Published in final edited form as:

Bull Math Biol. 2012 November ; 74(11): 2752–2777. doi:10.1007/s11538-012-9776-3.

Hypoxia Inducible Factors-Mediated Inhibition of Cancer by GM-CSF: A Mathematical Model

Duan Chen,

Mathematical Biosciences Institute, The Ohio State University, Columbus, OH, USA

Julie M. Roda,

Division of Pulmonary, Allergy, Critical Care & Sleep Medicine, College of Medicine, The Ohio State University, Columbus, OH, USA

Clay B. Marsh,

Division of Pulmonary, Allergy, Critical Care & Sleep Medicine, College of Medicine, The Ohio State University, Columbus, OH, USA

Timothy D. Eubank, and

Division of Pulmonary, Allergy, Critical Care & Sleep Medicine, College of Medicine, The Ohio State University, Columbus, OH, USA

Avner Friedman

Mathematical Biosciences Institute, The Ohio State University, Columbus, OH, USA

Department of Mathematics, The Ohio State University, Columbus, OH, USA

Duan Chen: chen.906@mbi.osu.edu

Abstract

Under hypoxia, tumor cells, and tumor-associated macrophages produce VEGF (vascular endothelial growth factor), a signaling molecule that induces angiogenesis. The same macrophages, when treated with GM-CSF (granulocyte/macrophage colony-stimulating factor), produce sVEGFR-1 (soluble VEGF receptor-1), a soluble protein that binds with VEGF and inactivates its function. The production of VEGF by macrophages is regulated by HIF-1 α (hypoxia inducible factor-1 α), and the production of sVEGFR-1 is mediated by HIF-2 α . Recent experiments measured the effect of inhibiting tumor growth by GM-CSF treatment in mice with HIF-1 α -deficient or HIF-2 α -deficient macrophages. In the present paper, we represent these experiments by a mathematical model based on a system of partial differential equations. We show that the model simulations agree with the above experiments. The model can then be used to suggest strategies for inhibiting tumor growth. For example, the model qualitatively predicts the extent to which GM-CSF treatment in combination with a small molecule inhibitor that stabilizes HIF-2 α will reduce tumor volume and angiogenesis.

Keywords

Free boundary model; Tumor growth

1 Introduction

Macrophages play an important role in tumor growth and progression (Pollard 1997). In murine models, breast tumor cells can secrete factors that usurp normal macrophage antitumor function, like macrophage colony-stimulating factor (M-CSF), and induce a phenotype switch to pro-tumor “tumor-associated macrophages” or TAMs that can help aid in tumor progression. These TAMs, recruited by monocyte chemoattractant protein-1 (MCP-1/CCL2), secrete vascular endothelial growth factor (VEGF), thereby promoting angiogenesis and tumor growth. Interestingly, a second growth factor, granulocyte/macrophage colony-stimulating factor (GM-CSF), has been shown to “re-educate” TAMs back into a more tumor-fighting phenotype. Treating macrophages with GM-CSF induces the production of soluble VEGF receptor-1 (sVEGFR-1), which binds and sequesters VEGF, neutralizing its proliferative effects on endothelial cells. Thus, GM-CSF tends to reverse the pro-tumor “education” of macrophages (Eubank et al. 2003, 2004, 2009). The transcriptional response to hypoxia is driven by a family of hypoxia inducible factors (HIFs). The alpha subunits (HIF-1 α and HIF-2 α) are constitutively-expressed but are rapidly degraded under normoxic conditions. Since oxygen is the rate-limiting cofactor in the hydroxylation reaction leading to degradation, the HIF proteins accumulate when the prolyl hydroxylase domain (PHD) proteins lack the oxygen required to hydroxylate the HIF- α subunits. HIF-2 α is hypothesized to mediate the anti-angiogenic process induced by GM-CSF. Recently, the role of HIF-1 α and HIF-2 α in melanoma has been considered in the absence and presence of GM-CSF. Transgenic mice with macrophages deficient in HIF-1 α or HIF-2 α , and control mice with functional HIF- α s, were observed for tumor growth over 15–19 days (Roda et al. 2011, 2012). The following conclusions were observed: (i) under hypoxic conditions, HIF-1 α mediates the production of VEGF in response to GM-CSF; HIF-1 α deletion substantially reduces VEGF production. (ii) HIF-2 α mediates the production of sVEGFR-1 in response to GM-CSF; HIF-2 α deletion significantly reduces the production of sVEGFR-1, but has no effect on VEGF production.

In the present paper, we develop a mathematical model of a tumor that includes the interactions among tumor cells (live or dead), macrophages, endothelial cells, and the cytokines, such as M-CSF, GM-CSF, VEGF, sVEGFR-1, MCP-1/CCL2, and oxygen. We also include the effects of HIF-1 α and HIF-2 α in GM-CSF-treated or untreated tumor growth. The model builds on previous work by Szomolay et al. (2012), but it includes new important features: the transcription factors HIF-1 α and HIF-2 α , and the introduction of a velocity $\vec{v}$, which underlies the movement of cells and cytokines. The boundary of the tumor moves with velocity $\vec{v}$ in the direction of the normal. It is shown that the model simulations are in agreement with the experimental results of Roda et al. (2011). The model is then used to predict the growth of the tumor volume under partial blocking of HIF-1 α or stabilization of HIF-2 α , with or without injection of GM-CSF. These results could be used to evaluate the benefit of therapeutic intervention targeting HIF-1 α inhibition (Semenza 2003) or novel compounds that cause HIF-2 α stabilization.

2 Mathematical Model

In this section, we describe a mathematical model representing the experiments of Roda et al. (2011, 2012), by a system of differential equations and discuss the parameters of the system. Although there are a wide variety of cell species and chemical components in a tumor, we only focus our interests on major cells such as live/dead tumor cells, macrophages, and endothelial cells (EC). The cytokines include M-CSF, MCP-1/CCL2, GM-CSF, VEGF, sVEGFR-1, as well as oxygen molecules. Figure 1 shows the cells-cytokines-oxygen interaction pathways on which a system of differential equations are built. Notations of densities of cell species and cytokines are listed in Table 1.

This is a two-phase free boundary model as shown in Fig. 2: the tumor is modeled as a growing continuum $\Omega(t)$ with boundary $\partial\Omega(t)$, both of which evolve in time.

The tumor region $\Omega(t)$ is contained in a fixed domain D and the region $D \setminus \Omega(t)$ represents the healthy tissue. Both live and dead tumor cells are assumed to be in $\Omega(t)$ while macrophages and EC are assumed to be in both tumor and healthy tissue; cytokines and oxygen molecules can diffuse throughout the whole domain D . Because of the internal pressure (induced by abnormal proliferation of tumor cells), there exists a macroscopic velocity field $\vec{v}$ in $\Omega(t)$, while $\vec{v} \equiv 0$ in $D \setminus \Omega(t)$. The tumor size is determined by the volume of $\Omega(t)$.

2.1 Governing Equations of Cells, Cytokines, and Oxygen

All cells are assumed to follow the general continuity equation:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{v}) = G,$$

where ρ may represent densities of live/dead tumor cells, endothelial cells (EC), and macrophages. The function G includes proliferation, apoptosis or clearance of cells, and chemotaxis terms related to cytokines, as appropriate. We note that some authors included a diffusion term for cells (e.g., Owen et al. 2004; Plank et al. 2004) in the above equation, while other neglect it since the diffusion is small, as in Ward and King (1997, 1999), Pettet et al. (2001).

The equation of live tumor cells density is given by

$$\frac{\partial c}{\partial t} + \nabla \cdot (c \vec{v}) = \underbrace{\lambda_1(w)c \left(1 - \frac{c}{c^*}\right)}_{\text{proliferation}} - \underbrace{\lambda_2(w)c}_{\text{dying by necrosis}} - \underbrace{\mu_c c}_{\text{apoptosis}}; r \in \Omega(t), \quad (1)$$

where c^* is the carrying capacity of all the cells, and a logistic growth is assumed for live tumor proliferation. The second and third terms in Eq. (1) account for tumor death due to necrosis and apoptosis, respectively. The proliferation and the necrotic death rates of tumor cells depend on the oxygen level. Equation (1) is an approximation of complex metabolism of cell evolution, and for simplicity, we take $\lambda_1(w)$ and $\lambda_2(w)$ in the forms as indicated by Fig. 3 and use the following piecewise linear approximations:

$$\lambda_1(w) = \begin{cases} 0 & \text{if } w < w_h, \\ \lambda_1(w - w_h)/(w_0 - w_h) & \text{if } w_h \leq w \leq w_0, \\ \lambda_1 & \text{if } w > w_0, \end{cases}$$

$$\lambda_2(w) = \begin{cases} \lambda_2 & \text{if } w < w_n, \\ \lambda_2(w_h - w)/(w_h - w_n) & \text{if } w_n \leq w \leq w_h, \\ 0 & \text{if } w > w_h, \end{cases}$$

where w_0 is the normal oxygen level, and $[0, w_n]$, $(w_n, w_h]$ and $(w_h, w_0]$ are ranges of oxygen in necrotic, extremely hypoxic, and hypoxic levels, respectively.

The evolution equation for density of dead tumor cells is:

$$\frac{\partial b}{\partial t} + \nabla \cdot (b \vec{v}) = \underbrace{\lambda_2(w)c + \mu_c c}_{\text{gain from live tumor cells}} - \underbrace{\mu_b \frac{w}{w_0} mb}_{\text{clearance by macrophages}}; \quad r \in \Omega(t), \quad (2)$$

dead tumor cells are obtained from necrosis and apoptosis of live tumor cells, and they are cleared by macrophages at rate $\mu_b w/w_0$ (Szomolay et al. 2012).

The macrophages are recruited by their chemoattractants MCP-1/CCL2 (Owen et al. 2004) and by GM-CSF (Eubank et al. 2009). Therefore, the equation of macrophage density is formulated as

$$\frac{\partial m}{\partial t} + \nabla \cdot (m \vec{v}) = \underbrace{-\nabla \cdot (k_p m \nabla p)}_{\text{chemotaxis by MCP1}} - \underbrace{\nabla \cdot (k_g m \nabla g)}_{\text{chemotaxis by GMCSF}}; \quad r \in D, \quad (3)$$

where the chemotactic coefficients k_p of MCP-1/CCL2 and k_g of GM-CSF are assumed to be constant. Equation (3) is a simplification of the macrophage population. As tumors progress, there are both M1 and M2-type macrophages. M1 macrophages infiltrate the microenvironment to help fight the tumor and M2-type to help promote tumor growth and progression. However, under the influence of tumor and GM-CSF, they may change their phenotypes from M1 to M2 phenotype and vice versa; functions of these two phenotypes are modeled in equations of VEGF and sVEGFR-1, respectively. Also, since all macrophages are subject to chemotaxis as in Eq. (3), for simplicity we do not consider the two types separately.

The vascular system is viewed as density of EC, which is recruited by the chemoattractant VEGF, so that the equation of EC density is written as

$$\frac{\partial e}{\partial t} + \nabla \cdot (e \vec{v}) = \underbrace{-\nabla \cdot (k_h e \nabla h)}_{\text{chemotaxis by VEGF}}; \quad r \in D, \quad (4)$$

where we assume that the chemotactic coefficient k_h is a constant. We note that in some models, such as Chaplain (1995), Szomolay et al. (2012), other source terms such as mitosis, proliferation, and anastomosis are included, while in other tumor-induced angiogenesis models (Anderson and Chaplain 1998), birth and death terms of EC are omitted since there is no proliferation at the sprout tips and since EC have a long half-life (in the order of months) (Pawelitz and Knierim 1989). Here, we take the latter approach and focus on the chemotaxis of EC.

In contrast to the equations of cell densities, the equations of the cytokines and oxygen molecules in the tumor include diffusion terms in the following form:

$$\frac{\partial \Phi}{\partial t} + \nabla \cdot (\Phi \vec{v}) = \nabla \cdot (D_\Phi \nabla \Phi) + F,$$

where Φ represents densities of cytokines M-CSF, MCP-1/CCL2, VEGF, sVEGFR-1, and GM-CSF, as well as oxygen. The diffusion coefficient D_Φ is assumed to be constant and function F may include cytokines production, decay, binding, oxygen delivery/uptaking, and other reactions. We note that whereas in the case of cells we ignored diffusion term (as explained above in the case of the general equation of the cells), for cytokines and oxygen molecules whose size is very small, the diffusion terms are included.

The M-CSF is secreted by tumor cells, so the equation of M-CSF density is given by

$$\frac{\partial q}{\partial t} + \nabla \cdot (q \vec{v}) = \nabla \cdot (D_q \nabla q) + \underbrace{\lambda_3 c}_{\text{secreted by tumor}} - \mu_q q, \quad (5)$$

where λ_3 is secretion rate of M-CSF by tumor cells; λ_3 is assumed to be a constant since there is no hypoxia regulatory element for M-CSF promoters (Oren et al. 2001). The decay rate of the M-CSF is a constant μ_q .

As a potent chemoattractive factor, the MCP-1/CCL2 is secreted by macrophages (Kanda et al. 2006), in response to M-CSF binding the M-CSF receptor. This process is saturated due to limited M-CSF receptors (Curry et al. 2008; Eubank et al. 2003; Leonard et al. 1991; Varney et al. 2005). Based on these facts, the equation of MCP-1/CCL2 density is set up as

$$\frac{\partial p}{\partial t} + \nabla \cdot (p \vec{v}) = \nabla \cdot (D_p \nabla p) + \underbrace{\lambda_4(w) \frac{q}{q+q_0} m}_{\text{secreted by macrophages}} - \mu_p p, \quad (6)$$

where q_0 is a parameter for the saturation effect and μ_p is a constant decay rate of MCP-1/CCL2. The MCP-1/CCL2 production rate by macrophages is oxygen-dependent: the production is inhibited by hypoxia to the extent of 2.3 to 2.8 fold compared to normoxia (Bosco et al. 2004). Thus, the production rate function is taken as

$$\lambda_4(w) = \begin{cases} 0 & \text{if } w < w_n, \\ 0.4\lambda_4 & \text{if } w_n \leq w \leq w_h, \\ \lambda_4 & \text{if } w > w_h. \end{cases}$$

VEGF is produced directly by tumor cells and by macrophages associated with M-CSF (Eubank et al. 2009). Macrophages also secrete sVEGFR-1, which sequesters VEGF by binding with it to form sVEGFR-VEGF compound (Roda et al. 2011); hence, sVEGFR-1 serves as a potent antagonist of VEGF signaling. The equation of VEGF density can then be written as

$$\begin{aligned} \frac{\partial h}{\partial t} + \nabla \cdot (h \vec{v}) = & \nabla \cdot (D_h \nabla h) + \underbrace{\lambda_5(w)c}_{\text{secreted by tumor}} + \underbrace{\theta_1 \lambda_6(w) \frac{q}{q+q_0} m}_{\text{secreted by macrophages}} \\ & - \underbrace{\bar{\mu}_s sh}_{\text{binding to sVEGFR1}} - \mu_h h, \end{aligned} \quad (7)$$

where $\bar{\mu}_s$ is the binding rate of VEGF to sVEGFR-1, and μ_h is the decay rate of VEGF, respectively. For model simplicity, we neglect the unbinding of the sVEGFR-VEGF compound.

For the production rate of VEGF by tumor cells, it is assumed that cells only secrete VEGF when the oxygen level is above the necrotic level w_n , and that the secretion rate arises with increasing oxygen level, under hypoxia, and then falls off when oxygen exceeds certain

hypoxic level. The VEGF secretion rate under normoxic conditions varies between $\frac{1}{4}$ to $\frac{1}{2}$ the level under hypoxic conditions (Braunstein et al. 2007; Koshikawa et al. 2003; Rapella et al. 2002; Shang et al. 2006). Based on these facts, we set $\lambda_5(w) = \lambda_5 \phi(w)$ and

$$\phi(w) = \begin{cases} 0 & \text{if } w < w_n, \\ (w - w_n)/(w^* - w_n) & \text{if } w_n \leq w < w^*, \\ 1 - 0.7(w - w^*)/(w_0 - w^*) & \text{if } w^* < w \leq w_0, \\ 0.3 & \text{if } w > w_0 \end{cases}$$

where $w^* \in (w_n, w_0)$ represents the threshold at which the hypoxic effect is maximal.

The secretion rate of VEGF by macrophages under M-CSF signaling has a saturation effect due to the c-macrophage receptor that activates the signal transduction pathway. Therefore, the production of VEGF from macrophages is modeled as the third term of Eq. (7), with q_0 being a scaling factor of M-CSF. The secretion rate $\lambda_6(w)$ is assumed to have the same oxygen-dependence as that of $\lambda_5(w)$, i.e., $\lambda_6(w) = \lambda_6 \phi(w)$. Furthermore, the transcription factor HIF1- α is reported to be responsible for the production of VEGF by macrophages (Roda et al. 2011). In the experiments with HIF-1 α -deficient macrophages, the production of VEGF was greatly reduced. This is accounted by taking θ_1 to be much smaller than 1 for mice with HIF-1 α -deficient macrophages, whereas for mice with normal macrophages $\theta_1 = 1$.

Macrophages also secrete sVEGFR-1 and the production is greatly enhanced by GM-CSF (Eubank et al. 2004). Equation (8) for sVEGFR-1 includes sVEGFR-1 production, binding of sVEGFR-1 to VEGF, and sVEGFR-1 decay terms,

$$\frac{\partial s}{\partial t} + \nabla \cdot (s \vec{v}) = \nabla \cdot (D_s \nabla s) + \underbrace{\theta_2 \lambda_7 \frac{g + \nu g_0}{g + g_0} m}_{\text{secreted by macrophages}} - \underbrace{\bar{\mu}_h h s}_{\text{binding to VEGF}} - \mu_s s. \quad (8)$$

As concluded in Roda et al. (2011), the transcription factor HIF-2 α supports the production of sVEGFR-1 by macrophages. Thus, θ_2 is smaller than one for mice with HIF-2 α -deficient macrophages and $\theta_2 = 1$ for normal mice. HIF-2 α is highly unstable, but its concentration can be increased by chemical stabilization; the effect of HIF-2 α stabilization is represented by $\theta_2 > 1$. Since macrophages secrete relatively small amounts of sVEGFR-1 when they are not stimulated by GM-CSF (Eubank et al. 2004), we have the additional small factor ν in the second term of Eq. (8). Based on the study of comparison of sVEGFR-1 production with and without GM-CSF simulation in Eubank et al. (2004), we estimate $\nu = 0.05$. The parameter g_0 is for the saturation effect. Further, extreme hypoxia also increases sVEGFR-1 production as shown in Eubank et al. (2011), but the production of sVEGFR-1 relies more significantly on the GM-CSF treatment, thus for simplicity, we neglect the oxygen-level dependence of the λ_7 .

Oxygen is delivered by EC and diffuses from the vasculature, while at the same time, it is consumed by tumor cells and macrophages. Hence, the oxygen density is governed by the equation

$$\frac{\partial w}{\partial t} + \nabla \cdot (w \vec{v}) = \nabla \cdot (D_w \nabla w) + \underbrace{\lambda_8 e}_{\text{delivered by EC}} - \underbrace{\lambda_9 m w}_{\text{uptake by macrophages}} - \underbrace{\lambda_{10} c w}_{\text{uptake by tumor cells}}, \quad (9)$$

where λ_8 is the delivery rate of oxygen by EC; λ_9 and λ_{10} are the oxygen consumption rates by macrophages and tumor cells, respectively. For simplicity, we assume that oxygen-delivery by red blood cells is proportional to the density of endothelial cells.

Finally, the density of GM-CSF depends on its diffusion coefficient D_g , injection $f(t)$, and decay rate μ_g ,

$$\frac{\partial g}{\partial t} + \nabla \cdot (g \vec{v}) = \nabla \cdot (D_g \nabla g) + \underbrace{f(t)}_{\text{injection}} - \mu_g g, \quad (10)$$

where $f(t)$ is simply modeled as a periodic (with respect to time) constant function, depending on the dosing regimen.

2.2 Radial Symmetry Simplification of the System

For simplicity, the tumor is assumed to be a spherical model, hence D is a closed, fixed spherical domain with radius $r = L$ and $\Omega(t)$ is a spheroid with time-dependent radius $r = R(t)$. The endothelial cells, macrophages, cytokines, and oxygen move in the domain $[0, L]$, but the tumor cells are confined to the tumor region $r < R(t)$. Assuming radial symmetry, i.e., $\vec{v} = v \hat{e}_r$, Eqs. (1) to (10) are transformed into:

$$\frac{\partial c}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 c v) = \lambda_1(w) c \left(1 - \frac{c}{c^*}\right) - \lambda_2(w) c - \mu_c c, \quad 0 < r < R(t); \quad (11)$$

$$\frac{\partial b}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 b v) = \lambda_2(w) c + \mu_c c - \mu_b \frac{w}{w_0} m b, \quad 0 < r < R(t); \quad (12)$$

$$\frac{\partial m}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 m v) = -\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 k_p m \frac{\partial p}{\partial r} \right) - \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 k_g m \frac{\partial g}{\partial r} \right), \quad 0 < r < L; \quad (13)$$

$$\frac{\partial e}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 e v) = -\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 k_h e \frac{\partial h}{\partial r} \right), \quad 0 < r < L; \quad (14)$$

$$\frac{\partial q}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 q v) = D_q \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial q}{\partial r} \right) + \lambda_3 c \chi_{\{r \leq R(t)\}} - \mu_q q, \quad 0 < r < L; \quad (15)$$

$$\frac{\partial p}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 p v) = D_p \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial p}{\partial r} \right) + \lambda_4(w) \frac{q}{q+q_0} m - \mu_p p, \quad 0 < r < L; \quad (16)$$

$$\begin{aligned} \frac{\partial h}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 h v) = & D_h \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial h}{\partial r} \right) + \lambda_5(w) c \chi_{\{r \leq R(t)\}} \\ & + \theta_1 \lambda_6(w) \frac{q}{q+q_0} m - \bar{\mu}_s s h - \mu_h h; \quad 0 < r < L; \end{aligned} \quad (17)$$

$$\begin{aligned} \frac{\partial s}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 s v) = & D_s \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial s}{\partial r} \right) + \theta_2 \lambda_7 \frac{q+\nu q_0}{g+g_0} m \\ & - \bar{\mu}_h s h - \mu_s s, \quad 0 < r < L; \end{aligned} \quad (18)$$

$$\frac{\partial w}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 w v) = D_w \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial w}{\partial r} \right) + \lambda_8 e - \lambda_9 m w - \lambda_{10} c w, \quad 0 < r < L; \quad (19)$$

$$\frac{\partial g}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 g v) = D_g \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial g}{\partial r} \right) + f(t) - \mu_g g, \quad 0 < r < L. \quad (20)$$

Notice that equations for live and dead tumor cells hold only in the tumor region $[0, R(t)]$, while the equations for the other cells and all the cytokines, as well as oxygen, take place in the entire fixed domain $[0, L]$.

2.3 Interface, Initial, and Boundary Conditions

Interface Condition—We assume that due to the internal pressure and moving boundary, cells in the tumor distribute themselves in such a way that the total cell density in the tumor is more or less constant (Ward and King 1997), so that $c + b + m + e = \text{constant}$. Also, tumor cells, macrophages, and EC occupy only a fraction (up to 90 %) of the tumor, according to experimental observation (Eubank et al. 2009), hence we take $c + b + m + e \equiv \theta_3 c^*$, where θ_3 represents the volume fraction of the cells in the tumor. Adding Eqs. (11) through (14), we obtain

$$\begin{aligned} \frac{\partial}{\partial r} (r^2 \theta_3 c^* v) = & r^2 \left[\lambda_1(w) c \left(1 - \frac{c}{c^*}\right) - \mu_b \frac{w}{w_0} m b \right] \\ & - \left[\frac{\partial}{\partial r} \left(r^2 k_p m \frac{\partial p}{\partial r} \right) + \frac{\partial}{\partial r} \left(r^2 k_g m \frac{\partial g}{\partial r} \right) + \frac{\partial}{\partial r} \left(r^2 k_h e \frac{\partial h}{\partial r} \right) \right], \end{aligned}$$

so that

$$\begin{aligned} v(r, t) = & \frac{1}{\theta_3 c^* r^2} \int_0^r \zeta^2 \left[\lambda_1(w) c \left(1 - \frac{c}{c^*}\right) - \mu_b \frac{w}{w_0} m b \right] d\zeta \\ & - \frac{1}{\theta_3 c^*} \left(k_p m \frac{\partial p}{\partial \zeta} + k_g m \frac{\partial g}{\partial \zeta} + k_h e \frac{\partial h}{\partial \zeta} \right) \Big|_{\zeta=r}. \end{aligned} \quad (21)$$

We assume that the velocity of the free boundary $r = R(t)$ of the tumor is equal to $v(R(t), t)$,

$$\begin{aligned} \dot{R}(t) = v(R(t), t) = & \frac{1}{\theta_3 c^* R^2(t)} \int_0^{R(t)} \zeta^2 \left[\lambda_1(w) c \left(1 - \frac{c}{c^*}\right) - \mu_b \frac{w}{w_0} m b \right] d\zeta \\ & - \frac{1}{\theta_3 c^*} \left(k_p m \frac{\partial p}{\partial \zeta} + k_g m \frac{\partial g}{\partial \zeta} + k_h e \frac{\partial h}{\partial \zeta} \right) \Big|_{\zeta=R(t)}. \end{aligned} \quad (22)$$

Initial Conditions—The initial radius of the tumor is denoted by R_0 . According to Eubank et al. (2009), tumors become palpable when their volume reaches 1 to 2 mm³; we take initial tumor volume as 1.0 mm³, which corresponds to initial radius $R_0 = 0.625$ mm.

For cells, we assume the following initial conditions:

$$c(r, 0) = c_0, \quad b(r, 0) = 0, \quad \text{if } r \in [0, R_0]; \quad (23)$$

$$m(r, 0) = m_0, \quad e(r, 0) = \frac{e_0}{1 + e^{(5R_0 - r)/\varepsilon}}, \quad r \in [0, L]; \quad (24)$$

where $\varepsilon > 0$ is small. The parameters c_0 and m_0 are the average densities of live cells and macrophages in the tumor, and e_0 is the average density of EC in the healthy tissue.

All other cytokines are assumed to have zero initial values, i.e.,

$$q(r, 0) = p(r, 0) = s(r, 0) = h(r, 0) = g(r, 0) = 0, \quad r \in [0, L]. \quad (25)$$

Finally, the initial oxygen level is assumed to have the following form:

$$w(r, 0) = w^* + \frac{r^2}{L^2}(w_0 - w^*), \quad r \in [0, L]. \quad (26)$$

Boundary Conditions—At $r = 0$, zero flux boundary conditions for w , q , p , s , h , g , and at $r = L$, zero flux boundary conditions for q , p , s , h , g , except that $w(L, t) = w_0$.

2.4 Parameters

2.4.1 Parameters Estimation

System Scaling Parameters: For computational convenience, all variables and parameters are nondimensionalized by the system scaling parameters, which are determined based on experimental conditions.

Tumor growth is recorded until they reach a size of 20 mm in any dimension (Roda et al. 2011); accordingly, we choose the length scale $L_0 = 0.5$ cm. Tumor development is monitored for 2 to 3 weeks, so we set the time scale as $\tau = 2.5 \times 10^5$ seconds, which is approximately 2.9 days.

For simplicity, live/dead tumor cells and macrophages are assumed to have the same volume, which is typically 10^{-9} cm³ (Ward and King 1997; Li 1982). Based on the observation in Eubank et al. (2009) that macrophages take up to 20 % of the tumor volume (Vincensini et al. 2007), we choose scaling parameters for live tumor cells to be $c_0 = 7.2 \times 10^8$ cell/cm³, for dead tumor cells to be $b_0 = 7.2 \times 10^8$ cell/cm³, and for macrophages to be $m_0 = 2.0 \times 10^8$ cell/cm³, as in Szomolay et al. (2012).

According to Less et al. (1991), the average radius of the blood capillaries in normal tissue is 5 μ m and parallel capillaries are 50 μ m apart. Hence, the volume enclosed by the

capillaries is less than $\frac{1}{100}$ of the total tumor volume. Since 10^9 cells fill in one cm³, the EC only occupies a fraction of volume of the capillaries; we correspondingly choose the EC scaling density to be $e_0 = 2.5 \times 10^6$ cell/cm³, as in Szomolay et al. (2012).

It was reported in Owen and Sherratt (1997), Kuratsu et al. (1993) that MCP-1 concentrations were in the range of 0.2 to 6.3 ng/ml. Levels of M-CSF in breast tumors were measured to be in the range of 134.5 to 1175.1 pg/ml in Lawichi et al. (2006). VEGF and sVEGFR-1 levels were reported to be in the ranges of 20–1000 pg/ml and 10–8000 pg/ml in Eubank et al. (2004). Accordingly, we set the scaling parameters for cytokines MCP-1/CCL2, M-CSF, VEGF sVEGFR-1, and GM-CSF as $p_0 = q_0 = s_0 = h_0 = g_0 = 1.0 \times 10^{-9}$ g/cm³.

Proliferation/Migration/Death Rate of Cells: According to Qian et al. (2009), the doubling time of tumor cells is 17.5 hours; then we have the proliferation time of live tumors

$\frac{\ln 2}{17.5} \text{ h}^{-1} = \frac{\ln 2}{17.5} \times \frac{1}{3600} \text{ s}^{-1} = 1.10 \times 10^{-5} \text{ s}^{-1}$. Since the proliferation model used in the present paper is a logistic growth, the corresponding parameter should be increased; we take the proliferation rate λ_1 of live tumor cells to be in the range of $\lambda_1 = 2 \times 10^{-5}$ to 8×10^{-5}

s^{-1} . The maximum necrosis rate of cancer cells is taken as $\lambda_2 = 9.63 \times 10^{-6} s^{-1}$, which is comparable to λ_1 (Owen et al. 2004; Szomolay et al. 2012).

As in Szomolay et al. (2012), the death rate of live tumor cells due to apoptosis is assumed to be smaller than the proliferation rate; we estimate it to be between 1/4 to 3/4 of $9.63 \times 10^{-6} s^{-1}$, and this gives μ_c a range of 2.4×10^{-6} to $7.22 \times 10^{-6} s^{-1}$. The rate by which live tumor cells die due to apoptosis is assumed to be the same as the clearance rate of dead cells by macrophages at normoxic conditions (i.e., $w = w_0$), so that $\mu_b m_0 \approx \mu_c$; hence, $\mu_b = 1.0 \times 10^{-14}$ to $5.0 \times 10^{-14} cm^3 cell^{-1} s^{-1}$.

Migrations of macrophages and EC depend not only on velocity, but also on the chemotactic coefficients of MCP-1/CCL2, GM-CSF, and VEGF. The chemotactic coefficient of VEGF has a wide range 1 to $100 cm^5 g^{-1} s^{-1}$, according to Pettet et al. (1996), Schugart et al. (2008), Stokes and Lauffenburger (1991); here, we restrict the range to 20 to $80 cm^5 g^{-1} s^{-1}$. As in Owen and Sherratt (1997), Szomolay et al. (2012), the chemotactic coefficient of MCP-1/CCL2, multiplied by its molecular weight, is taken to be in the range of 20 to 60 cm^2/sM ; $1 M = 1 mol/liter = N_A/1000 cm^3$, where $N_A \approx 6.02 \times 10^{23}$ is the Avogadro constant. Considering that the molecular weight of MCP-1 is 10 kDa = $10 \times 10^3 \times 1.66 \times 10^{-24} g$ (Papayianni et al. 2002), we have

$$\frac{cm^2}{sM \times 10 kDa} = \frac{cm^2}{s \times \frac{N_A}{1000 cm^3} \times 10 \times 10^3 \times 1.66 \times 10^{-24} g} \approx 0.1 cm^5 g^{-1} s^{-1};$$

hence, k_p is in the range of 2 to $6 cm^5 g^{-1} s^{-1}$. Finally, we take a comparable (but with slightly wider range) chemotactic coefficient for GM-CSF: $k_g = 1$ to $30 cm^5 g^{-1} s^{-1}$.

Production/Decay Rate of Cytokines: The production rates of the cytokines, $\lambda_3, \lambda_4, \lambda_5, \lambda_6, \lambda_7$ of MCP-1/CCL2, M-CSF, VEGF, and sVEGFR-1 are estimated to have the same order of magnitude, as in Owen and Sherratt (1997), Szomolay et al. (2012).

First, it was observed in Pyaskovskaya et al. (2008) that the VEGF production rates decreased from 35 to 5 pg/ 10^6 cells per hour, or 9.7 to 1.3 fg/ 10^6 cells per second, in unfed culture for Lewis lung carcinoma over 5 days. Accordingly, we take the range of λ_5 to vary from 10^{-21} to $10^{-20} gs^{-1} cell^{-1}$. According to Vicioso et al. (2006), the amount of VEGF secreted by macrophages is at least 2 to 3 times the amount secreted by tumor cells, and the production rates from tumor cells and macrophages are both elevated by hypoxia. For simplicity, we assume that the production rates are increased by the same order in tumors and macrophages, under hypoxia. The choice of λ_6 is linked to λ_5 roughly by relation $3\lambda_5 c_0 \approx \lambda_6 m_0$ as in Szomolay et al. (2012). It is reported in Wu et al. (2010) that the secretion rates of sVEGFR-1 and VEGF are similar in some tissues, so we take $\lambda_7 \approx \lambda_6$.

By Utting et al. (2010), the mRNA levels of VEGF and M-CSF from osteoclast are comparable. Adjusting for hypoxic condition, we take λ_3 to be in the range of $0.3\lambda_5$ to $3\lambda_5$. Finally, we compare MCP-1/CCL2 production rate λ_4 to the production rate of VEGF, λ_6 , taking $\lambda_4 \approx 0.3\lambda_6$ to $3\lambda_6$.

M-CSF has a half-life of 3–4 hours (Tang et al. 2000); accordingly, we take $\mu_q = \frac{\ln 2}{4} \frac{1}{3600}$ to $\frac{\ln 2}{3} \frac{1}{3600} s^{-1}$, or $\mu_q = 4.8 \times 10^{-5}$ to $6.4 \times 10^{-5} s^{-1}$. As in Owen and Sherratt (1997), the MCP-1/CCL2 decay rate is taken as $\mu_p = 2.0 \times 10^{-5} s^{-1}$. The decay rate of VEGF is $0.456 h^{-1}$, according to Plank et al. (2004), so we take $\mu_h = 1.26 \times 10^{-4} s^{-1}$. The half-life of

sVEGFR-1 has been reported in Wathen et al. (2011) to vary between 3.4 hours and 29 hours; correspondingly, we take $\mu_s = 6.63 \times 10^{-6}$ to $5.60 \times 10^{-5} \text{ s}^{-1}$. The half-life of GM-CSF is between 12 and 14 hours, so we take $\mu_g = 1.38 \times 10^{-5}$ to $1.93 \times 10^{-5} \text{ s}^{-1}$. In Wu et al. (2009), the binding rate of VEGF to sVEGFR-1 is reported to be $3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ in *in vitro* measurements, and the molecular weights for VEGF and sVEGFR-1 are 46 kDa and 220 kDa, respectively. Accordingly, we can derive

$$\bar{\mu}_h = \frac{3.0 \times 10^7}{\frac{N_A}{1000 \text{ cm}^3} \times \text{s} \times 46 \times 10^3 \times 1.66 \times 10^{-24} \text{ g}} = 6.52 \times 10^5 \text{ cm}^3 \text{ g}^{-1} \text{ s}$$

and

$$\bar{\mu}_s = \frac{3.0 \times 10^7}{\frac{N_A}{1000 \text{ cm}^3} \times \text{s} \times 220 \times 10^3 \times 1.66 \times 10^{-24} \text{ g}} = 1.36 \times 10^5 \text{ cm}^3 \text{ g}^{-1} \text{ s}.$$

Finally, we take $\mu_s^- = 1.0 \times 10^5$ to $2.0 \times 10^5 \text{ cm}^3 \text{ g}^{-1} \text{ s}^{-1}$ and $\mu_h^- = 2.0 \times 10^5$ to $8.0 \times 10^5 \text{ cm}^3 \text{ g}^{-1} \text{ s}^{-1}$.

Oxygen Delivery/Consumption and Hypoxic/Necrotic Threshold: In a normal breast tissue, the mean oxygen pressure is 52 mm Hg (Vaupel et al. 2003); in the breast tumor of patients, the mean oxygen pressure is in the range of 3–15 mm Hg (Vaupel et al. 2003). Based on these data, we take the oxygen pressure in normal breast tissue as 52 mm Hg, maximal oxygen pressure in hypoxic region of tumor as 12 mm Hg, and maximal pressure in dead/necrotic region tumor as 4 mm Hg, as in Szomolay et al. (2012). Here, we assume that the oxygen density is proportional to its pressure. Since the oxygen pressure in air is 160 mm Hg and $1.4 \times 10^{-3} \text{ g/cm}^3$ (Gordon 2003; Leitzel et al. 2007), we get the normal oxygen level as $w_0 = 52/160 \times 1.43 \times 10^{-3} \text{ g/cm}^3 = 4.65 \times 10^{-4} \text{ g/cm}^3$, which is also taken as the scaling factor of oxygen level. Consequently, the thresholds for hypoxic and necrotic conditions are $w_h = 1.0 \times 10^{-4} \text{ g/cm}^3$ and $w_n = 3.57 \times 10^{-5} \text{ g/cm}^3$, respectively. Finally, we estimate $w^* = 1.69 \times 10^{-4} \text{ g/cm}^3$.

The oxygen consumption rate by tumor cells was estimated in Girgis-Gabardo and Hassell (2008) to be $6.2 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1}$ per cell. Taking into account that fact that older tumors have smaller oxygen consumption rate (Butterworth A and Cater 1967), we choose the value of parameter λ_{10} in range of $\lambda_{10} = 1.0 \times 10^{-13}$ to $1.0 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1} \text{ cell}^{-1}$. In Nalwaya and Deen (2008), the oxygen consumption rates in a typical mammalian cells, the macrophage-like RAW264.7 cells, are reported to be $32 \pm 3 \text{ pmol}/(10^6 \text{ cells} \times \text{s})$. Assuming that one mole of oxygen molecules takes the volume of 22.4 litres, we get the consumption rate of

$$(32 \pm 3) \times \frac{10^{-12} \times 22.4 \times 10^3 \text{ cm}^3}{10^6 \text{ cell} \times \text{s}} = 6.5 \times 10^{-13} \text{ to } 7.8 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1} \text{ cell}^{-1}$$

after unit conversion; we shall take λ_9 in range of 1.0×10^{-13} to $1.0 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1} \text{ cell}^{-1}$.

To determine the delivery rate of oxygen by EC, we assume that the oxygen is approximately at a constant level when tumor cells are absent, i.e., $\lambda_8 e_0 = \lambda_9 m_0 w_0$; hence, λ_8 is in the range of 6.0×10^{-15} to $2.4 \times 10^{-14} \text{ g s}^{-1} \text{ cell}^{-1}$.

Diffusion Coefficients of Cytokines and Oxygen: In Owen and Sherratt (1997), the diffusion coefficient of MCP-1/CCL2 was $2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The diffusivities of VEGF and sVEGFR-1 were reported as $1.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and $6.5 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, respectively (Gabhann and Popel 2004). The diffusion rate of oxygen was taken as $2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Macdougall and McCabe 1967). Accordingly, we take the diffusion coefficients of the cytokines to be $D_q = 2.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $D_p = 2.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $D_s = 1.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $D_h = 1.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $D_w = 2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $D_g = 1.0 \times 10^{-6}$ to $3.0 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

2.4.2 System Nondimensionalization and Suggested Parameter Values—The variables and parameters of the system are nondimensionalized as follows:

$$\begin{aligned} \hat{r} &= \frac{r}{L_0}, \quad \hat{t} = \frac{t}{\tau}, \\ \hat{c} &= \frac{c}{c_0}, \quad \hat{b} = \frac{b}{b_0}, \quad \hat{m} = \frac{m}{m_0}, \quad \hat{e} = \frac{e}{e_0}, \\ \hat{q} &= \frac{q}{q_0}, \quad \hat{p} = \frac{p}{p_0}, \quad \hat{g} = \frac{g}{g_0}, \quad \hat{w} = \frac{w}{w_0}, \quad \hat{h} = \frac{h}{h_0}, \quad \hat{s} = \frac{s}{s_0}, \\ &\{\hat{\mu}_c, \hat{\mu}_b, \hat{\mu}_q, \hat{\mu}_p, \hat{\mu}_s, \hat{\mu}_w, \hat{\mu}_h, \hat{\mu}_g\} \\ &= \{\mu_c, \mu_b, m_0, \mu_q, \mu_p, \mu_s, \mu_w, \mu_h, \mu_g\} \tau, \\ \{\hat{D}_q, \hat{D}_p, \hat{D}_h, \hat{D}_s, \hat{D}_w, \hat{D}_g\} &= \{D_q, D_p, D_h, D_s, D_w, D_g\} \frac{\tau}{L_0^2}, \\ \{\hat{k}_p, \hat{k}_g, \hat{k}_h\} &= \{k_p, k_g, k_h\} \frac{\tau}{L_0^2}, \\ \{\hat{\lambda}_1(\hat{w}), \hat{\lambda}_2(\hat{w}), \hat{\lambda}_3(\hat{w}), \hat{\lambda}_4(\hat{w}), \hat{\lambda}_5(\hat{w})\} \\ &= \tau \left\{ \lambda_1(w), \lambda_2(w), \frac{c_0}{q_0} \lambda_3(w), \frac{m_0}{p_0} \lambda_4(w), \frac{e_0}{h_0} \lambda_5(w) \right\}, \\ \{\hat{\lambda}_6(\hat{w}), \hat{\lambda}_7, \hat{\lambda}_8, \hat{\lambda}_9, \hat{\lambda}_{10}\} &= \tau \left\{ \frac{m_0}{h_0} \lambda_6(w), \frac{m_0}{s_0} \lambda_7, \frac{e_0}{w_0} \lambda_8, m_0 \lambda_9, c_0 \lambda_{10} \right\}, \\ \{\hat{w}_n, \hat{w}^*, \hat{w}_h\} &= \frac{1}{w_0} \{w_n, w^*, w_h\} \end{aligned}$$

and the values utilized in simulations are summarized in Table 2, in original and in dimensionless forms.

2.5 Sensitivity Analysis

In the model, some parameters are obtained from experimental measurements while others are estimated. In order to check whether they significantly affect the simulation results, we have implemented sensitivity analysis on the most relevant parameters in Table 2; these parameters are listed in Table 3, together with their baselines and ranges. For each parameter, we have used Latin hypercube sampling to generate 200 samples and calculated the partial rank correlation coefficients (PRCC), with respect to the tumor radius at a given time. The sensitivity analysis was carried out based on the method in Marino et al. (2008), using the Matlab files available at <http://malthus.micro.med.umich.edu/lab/usadata/>.

Among the tested parameters, eight of them are statistically significant (p-value < 0.01) at day 7 ($\lambda_1, \lambda_5, \lambda_9, \lambda_{10}, \mu_c, \mu_s, k_p, \theta_3$) and six at day 15 ($\lambda_1, \lambda_5, \lambda_9, \lambda_{10}, \mu_c, \theta_3$). Figure 4 shows the statistically significant PRCC values (p-value < 0.01) of the parameters with respect to $R(t)$ at day 7 (a) and day 15 (b). The positive PRCC indicates that an increase in the corresponding parameter value will increase the tumor radius while the negative PRCC indicates the opposite. Specifically, we can conclude that the tumor radius $R(t)$ is positively correlated to $\lambda_1, \lambda_5, \mu_c, k_p$, and θ_3 , and negatively correlated to λ_9, λ_{10} , and μ_s .

3 Results and Discussion

All simulations were carried out with MATLAB (version R2011a Mathworks). The model equations of parabolic type were numerically solved using pdepe (MATLAB function for initial-boundary value problems for parabolic-elliptic PDEs in 1D), and the equations of hyperbolic type were solved by the semi-Lagrangian scheme.

We recall the following experimental facts reported in Roda et al. (2011, 2012): (i) GM-CSF stimulates macrophages to secrete large amounts of sVEGFR-1, which reduces biologically active VEGF available for angiogenesis, resulting in suppression of tumor growth. (ii) Tumor growth is inhibited by GM-CSF treatment in mice with HIF-1 α -deficient macrophages. (iii) GM-CSF treatment fails to inhibit tumor growth in mice with HIF-2 α -deficient macrophages. In Fig. 5, we compare simulation results of our model with the experimental results, regarding the effects of GM-CSF treatment and HIFs knockout, stated in (i)–(iii). The experimental data in Figs. 5(a) and 5(b) are taken from Roda et al. (2011), in which tumor sizes are recorded for 15 and 19 days, respectively. All parameters are fixed and listed in Table 2, except that λ_1 and θ_3 are adjusted to fit part of the experimental data in each figure. Tumor volumes are recorded in units of mm³ (vertical axis), against the evolving time in days (horizontal axis), in the absence and presence of GM-CSF, with and without HIF-1 α /HIF-2 α knockout (KO). The solid lines in various colors are simulation results while the dots with error bars are experimental measurements.

Figure 5(a) shows tumor growth of mice with normal and HIF-1 α -knockout macrophages, with or without GM-CSF treatments. GM-CSF treatment (injection of 100 ng GM-CSF in 50 μ L given three times a week) alone can effectively inhibit the tumor growth: the tumor volume is reduced to roughly one third of the tumor without GM-CSF treatment after 15 days; HIF-1 α knockout also can suppress tumor development: the tumor volume decreases to half of that of mice with normal macrophages, even without GM-CSF injection. With combined GM-CSF treatment and HIF-1 α knockout, the tumor volume is reduced to one fifth of the volume of untreated tumor. In this figure, $\lambda_1 = 2.41 \times 10^{-5} \text{ s}^{-1}$ and $\theta_3 = 0.9$ were taken to fit (manually) curves denoted by “w/o GM-CSF” and “w/GM-CSF” to the corresponding experimental data, then their values are used for the rest of the simulations by setting $\theta_1 = 0.1, 1$ for mice with HIF-1 α -deficient and normal macrophages, respectively.

Figure 5(b) displays the effect of HIF-2 α knockout. Even with GM-CSF treatment, the tumor growth is not inhibited if HIF-2 α is knocked out; the tumor volume is slightly smaller than the one without GM-CSF treatment. Furthermore, without GM-CSF treatment, the tumor volume is even slightly increased in mice with HIF-2 α knockout. The reason is that the small amounts of natural secretion of sVEGFR-1 (without GM-CSF) is suppressed by HIF-2 α knockout, so that more VEGF become active. Similarly, we chose $\lambda_1 = 1.68 \times 10^{-5} \text{ s}^{-1}$ and $\theta_3 = 0.87$ (all the parameters are the same as used for Fig. 5(a)), to fit curves denoted by “w/o GM-CSF” and “w/GM-CSF” to the corresponding experimental data, then their values are used for the rest of the simulations by setting $\theta_2 = 0.1, 1$ for mice with HIF-2 α -deficient and normal macrophages, respectively.

For the rest of simulations, we used fixed values for all parameters, which are listed in Table 2.

In Fig. 6, we calculated relative densities of active VEGF (a) and sVEGFR-1 (b) averaged in tumor region under different treatments. The white and black bars are for tumors without and with GM-CSF treatments, respectively. The horizontal axis indicates the cases of mice with normal, HIF-1 α -knockout, and HIF-2 α -knockout macrophages. It was concluded experimentally in Roda et al. (2011) and computationally by our model simulations that HIF-1 α facilitates macrophage production of VEGF, whereas HIF-2 α controls sVEGFR-1 production. Based on this conclusion we set up the model and our simulations show that, without GM-CSF treatment, the density of VEGF is reduced with HIF-1 α knockout, while there is no significant change in density of sVEGFR-1 (actually the density is slightly enhanced since there is less VEGF to bind sVEGFR-1 and leaving more bioavailable); on the other hand, HIF-2 α knockout greatly reduces density of sVEGFR-1 and have no effect on VEGF density. The situation is relatively complicated when large amounts of sVEGFR-1

is produced with GM-CSF. HIF-1 α knockout greatly reduces production of VEGF, so there is much less VEGF-sVEGFR-1 interactions, so that the density of free sVEGFR-1 increases considerably. Similarly, HIF-2 α knockout decreases the secretion of sVEGFR-1 and as a result, the density of active VEGF is enhanced.

In Fig. 7, we showed the simulated EC density (dimensionless, e/e_0) in the whole domain $[0, L]$ as time evolves, for non-inhibited tumor (a), and tumor inhibited by GM-CSF and HIFs treatments (b). In each figure, the density of EC is represented by the color intensity, in which dark blue is for low while red is for high densities of EC. The horizontal axis is the distance to the tumor center in units of mm, the vertical axis is the time in days, and the tumor boundary $R(t)$ is labeled by the white curve. It can be seen that at day 0, according to the initial condition (23), EC density is low (dark blue) inside and near the tumor while the average EC density (light blue) only presents in the healthy tissue away from the tumor (from $r = 3$ mm). Since EC is governed by the hyperbolic equation (4) and since the velocity v vanishes in the healthy tissue, the change of the light blue area in time outside the tumor (right to the tumor boundary) represents the “traveling” of EC by chemotaxis. On the other hand, the color change inside the tumor (left to the tumor boundary) represents more complicated EC motions under both chemotaxis and the velocity field v . In Fig. 7(a), there shows an obvious chemotaxis of EC: after about seven days, EC (originally at $r = 3$ mm) enter the tumor by chemotaxis due to VEGF secreted by tumor cells and TAM, then they accumulate, redistribute, and concentrate themselves near the tumor boundary. However, as displayed in Fig. 7(b), when the function of VEGF is inactivated by sVEGFR-1, the chemotaxis is suppressed (the light blue area stays at $r \approx 3$ mm). In both cases, EC density remains somewhat constant far away from the tumor boundary, because the proliferation and death of EC are neglected in our model, and the velocity is small in the center of tumor or there is no velocity field in the healthy tissue.

We next use our model to explore the effects of different dosing protocols of GM-CSF, degradation of HIF-1 α , or stabilization of HIF-2 α (by a small molecule inhibitor such as prolyl hydroxylase domain protein 3, or PHD3) on tumor reduction. In the experiments reported in Roda et al. (2011, 2012), an injection of 100 ng GM-CSF in 50 μ L was given three times a week. In Fig. 8(a), we display simulations for injection of double dose (200 ng), half dose (50 ng), and quarter dose (25 ng) at the same frequency. As expected, reduction of GM-CSF injection increases tumor growth. After 15 days, 100 ng injection of GM-CSF decreases the tumor volume to 35.8 % of the tumor without GM-CSF treatment, while 50 ng and 25 ng injections only reduce the tumor volume to 43.4 % and 56.6 %, respectively. On the other hand, a double dose (200 ng) of the GM-CSF injection reduces the tumor volume to 32.8 %.

Keeping the total amount (600 ng) of GM-CSF unchanged during the 2-week period, we simulate the effect of injecting GM-CSF at different frequencies, weekly (300 ng each time), three times a week (100 ng each time), and daily (40 ng each time). The results in Fig. 8(b) suggest that more frequent GM-CSF treatment will achieve better effect in reducing tumor volume. Daily injection can reduce tumor volume to 27 % of the one without GM-CSF treatment, while three times a week or weekly injection can decrease tumor volume to 35.8 % or 73.7 %, respectively.

HIFs undergo degradation and stabilization in macrophages, depending on the oxygen concentration and drug treatments. The mechanism of HIFs dynamics is beyond the scope of the current work, but we next explore how degradation of HIF-1 α and HIF-2 α stabilization affects tumor growth. Since the parameter $\theta_1 = 0.1$ is considered as totally HIF-1 α deficient and $\theta_1 = 1$ is for normal HIF-1 α available in macrophages, simulations are performed at $\theta_1 = 0.73$, $\theta_1 = 0.55$ as 30 % and 50 % degradation of HIF-1 α , respectively. Figure 9(a)

represents the simulated tumor volume at day 15: without GM-CSF treatment (black bars), 30 %, 50 %, and 100 % degradation of HIF-1 α can reduce tumor volume to 88.6 %, 78.7 %, and 53.6 % of tumors from mice with normal macrophages, after 15 days. Combined with GM-CSF treatment (white bars), degradation of HIF-1 α with the same percentages can reduce tumor volume to 28.7 %, 25.5 %, and 21.1 % of tumors from mice without any treatment, respectively.

HIF-2 α mediates production of sVEGFR-1 which can inhibit angiogenesis. Figure 9(b) displays the simulated tumor volume after 15 days with macrophages that undergone HIF-2 α stabilization. We express the effect of stabilizing HIF-2 α by the value of θ_2 in Eq. (8); θ_2 represents the magnitude of HIF-2 α after stabilization comparing to the normal case. As indicated in black bars, without GM-CSF, there is no obvious change in tumor volumes unless $\theta_2 = 5$. With $\theta_2 = 1.5, 2$, and 5 as HIF-2 α stabilization, tumor volume is reduced to 96.7 %, 94.2 %, and 74.2 % of the one with normal macrophages. On the other hand, combined with GM-CSF injection, stabilizing HIF-2 α promises much better results in reducing tumor volume, as seen in white bars. Stabilizing HIF-2 α at levels measured by $\theta_2 = 1.5, 2$, and 5 can reduce tumor volume to 72.7 %, 66.6 %, and 61.1 % of the one with GM-CSF injection alone.

4 Conclusion

GM-CSF has been shown experimentally to slow tumor growth in a mouse model of breast cancer. In response to low oxygen, both tumor cells and tumor-associated macrophages produce VEGF, which mobilizes endothelial cells in the cellular microenvironment and promotes angiogenesis. Stimulated by GM-CSF, macrophages secrete large amounts of sVEGFR-1 that binds to and inactivates VEGF. Thus, GM-CSF treatment serves a role in suppression of angiogenic activities in breast cancer, resulting in lowering oxygen tension, reducing blood density, and metastatic activities. The HIF transcription factors (HIF-1 α and HIF-2 α) whose stabilization is traditionally reported to be beneficial to tumor progression, can mediate the inhibition of tumor growth and angiogenesis when HIF-1 α is knocked out and HIF-2 α is stabilized under hypoxia in combination with GM-CSF. It was determined experimentally (Roda et al. 2011, 2012) that HIF-1 α in macrophages controls the production of VEGF while HIF-2 α is responsible for the secretion of sVEGFR-1.

In this work, we developed a mathematical model which simulates the anti-angiogenic activity of GM-CSF treatment mediated by HIF-2 α . The model is based on a coupled system of partial differential equations, which describe interactions among the main components involved in tumor angiogenesis, with GM-CSF treatment, VEGF regulation by sVEGFR-1, and HIF-2 α stabilization. Tumor growth is modeled as a free boundary and its evolution depends on various types of cells in the tumor microenvironment. Some important components, such as VEGF, sVEGFR-1, endothelial cells, and oxygen level, are quantitatively presented to help demonstrate the processes. The model is validated by comparison of simulations with experimental data utilizing GM-CSF treatment in mice with macrophage-specific deletions of HIF-1 α or HIF-2 α from homozygous LysMcre-HIF-1 $\alpha^{\text{fl/fl}}$ or LysMcre-HIF-2 $\alpha^{\text{fl/fl}}$ mice in combination with an orthotopic injection model of breast cancer. Our simulation of the model based on actual data suggests treatment with GM-CSF in combination with a small molecule inhibitor that stabilizes HIF-2 α may be considered as a potential therapeutic approach to reduce tumor growth and angiogenesis. And further, our model can be utilized to predict the optimal dosage/schedule of GM-CSF treatment, as well as the effects of combination therapy using a compound specific for the stabilization of HIF-2 α .

Acknowledgments

This work was supported in part by National Science Foundation Award 0635561, NCI 5R00CA131552 (T.D.E.), and R01 HL067167 (C.B.M.).

References

- Anderson A, Chaplain MAJ. Continuous and discrete mathematical models of tumor-induced angiogenesis. *Bull Math Biol.* 1998; 60:857–900. [PubMed: 9739618]
- Bosco MC, Puppo M, Pastorino S, Mi Z, Melillo G, Massazza A, Rapisarda S, Varesio L. Hypoxia selectively inhibits Monocyte Chemoattractant Protein-1 production by macrophages. *J Immunol.* 2004; 172:1681–1690. [PubMed: 14734750]
- Braunstein S, Karpisheva K, Pola J, Goldberg C, Hochman T, Yee H, Cangiarella J, Arju R, Formenti SC, Schneider RJ. A hypoxia-controlled cap-dependent to cap-independent translation switch in breast cancer. *Mol Cell.* 2007; 28(3):501–512. [PubMed: 17996713]
- Breward CJW, Byrne HM, Lewis CE. Modeling the interactions between tumour cells and a blood vessel in a microenvironment within a vascular tumour. *Eur J Appl Math.* 2001; 12:529–556.
- Butterworth A E, Cater D. Effect of lysolecithin on oxygen uptake of tumour cells polymorphonuclear leucocytes lymphocytes and macrophages in vitro. *Br J Cancer.* 1967; 21(2):373–389. [PubMed: 4291113]
- Caldwell J, Locey B, Clarke MF, Emerson SG, Palsson BO. Influence of medium exchange schedules on metabolic, growth, and GM-CSF secretion rates of genetically engineered NIH-3T3 cells. *Biotechnol Prog.* 1991; 7:1–8. [PubMed: 1366977]
- Casciari JJ, Sotirchos SV, Sutherland RM. Glucose diffusivity in multicellular tumor spheroids. *Cancer Res.* 1988; 48:3905–3909. [PubMed: 3383189]
- Chaplain M. The mathematical modeling of tumor angiogenesis and invasion. *Acta Biotheor.* 1995; 43:387–402. [PubMed: 8919350]
- Chen Y, Cairns R, Papandreou I, Koong A, Denko NC. Oxygen consumption can regulate the growth of tumors, a new perspective on the warburg effect. *PLoS ONE.* 2009; 4(9):27033.
- Curry J, Eubank T, Roberts R, Wang Y, Pore N, Maity A, Marsh C. M-CSF signals through the MARK/ERK pathway via Sp1 to induce VEGF production and induces angiogenesis in vivo. *PLoS ONE.* 2008; 3(10):e3405. [PubMed: 18852899]
- Eubank T, Galloway M, Montague C, Waldman W, Marsh C. M-CSF induces vascular endothelial growth factor production and angiogenic activity from human monocytes. *J Immunol.* 2003; 175(5):2637–2643. [PubMed: 12928417]
- Eubank T, Roberts RD, Galloway M, Wang Y, Cohn D, Marsh C. GM-CSF induces expression of soluble VEGF receptor-1 from human monocytes and inhibits angiogenesis in mice. *Immunity.* 2004; 21:831–842. [PubMed: 15589171]
- Eubank T, Roberts RD, Khan M, Curry J, Nuovo GJ, Kuppusamy P, Marsh C. Granulocyte macrophage Colony-Stimulating factor inhibits breast cancer growth and metastasis by invoking an anti-angiogenic program in tumor-educated macrophages. *Cancer Res.* 2009; 69(5):2133–2140. [PubMed: 19223554]
- Eubank T, Roda JM, Liu H, O’Neil T, Marsh C. Opposing roles for HIF-1 α and HIF-2 α in the regulation of angiogenesis by mononuclear phagocytes. *Blood.* 2011; 117(1):323–331. [PubMed: 20952691]
- Gabhann FM, Popel AS. Model of competitive binding of vascular endothelial growth factor and placental growth factor to VEGF receptors on endothelial cells. *Am J Physiol Heart Circ Physiol.* 2004; 286:H153–H164. [PubMed: 12714333]
- Girgis-Gabardo A, Hassell J. Scale-up of breast cancer stem cell aggregate cultures to suspension bioreactors. *Biotechnol Prog.* 2008; 22:801–810. [PubMed: 16739964]
- Gordon S. Alternative activation of macrophages. *Nat Rev Immunol.* 2003; 1:23–35. [PubMed: 12511873]

- Grabhann FM, Popel AS. Model of competitive binding of vascular endothelial growth factor and placental growth factor to VEGF receptors on endothelial cells. *Am J Physiol Heart Circ Physiol*. 2003; 286:H153–H164. [PubMed: 12714333]
- Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest*. 2006; 116(6):1494–1505. [PubMed: 16691291]
- Koshikawa N, Iyozumi A, Gassmann M, Takenaga K. Constitutive upregulation of hypoxiainducible factor 1 α mRNA occurring in highly metastatic lung carcinoma cells leads to vascular endothelial growth factor over-expression upon hypoxic exposure. *Oncogene*. 2003; 22:6717–6724. [PubMed: 14555985]
- Kuratsu J, Yoshizato K, Yoshimura T, Leonard EJ, Takeshima H, Ushio Y. Quantitative study of monocyte chemoattractant Protein-1 (MCP-1) in cerebrospinal fluid and cyst fluid from patients with malignant glioma. *J Natl Cancer Inst*. 1993; 85:1836–1839. [PubMed: 8230263]
- Lawichi S, Szmikowski M, Wojtukiewicz M. The pretreatment plasma level and diagnostic utility of M-CSF in benign breast tumor and breast cancer patients. *Clin Chim Acta*. 2006; 371:112–116. [PubMed: 16631152]
- Leitzel, K.; Ettenberg, S.; Walsh, R.; Abraham, J.; Modur, V.; Braendle, E.; Evans, D.; Ali, S.; Demers, L.; Lipton, A. Elevated serum M-CSF level predicts reduced survival in metastatic breast cancer patients. *J Clin Oncol*; ASCO annual meeting proceedings I; 2007.
- Leonard E, Skeel A, Yoshimura T, Rankin J. Secretion of monocyte chemoattractant protein (MCP-1) by human mononuclear phagocytes. *Adv Exp Med Biol*. 1991; 351:55–64. [PubMed: 7942299]
- Less J, Skalak T, Sevic E, Jain R. Microvascular architecture in a mammary-carcinoma—branching patterns and vessel dimensions. *Cancer Res*. 1991; 51:265–273. [PubMed: 1988088]
- Li C. The glucose distribution in 9L rat brain multicell tumour spheroid and its effect on cell necrosis. *Cancer*. 1982; 50:2066–2073. [PubMed: 7127250]
- Macdougall JDB, McCabe M. Diffusion coefficient of oxygen through tissues. *Nature*. 1967; 215:1173–1174. [PubMed: 6061810]
- Marino S, Hogue I, Ray C, Kirschner D. A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J Theor Biol*. 2008; 254:178–196. [PubMed: 18572196]
- Melillo G, Sausville E, Cloud K, Lahusen T, Varesio L, Senderowicz A. Flavopiridol, a protein kinase inhibitor, down-regulates hypoxic induction of vascular endothelial growth factor expression in human monocytes. *Cancer Res*. 1999; 59(21):5433–5437. [PubMed: 10554012]
- Nalwaya N, Deen W. Nitric oxide, oxygen, and superoxide formation and consumption in macrophage cultures. *Chem Res Toxicol*. 2008; 18:486–493. [PubMed: 15777088]
- Oren H, Duman N, Abacioglu H, Ozkan H, Irken G. Association between serum macrophage Colony-Stimulating factor levels and monocyte and thrombocyte counts in healthy, hypoxic, and septic term neonates. *Pediatrics*. 2001; 108(2):329–332. [PubMed: 11483796]
- Owen MR, Sherratt JA. Pattern formation and spatiotemporal irregularity in a model for macrophage tumour interactions. *J Theor Biol*. 1997; 189(1):63–80. [PubMed: 9398504]
- Owen MR, Byrne HM, Lewis CE. Mathematical modeling of the use of macrophages as vehicles for drug delivery to hypoxic tumour sites. *J Theor Biol*. 2004; 226:377–391. [PubMed: 14759644]
- Papayianni A, Alexopoulos E, Giamalis P, Gionanlis L, Belechri A, Koukoudis P, Memmos D. Circulating levels of ICAM-1, VCAM-1, and MCP-1 are increased in haemodialysis patients: association with inflammation, dyslipidaemia, and vascular events. *Nephrol Dial Transplant*. 2002; 17:435–441. [PubMed: 11865089]
- Pawelcz N, Knierim M. Tumor-related angiogenesis. *Crit Rev Oncol /Hematol*. 1989; 9:197–242.
- Pettet G, Byrne H, McElwain D, Norbury J. A model of wound-healing angiogenesis in soft tissue. *Math Biosci*. 1996; 136:35–63. [PubMed: 8755336]
- Pettet G, Please C, Tindall M. The migration of cells in multicell tumor spheroids. *Bull Math Biol*. 2001; 63:231–257. [PubMed: 11276525]
- Plank M, Sleeman B, Jones PF. A mathematical model of tumour angiogenesis, regulated by vascular endothelial growth factor and the angiopoietins. *J Theor Biol*. 2004; 229:435–454. [PubMed: 15246783]

- Pollard JW. Role of colony-stimulating factor-1 in reproduction and development. *Mol Reprod Dev.* 1997; 46:54–60. [PubMed: 8981364]
- Pyaskovskaya ON, Kolesnik DL, Kolobov AV, Vovyanok SI, Solyanik GI. Analysis of growth kinetics and proliferative heterogeneity of lewis lung carcinoma cells growing as unfed culture. *Exp Oncol.* 2008; 30(4):269–275. [PubMed: 19112423]
- Qian B, Deng Y, Hong Im J, Muschel RJ, Zou Y, Li J, Lang RA, Pollard JW. A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. *PLoS ONE.* 2009; 4(8):e6562. [PubMed: 19668347]
- Rapella A, Negrioli A, Melillo G, Pastorino S, Varesio L, Bosco M. Flavopiridol inhibits vascular endothelial growth factor production induced by hypoxia or picolinic acid in human neuroblastoma. *Int J Cancer.* 2002; 99:658–664. [PubMed: 12115498]
- Roda JM, Summer LA, Evans R, Philips GS, Marsh CB, Eubank TD. Hypoxia-inducible factor-2 α regulates GM-CSF-derived soluble vascular endothelial growth factor receptor 1 production from macrophages and inhibits tumor growth and angiogenesis. *J Immunol.* 2011; 187:1970–1976. [PubMed: 21765015]
- Roda J, Wang Y, Sumner L, Phillips G, Eubank T, Marsh C. Stabilization of HIF-2 α induces SVEGFR-1 production from Tumor-associated macrophages and enhances the Anti-tumor effects of GM-CSF in murine melanoma model. *J Immunol.* 2012; 189:3168–3177. [PubMed: 22869907]
- Schugart RC, Friedman A, Zhao R, Sen CK. Wound angiogenesis as a function of tissue oxygen tension. *Proc Natl Acad Sci USA.* 2008; 105(7):2628–2633. [PubMed: 18272493]
- Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev.* 2003; 3:721–732.
- Shang Z, Li Z, Li J. VEGF is up-regulated by hypoxic stimulation and related to tumor angiogenesis and severity of disease in oral squamous cell carcinoma: in vitro and in vivo studies. *Int J Oral Maxillofac Surg.* 2006; 35:533–538. [PubMed: 16388929]
- Stokes C, Lauffenburger D. Analysis of the roles of microvessel endothelial cell random motility and chemotaxis in angiogenesis. *J Theor Biol.* 1991; 152:377–403. [PubMed: 1721100]
- Szomolay B, Eubank T, Roberts R, Marsh C, Friedman A. Modeling the inhibition of breast cancer growth by GM-CSF. *J Theor Biol.* 2012; 303:141–151. [PubMed: 22763136]
- Tang S, Liu H, Rao Q, Geng Y, Zheng G, Zheng D, Wu K. Internalization and half-life of membrane-bound macrophage colony-stimulating factor. *Chin Sci Bull.* 2000; 45(18):1697–1703.
- Utting JC, Flanagan AM, Brandao-Burch A, Orriss IR, Arnett TR. Hypoxia stimulates osteoclast formation from human peripheral blood. *Cell Biochem Funct.* 2010; 28(5):374–380. [PubMed: 20556743]
- Varney M, Olsen KJ, Mosley R, Singh R. Paracrine regulation of vascular endothelial growth factor-expression during macrophage-melanoma cell interaction: role of monocyte chemotactic protein-1 and macrophage colony-stimulating factor. *J Interferon Cytokine Res.* 2005; 25(11):674–683. [PubMed: 16318581]
- Vaupel P, Mayer A, Briest S, Hockel M. Oxygenation gain factor: a novel parameter characterizing the association between hemoglobin level and the oxygenation status of breast cancers. *Cancer Res.* 2003; 63:7634–7637. [PubMed: 14633681]
- Vicioso L, Gonzalez F, Alvarez M, Ribelles N, Molina M, Marquez A, Perez L, Matilla A, Alba E. Elevated serum levels of vascular endothelial growth factor are associated with tumor-associated macrophages in primary breast cancer. *Am J Clin Pathol.* 2006; 125:111–118. [PubMed: 16482999]
- Vincensini D, Dedieu V, Eliat PA, Vincent C, Bailly C, de Certaines J, Joffre F. Magnetic resonance imaging measurements of vascular permeability and extracellular volume fraction of breast tumors by dynamic Gd-DTPA-enhanced relaxometry. *J Magn Reson Imaging.* 2007; 25:293–302.
- Ward J, King J. Mathematical modeling of avascular tumor growth. *IMA J Math Appl Med Biol.* 1997; 14:39–69. [PubMed: 9080687]
- Ward J, King J. Mathematical modeling of avascular tumor growth II: modeling growth saturation. *IMA J Math Appl Med Biol.* 1999; 16:171–211. [PubMed: 10399312]
- Wathen K, Sarvela J, Stenman F, Stenman U, Vuorela P. Changes in serum concentrations of soluble vascular endothelial growth factor receptor-1 after pregnancy. *Hum Reprod.* 2011; 26(1):221–226. [PubMed: 21097488]

- Williams M, Kelsey S, Newland A. GM-CSF and stimulation of monocyte/macrophage function in vivo relevance and in vitro observations. *Eur J Cancer*. 1999; 35(3):S1–S22.
- Wu FTH, Stefanini MO, Gabhann FM, Popel AS. A compartment model of VEGF distribution in humans in the presence of soluble VEGF receptor-1 acting as a ligand trap. *PLoS ONE*. 2009; 4(4):e5108. [PubMed: 19352513]
- Wu FTH, Stefanini MO, Gabhann FM, Kontos CD, Annex BH, Popel AS. VEGF and soluble VEGF receptor-1 (sFlt-1) distributions in peripheral arterial disease: an in silico model. *Am J Physiol Heart Circ Physiol*. 2010; 298:H2174–H2191. [PubMed: 20382861]

Fig. 1.

Cells-cytokines-oxygen interaction pathways in the process of tumor growth: live mammary tumor cells secrete M-CSF, which induces pro-angiogenic features from the macrophage. These tumor-educated macrophages also produce MCP-1/CCL2, a chemoattractant for monocytes, to recruit more macrophages into the tumor (*vertical double-ended arrows*). At hypoxia these macrophages, along with live tumor cells, secrete VEGF that induces endothelial cells to form new vessels and provide oxygen to the tumor. To the contrary, GM-CSF “re-educates” the tumor macrophages to produce sVEGFR-1, which sequesters and inhibit VEGF (*horizontal double-ended arrows*) thus suppressing angiogenesis. During this process, the transcription factor HIF-1 α in macrophages upregulates (*left dashed line*) VEGF production while HIF-2 α regulates (*right dashed line*) sVEGFR-1 production. Additionally, oxygen is consumed by both live tumor cells and macrophages. Dead tumor cells, either from necrosis or apoptosis, are removed by the tumor macrophages

Fig. 2.

The two-phase free boundary problem: the tumor region is modeled as $\Omega(t)$ enclosed by the curve $\partial\Omega(t)$ and it is contained in a domain D . The region $D \setminus \Omega(t)$ represents a healthy tissue. The tumor region $\Omega(t)$ and its boundary $\partial\Omega(t)$ evolve with respect to time while D keeps fixed. Both live and dead tumor cells are assumed to be in the tumor region $\Omega(t)$; macrophages and EC are assumed to be in both tumor and healthy tissue; cytokines and oxygen molecules can diffuse throughout the whole domain D . Abnormal proliferation of tumor cells generates internal pressure in $\Omega(t)$, resulting a velocity field $\vec{v} \neq 0$ (while $\vec{v} \equiv 0$ in $D \setminus \Omega(t)$). All cells are assumed to be convected under this macroscopic velocity field while their random motions are neglected because the small diffusion coefficients

Fig. 3.
Assumed oxygen level-dependent proliferation and necrosis rates of live tumor cells.
Horizontal: w_n , w_h , and w_0 are thresholds of necroxia, hypoxia, and normoxia, respectively

Fig. 4.

Statistically significant PRCC values (p-value < 0.01) for $R(t)$ at (a) day 7 and (b) day 15. The positive PRCC means that increasing the corresponding parameter leads to an increase in the tumor radius

Fig. 5.

Simulations compared with experimental data. *Horizontal axis* represents time in days and *vertical axis* is tumor volume in units of mm³; *colored solid lines* are model simulations, *solid dots with error bars* are experimental measurements. Label “w/o GM-CSF & HIF-1 α KO” means “without GM-CSF treatment, but with HIF-1 α KO”. Similar labels are used for GM-CSF and HIF-2 α treatments. **(a)** Tumor growth under HIF-1 α KO (or not) and GM-CSF treatment (or not); **(b)** tumor growth under HIF-2 α KO (or not) and GM-CSF treatment (or not) (Color figure online)

Fig. 6.

Simulations: comparisons of relative VEGF and sVEGFR-1 densities under different conditions. *White bars*, tumor growth without GM-CSF; *black bars*, tumor growth with GM-CSF. *Horizontal axis from left to right*: normal, HIF-1 α -KO, and HIF-2 α -KO macrophages. **(a)** Vertical axis represents relative VEGF density, **(b)** Vertical axis represents relative sVEGFR-1 density

Fig. 7.

Simulations: evolution of EC densities inside and outside of the tumor. *Horizontal axis*, distance from the tumor center in units of mm; *vertical axis*, time in days. Color intensity, dimensionless EC density e/e_0 . *White curve*: tumor boundary. Initially (day 0), the EC density is set to be low (*dark blue*) inside and around the tumor ($r = 0-3$ mm), while to be the average density e_0 (*light blue*) in the healthy tissue from 3 to 10 mm. **(a)** Without GM-CSF treatment, the EC move toward the tumor fast by chemotaxis in the first 7 days (from day 0 to day 7, the *light blue area* “travels” toward the tumor boundary). **(b)** with GM-CSF treatment, the chemotaxis is almost suppressed in the first 7 days (from day 0 to day 7, the *light blue area* stays at $r \approx 3$ mm), and afterward the EC starts to move toward tumor slowly (Color figure online)

Fig. 8.

Simulations: effects of different doses **(a)** and frequencies **(b)** of GM-CSF treatments in tumor growth inhibition. *Horizontal axis*, time in days; *vertical axis*, tumor volume in mm³. In **(a)**, tumor growth with 200 ng, 100 ng, 50 ng, and 25 ng GM-CSF three times a week are compared to growth without GM-CSF treatment. In **(b)**, tumor growth with daily, three times a week, and weekly injection of same total amount 600 ng of GM-CSF (Color figure online)

Fig. 9. Simulations: comparing tumor sizes at day 15 with extents of HIF-1 α degradation **(a)** and HIF-2 α stabilization **(b)**, with GM-CSF treatment or not. *Black*: without GM-CSF treatment; *white*: with GM-CSF treatment. *Vertical axis*, tumor volume in mm³ **(a)**: different percentages of HIF-1 α degradation; **(b)**: different extents of HIF-2 α stabilization

Table 1**Model variables and units**

Cells	
$c(r, t)$	live tumor cell density (cell/cm ³)
$b(r, t)$	dead tumor cell density (cell/cm ³)
$m(r, t)$	macrophage density (cell/cm ³)
$e(r, t)$	endothelial cell density (cell/cm ³)
Cytokines	
$q(r, t)$	M-CSF density (g/cm ³)
$p(r, t)$	MCP-1/CCL2 density (g/cm ³)
$g(r, t)$	GM-CSF density (g/cm ³)
$h(r, t)$	VEGF density (g/cm ³)
$s(r, t)$	sVEGFR-1 density (g/cm ³)
Oxygen	
$w(r, t)$	density (g/cm ³)

Table 2

Model parameter values and reference

Parameter	Dimensional	Dimensionless	Reference
μ_c	$4.80 \times 10^{-6} \text{ s}^{-1}$	1.20	Breward et al. (2001)
μ_b	$4.80 \times 10^{-14} \text{ cm}^3 \text{ cell}^{-1} \text{ s}^{-1}$	2.40	Breward et al. (2001) and estimated
μ_q	$4.80 \times 10^{-5} \text{ s}^{-1}$	12.00	Tang et al. (2000)
μ_p	$2.00 \times 10^{-5} \text{ s}^{-1}$	5.00	Owen and Sherratt (1997)
μ_s	$1.98 \times 10^{-5} \text{ s}^{-1}$	4.97	Wathen et al. (2011)
μ_s^-	$1.19 \times 10^5 \text{ cm}^3 \text{ g}^{-1} \text{ s}^{-1}$	29.7	Wu et al. (2009) and estimated
μ_h	$1.26 \times 10^{-4} \text{ s}^{-1}$	31.60	Plank et al. (2004)
μ_h^-	$3.57 \times 10^5 \text{ cm}^3 \text{ g}^{-1} \text{ s}^{-1}$	89.3	Wu et al. (2009) and estimated
μ_g	$1.60 \times 10^{-5} \text{ s}^{-1}$	4.00	Williams et al. (1999)
λ_1	$2.41 \times 10^{-5} \text{ s}^{-1}a$	6.00	Owen et al. (2004), Qian et al. (2009)
λ_2	$9.63 \times 10^{-6} \text{ s}^{-1}$	2.41	Owen et al. (2004), Qian et al. (2009) and estimated
λ_3	$3.20 \times 10^{-22} \text{ g s}^{-1} \text{ cell}^{-1}$	57.60	Utting et al. (2010), Oren et al. (2001) and estimated
λ_4	$1.92 \times 10^{-20} \text{ g s}^{-1} \text{ cell}^{-1}$	9.60×10^2	Bosco et al. (2004) and estimated
λ_5	$1.51 \times 10^{-21} \text{ g s}^{-1} \text{ cell}^{-1}$	2.72×10^2	Braunstein et al. (2007), Owen et al. (2004), Owen and Sherratt (1997), Pyaskovskaya et al. (2008) and estimated
λ_6	$1.83 \times 10^{-20} \text{ g s}^{-1} \text{ cell}^{-1}$	9.15×10^2	Melillo et al. (1999), Vicioso et al. (2006) and estimated
λ_7	$1.86 \times 10^{-20} \text{ g s}^{-1} \text{ cell}^{-1}$	5.43×10^2	Wu et al. (2010) and estimated
λ_8	$2.22 \times 10^{-14} \text{ g s}^{-1} \text{ cell}^{-1}$	30.00	estimated
λ_9	$5.61 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1} \text{ cell}^{-1}$	28.11	Girgis-Gabardo and Hassell (2008) and estimated
λ_{10}	$4 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1} \text{ cell}^{-1}$	72.00	Butterworth A and Cater (1967), Chen et al. (2009) and estimated
k_p	$6.00 \text{ cm}^5 \text{ g}^{-1} \text{ s}^{-1}$	6.00×10^{-3}	Owen et al. (2004)
k_g	$1.00 \text{ cm}^5 \text{ g}^{-1} \text{ s}^{-1}$	1.00×10^{-3}	estimated
k_h	$24.00 \text{ cm}^5 \text{ g}^{-1} \text{ s}^{-1}$	1.00×10^{-3}	Schugart et al. (2008)
D_p, D_q	$2.00 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	2.00	Casciari et al. (1988), Owen et al. (2004), Owen and Sherratt (1997) and estimated
D_p, D_s	$1.00 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	1.00	Schugart et al. (2008), Grabhann and Popel (2003), Gabhann and Popel (2004) and estimated
D_w	$2.00 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$	20.00	Macdougall and McCabe (1967) and estimated
D_g	$3.00 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$	3.00	Caldwell et al. (1991) and estimated
w_0	$4.65 \times 10^{-4} \text{ g cm}^{-3}$	1.00	Vaupel et al. (2003)
w_n	$3.57 \times 10^{-5} \text{ g cm}^{-3}$	0.077	Vaupel et al. (2003) and estimated
w^*	$1.69 \times 10^{-4} \text{ g cm}^{-3}$	0.36	estimated
w_h	$1.00 \times 10^{-4} \text{ g cm}^{-3}$	0.22	Vaupel et al. (2003) and estimated
θ_3	fraction number	0.90^b	estimated
c^*	$1.00 \times 10^9 \text{ cell cm}^{-3}$	1.28	Eubank et al. (2009)
c_0	$7.20 \times 10^8 \text{ cell cm}^{-3}$	1.00	Eubank et al. (2009), Vincensini et al. (2007)
m_0	$2.00 \times 10^8 \text{ cell cm}^{-3}$	1.00	Eubank et al. (2009)
e_0	$2.50 \times 10^6 \text{ cell cm}^{-3}$	1.00	estimated

Parameter	Dimensional	Dimensionless	Reference
q_0, p_0, s_0, h_0, g_0	$1.00 \times 10^{-9} \text{ g cm}^{-3}$	1.00	Scaling factor
τ	$2.50 \times 10^5 \text{ s}$	1.00	Scaling factor
L_0	0.50 cm	1.00	Scaling factor

^a A different value, $\lambda_1 = 1.68 \times 10^{-5}$ is used for Fig. 5(b)

^b A different value, $\theta_3 = 0.87$ is used for Fig. 5(b)

Table 3

Parameters chosen for sensitivity analysis and their ranges

Parameter	Minimum	Baseline	Maximum	Units
μ_c	2.40×10^{-6}	4.80×10^{-6}	7.20×10^{-6}	s^{-1}
μ_q	2.40×10^{-5}	4.80×10^{-5}	9.60×10^{-5}	s^{-1}
μ_p	1.00×10^{-5}	2.00×10^{-5}	4.00×10^{-5}	s^{-1}
μ_s	6.63×10^{-5}	1.98×10^{-5}	5.60×10^{-5}	s^{-1}
μ_s^-	1.00×10^5	1.19×10^5	2.00×10^5	$cm^3 g^{-1} s^{-1}$
μ_h	5.00×10^{-5}	1.26×10^{-4}	3.00×10^{-4}	s^{-1}
μ_g	1.38×10^{-5}	1.6×10^{-5}	1.93×10^{-5}	s^{-1}
λ_1	2.00×10^{-5}	2.41×10^{-5}	8.00×10^{-5}	s^{-1}
λ_2	5.00×10^{-6}	9.63×10^{-6}	2.00×10^{-5}	s^{-1}
λ_5	1.00×10^{-21}	1.51×10^{-21}	1.00×10^{-20}	$g s^{-1} cell^{-1}$
λ_9	1.00×10^{-13}	5.61×10^{-13}	1.00×10^{-12}	$cm^3 s^{-1} cell^{-1}$
λ_{10}	1.00×10^{-13}	4.00×10^{-13}	1.00×10^{-12}	$cm^3 s^{-1} cell^{-1}$
k_p	2.00	3.00	6.00	$cm^5 g^{-1} s^{-1}$
k_g	1.00	10.00	30.00	$cm^5 g^{-1} s^{-1}$
k_h	20	24	80	$cm^5 g^{-1} s^{-1}$
D_p, D_q	1.00×10^{-6}	2.00×10^{-6}	5.00×10^{-6}	$cm^2 s^{-1}$
D_h, D_s	5.00×10^{-7}	1.00×10^{-6}	2.00×10^{-6}	$cm^2 s^{-1}$
D_w	1.00×10^{-5}	2.00×10^{-5}	5.00×10^{-5}	$cm^2 s^{-1}$
D_g	1.00×10^{-6}	3.00×10^{-6}	5.00×10^{-6}	$cm^2 s^{-1}$
w^*	1.00×10^{-4}	1.69×10^{-4}	4.65×10^{-4}	$g cm^{-3}$
θ_3	0.85	0.9	0.95	fraction number