Mathematical analysis of Glioblastoma invasion in 3D

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Abstract: The most malignant brain cancer, so called Glioblastoma, has extremely poor outcome. It confounds the clinical management of glioblastomas due to the hight local invasiveness of these cancer cells. Furthermore the mechanisms governing invasion are poorly understood. Stein et al. proposed a continuum mathematical model of the dispersion behaviours based on a Swanson's model describing glioblastoma invasion. They conducted experiments on the patterns of growth and dispersion of U87 glioblastoma tumour spheroids in a three-dimensional collagen gel. They intended to identify and characterize discrete cellular mechanisms underlying invasive cell motility from the experimental data. The mathematical model reproduces an characteristic behaviour of the U87WT invasive cells that they have a strong radial directional motility bias away from the spheroid center. However in their experiments it is observed that the U87WT invasive cells sometimes exhibit more complicated behaviour than indicated by their model. We propose a mathematical model in a more general form of the radially biased component term of their model so that it covers more realistic behaviour of U87WT cells in the experiment. We show a rigorous mathematical analysis of our model and give computer simulation of cell motility in the experiment from our mathematical model.

Key–Words: Glioblastoma, 3D invasion, Tumour, radially biased motility, Collagen, Mathematical model, Mathematical analysis, Computer simulation, Existence of solution, Asymptotic behaviour of solution.

1 Introduction

In 2007 Stein et al. [18] presented results from their experiment where tumour spheroids are grown in three-dimensional collagen gels ([3], [4], [5]). They describe a continuum mathematical model that allows us to quantitatively interpret the data. Fitting the model to the experimental data indicates that Glioma cells invade in a more biased manner, away from the tumour spheroid and are shed from the spheroid at a great rate, suggesting lower cell-cell adhesion and they specified the extent of invasive cell population. When we follow to their mathematical model, the path of invasive cell radiates in a fixed direction and at a constant velocity. However it is observed that they sometimes exhibit more complicated and undirected behaviour, such as greatly turn around or turn back to one's path. In order to describe such kind of behaviour of cell motility we generalize their mathematical model by extending the radially biased component term.

The goal of this paper is to better understand the mechanisms governing invasive cell behaviour. We will show rigorous mathematical analysis of our model and give computer simulations of cell motility of our mathematical model, which includes the simulations in [18].

1.1 Mathematical models

Several mathematical models have been known in the literature for cell invasion ([1], [6], [21]). In the single model for core and invasive cell behaviour by Swanson et al. [17], tumour growth is described by a reaction-diffusion equation:

$$\frac{\partial u}{\partial t} = D\nabla^2 u + gu\left(1 - \frac{u}{u_{max}}\right) \qquad (1.1)$$

where cell concentration u move along undirected, random paths as a function of position and time, cells throughout the tumour are assumed to proliferate at a constant rate g until they reach a limiting density, u_{max}, the constant D is the diffusion (undirected motion); the larger D becomes, the more motile the cells. This model assumes spherical symmetry of the multicellular tumour spheroid. The single-population reaction-diffusion model has been used

with some success to describe how a tumour responds to chemotherapy and why surgical removal of GBM is usually not effective ([17]). This model is only applicable for tumours that are $> 1mm^3$ and it fails for smaller tumours.

In addition, Stein et al. [18] considered that the invasive cells are biased to move away from the center of the tumour spheroid at an average speed, v_i. It has been observed that invasive cells may follow directed paths away from the tumour spheroid . The cause of this bias is not known. It may be due to attraction toward nutrients in the environment, repulsion from waste products produced by the spheroid, or a re-alignment of the collagen gel as the cells move. They proposed the following equation for the evolution of the cell population, u_i

$$\frac{\partial u_i}{\partial t} = \underbrace{D\nabla^2 u_i}_{\text{diffusion}} - \underbrace{v_i \nabla_r \cdot u_i}_{\text{a radially biased component}}$$

$$+ \underbrace{s\delta(r - R(t))}_{\text{shedding invasive cells rate}} + \underbrace{gu_i\left(1 - \frac{u_i}{u_{max}}\right)}_{\text{proliferation}} \quad (1.2)$$

The behaviour of invasive cells can be described by four parameters: $\{D, v_i, s, g\}$. Invasive cells are introduced into the population through shedding from the core surface, s, and proliferation, g. Cell motility is modeled as having an undirected component, D, and a radially biased component, v_i. In the above equation, δ is the Dirac delta function, r is the spatial coordinate for the radial distance from the tumour center, and $R(t)$ is the radius of the core at time t. We take the core radius to be given by $R(t) = R_0 + v_c t$, where R_0 is the initial tumour radius, and v_c is the rate at which the core increases in radius.

In in vitro experiments of glioma tumour 3D invasion in collagen gel by Stein et. al. ([16; ChapterIV], [20]) , invasive cells with the radially biased motility away from the spheroid center make a progress initially and after that they often exhibit more complicated behaviour(see Figure1.(a)). It seems that such complicated behaviour of invasive cells can not be reproduced by their simulation as in Figure1.(b) or even using (1.2), because their radially biased component term of (1.2) is *linear*. In order to describe the *nonlinear* paths of such cell motility we propose a mathematical model generalized the radially biased component of their model to a *nonlinear* term. We also gain rigorous mathematical analysis of our model and give computer simulation of cell motility of the mathematical model.

(a) Cell paths from experiment: day 1   (b) Cell paths simulated: day 1
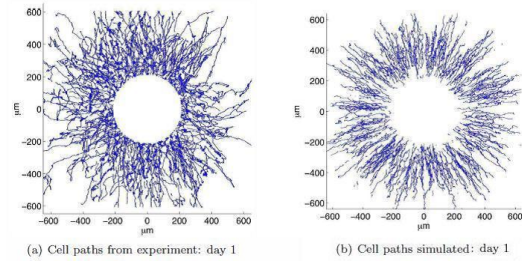
Figure 1: In vitro experiments of glioma tumour 3D invasion in collagen gel performed by Stein and coworkers in [16],[20].
(a) Cell trajectories from experiment (b) Simulation of cell trajectories.

1.2 Mathematical model generalized radially biased component

Now we consider the problem for the model by Stein et al. [18] for invasive cell population $u := u(x,t)$

$$(1.3) \begin{cases} \dfrac{\partial u}{\partial t} = D\nabla^2 u - v\nabla_r \cdot u + s\delta(r - R(t)) + gu\left(1 - \dfrac{u}{u_{max}}\right) \\ \qquad\qquad\qquad\qquad\qquad\qquad in\ \Omega \times (0, T) \\ \\ \dfrac{\partial}{\partial \nu} u|_{\partial\Omega} = 0, \\ \\ u|_{t=0} = u_0(x) \end{cases}$$

Since we especially focus on the behaviour of each cell of dispersing cells at the spheroid's edge, neglecting the effect of δ function and proliferation, that is, we consider

$$\frac{\partial u}{\partial t} = D\nabla^2 u - v\nabla_r \cdot u \quad (1.4)$$

Furthermore we generalize $\nabla_r \cdot u$ as follows. For $r = (r_1, \cdots, r_n)$ we have

$$\nabla_r \cdot u = (r_1, \cdots, r_n) \cdot (u_{x_1}, \cdots, u_{x_n})$$

putting $(u_{x_1}, \cdots, u_{x_n}) = \nabla u$

$$= \nabla u \cdot (r_1, \cdots, r_n)$$
$$= \nabla \cdot u(r_1, \cdots, r_n)$$
$$= \nabla \cdot u\nabla(x_1 r_1 + \cdots + x_n r_n).$$

Replacing $(x_1 r_1 + \cdots + x_n r_n)$ by $\log(\alpha + w)$ for a positive constant α it follows from $\nabla_r \cdot u$

$$\nabla \cdot (u\nabla \log(\alpha + w)).$$

Therefore (1.4) is extended to the following equation.

$$\frac{\partial u}{\partial t} = D\nabla^2 u - \nabla \cdot (u\nabla \log(\alpha + w)) \qquad (1.5)$$

(1.5) is considered by Othmer-Stevens [13] as a continuum model of reinforced random walks where w is called control species and $\log(\alpha + w)$ is, so called, a sensitivity function. Hence it is seen that (1.5) admits a randomwalking of the invasive cell along the direction and the velocity indicated by the radially biased component in (1.2). The following systems for (1.5) is applied to a understanding of tumour angiogenesis([2],[12]).

$$(1.6) \begin{cases} \dfrac{\partial u}{\partial t} = D\nabla^2 u - \nabla \cdot \chi_0(u\nabla \log(\alpha + w)) \\ \qquad\qquad\qquad\qquad in\ \Omega \times (0,\infty) \\[2ex] \dfrac{\partial w}{\partial t} = -kuw \\ \qquad\qquad\qquad\qquad in\ \Omega \times (0,\infty) \\[2ex] \dfrac{\partial}{\partial \nu} u|_{\partial\Omega} = 0 \\ \qquad\qquad\qquad\qquad on\ \partial\Omega \times (0,\infty) \\[2ex] u(x,0) = u_0(x) \qquad\quad in\ \Omega \end{cases}$$

where D is a positive constant, Ω is a bounded domain in R^n and $\partial\Omega$ is a smooth boundary of Ω and ν is the outer unit normal vector.

2 Mathematical analysis

In this section we review a known mathematical result related to our model and apply it to ours and Othmer-Stevens model, which plays an important role to carry out the computer simulation of (1.6).

2.1 Known result

In Kubo [7] the following initial Neumann-boundary value problems of nonlinear evolution equations is considered(see [8]-[11]).

$$(NE) \begin{cases} u_{tt} = D\nabla^2 u_t + \nabla \cdot (\chi(u_t, e^{-u})e^{-u}\nabla u) \\ \qquad\qquad\qquad in\ \Omega \times (0,\infty)\quad (2.1) \\ \dfrac{\partial}{\partial\nu}u\,|_{\partial\Omega} = 0 \qquad on\ \partial\Omega \times (0,\infty)\quad (2.2) \\ u(x,0) = u_0(x),\ \ u_t(x,0) = u_1(x)\ in\ \Omega\ (2.3) \end{cases}$$

where we denote

$$\frac{\partial}{\partial t} = \partial_t,\ \frac{\partial}{\partial x_i} = \partial_{x_i},\ i = 1,\cdots,n,$$

$$\nabla u = \mathrm{grad}_x u = (\partial_{x_1} u,\cdots,\partial_{x_n} u) \qquad (2.4)$$

Suppose that the assumption (A) holds.

(A) Let $B_{r+} = B_r \cap R \times R_+$, where B_r is a ball of radius r at 0 in R^2. For any constant $r > 0$ and $(s_1, s_2) \in B_{r+}$ there exist positive constants c_r, c'_r and δ_r such that for any integer $m \geq [n/2] + 3$

$$c_r(b - \delta_r) < \chi(s_1 + b, s_2) \in C^m(R \times R_+), \qquad (2.5)$$

$$\sup_{(s_1,s_2)\in B_{r+}} |\, (\partial_{s_1}^k \partial_{s_2}^l \chi)(s_1 + b, s_2)\,| \leq c'_r b,$$
$$0 \leq k + l \leq m. \qquad (2.6)$$

Now let us introduce function spaces. First, $H^l(\Omega)$ denotes the usual Sobolev space $W^{l,2}(\Omega)$ of order l on Ω. For functions $h(x,t)$ and $k(x,t)$ defined in $\Omega \times [0,\infty)$, we put

$$(h, k)(t) = \int_\Omega h(x,t)k(x,t)dx,$$

$$\|h\|_l^2(t) = \sum_{|\beta|\leq l} \|\partial_x^\beta h(\cdot,t)\|_{L^2(\Omega)}^2.$$

The eigenvalues of $-\Delta$ with the homogeneous Neumann boundary conditions are denoted by

$$\{\lambda_i | i = 1, 2, \cdots\},$$

which are arranged as

$$0 < \lambda_1 \leq \lambda_2 \leq \cdots \to +\infty,$$

and $\varphi_i = \varphi_i(x)$ indicates the L^2 normalized eigenfunction corresponding to λ_i.

For a non-negative integer l, we set $W^l(\Omega)$ as a closure of $\{\varphi_1, \varphi_2, \cdots, \varphi_n, \cdots\}$ in the function space $H^l(\Omega)$. Taking $\lambda_1 \neq 0$ into account, it is noticed that we have $\int_\Omega h(x) = 0$ for $h(x) \in W^l(\Omega)$, which enables us to use Poincare's Inequlaity. Then the following result is obatined in Kubo[11].

Theorem 1. *Assume that (A) holds and $(h_0(x), h_1(x)) \in W^{m+1}(\Omega) \times W^m(\Omega)$ for $h_0(x) = u_0(x) - a$ and $h_1(x) = u_1(x) - b$. For sufficiently large a and any $b > 0$ there is a solution $u(x,t)(= a + bt + v(x,t)) \in \bigcap_{i=0}^{1} C^i([0,\infty); H^{m-i}(\Omega))$ to (NE) such that for*

$$\overline{u}_1 = |\Omega|^{-1}\int_\Omega u_1(x)dx$$

$$\lim_{t\to\infty} \|u_t(x,t) - \overline{u}_1\|_{m-1} = 0. \qquad (2.7)$$

2.2 Application

We apply theorem 1 to our problem (1.6) following to the method by Levin and Sleeman [12]. Put $\log w(x,t) = -\int_0^t u(x,\tau)d\tau = U(x,t)$ in the second equation of (1.6), then (1.6) are reduced to

$$U_{tt} = D\Delta U_t + \nabla \cdot (\frac{\chi_0 e^{-U}}{1 + \alpha e^{-U}} U_t \nabla U)$$

which is regarded as the same type of equation as (2.1) and satisfies the condition (A). Therefore it is evident that Theorem 1 holds for (1.6) and it implies that there exists a classical solution $u(x,t)$ to (1.6) such that

$$\lim_{t\to\infty} \|u(x,t) - \overline{u}_0\|_{m-1} = 0.$$

3 Computer simulation

It is possible to carry out computer simulation of Othmer-Stevens model by reinforced random walk([13]). Since our model (1.6) can be considered as a special case of Othmer-Stevens model, simulations of (1.6) by using reinforced radomwalk are shown in Fig.3 and Fig.4. Also, simulation for a mathematical model of in vitro experiment for endothelial cell migration is given by [15],[19] in the similar way.

The following picture is a photo of an experiment in vitro of glioma tumour 3D invasion in collagen gel performed by Eke and coworkers in [4]. We choose three characteristic paths of each single invasive cell from the experiment and draw them as solid lines on the picture. In figure 4, we intend to reproduce the three solid lines by using 3D simulations of the model (1.6).
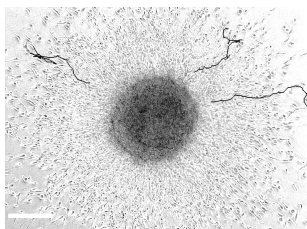

Figure 2: A photo in $x - y$ plain of an experiment in vitro of U87WT glioma tumour 3D invasion in collagen gel performed by Eke and coworkers in [4], which is similar to the experiment in [16], [18], [20].

3.1 3D simulation of a path of a single cell from (1.6)

Figure 3: Simulation of a single path of a tip cell in the cuboid domain from various viewpoints.

3.2 2D simulation from (1.6) of Glioblastoma 3D invasion in vitro experiment

Figure 4: Simulation from (1.6) of three paths of each cell in $x - y$ plain for 3D cell invasion in vitro experiment of U87WT.

The path (a) indicates that the cell initially radiates rather linearly and after that turns around. In the path (b) it is observed that the cell exhibits more complicated behaviour than (a). In the path (c) Once the cell arrives at the edge of the sphere consist of invasive cells, it turns back to one's way.

4 Conclusions

The data of the experiment provides clear evidence that the tumour spheroid cells move away from it at a constant rate initially in the radial direction and after that the radial velocity bias decrease. It seems to be important to gain the understanding of the mechanism of invasion in these in vitro experiments so that their usefulness in understanding the in vitro situation can be understood. However in the mathematical model of Stein et al. [18] the radially biased component implies that the cell motility with a constant velocity and a fixed direction is different from real cell paths observed in the experiment. The cause of radial bias is not known. Therefore we propose a mathematical model generalized and improved the radially biased component term of the model of [18] so that it covers more realistic motility as observed in the experiment of Eke et. al. [4]. For this purpose we choose three characteristic paths of U87WT cell. We show a rigorous mathematical analysis of our model and give results of computer simulation of solid lines in Figure 2 for our mathematical model, which seems to realize more realistic invasive cell behaviour.

3.1 3D simulation a single path of a tip cell from (1.6)
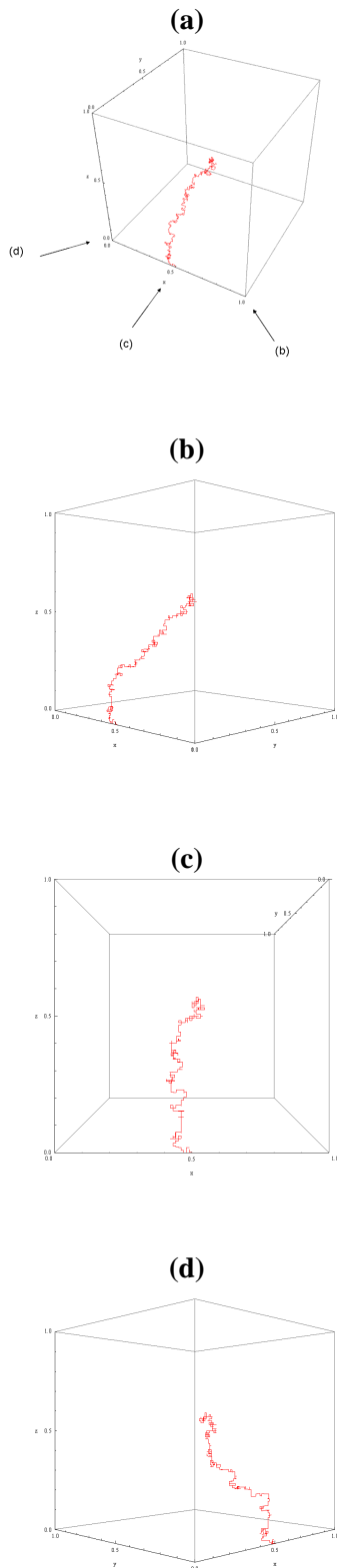
(a)

(b)

(c)

(d)

Figure 3: Simulation of a single path of a tip cell in the cuboid domain from various viewpoints.

3.2 2D simulation from (1.6) of Glioblastoma 3D invasion in vitro experiment

We choose three characteristic paths of a single cell of U87WT and by the method as obtained Figure 3 we have simulations of them from our model (1.6) in Figure 4.
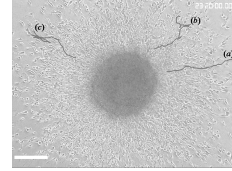

Figure 2': In three solid lines, the path (a) indicates that the cell initially radiates and after that turns around greatly. In the path (b) it is observed that the cell changes the direction greatly several times. In the path (c) Once the cell arrives at the edge of the sphere consist of invasive cells, it suddenly turns back to one's way.
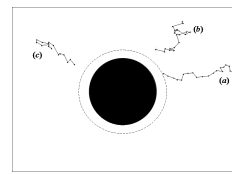

Figure 4: Simulations of the path of each cell are corresponding to the solid lines (a)-(c) in Figure 2' respectively. Compared with Figure 1. (b), our simulations seem to be much closer to (a)-(c) in Figure 2'.

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