the tumor micro-environment may lead, when cell adhesion forces are relatively weak, to progression of a tumor shape instability. Here we further investigate the effects that nutrient heterogeneity may have on inducing perturbations of tumor morphology leading to infiltration into the surrounding host tissue. We use parameters consistent with modeling glioma growth (Zheng et al., 2005; Frieboes et al., 2006) via a fully two-dimensional simulation in vivo. Glioma progression is characterized by unscheduled glial cell proliferation and infiltration of normal brain tissue (Merlo, 2003). Although there is abundant information about the biological and clinical behavior of these tumors and the genetic pathways involved (Maher et al., 2001), a quantitative link between genotype and disease progression remains elusive. Glioma development exhibits a complex interaction of multiple, nonrandom genetic events that include activation of proto-oncogenes and inactivation of tumor suppressor genes (Benjamin et al., 2003).

For simplicity, here we assume that the tumor cell population contains two transformed species, each characterized by a genotype consisting of a set of oncogenes and a set of tumor suppressor genes. We assume that a set of oncogenes can increase uptake of nutrient twice the normal rate, and a set of mutated tumor suppressor genes down-regulates the apoptosis rate by about 10%. These rates affect the simulated tumor species evolution as described in §2.2. We denote Species 2 as the more aggressive species. Physical parameters are shown in Tab. 7. In this initial study, we assume that nutrient levels remain sufficiently high so that the rate of cell apoptosis dominates the rate of cell necrosis.

The computational domain is $\Omega = [0, 160] \times [0, 160]$, representing $16 \times 16$ mm$^2$, the mesh size is $256 \times 256$, and $\epsilon = 0.2$. The spatial resolution of the interface is half of what is recommended in §4.2, Part I—there are only two or three mesh points on the tumor interface—in order to accommodate a larger domain size so that disease progression can be studied over a sufficiently long simulation time to represent 10 months in vivo. As a result, the tumor boundary may appear squarish (Figs. 8b and 8f). Shorter simulation times may not match disease progression observed clinically (Maher et al., 2001). Results herein are expected to be qualitatively similar to those obtained at a higher resolution because nutrient heterogeneity, which is hypothesized to be the primary mechanism of morphological instability, is adequately resolved. This constraint imposed by the current numerical solution will be significantly alleviated in the future with the inclusion of an adaptive mesh technology (Part I, §5).

At the beginning, Fig. 8a, a small tumor grows with only one cell species,
Species 1. The morphology is stable and compact. Species 2 is not present and nutrient is homogeneously distributed in the tumor micro-environment. In Fig. 8b we see that the growth is still compact after about two months’ time, when Species 2 is still not present. Nutrient continues to be uniformly maintained. Fig. 8c shows the tumor after four months, when the growth of the second species, generated by random mutation from Species 1 as described in §2.2, begins to deform the tumor mass. This distension represents the tumor mass response to the perturbation in the nutrient field caused by Species 2, which introduces heterogeneity in the tumor micro-environment by uptaking nutrient at twice the rate of Species 1 and dying at a 10% lower rate.

At about six months’ time we simulate a uniform therapy by increasing the two species’ apoptosis rates $\lambda_1^A$ and $\lambda_2^A$ by 50% for a simulated time of 10 days. At the beginning of therapy, Fig. 8d, Species 2 is fingerling throughout the tumor as its proliferation is starting to compromise tumor compactness. The diffusional shape instability driven by nutrient gradients and triggered by the perturbation introduced by Species 2 is developing similarly to the in vivo cases presented in §3. Significant heterogeneity in nutrient is present, and tumor growth at this point is very unstable. Significant cell death also develops as a consequence of increased nutrient uptake by Species 2. Without the application of therapy, similar results to those in the previous section will eventually be obtained (results not shown), in which the tumor morphology is observed to evolve into a complicated shape that maximizes access to nutrient.

After therapy, however, the tumor mass regresses, although both Species 1 and 2 are still proliferating two months later as shown in Fig. 8e. A partial regression of tumor mass and eventual regrowth correspond to the results obtained by Sinek et al. (2004), where initial tumor shrinkage and later recovery were seen during the modeling of uniform chemotherapeutic drug application. Indeed, the effects of therapy are short-lived, since at ten months, Fig. 8f, the tumor is seen to have resumed growth and infiltration of surrounding tissue, driven by aggressive proliferation of both species. Paradoxically, one of the consequences of therapy has been an increased invasiveness of the two species, shown as extensive fingering into healthy tissue and perhaps due to the therapy-induced exacerbation of nutrient heterogeneity. This result corresponds to experimental observations in vivo and in vitro (Young et al. (1988), Cairns et al. (2001), Rofstad et al. (2002)), where hypoxia was found to trigger aggressive tumor invasion.

Thus, in almost a year of simulated time, the surrounding host tissue has been severely compromised by the invasive tumor morphology. Genetic mutations that lead to increases in nutrient consumption in certain regions of a tumor create nutrient inhomogeneity in the micro-environment. This inhomogeneity may destabilize the tumor morphology, reinforcing some regions that are favored for proliferation and others for cell death (Cristini et al.,
A genotype that aggressively consumes nutrient is therefore predicted to be a powerful driver of unstable tumor morphologies, leading to invasion of surrounding tissue as is shown in the simulation. This process involves growth-generated shape instabilities that combine with genotype-generated morphological instabilities to foment tumor spread via invasive fingering. The process is self-reinforcing based on nutrient heterogeneity because of the way that the model incorporates the effects of mutations as a function of nutrient consumption. The model also predicts that application of therapy, even in a uniform manner, may destabilize tumor morphology and lead to increased invasive potential by selecting tumor regions for optimal proliferation.

The unrestrained growth of certain cancers, such as gliomas, imposes an increased nutrient requirement that is probably due to higher rates of cell proliferation. This condition occurs via genetic transformations that affect cell proliferation and nutrient uptake (Benjamin et al., 2003). This growth can lead to hypoxic tumor regions and development of necrosis, which in turn trigger angiogenesis (Principi et al., 2003) and enhanced invasive capability. Indeed, among gliomas, glioblastomas are the most aggressive, and the only brain tumors that develop necrosis (Principi et al., 2003). Our modeling results (Fig. 8a–Fig. 8f) are in good qualitative agreement with typical glioblastoma characteristics observed in vivo through magnetic resonance imaging. Based on parameters calibrated from in vitro glioblastoma growth (Frieboes et al., 2006), the model predicts that strong infiltration of host tissue by the tumor follows the onset of significant cell death, and that a tumor size of about 1.2 cm diameter is reached within six months. Results presented here provide a theoretical explanation for the observed biological phenomena, supporting the hypothesis that nutrient heterogeneity introduced in the tumor microenvironment by genetic mutations affecting nutrient uptake and apoptosis could be the mechanism by which gliomas progress to become glioblastomas.

5 Summary and Future Work

We provide computational evidence that tumor morphological stability may be a function of the nutrient profile in the micro-environment and of cell differential proliferation due to nutrient inhomogeneity. In two dimensions we show that beginning with an ellipsoidal tumor shape, complex morphologies develop as tumors grow and form a necrotic core. We also show that, depending upon the sizes of nutrient gradients exterior to the tumors, very different shape instabilities could occur. For example, if nutrient gradients are low, morphologies are characterized by recursive branching, and if nutrient gradients are high, they are characterized by accelerated tip growth and tumor elongation. In three dimensions, starting with a four-plus-two mode initial tumor interface, the instability that develops is similar. In the case of low nutrient
gradients, recursive branching occurs as in the 2-D case but in a spatially more complex manner. Using a fully two-dimensional simulation containing two cell genotypic species, with one of them representing higher cell nutrient uptake and a lower cell death rate, we show that tumor morphology can become unstable and exhibit aggressive fingering into surrounding tissue, predicting disease progression and morphologies seen clinically. This instability is caused by uneven rates of nutrient uptake and death of the two cell species, which generate inhomogeneous gradients of nutrient in an environment containing heterogeneous nutrient profiles. We also showed that morphological instability can be exacerbated through the uniform application of drug therapy, which may further nutrient heterogeneity by selecting for more aggressive cell species. Therapy is seen to augment the invasive potential, perhaps due to an increase in hypoxia. These results support vascular normalization (Jain, 2001b, 2005) and maintenance of cell adhesion as means to enhance tumor compactness and facilitate tumor resection (Cristini et al., 2005).

In future work we plan to model the effect of specific genetic mutations on tumor morphology to explore postulated quantitative links between genotype and morphology, and to study the effects of therapy in the tumor micro-environment. It is unclear which phenotypic characteristics correspond with which karyotypic abnormalities, such as the ability to invade or develop resistance to cytotoxic agents (Benjamin et al., 2003). Here we established a relationship between glioma morphology and genotype via inhomogeneity of the mass exchange and nutrient uptake rates. From a phenomenological standpoint, these inhomogeneities, which are genetic in origin, are the direct cause of tumor-level invasiveness. While this is consistent with observation, further study is needed to establish a definitive causal link. By obtaining experimental in vitro and in vivo tumor data, model parameters can be set more accurately and the model formulation may be improved. The study of complex tumor morphologies and their response to therapy based on these parameters may then ultimately translate into clinical application by providing a means to enable more accurate selection of treatment on a patient-specific basis.

Acknowledgements

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References

Jain, R., 2005. Normalization of tumor vasculature: An emerging concept in
**List of Tables**

1. Mechanical response and nutrient parameters. Parameters with an overbar are dimensional and are defined in Wise et al. (2006). The parameter $\mathcal{L}$, used to nondimensionalize the space, is the diffusion length scale (Zheng et al., 2005; Wise et al., 2006).

2. Nondimensional mass transfer rates for the viable/necrotic model. Parameters carrying an overbar are the corresponding dimensional parameters; $\bar{\lambda}_M$ is the dimensional mitosis rate of viable cells.

3. Nondimensional mass transfer rates for the model with two viable species. Parameters carrying an overbar are the corresponding dimensional parameters. $\bar{\lambda}_M^{(2)}$ is the dimensional mitosis rate and $\bar{\nu}_U^{(2)}$ is the dimensional nutrient uptake rate for the second viable species.

4. Nondimensional parameters used in the two-dimensional numerical simulations shown in Figs. 2 and 3a-3c.

5. Nondimensional parameters used in the three-dimensional numerical simulation shown in Figs. 5a-5d. The steady-state, radially-symmetric tumor with these parameters will have a radius of about 3.8.

6. Nondimensional parameters used in the three-dimensional numerical simulation shown in Fig. 6. These parameters are chosen such that the corresponding steady-state, radially-symmetric tumor will also have a radius of about 4.3. Compare with those in Tab. 5

7. Nondimensional parameters used in the two-dimensional numerical simulation of two cell species shown in Fig. 8.
<table>
<thead>
<tr>
<th>Rate of nutrient transfer from vasculature to healthy tissue</th>
<th>$\nu_H^P = \tilde{\nu}_H^P / \tilde{\nu}_U$</th>
<th>Rate of nutrient transfer from vasculature to tumor tissue</th>
<th>$\nu_T^P = \tilde{\nu}_T^P / \tilde{\nu}_U$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient level in the blood</td>
<td>$n_C = \tilde{n}<em>C / \tilde{n}</em>\infty$</td>
<td>Diffusivity in healthy tissue</td>
<td>$D_H = \tilde{D}_H / \tilde{D}_T$</td>
</tr>
<tr>
<td>Surface tension</td>
<td>$\gamma = \tilde{\gamma} / (L^2 \tilde{p}_T)$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1
Mechanical response and nutrient parameters. Parameters with an overbar are dimensional and are defined in Wise et al. (2006). The parameter $L$, used to nondimensionalize the space, is the diffusion length scale (Zheng et al., 2005; Wise et al., 2006).
<table>
<thead>
<tr>
<th>Rate of apoptosis</th>
<th>$\lambda_A = \bar{\lambda}_A / \bar{\lambda}_M$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of necrosis</td>
<td>$\lambda_N = \bar{\lambda}_N / \bar{\lambda}_M$</td>
</tr>
<tr>
<td>Rate of lysing</td>
<td>$\lambda_L = \bar{\lambda}_L / \bar{\lambda}_M$</td>
</tr>
</tbody>
</table>

Table 2
Nondimensional mass transfer rates for the viable/necrotic model. Parameters carrying an overbar are the corresponding dimensional parameters; $\bar{\lambda}_M$ is the dimensional mitosis rate of viable cells.
Rate of apoptosis in $i^{th}$ species

\[ \lambda^{(i)}_A = \frac{\bar{\lambda}^{(i)}_A}{\bar{\lambda}^{(2)}_M} \]

Rate of necrosis in $i^{th}$ species

\[ \lambda^{(i)}_N = \frac{\bar{\lambda}^{(i)}_N}{\bar{\lambda}^{(2)}_M} \]

Rate of mitosis in first species

\[ \lambda^{(1)}_M = \frac{\bar{\lambda}^{(1)}_M}{\bar{\lambda}^{(2)}_M} \]

Rate of nutrient uptake by first species

\[ \nu^{(1)}_U = \frac{\bar{\nu}^{(1)}_U}{\bar{\nu}^{(2)}_U} \]

<table>
<thead>
<tr>
<th>Rate of apoptosis in $i^{th}$ species</th>
<th>$\lambda^{(i)}_A = \frac{\bar{\lambda}^{(i)}_A}{\bar{\lambda}^{(2)}_M}$</th>
<th>Rate of necrosis in $i^{th}$ species</th>
<th>$\lambda^{(i)}_N = \frac{\bar{\lambda}^{(i)}_N}{\bar{\lambda}^{(2)}_M}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of mitosis in first species</td>
<td>$\lambda^{(1)}_M = \frac{\bar{\lambda}^{(1)}_M}{\bar{\lambda}^{(2)}_M}$</td>
<td>Rate of nutrient uptake by first species</td>
<td>$\nu^{(1)}_U = \frac{\bar{\nu}^{(1)}_U}{\bar{\nu}^{(2)}_U}$</td>
</tr>
</tbody>
</table>

Table 3

Nondimensional mass transfer rates for the model with two viable species. Parameters carrying an overbar are the corresponding dimensional parameters. $\bar{\lambda}^{(2)}_M$ is the dimensional mitosis rate and $\bar{\nu}^{(2)}_U$ is the dimensional nutrient uptake rate for the second viable species.
Table 4
Nondimensional parameters used in the two-dimensional numerical simulations shown in Figs. 2 and 3a-3c.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu^H_T = \nu^H_T/\nu_U$</td>
<td>0.0</td>
</tr>
<tr>
<td>$\nu^T_T = \nu^T_T/\nu_U$</td>
<td>0.0</td>
</tr>
<tr>
<td>$D_H = D_H/D_T$</td>
<td>$1.0 \times 10^3$</td>
</tr>
<tr>
<td>$n_N = n_N/n_\infty$</td>
<td>0.6 (Figs. 2a and 3)</td>
</tr>
<tr>
<td>$\lambda_A = \lambda_A/\lambda_M$</td>
<td>0.0</td>
</tr>
<tr>
<td>$\lambda_N = \lambda_N/\lambda_M$</td>
<td>3.0</td>
</tr>
<tr>
<td>$\lambda_L = \lambda_L/\lambda_M$</td>
<td>1.0</td>
</tr>
<tr>
<td>$\gamma = \bar{\gamma}/(L^2 p_T)$</td>
<td>0.0</td>
</tr>
<tr>
<td>$M$</td>
<td>5.0</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>0.1</td>
</tr>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
</tr>
<tr>
<td>$\nu_P^H = \tilde{\nu}_P^H / \tilde{\nu}_U$</td>
<td>0.0</td>
</tr>
<tr>
<td>$D_H = \tilde{D}_H / \tilde{D}_T$</td>
<td>$1.0 \times 10^3$</td>
</tr>
<tr>
<td>$\lambda_A = \tilde{\lambda}_A / \tilde{\lambda}_M$</td>
<td>0.0</td>
</tr>
<tr>
<td>$\lambda_L = \tilde{\lambda}_L / \tilde{\lambda}_M$</td>
<td>1.5</td>
</tr>
<tr>
<td>$M$</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 5
Nondimensional parameters used in the three-dimensional numerical simulation shown in Figs.5a-5d. The steady-state, radially-symmetric tumor with these parameters will have a radius of about 3.8.
Table 6
Nondimensional parameters used in the three-dimensional numerical simulation shown in Fig. 6. These parameters are chosen such that the corresponding steady-state, radially-symmetric tumor will also have a radius of about 4.3. Compare with those in Tab. 5.
Table 7
Nondimensional parameters used in the two-dimensional numerical simulation of two cell species shown in Fig. 8.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu_P^H$</td>
<td>$\bar{\nu}_P^H / \bar{\nu}_U$</td>
</tr>
<tr>
<td>$\nu_U^{(1)}$</td>
<td>$\bar{\nu}_U^{(1)} / \bar{\nu}_U^{(2)}$</td>
</tr>
<tr>
<td>$\nu_U^{(2)}$</td>
<td>$\bar{\nu}_U^{(2)} / \bar{\nu}_U^{(2)}$</td>
</tr>
<tr>
<td>$\lambda_A^{(1)}$</td>
<td>$\bar{\lambda}_A^{(1)} / \bar{\lambda}_A^{(1)}$</td>
</tr>
<tr>
<td>$\lambda_A^{(2)}$</td>
<td>$\bar{\lambda}_A^{(2)} / \bar{\lambda}_A^{(2)}$</td>
</tr>
<tr>
<td>$\lambda_N^{(1)}$</td>
<td>$\bar{\lambda}_N^{(1)} / \bar{\lambda}_M^{(1)}$</td>
</tr>
<tr>
<td>$\lambda_N^{(2)}$</td>
<td>$\bar{\lambda}_N^{(2)} / \bar{\lambda}_M^{(2)}$</td>
</tr>
<tr>
<td>$\lambda_M^{(2)}$</td>
<td>$\bar{\lambda}_M^{(1)} / \bar{\lambda}_M^{(1)}$</td>
</tr>
<tr>
<td>$D_H$</td>
<td>$\bar{D}_H / \bar{D}_T$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>$\bar{\gamma} / (L^2 p_T)$</td>
</tr>
</tbody>
</table>
List of Figures

1 Human glioblastoma tumor growing in mice treated with anti-angiogenic therapy. Unlike untreated mice, the tumor is surrounded by numerous smaller satellite tumors as well as individual cells that have separated from the main tumor to co-opt existing vasculature. Adapted from Kunkel et al. (2001).

2 Two-dimensional, radially symmetric steady state solutions, shown at nondimensional time \( t = 150 \). In (a) \( n_N = 0.6 \) and the final radius is about 3.75, and in (b) \( n_N = 0.2 \), with final radius 6.0.

3a Two-dimensional tumor at time \( t = 5 \) (left, top and bottom). Nutrient concentration \( n \) and pressure \( p \) with two tumor cell density contours superimposed (outer solid contour line is tumor interface \( \Sigma_T \), inner dashed contour is the line of \( \rho_D = 0.5 \)). The initial \( \Sigma_T \) (not shown) is a perturbed circle of radius \( \approx 3.5 \). At \( t = 20 \) (right, top and bottom), the tumor begins to elongate as a consequence of diffusional instability.

3b Two-dimensional tumor at time \( t = 30 \) (left, top and bottom), continuing the evolution from Fig. 3a. The tumor forms symmetrical bulbs and begins to pinch in the middle. At \( t = 40 \) (right, top and bottom), the same instability repeats on the sub-bulbs.

3c A close-up view of the pressure field shown at \( t = 45 \) (left); and the nutrient and pressure fields at \( t = 47.5 \) (right, top and bottom), concluding the evolution started in Fig. 3a. Superimposed on the pressure plot on the left are the tumor cell density contours (as in Fig. 3a and Fig. 3b) and the cell velocity field. This shows that cells at the tip of the bulb have a net outward velocity due to higher proliferation because of better access to nutrient, while the lower proliferation of cells in the invaginated, lower nutrient regions leads to mass involution. By \( t = 47.5 \) the tumor has evolved by diffusional instability into a complicated, but highly symmetric shape. Four bulbs have formed.
Top: Human pancreatic tumor xenograft growing *in vivo* as seen through a dorsal window in a mouse model. The outer boundary of the tumor is delineated revealing growing low-wave-number fluctuations (roughly, a nearly symmetrical mode 3). Bottom: pH gradients measured along radii emanating from the center of the tumor. While levels are fairly uniform outside the tumor, a decrease and large gradients are detected inside the tumor mass, suggesting corresponding variation in nutrient levels. According to our mathematical model, these gradients trigger diffusional shape instability. Values were measured in six sectors, denoted by the angles. Adapted from (Gatenby et al., 2006).

5a The initial conditions for a growing three-dimensional *in vitro* tumor. The tumor interface $\Sigma_T$ is a four-bulbed shape, and the growth parameters are in Tab. 5. The computational domain is $\Omega = [0, 20] \times [0, 20] \times [0, 20]$. Contour plots are shown for the nutrient and pressure fields along the $x = 10$ slice though $\Omega$, with $\Sigma_T$ superimposed on the pressure plot. Initially there are no necrotic cells in the tumor, and the high pressure field surrounding the tumor indicates proliferation of tumor cells.

5b Snapshot of the growing *in vitro* tumor at time $t = 12.5$. Superimposed on the pressure plot are the iso-contours $\rho_T = 0.5$, and $\rho_D = 0.5$. The tumor has developed a necrotic core and grown in size, while maintaining its four-bulbed perturbed shape.

5c Snapshot of the *in vitro* tumor at $t = 22.5$. As the diffusional instability develops, the tumor continues to grow at its outer bulbs but begins to collapse in the center.

5d Snapshot of the *in vitro* tumor at $t = 32.5$. The tumor evolves to very complicated shape that is the 3D analogue of that in Fig. 3c for a 2D tumor. The four outer bulbs have each split into two sub-bulbs, and the center of the tumor has continued to collapse.
6 Snapshot of a growing in vivo tumor at time $t = 45$. The parameters for the simulation are given in Tab. 6. The initial shape of the tumor boundary for this simulation is the same four-bulbed shape as the one in Fig. 5a. Since the nutrient diffusion coefficient is equal to that inside the tumor, large nutrient gradients develop outside. Rather than growing into a highly folded shape, the tumor elongates to reach the high levels of nutrient at $\Sigma_\infty$.

7 Left, top and bottom: human glioblastoma spheroids growing in culture. Low wave-number fluctuations on the tumor surface are clearly visible in the top photograph, while recursive sub-bulbing is observed in the bottom photograph. Bar = 130 $\mu$m. Adapted from Frieboes et al. (2006). Right: transformed human mammary epithelial cells growing in culture form a complicated structure characterized by recursive acinus formation. Adapted from Debnath et al. (2002). The model predicts that the morphologies in these cases are caused by gradients of nutrient in the micro-environment that favor subsets of tumor cells for proliferation, as simulated in two and three dimensions in the previous figures.

8a Upper left: initial condition ($t = 0$) of a small glioma simulated growing in vivo with only one cell species, Species 1 ($c_1$ denotes Species 1 cell density). Upper right: Species 2 is not present, as there have not occurred mutations to generate it from Species 1 ($c_2$ denotes Species 2 cell density). Bottom: nutrient is uniformly distributed around the tumor.

8b Upper left: simulated glioma growth is compact at $t = 60$ (ca. two months). Upper right: Species 2 is not yet present. Bottom: nutrient continues to be uniformly distributed.

8c Upper left: Simulated glioma at $t = 120$ (ca. four months), when the second species, generated by mutation from Species 1, begins to deform the tumor mass. Upper right: Species 2 is exerting its presence on the tumor morphology (see lower left tumor corner) by uptaking nutrient at twice the rate of Species 1 and dying at a 10% slower rate. Bottom: Nutrient heterogeneity starts to be introduced by the growth of Species 2.
8d Simulation of therapy is started at $t = 180$ (ca. six months) by increasing apoptosis rate for both species by a factor of two for ten days, i.e., for $180 \leq t \leq 190$. Upper left: At the start of therapy, tumor morphology is already visibly affected by the aggressive proliferation of Species 2. Upper right: Species 2 is fingering throughout the tumor. Bottom: heterogeneity in nutrient is present where Species 2 proliferates the most. Note low level of nutrient that has developed at tumor lower left corner, where most of Species 2 resides.

8e Upper left: at $t = 240$ (ca. eight months) the tumor shows regression as a result of therapy. Upper left: Species 1 exhibits the most regression. Upper right: regression of Species 2 within the tumor mass. Bottom: nutrient profile continues to reflect inhomogeneity caused by presence of the two species and as a result of therapy.

8f Tumor resumes growth after therapy at $t = 300$ (ca. ten months). Upper left: Species 1 becomes more invasive, fingering into host tissue. Upper right: The overall invasive phenotype follows Species 2 due to its aggressive proliferation. Bottom: nutrient inhomogeneity is present throughout the tumor as a consequence of increased nutrient uptake by Species 2.
Fig. 1. Human glioblastoma tumor growing in mice treated with anti-angiogenic therapy. Unlike untreated mice, the tumor is surrounded by numerous smaller satellite tumors as well as individual cells that have separated from the main tumor to co-opt existing vasculature. Adapted from Kunkel et al. (2001).
Fig. 2. Two-dimensional, radially symmetric steady state solutions, shown at nondimensional time $t = 150$. In (a) $n_N = 0.6$ and the final radius is about 3.75, and in (b) $n_N = 0.2$, with final radius 6.0.
Fig. 3a. Two-dimensional tumor at time $t = 5$ (left, top and bottom). Nutrient concentration $n$ and pressure $p$ with two tumor cell density contours superimposed (outer solid contour line is tumor interface $\Sigma_T$, inner dashed contour is the line of $\rho_D = 0.5$). The initial $\Sigma_T$ (not shown) is a perturbed circle of radius $\approx 3.5$. At $t = 20$ (right, top and bottom), the tumor begins to elongate as a consequence of diffusional instability.
Fig. 3b. Two-dimensional tumor at time $t = 30$ (left, top and bottom), continuing the evolution from Fig. 3a. The tumor forms symmetrical bulbs and begins to pinch in the middle. At $t = 40$ (right, top and bottom), the same instability repeats on the sub-bulbs.
Fig. 3c. A close-up view of the pressure field shown at $t = 45$ (left); and the nutrient and pressure fields at $t = 47.5$ (right, top and bottom), concluding the evolution started in Fig. 3a. Superimposed on the pressure plot on the left are the tumor cell density contours (as in Fig. 3a and Fig. 3b) and the cell velocity field. This shows that cells at the tip of the bulb have a net outward velocity due to higher proliferation because of better access to nutrient, while the lower proliferation of cells in the invaginated, lower nutrient regions leads to mass involution. By $t = 47.5$ the tumor has evolved by diffusional instability into a complicated, but highly symmetric shape. Four bulbs have formed.
Fig. 4. Top: Human pancreatic tumor xenograft growing *in vivo* as seen through a dorsal window in a mouse model. The outer boundary of the tumor is delineated revealing growing low-wave-number fluctuations (roughly, a nearly symmetrical mode 3). Bottom: pH gradients measured along radii emanating from the center of the tumor. While levels are fairly uniform outside the tumor, a decrease and large gradients are detected inside the tumor mass, suggesting corresponding variation in nutrient levels. According to our mathematical model, these gradients trigger diffusional shape instability. Values were measured in six sectors, denoted by the angles. Adapted from (Gatenby et al., 2006).
Fig. 5a. The initial conditions for a growing three-dimensional *in vitro* tumor. The tumor interface $\Sigma_T$ is a four-bulbed shape, and the growth parameters are in Tab. 5. The computational domain is $\Omega = [0, 20] \times [0, 20] \times [0, 20]$. Contour plots are shown for the nutrient and pressure fields along the $x = 10$ slice though $\Omega$, with $\Sigma_T$ superimposed on the pressure plot. Initially there are no necrotic cells in the tumor, and the high pressure field surrounding the tumor indicates proliferation of tumor cells.
Fig. 5b. Snapshot of the growing *in vitro* tumor at time $t = 12.5$. Superimposed on the pressure plot are the iso-contours $\rho_T = 0.5$, and $\rho_D = 0.5$. The tumor has developed a necrotic core and grown in size, while maintaining its four-bulbed perturbed shape.
Fig. 5c. Snapshot of the \textit{in vitro} tumor at $t = 22.5$. As the diffusional instability develops, the tumor continues to grow at its outer bulbs but begins to collapse in the center.
Fig. 5d. Snapshot of the *in vitro* tumor at $t = 32.5$. The tumor evolves to very complicated shape that is the 3D analogue of that in Fig. 3c for a 2D tumor. The four outer bulbs have each split into two sub-bulbs, and the center of the tumor has continued to collapse.
Fig. 6. Snapshot of a growing in vivo tumor at time $t = 45$. The parameters for the simulation are given in Tab. 6. The initial shape of the tumor boundary for this simulation is the same four-bulbed shape as the one in Fig. 5a. Since the nutrient diffusion coefficient is equal to that inside the tumor, large nutrient gradients develop outside. Rather than growing into a highly folded shape, the tumor elongates to reach the high levels of nutrient at $\Sigma_{\infty}$. 
Fig. 7. Left, top and bottom: human glioblastoma spheroids growing in culture. Low wave-number fluctuations on the tumor surface are clearly visible in the top photograph, while recursive sub-bulbing is observed in the bottom photograph. Bar = 130 µm. Adapted from Frieboes et al. (2006). Right: transformed human mammary epithelial cells growing in culture form a complicated structure characterized by recursive acinus formation. Adapted from Debnath et al. (2002). The model predicts that the morphologies in these cases are caused by gradients of nutrient in the micro-environment that favor subsets of tumor cells for proliferation, as simulated in two and three dimensions in the previous figures.
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