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Biological Inferences from a Mathematical Model for Malignant Invasion

Abstract

Invasive cells show changes in adhesion, motility and the protease-antiprotease balance. In this paper the authors derive a model based on a continuum approach that describes the behaviour of the invasive cells. The invasive cells are studied in the context of their interaction with normal cells, noninvasive tumour cells, ECM proteins and the proteases. The authors briefly describe the methods of mathematical analysis used and then go on to highlight the biological inferences drawn from the mathematical analysis. Based on the results from the modelling the authors suggest that the movement of cells under the simultaneous effects of a haptotactic gradient and a concomitantly created chemotactic gradient is oscillatory both with respect to the speed of invasion and the wave profile of the invasive cells. They further demonstrate that the average speed of invasion can be computed as a measure of the phenotypic properties of the cell and the matrix. They use the model to suggest an intuitive explanation for the occurrence of noninvasion with high protease expression on the basis of chemotactic gradients that prevent invasion. The authors have studied the effect of the diffusivity of the protease on an invading cell and shown that increase in diffusivity initially results in enhanced invasion, but extreme increases in protease diffusivity can result in noninvasion.

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Introduction

The essential pathology of cancer involves the transformation of a single cell (or sometimes a group of cells) into an actively growing and invading group of cells that appear to have escaped normal control mechanisms. Both growth and invasion are features of normal cells; however; these factors are tightly regulated in a spatial and temporal fashion. Principally, cancer may be viewed as a breakdown of this spatio-temporal regulation.

The sequence of steps by which a cancerous cell leaves its primary site and enters adjoining tissue is called malignant invasion. This invasion may transport the malignant cell to a nearby blood vessel, lymph vessel or body cavity whence it is carried to a distant site. If the malignant cell survives this journey through the blood or lymph and eventually finds another suitable site to thrive, it establishes a colony of cells, away from the primary site, called a secondary or a metastatic growth. To successfully invade neighbouring tissue, the cells acquire several phenotypic alterations which can be conveniently classified into three sets of changes: (1) Changes in cell adhesion (attachment). (2) Changes in protease-antiprotease axis (proteolysis). (3) Enhanced motility of cells (migration).

It is through the repetitive cycling of these three steps, attachment, proteolysis and migration, that tumour cells accomplish the processes necessary for successful invasion. Historically, this sequence of steps has been called the three-step hypothesis [1] which we will describe from a mathematical standpoint in this paper. The essential object of the model is to explore the ways in which different combinations of these steps can conspire to produce an invasive phenotype. We first describe in detail the formulation of the model and then briefly mention the methods of analyis used to study the model. We conclude by using the model solutions to draw biological inferences about the nature of malignant invasion in a fashion that can be readily verified through laboratory experiments.

There has been a recent increase in the interest in malignant invasion because of the possibility of using anti-invasion and anti-metastatic strategies as a means of therapy in cancer. Jiang and Mansel [2] recently reviewed progress on this front while Hart and Vile [3] have discussed the possibility of targeted gene therapy as an anti-invasive strategy. As we begin to appreciate the different pathways involved in the mediation of invasion and the possibility of selectively targeting these pathways using blocking antibodies or competitive inhibitors, newer targets in the fight against invasion are presenting themselves. For instance Bennett et al. [4] and Aznavoorian et al. [5] have demonstrated the differences in the pathways in intracellular signaling. All this makes it possible to be highly selective in the choice of therapeutic targets.

Approaches to Cancer Modelling

The general area of oncology has been a very productive one for mathematical modelling. In order to introduce our work we will briefly review previous modelling in the areas of avascular tumour growth, tumour immunology and angiogenesis. Our approach to modelling draws heavily on previous work in these areas. Avascular tumour growth was first modelled more than 30 years ago, highlighting that the size of an avascular tumour is limited by the diffusion of nutrient into it, and that this size is tightly regulated by chemical inhibiton of mitosis [6, 7]. More recent work has extended these early models to include more general geometries and spatial

nonuniformities [8–12]. The multicell spheroid in vitro assay is an ideal test case for such modelling, and was considered explicitly in the modelling of McElwain and Pettet [13]. The role of different mechanisms of oncogene function has also attracted interest as a modelling problem [14, 15], demonstrating a fundamental difference between mutations that affect the cell response to mitotic regulators, and those that affect the cellular production of such regulators. Interactions between different growth control mechanisms have been modelled in detail by Michelson and Leith [16, 17].

Tumour immunology raises a wide range of interesting modelling questions, which have recently been surveyed in the book of Adam and Bellomo [18]. Early models in this area were based on representations of predator-prey systems in ecology [19, 20]; more recent work has focussed on specific tumour-immune interactions [21] and on the importance of spatial variation in the immune response [22, 23].

The modelling of tumour angiogenesis has been an active area of research in recent years. The main focus of interest has been the way in which tumour angiogenesis factors coordinate capillary ingrowth. The basic ingredient of endothelial cell chemotaxis has been modelled extensively by Chaplain and coworkers [24, 25]. Their modelling accounts for the main observed events associated with endothelial cells during the process of angiogenesis, i.e. endothelial cell migration and proliferation. Byrne and Chaplain [26] have recently extended this model, describing the increasing speed of a vascular wave front, and the evolution of an increasingly developed vascular network behind the leading capillary tip front (the brush-border effect).

By contrast, tumour invasion is a relatively new area for mathematical modelling, with recent work by Tracqui [27] and Gatenby [28], as well as by our own group [29]. The area has tremendous potential for modelling, which we will highlight in this paper.

The Aim of Modelling

Mathematical modelling can be used to make both qualitative and quantitative predictions. In the former case, one uses a theoretical framework as a mechanism to test biological hypotheses; in our application the hypotheses are at the cellular and molecular level, and the aim of the model is to explore the macroscopic implications of these hypotheses. In particular, such a model can be used to make predictions about aspects of the system that are difficult to address experimentally for logistical or financial reasons. Quantitative predictions use the same type of modelling framework, but require much more detailed data inputs, to enable parameter values to be accurately estimated. In this paper, we develop a model which is used for qualitative prediction. In order to meet our long-term aim of making such predictions quantitative, an experimental programme is currently underway to determine the necessary parameter values.

The Invasive Phenotypes and Their Modelling

We derive a one-dimensional model for invasion based on a continuum approach. The full mathematical description of the model is provided in the 'Appendix'; in this section we concentrate on the principles of the derivation rather than their explicit mathematical forms. The key variables involved in our model are the concentration of the invasive cells which we call $u(x, t)$ (implying the concentration of u changes with respect to

space x and time t), noninvasive tumour cells $m(x, t)$, normal cells $n(x, t)$, a generic ECM protein $c(x, t)$ (e.g. collagen, fibronectin, laminin), a generic protease $p(x, t)$ and the resultant product of proteolytic digestion of the ECM protein $s(x, t)$. We cast the behaviour of each of these variables in terms of the three-step hypothesis of malignant invasion.

Changes in Motility

The net displacement of a cell is a consequence of its random movement (chemokinesis) and directed movement (chemotaxis and haptotaxis). In a mathematical formulation this may be written as:

Net change in tumour cell density =
Chemokinetic movement +
Chemotactic movement +
Haptotactic movement

We now discuss the approach that we use to model each of these types of movement. We also discuss the mechanisms involved in the genesis of the gradients that induce and guide movement of the tumour cells.

Chemokinesis. A variety of agents appear to stimulate motile responses in tumour cells in vitro. These include host-derived scatter factors [30, 31], growth factors [5, 32], components of extracellular matrix [33, 34], hyaluron [35] and tumour scatter factors [36]. Motility stimulation by each of these factors involves a combined enhancement of random and directed movement. The former is known as chemokinesis; we consider directed movement separately. These stimuli could provide tumour cells with multiple opportunities for transiting different microenvironments during the metastatic process.

In order to model the enhanced random movement of invasive cells we study the changes prompted by an increase in the diffusion coefficient of the cell (which is, Γ_i (u, n, m) in equations 1a–c) An augmented diffusion coefficient will cause the invasive clones of cells to migrate faster than the noninvasive cells, simultaneously displacing the normal cells that hinder the path of invasion. This solution behaviour of the system in equation 1 is shown in figure 1. Here a group of cells which have decreased contact inhibition (m and u cells) shape the gradual evolution of a benign tumour which grows at the expense of the surrounding normal tissue. Halfway through the simulation, we introduce a mutation in the u cells which makes them chemokinetically more active. This may be a consequence of either increased production of chemokinetic factors (for example, scatter factor) or increased sensitivity to the effects of a chemokinetic agent. The result of such a mutation is a rapid increase in the number of invasive u cells, at the expense of all other cell types.

Chemotaxis. Tumour cells can respond to chemicals by migrating up chemical gradients. If the chemicals concerned are diffusible, the cellular movement is known as chemotactic. However, if the chemicals are fixed in the surrounding tissue the motile response of the invasive cell is termed haptotactic movement. The use of ECM macromolecules as attractants in motility assays has shown that there is a fundamental difference between chemotaxis and haptotaxis with respect to post-receptor signal trasduction events [5]. The differential sensitivity of haptotaxis and chemotaxis to pertussis toxin in A2058 melanoma cells is further proof of their difference.

We model the chemotactic movement of cells as responsiveness to positive gradients of soluble ECM molecules ($\partial s/\partial x$ in equation 1c). The responsiveness is modified by a function $\Psi(s)$ which is included to account for the dynamics of the receptor-ligand interaction). Crucially, the chemoattractant is soluble and hence diffuses throughout the surrounding tissue ($\partial^2 s/\partial x^2$ in equation 1e).

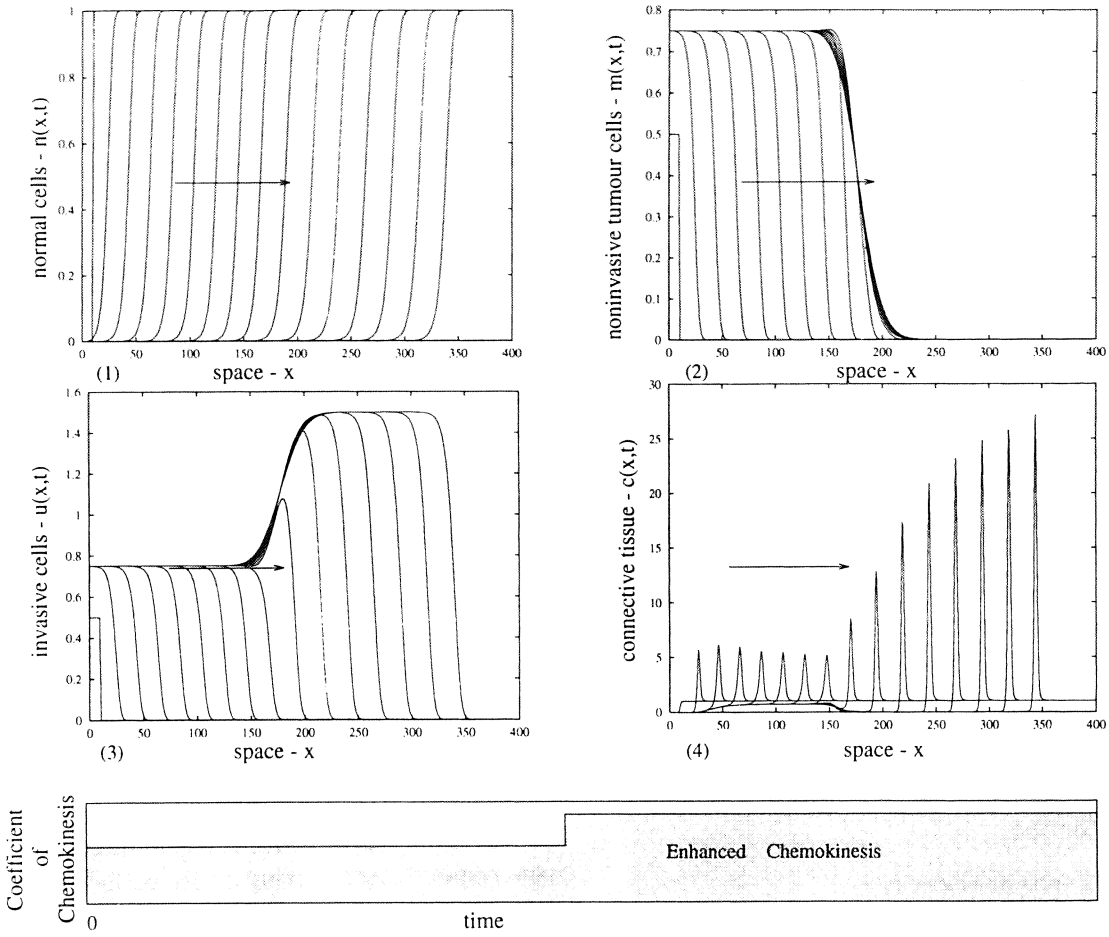

Fig. 1. Numerical solution of (1) illustrating the effects of a mutation producing enhanced chemokinesis resulting in an enhanced migration and proliferation of the invasive cells. The change in the coefficient of chemokinesis with time is shown in the box below. The invasive cells (frame 3) by their aggressive proliferation combined with enhanced chemokinesis displace the normal cells (frame 1) as also outproliferate the noninvasive clones (frame 2). An incidental feature seen here is the accumulation of connective tissue on the surface of the tumour reminiscent of the formation of a tissue capsule (frame 4). The details of the bifurcation that gives rise to capsule formation is described elsewhere [42]. The functional forms used in this simulation are as described in the 'Appendix'. The parameter values used in this simulation are $k_1 = 1$, $k_2 = 1$, $k_3 = 1$, $k_4 = 1.5$, $k_5 = 1.5$, $k_7 = 0$, $k_8 = 1$, $K = 30$, $D_s = 1$, $k_{11} = 0$, $k_{12} = 1$, $k_{13} = 0$, $k_{14} = 0$, $D_p = 0$, $k_{16} = 0$, $k_{17} = 0$, $k_{18} = 1$, $k_{19} = 1$, $k_{20} = 0$, $k_{21} = 1$, $k_{25} = 0$, $k_{26} = 1$, $k_{27} = 30$ and $k_{28} = 45$.

Haptotaxis. Most connnective tissue in the body, particularly the ECM molecules that invasive cells lyse during invasion, are fixed to the surrounding tissue. For example, the laminin that melanoma cells interact with is predominantly fixed to the basement membrane. Thus, by the definition of haptotaxis as responsiveness to gradients of fixed or nondiffusible chemicals, the directional movement of invasive cells in response to ECM protein

gradients is haptotactic. Much recent interest has been focussed on the characterisation of such motion [37]. While the demonstration of haptotaxis in an in vivo situation has not been made, our understanding of the architecture of tissues suggest that haptotaxis is a major component of directed movement.

Changes in Adhesion

Normal cells express adhesion receptors on their surface that cause them to stick to neighbouring cells and to the connective tissue in which they are embedded. When cells stick to cells of their own kind the adhesion is termed *homotypic*. Adhesion to other cell types or connective tissue is known as *heterotypic*. Invasion is attendant with changes in both homotypic and heterotypic adhesion [1]. Decreased homotypic adhesion allows cancerous cells to break away from the parent tumour and invade surrounding tissue. Increased heterotypic adhesion allows cells to attach to and thereby anchor themselves to connective tissue elements giving them time to degrade the connective tissue and thereafter move on. In a mathematical formulation this may be written as:

Net cellular adhesivity =
Homotypic adhesion + Heterotypic adhesion

A number of specific cell-surface-associated molecules that modulate cell-matrix and cell-cell interactions have been characterised. These include the integrins, cadherins, Ig superfamily and CD44 [1]. The integrins exemplify the complex role that cell-surface receptors play in invasion. To date at least 20 different integrins have been identified. Originally, the integrins were identified as receptors for extracellular matrix proteins such as collagen, fibronectin, laminin and vitronectin. However, some integrins may also function as cell-cell adhesion molecules. More recently, it has been shown by Seftor et al. [38] that signal transduction through $\alpha_v\beta_3$ integrin receptors may modulate the production of proteolytic enzymes. Hence, though the three-step hypothesis is a convenient conceptual framework to study separate aspects of the invasive phenotype, these processes form part of a continuum, with one step triggering another.

In order to model the changes in motility accruing from altered adhesion, we incorporate into the diffusion coefficient a function Γ_i (n, m, u), $i = n, m, u$ (see equations 1a–c) which accounts for the variable expression of adhesion molecules on the surface of normal and tumour cells. By choosing suitable functional forms of Γ, we modulate the expression of both homotypic and heterotypic receptors. In figure 2 we present a simulation showing the effects of decreased homotypic adhesion on invasive cells. In this simulation the early behaviour of the system recapitulates the behaviour of a benign tumour. As the simulation progresses we introduce a mutation in the u cells which causes decreased homotypic adhesion. This causes the u cells to become the dominant species in the invasive wavefront. As in figure 1, the connective tissue responds by accreting on the surface of the tumour.

Changes in the Protease-Antiprotease Axis

Reciprocal changes in the balance between proteases and anti-proteases can give rise to excessive matrix degradation in the vicinity of the tumour. Experimental results consistently localise proteases to the stromal fibroblasts immediately adjacent to the site of tumour invasion [39]. This suggests that invasive tumour epithelia release a stimulus for induction of fibroblast synthesis of the proteases. A tumour-derived collagenase stimulatory factor has been characterised and partially sequenced [40]. This tumour-cell-derived agent also stimulates the synthesis and secretion of

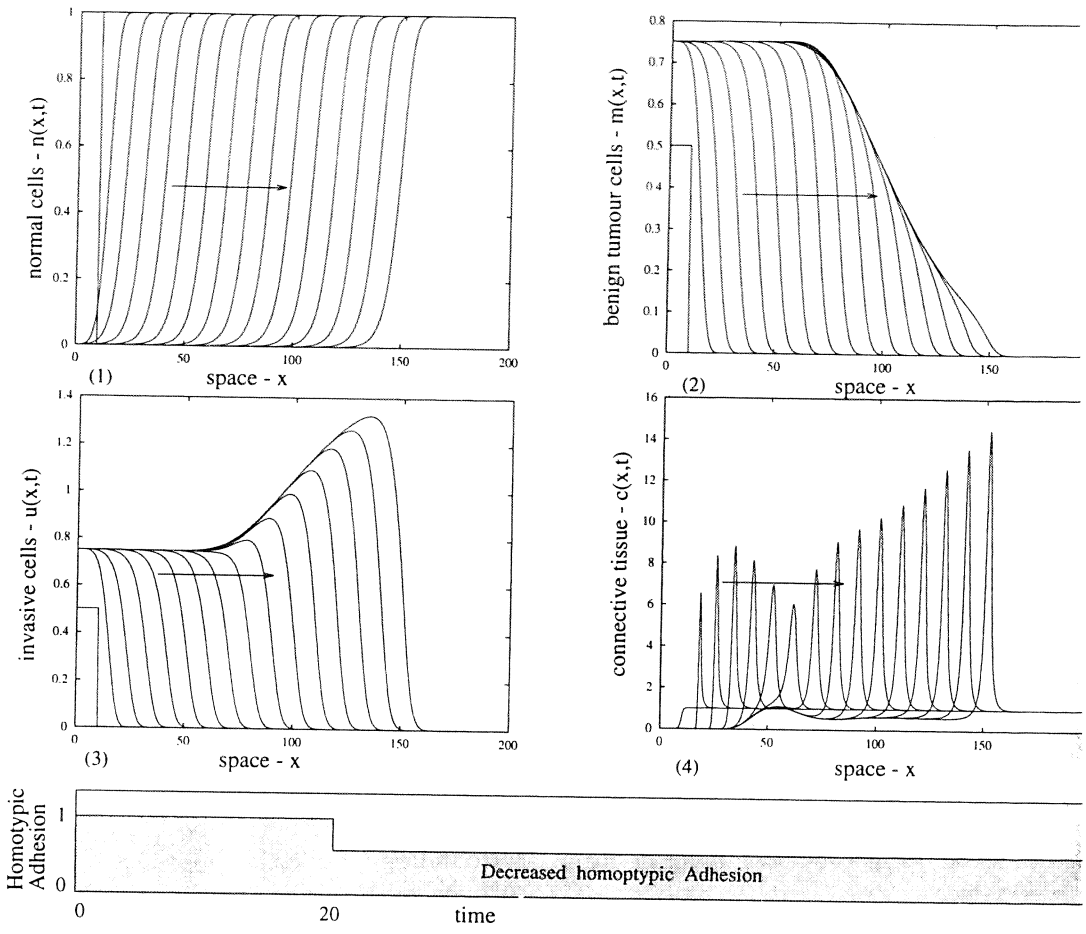

Fig. 2. Numerical solution of (1) illustrating the effects of a mutation resulting in the los of homotypic adhesion. The change in homotypic adhesion as the tumour evolves is shown i the box below. As a consequence of the mutation the invasive cells move out of the primar tumour more aggressively (frame 3) and become the dominant species displacing the norma cells (frame 1) and the noninvasive cells (frame 2). The functional forms used in this simula tion are as described in the 'Appendix'. The parameter values used here are $k_1 = 1$, $k_2 = 1$, $k_3 =$ 1, $k_4 = 1.5$, $k_5 = 1.5$, $k_6 = 1$, $k_7 = 0$, $k_8 = 1$, $K = 30$, $D_s = 1$, $k_{11} = 0$, $k_{12} = 1$, $k_{13} = 0$, $k_{14} = 0$, $D_p = 0$ $k_{16} = 0$, $k_{17} = 0$, $k_{18} = 2$, $k_{19} = 1$, $k_{21} = 1$, $k_{25} = 1$, $k_{26} = 1$, $k_{27} = 15$ and $k_{28} = 25$.

the proteases gelatinase A and stromelysin from human fibroblasts in culture.

Once the proteases are released they act on the various connective tissue elements and break them down. For example, the interstitial collagenases degrade the triple helical domains of fibrillar collagens. In normal conditions the activity of the proteases is kept in check by the release of protease inhibitors by the tissues of the body. Some of the best stud-

ed anti-proteases [41] are the tissue inhibitors of metalloproteases (TIMPs).

The changes in the protease-anti-protease ıxis may be represented in a mathematical ormulation as:

Net change in protease =
Protease production +
Protease neutralisation by antiproteases +
Protease diffusion

In order to model the behaviour of the pro-ease we used two functions. An interesting ıodelling challenge is to confine the protease) the interface of the advancing tumour and ıe surrounding tissue. We do this by using a ınction ($l_1(u, c)$ in equation 1f) which is a roduct of the local concentration of the inva-ve cells and the connective tissue. The resul-nt profile of the protease is that of a pulse ave, the peak concentration of which lies at e juxtaposition of the tumour and the sur-ıunding tissue. A second function accounts r the degradation or neutralisation of the oteases by antiproteases ($l_2(n, m, u, p)$ in 1f). ıhanced proteolysis can hence be brought ıout either by an increase in l_1 or by a crease in l_2.

Methods – Solution Techniques

Apart from the continuum modelling ethods used in this paper there are a number other computationally efficient modelling ethods such as cellular automata and cou-d map lattice methods. The method we ve chosen presents two particular advan-es. In addition to computational analysis, continuum model lends itself to mathe-tical analysis which facilitates the deduc-n of general results in the form of mathe-tical formulae that describe the gross be-ıiour of an invading tumour in terms of the enotypic parameters that govern the un-lying cell behaviour. Where the com-plexity of the system makes it difficult to pur-sue analytical methods we were able to study reduced submodels [29, 42]. Besides making analysis more thorough, this method permits us to focus on a few interactions at a time, and enables us to identify which interactions are crucial and which peripheral to the invasive phenotype.

Our approach to the model consists of three stages. We first reduce the model in equation 1 to separate submodels that high-light a particular set of invasive interactions. We analyse each of these models using a batte-ry of analytical techniques. In the final stage we solve the whole model computationally and examine it for the features deduced from the submodels. Here we focus on the full mod-el, but we will describe briefly the analytical results obtained from the submodels.

Since invading cells behave like a front of cells moving as a wave, our analytical focus is on understanding the nature of this wave and the changes occurring at the interface between the invading tumour and the receding tissue surrounding it. A convenient way to study such behaviour is to 'move' alongside the wave at its presumed speed (which is achieved mathematically by a change of coordinates) and then to infer the speed of the wave. This gives a measure of the invasiveness of the tumour. This method is called travelling wave analysis. We have used this method to study in detail a submodel in which only invasive tumour cells, ECM and protease are consid-ered and any other interactions which are present in the full model are neglected; details of this analysis are given in Perumpanani et al. [29]. The basic result of this work is a for-mula for the invasion wave speed in terms of the model parameters, for example describing the way in which invasion speed increases with mitotic rate or integrin expression on the cell surface.

We have also studied a submodel that focusses on the interaction between the benign tumour cell population and ECM. The main interest here is the formation of a capsule around a developing tumour. In the simulations of the full model shown in figures 1 and 2, we can see the development of an increasingly dense band of ECM, corresponding to a developing capsule. We were able to study this second submodel using a mathematical technique known as the method of characteristics. This technique enables the progression of the ECM solution to be followed as the tumour cell wave moves forward. In particular, we determined an expression for a critical value of the speed of tumour growth, such that a capsule only forms at speeds above this critical speed. The details of this analysis are given in Perumpanani et al. [42]. We were also able to use the submodel to study the development of nodularity in tumours.

Key Biological Implications of the Modelling

We will now highlight some of the key biological inferences obtained by the application of the above methods.

The average speed of invasion can be computed as a function of the phenotypic properties of the cell and the matrix. As described above, we have used a reduced version of the full model (1) to determine the average speed of invasion in terms of the invasive cell kinetics (doubling time and carrying capacity) and the coefficient of haptotaxis. A detailed description of this analysis is available in Perumpanani et al. [29].

The occurrence of noninvasion with high protease expression can be explained on the basis of chemotactic gradients that prevent invasion. The products of proteolytic degradation left behind by the invading cell are much smaller than the parent molecule and hence more readily diffusible. They can exert a chemotactic pull on the invading cell which opposes the direction of invasion. This provides a possible explanation for the observation of Tsuboi and Rifkin [43] that cells producing large amounts of protease become noninvasive. The model finding is based on the fact that excessive production of protease can cause the degradation of large amounts of ECM. This exerts a chemotactic pull, preventing invasive spread of the cell.

Increased protease diffusion can result in noninvasion. A small increase in the diffusivity of the protease (D_p in equation 1f) may dramatically increase the invasiveness of the tumour. However, as shown in figure 3, a large increase in the diffusivity may lead to a reduction in the speed of invasion. An intuitive biological explanation for this phenomenon is that large increases in protease diffusivity obliterate the ECM gradients which guide the movement of the invasive cells.

The movement of cells under the simultaneous effects of a haptotactic gradient and a concomitantly created chemotactic gradient is oscillatory both with respect to the speed of invasion and the wave profile of the invasive cells. An effect that has hitherto not been addressed either experimentally or theoretically is the movement of cells under multiple spatial gradients. Protease production by an invading cell results in matrix degradation that can give rise to haptotactic gradients and, thereby, induce cellular migration. However, the proteolysed components can themselves exert a chemotactic pull on the invading cells [44]. As a result, tumour cells could detect and respond to the soluble fragments as well as to insoluble intact matrix molecules. In vivo this amounts to the invading cell being held between a forward-pulling haptotactic gradient and a retarding, or restraining, chemotactic

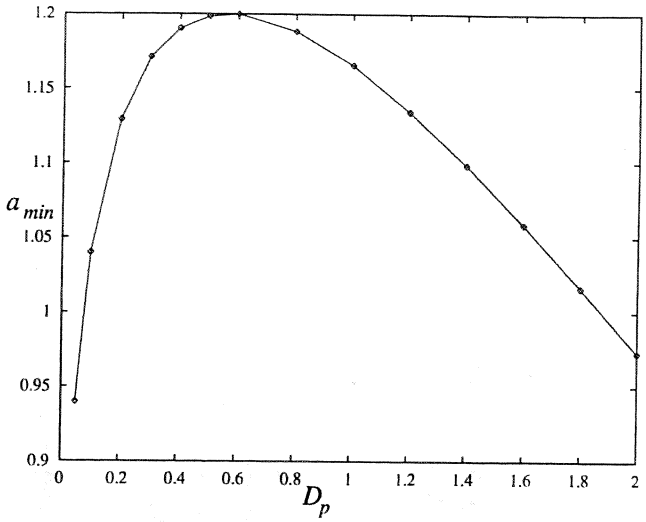

Fig. 3. Computed minimum wave velocities for a finite difference solution of (1) for various values of the protease diffusion coefficient D_p. As D_p increases the wavespeed initially increases and then decreases. The parameter values used in this simulation are k_4 = 1.72, k_5 = 1, k_6 = 4.0, k_8 = 0.345, k_9 = 0, k_{11} = 5.0, k_{14} = k_{13} = 0.346, k_{12} = 0 and k_{21} = 0. n, m and s are identically zero throughout the domain.

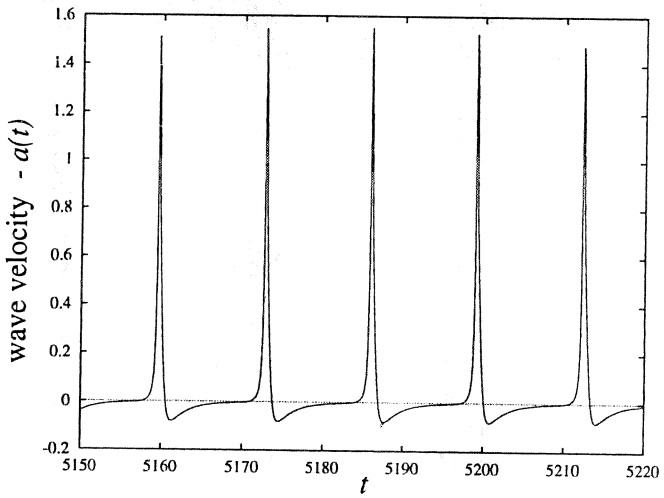

Fig. 4. An illustration of the change in the velocity of invasion against time. The velocity of the u wave is measured as the rate of translation of a fixed concentration in the spatial profile of u (x, t). In this simulation the fixed concentration we chose was u = 0.5. In a periodic fashion the wave moves forwards and then backwards with intervening periods when it is stationary. The parameter values we used were k_4 = 1, k_5 = 1, k_6 = 0, k_7 = 0.1, k_{16} = 50.0, k_8 = 3, k_{21} = 1, D_s = ., k_{11} = 20, k_{12} = 17, k_{13} = 0.5, k_{14} = 0.2 and k_{15} = 0.

gradient. Our mathematical investigations show that the invasive movement of such a cell (such as A2058 melanoma cell) will be oscillatory (fig. 4). While the net translation of the cell is in the forward direction, there is an initial phase of rapid movement, followed by a period of deceleration (which is sometimes strong enough to produce movement in the negative direction), before the resumption of motion in the forward movement.

Appendix

Here, we describe the translation of the invasive phenotypes and their modelling described in 'The invasive phenotypes and their modelling' section into a system of six coupled partial differential equations using the mathematical notation introduced earlier. Each equation describes both the behaviour of the state variable (invasive cells, ECM, proteases, etc.) and its coupling with other elements (e.g. the protease causing ECM degradation). Using undefined functions to describe the key phenotypes we write the model as:

$$\frac{\partial n}{\partial t} = nf_1(n, m, u) + \frac{\partial}{\partial x}\left[\Theta(c)\left(\Gamma_n(u, m, n)\frac{\partial u}{\partial x}\right)\right] \quad (1a)$$

$$\frac{\partial m}{\partial t} = mf_2(n, m, u) + \frac{\partial}{\partial x}\left[\Theta(c)\left(\Gamma_n(u, m, n)\frac{\partial m}{\partial x}\right)\right] \quad (1b)$$

$$\frac{\partial u}{\partial t} = uf_2(n, m, u) +$$

$$\frac{\partial}{\partial x}\left[\Theta(c)\left(\Gamma_u(u, m, n)\frac{\partial u}{\partial x} - u\chi(c)\frac{\partial c}{\partial x} - u\Psi(s)\frac{\partial s}{\partial x}\right)\right] \quad (1c)$$

$$\frac{\partial c}{\partial t} = g(p, c) + \frac{\partial}{\partial x}K\left[c\,\Theta(c)\left(\Gamma_n\left(\frac{\partial u}{\partial x} + \frac{\partial m}{\partial x}\right) +\right.\right.$$

$$\left.\left.\Gamma_u\frac{\partial u}{\partial x} - u\chi(c)\frac{\partial c}{\partial x} - u\Psi(s)\frac{\partial s}{\partial x}\right)\right] \quad (1d)$$

$$\frac{\partial s}{\partial t} = h(p, c) + D_s\frac{\partial^2 s}{\partial x^2} \quad (1e)$$

$$\frac{\partial p}{\partial t} = l_1(u, c) - l_2(u, p, c) + D_p\frac{\partial^2 p}{\partial x^2} \quad (1f)$$

The model described in equation 1a is a framework model which we have used to study a wide range of invasive interactions. In this article we describe a limited range of interactions which highlight particular biological results. To this end we have chosen the simplest functional forms possible in order to keep the model mathematically simple. We use the notation k_i where $i = 1, 2, 3, \ldots$ to denote the various phenotypic constants in the model. Equation 1a describes the evolution of the normal cell population. These cells proliferate locally (nf_1) and are capable of minimal degree of random movement. In our simulations we use the logistic growth curve, i.e. $f_1 = k_1(k_2 - n - m - u)$, which Vaidya and Alexandro [45] have shown to be appropriate. There is an upper ceiling k_2 limiting the local increase in cell density. This ceiling is called the 'carrying capacity' and can be changed to reproduce the effects of contact inhibition. For example, a reduction in the carrying capacity corresponds to an increase in contact inhibition: cells have lower affinities for one another and therefore tend to spread over a large region. The diffusion coefficient of the normal cells is composed of two functions Γ_n (which we discuss later) and $\Theta(c)$ which represents the effects of accumulating connective tissue retarding the movement of cells. To model this we used a decreasing ramp function of the form

$$\Theta(c) = \begin{cases} k_{26} & 0 < c < k_{27} \\ \dfrac{k_{28} - c}{k_{28} - k_{27}} & k_{27} < c < k_{28} \\ 0 & k_{28} < c \end{cases} \quad (2)$$

When the concentration of connective tissue at the periphery of the growing tumour is small (that is, less than k_{27}) the movement of cells is unimpeded ($k_{26} = 1$). When the concentration of connective tissue crosses a threshold (k_{28}) the accumulation of connective tissue is sufficient to prevent any further movement of cells. For intermediate concentrations of connective tissue the movement varies linearly with the concentration of the connective tissue.

Decreased contact inhibition is incorporated in equation 1b where the benign tumour cells m show logistic growth ($f_2 = k_4(k_5 - n - m - u)$) with an increased carrying capacity (i.e. $k_5 > k_2$). Similarly the invasive cells described in equation 1c possess an increased carrying capacity and hence their proliferation is also described by f_2.

To model changes in the chemokinesis and homotypic adhesion we used the functional forms:

$$\Gamma_n = k_3\frac{k_{18}}{k_{19} + k_{25}(k_{25}n + k_{25}m + k_{20}u)}$$
$$\Gamma_u = k_6\frac{k_{18}}{k_{19} + k_{20}(k_{25}n + k_{25}m + k_{20}u)} \quad (3)$$

where k_{25} and k_{20} parametrise the expression of cell adhesion molecules by noninvasive cells (n and m) and the invasive cells (u), respectively. A decrease in k_{20} will marginally increase the diffusion coefficient of the noninvasive cells, but a much greater increase in the diffusion coefficient of the invasive cells will occur. Changes in k_3 and k_6 represent changes in the chemokinetic movement of the cell.

Responsiveness to fixed gradients (haptotaxis modulated by $\chi(c)$) and soluble gradients (chemotaxis

modulated by $\Psi(s)$) represent the two modalities of directed movement. The functions $\chi(c)$ and $\Psi(s)$) represent the kinetics of receptor-ligand interactions which bring about directed movement. In this article we have used simple constant forms ($\Psi(s) = k_{16}$ and $\chi(c) = k_{17}$) to study the gradient sensitivity of the invasive cells.

The behaviour of the ECM is described in equation 1d where the local changes produced by proteolysis are described by $g(p, c)$ which is a decreasing function of p. Specifically, we take $g = -k_8pc$. The expansive growth of the tumour pushing the surrounding connective tissue [46] is modelled by the convective flux of c being proportional to the flux of the cellular elements described previously. Equation 1e models the production of proteolysed ECM through $h(p, c)$ which is proportional to the degradation of c (i.e. is $h(p, c) = k_{21}g(p, c)$). Since the proteolysed ECM elements are much smaller than the parent molecule, they are capable of random movement modelled simply as Fickian diffusion with a constant diffusion coefficient D_s. Finally, we describe the behaviour of the proteases in equation 1f. The tightly regulated production of the protease at the interface of the tumour and the ECM is modelled by $l_1(u, c)$ (in our simulations we use $l_1 = k_1uc$) and its removal (anti-protease neutralisation and natural decay) modelled by $l_2(p, u, c)$ (in our simulations we use $l_2 = -k_{12}p - k_{13}pu - k_{14}pc$). Since proteases are biochemicals with much smaller molecular weights than cells, their random movement is modelled by the diffusion term with a constant diffusion coefficient D_p. For secreted proteases (e.g. MMP-2) $D_p > 0$ and for surface expressed proteases (e.g. uroplasminogen activator) $D_p = 0$.

Boundary Conditions

In our analysis we consider a spatial domain which is the whole real line. In the context of malignant invasion we are in most cases studying a domain which is much larger than the size of the tumour, since the tumour is localised to one part of the tissue. In our simulations we use very large spatial domains such that the effects from the boundary are minimal. We use zero flux boundary conditions because the interest here is in the behaviour of an isolated system free from any external inputs.

Initial Conditions

In our simulations shown in figures 1 and 2, we solved the system (1) using step function initial conditions. The total cell density through the whole domain ($0 < x < L$) was uniform. Both the benign and invasive cells were initially confined near $x = 0$ and the remainder of the domain had normal cells $n(x, t)$. Connective tissue $c(x, t)$, was present outside the tumour in a uniform distribution. There was no protease ($p(x, t)$) or solubilised ECM ($s(x, t)$) to start with.

$$n(x, 0) = \begin{cases} 0 & 0 < x < 10 \\ 1 & 10 \leq x \end{cases}$$

$$m(x, 0) = \begin{cases} 0.5 & 0 < x < 10 \\ 0 & 10 \leq x \end{cases}$$

$$u(x, 0) = \begin{cases} 0.5 & 0 < x < 10 \\ 0 & 10 \leq x \end{cases}$$

$$c(x, 0) = \begin{cases} 0 & 0 < x < 10 \\ 1 & 10 \leq x \end{cases}$$

$$s(x, 0) = 0$$

$$p(x, 0) = 0 \qquad (4)$$

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