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Modelling acidosis and the cell cycle in multicellular tumour spheroids

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ABSTRACT

A partial differential equation model is developed to understand the effect that nutrient and acidosis have on the distribution of proliferating and quiescent cells and dead cell material (necrotic and apoptotic) within a multicellular tumour spheroid. The rates of cell quiescence and necrosis depend upon the local nutrient and acid concentrations and quiescent cells are assumed to consume less nutrient and produce less acid than proliferating cells. Analysis of the differences in nutrient consumption and acid production by quiescent and proliferating cells shows low nutrient levels do not necessarily lead to increased acid concentration via anaerobic metabolism. Rather, it is the balance between proliferating and quiescent cells within the tumour which is important; decreased nutrient levels lead to more quiescent cells, which produce less acid than proliferating cells. We examine this effect via a sensitivity analysis which also includes a quantification of the effect that nutrient and acid concentrations have on the rates of cell quiescence and necrosis.

1. Introduction

Multicellular tumour spheroids (MCTS) are three-dimensional cellular aggregates which mimic many of the characteristics of *in vivo* avascular tumours (Mueller-Klieser, 1997). They have been the focus of research for experimentalists and applied mathematicians over the past 30 years (Araujo and McElwain, 2004; Roose et al., 2007). Whilst able to mimic many characteristics of *in vivo* tumours, for instance the observed spatial variation in oxygen and glucose concentrations from the outer to inner regions (Kunz-Schugart et al., 1998; Mueller-Klieser, 1997), MCTS are not widely used in cancer drug discovery, due to the cell culture techniques being more complex than standard 2D monolayers (Tung et al., 2011).

Initial mathematical modelling work in the area focused on simple models describing MCTS growth in the context of nutrient delivery to the tumour (Burton, 1996; Greenspan, 1972). With time and further experimental understanding a number of continuum mathematical models have been developed which have focused on the effect certain biological mechanisms (biochemical and biomechanical) have on MCTS development (see, for example Breward et al., 2002; Netti et al., 1995; Ward and King, 1997). The work presented here involves mathematical modelling of two different aspects of tumour growth; the cell cycle and acidosis and the effects both of these have on MCTS growth.

The cell cycle is a series of tightly regulated biochemical events which control the growth and development of a cell. To summarise: cells grow during G_1 phase before entering a period during which their DNA is synthesised (S-phase). G_2, a short period following S-phase, allows the cell time to prepare for cell division, involving splitting of the DNA spindle and physical division of the cell in two (M-phase). The newly generated cells may enter a period of extended time without further proliferation. Such cells are defined to have entered the quiescent G0 phase. In tumour biology entering such a phase is usually driven by factors external to the cell, for instance a decrease in growth factors or nutrient deprivation. Cells may undergo two basic forms of cell death; apoptosis or necrosis. Apoptosis is a decision by a cell to commit cell 'suicide'. In doing so the cell shrinks to form an apoptotic body which is removed by the immune system. Physiological events, such as decreased nutrient concentration within the tumour or high acidity, can have harmful effects on quiescent cells and may eventually lead to necrosis; the breaking down of the cellular wall and release of cell contents into the extracellular environment.

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Recent experimental and mathematical modelling work has elucidated the importance of pH levels on tumour morphology. Noninvasive magnetic resonance (MR) techniques have been developed to measure both intracellular pH (pH_I) and extracellular pH (pH_X) of human and animal tissues (Gillies et al., 2004, 2002). Virtually all tumour pH data to date show an acidic pH_X and alkaline pH_I relative to normal tissue. Moreover, it is found that the pH_X becomes more acidic as the tumour grows, consistent with reduced perfusion (Gillies et al., 2002). Clinical specimens have shown that these changes have a molecular basis in upregulation of glucose transporter 1 and the Na^+/H^+ exchanger (Gatenby et al., 2007); the developed mathematical models (Smallbone et al., 2007) have shown excellent agreement with experimental data on the distribution of upregulated cells.

There exists a growing number of mathematical models in the tumour literature which have been developed to understand the role of the cell cycle in tumour growth. These include dynamic models which encorporate simple (Bajzer et al., 1997; Bertuzzi et al., 1981; Cojocaru and Agur, 1992; Hillen et al., 2010) or complex (Zhao and Ricci, 2010) descriptions of the cell cycle, the spatiotemporal distribution of the cell cycle state within MCTS (Billy et al., 2009; Jeon et al., 2010; Mahmood et al., 2011; Tindall and Please, 2007; Tindall et al., 2008) and therapeutic interventions (Billy et al., 2009; Zhao and Ricci, 2010). To our knowledge no mathematical model currently exists which has considered how nutrient and acidosis levels affect the spatiotemporal cell cycle state of tumour cells and dead cell material within an avascular tumour.

In this paper we consider a mathematical model of a MCTS which includes a simple model of the cell cycle, where cells are considered to exist in either a proliferating, quiescent or dead cell (due to necrosis or apoptosis) state. The model includes a description of the nutrient and acid concentration within the MCTS. In what follows, the effect these have on the distribution of proliferating cells, quiescent cells and dead cell material and the overall tumour size, is investigated.

2. Model formulation

Let $P(\mathbf{x},t)$, $Q(\mathbf{x},t)$ and $M(\mathbf{x},t)$ represent the density of proliferating cells, quiescent cells and dead cell material per unit volume, respectively, whose mass conservation is described by

$$P_t+\nabla\cdot(\mathbf{u}P)=(K_B(C)-K_Q(C,H)-K_A)P+K_P(C)Q, \tag{1}$$

$$Q_t+\nabla\cdot(\mathbf{u}Q)=K_Q(C,H)P-(K_D(C,H)+K_P(C))Q, \tag{2}$$

$$M_t+\nabla\cdot(\mathbf{u}M)=K_AP+K_D(C,H)Q-\lambda M, \tag{3}$$

where $\mathbf{u}(\mathbf{x},t)$ represents the local velocity of the cells and K_I ($I=B,P,Q,D,A$) are cell cycle transition, or birth/death rates, which we assume are dependent upon the local diffusible nutrient $C(\mathbf{x},t)$ and extracellular hydrogen ion (acid) concentration $H(\mathbf{x},t)$. We assume that dead cell material is lost from the tumour at a constant rate λ (as first observed in Greenspan, 1972).

We note that our work differs from other models by explicitly accounting for proliferating cells, quiescent cells and dead cell material. Comparative papers (Ward and King, 1997, 1998) only account for live and dead cells within an MCTS. Although we have recently considered the effect of different spatial velocities, dependent upon the cell cycle state of the cell and the cell's local extracellular nutrient gradient (Tindall et al., 2008), we have here, for simplicity, assumed that all cells move with the same spatial velocity. This assumption reduces the complexity of having to account for varying cell cycle state structures, as a result of the varying chemotactic response to the local environment within the tumour.

We will take simple expressions for the K_I which capture the qualitative behaviour:

$$K_B(C)=\overline{k}_BC, \tag{4}$$

$$K_P(C)=\overline{k}_PC, \tag{5}$$

$$K_Q(C,H)=\overline{k}_Q(C_\infty-C)+\overline{k}'_Q(H-H_\infty), \tag{6}$$

$$K_D(C,H)=\overline{k}_D(C_\infty-C)+\overline{k}'_D(H-H_\infty), \tag{7}$$

$$K_A=\overline{k}_AC_\infty. \tag{8}$$

Here $K_B(C)$ represents the rate of cell birth, $K_P(C)$ is the rate of cell transfer from the quiescent to proliferating compartments, $K_Q(C,H)$ is the rate at which cells move from the proliferating to quiescent compartment (quiescence), $K_D(C,H)$ is the rate of cell death from the quiescent cell compartment (necrosis), $K_A(C)$ is cell death from the proliferating cell compartment (apoptosis) and C_∞ and H_∞ denote the concentration, respectively, of nutrient and acid at the tumour boundary which are assumed to be constant.

Acidification leads to death of normal cells due to activation of p53-dependent apoptosis pathways, as well as loss of function of critical pH-sensitive genes (Park et al., 1999; Williams et al., 1999). Tumour cells, however, may be relatively resistant to acidic pH_X. Whilst normal cells die in environments with a persistent pH below about 7, tumour cells continue to proliferate in a relatively acidic medium (pH 6.8) (Casciari et al., 1992). Beyond this point quiescence and eventually necrosis occur (Patel et al., 2001). This biological knowledge is reflected in the monotonic increase of quiescence K_Q and necrosis K_D with H.

Given that the rate of diffusion of nutrient throughout the spheroid is rapid compared to the time scale of growth, we adopt the standard quasi steady-state assumption (Ward and King, 1997):

$$D_C\nabla^2C=\sigma_C(P+\epsilon_CQ)C. \tag{9}$$

This equation has two nutrient consumption terms, one relating to proliferating cells (σ_C) and the other to quiescent cells ($\sigma_C\epsilon_C$). Here D_C is the nutrient diffusion coefficient.

In the case of acid diffusion throughout the spheroid, we also make a quasi steady-state assumption:

$$D_H\nabla^2H=-(P+\epsilon_HQ)(\sigma_H+\sigma'_H(C_\infty-C)), \tag{10}$$

where the acid diffuses at a rate D_H and σ_H and $\sigma_H\epsilon_H$ represent the production of acid by proliferating and quiescent cells, respectively. Note that $C\leq C_\infty$ and $H\geq H_\infty$, given the respective boundary conditions and application of the maximum principle.

In Eqs. (9) and (10), $\epsilon_C\ll1$ and $\epsilon_H\ll1$, representing the fact that quiescent tissue is essentially metabolically inactive, consuming significantly less oxygen than its proliferating counterpart and producing significantly fewer hydrogen ions. Tumours rely on anaerobic metabolism and hence produce acid at a rate σ_H under normoxic conditions (the Warburg (1930) effect); nonetheless, as oxygen levels decrease, acid production increases linearly at rate σ'_H (the Pasteur effect, Racker, 1974). Whilst more complex descriptions of tumour metabolism are possible (see, for example Bertuzzi et al., 2007; Forbes et al., 2006), in this form the size of the parameter space remains tractable.

We adopt the common assumption that the tumour is spherical and thus we will consider solutions in the one-dimensional spherical polar coordinates regime (see Section 4). This assumption allows us to determine the motion of the cells by noting that