

MODELLING ASPECTS OF VASCULAR CANCER DEVELOPMENT

PHILIP K. MAINI

*Centre for Mathematical Biology, Mathematical Institute
University of Oxford, 24-29 St Giles', Oxford OX1 3LB, United Kingdom
E-mail: maini@maths.ox.ac.uk*

TOMÁS ALARCÓN

*Bioinformatics Unit, Department of Computer Science
University College London, Gower Street, London WC1E 6BT
United Kingdom*

HELEN M. BYRNE

*Centre for Mathematical Medicine, Division of Applied Mathematics
University of Nottingham, Nottingham NG7 2RD
United Kingdom*

The modelling of cancer provides an enormous mathematical challenge because of its inherent multi-scale nature. For example, in vascular tumours, nutrient is transported by the vascular system, which operates on a tissue level. However, it also affects processes occurring on the molecular level. Molecular and intra-cellular events in turn affect the vascular network and therefore the nutrient dynamics. Our approach is to model, using partial differential equations, processes on the tissue level, and couple these to the intra-cellular events (modelled by ordinary differential equations) via cells modelled as automaton units. Thus far, within this framework, we have investigated the effects on tumour cell dynamics of structural adaptation at the vessel level, have explored certain drug protocol treatments, and have modelled the cell cycle in order to account for the possible effects of p27 in hypoxia-induced quiescence in cancer cells. We briefly review these findings here.

1. Introduction

Cancer is one of the biggest killers in the Western World. There has been a huge amount of experimental and medical research into this disease and for certain cancers cure rates have improved. Unfortunately, however, we still do not have an understanding of how this disease progresses and how the myriad processes involved conspire to initiate cancer and the growth

of tumours. In comparison to experimental research in this area, there has been relatively little theoretical work on cancer growth. It is now slowly being recognised that mathematical modelling may help us to extract the full potential of the vast amounts of data being generated in the laboratory and provide a framework in which to interpret these results¹. Modelling cannot find a cure for cancer, but it may allow experimental work to be directed in more efficient ways.

The ultimate challenge in the modelling of biological systems in general is to integrate the huge amount of experimental information being generated at the many different scales that make up a biological system. The traditional “top-down” approach does not capitalise on lower level data, while the “bottom-up” approach runs the risk of being too unwieldy and simply replacing a biological system we do not understand by a computational system we do not understand. Moreover, we must take into account the reality that many parameters are unknown and information is only partial. At the moment, it is an open question as to whether mathematics can meet this challenge. Equally, the best way to implement such an approach remains to be established.

In this paper we briefly review our recent attempt to build an integrated model of tumour growth. In Section 2 we present a very brief overview of tumour growth and then in Section 3 we outline our modelling approach, which uses a hybrid cellular automaton framework. Our philosophy is to start with a model which is comprised largely of “black boxes” or modules, which are represented at the outset by simple imposed rules. This is very much a macroscale level approach. We then aim to “zoom in” on particular modules as more experimental data becomes available and develop more realistic models. We illustrate this in Section 4 with a model for the G1/S transition in the cell cycle and in Section 5 with a simple model for pH.

2. Brief biological background

Under normal conditions, cell division and growth are tightly regulated by proliferation (division) and apoptotic (self-induced cell death) signals. However, in cancer, it is thought that a series of mutations (see, for example, Michor *et al.*²) within a cell leads to it escaping from these controls and this, in turn, can lead to an uncontrolled growth of tissue. Initial growth of a tumour has been studied in the laboratory using multi-cellular spheroids. The growth of this tissue is diffusion-limited as its main nutrient is oxygen and it has no active transport mechanisms. It develops a pattern typically

composed of an inner necrotic (dead) core, surrounded by a quiescent region (live cells which are not dividing), and an outer rim of proliferative cells. The growth rate greatly diminishes when the spheroid reaches about 1 mm in diameter and at this stage, if the tumour is to continue to grow significantly it needs a vascular system to provide it with nutrient. There is now quite a substantial amount of literature on the mathematical modelling of avascular tumour growth, ranging from very simple models which consider the dynamics of cell populations, to more sophisticated models ranging from those which delve into the microscopic levels of biochemical control of nutrient uptake, to those which consider the tumour mass as a multi-phase material modelled via the techniques of continuum mechanics. Other approaches include individual-based-models which consider cells as independent units and define equations or rules on how each unit grows, divides, moves, etc. References are too numerous to mention here so we simply refer the interested reader to the review by Roose *et al.*³ and references therein.

To gain access to more nutrient, the tumour cells secrete what are known as Tumour Angiogenesis Factors (TAFs) which diffuse into the surrounding normal tissue and, on reaching normal blood vasculature, initiate a series of events, the net result of which is that cells lining the vessel walls break away and begin to migrate chemotactically towards the tumour. On approaching the tumour they join up via the process of anastomosis establishing a blood supply for the tumour. This was first shown by the classical experiment of Folkman⁴. As with avascular tumours, there is now a quite substantial amount of modelling literature on the interaction of TAFs with the vessel lining, the formation of the angiogenic network and its chemotactic response. We refer the reader to the review by Mantzaris *et al.*⁵.

As the tumour mass now begins to grow out further it produces proteases that can degrade the extracellular material surrounding it, giving the tumour space to move. Cells can also break off from the main (or primary) tumour mass and enter the blood supply, leading to the process of metastasis and the formation of often fatal secondary tumours.

There are several reviews describing the process of nutrient consumption and diffusion inside tumours and we refer the reader to the papers^{6,7}.

3. Cellular automaton model

As mentioned in the previous section, there is a growing literature on the mathematical modelling of various aspects of tumour growth. However,

there is little theoretical work to date on how blood is delivered to tissue, how tissue demands are met by the structural adaptation of the blood network, and how spatial heterogeneity affects tumour dynamics. If we wish eventually to develop a model which allows us to explore different drug delivery protocols for therapy, then it is important that we understand these aspects. This was the motivation for developing the modelling framework below (we refer the reader to the original paper⁸ for full details).

We consider for simplicity a vascular structure which is composed of a regular hexagonal network embedded in a two-dimensional $N \times N$ lattice composed of normal cells, cancer cells, and space into which cells can divide. We impose a pressure drop across the vasculature, assuming that blood flows into the idealised “tissue” through a single inlet vessel and drains through a single outlet vessel. To compute the flow of blood through each vessel we use the Poiseuille approximation, and, given the initial network configuration (that is, radii and lengths) we compute the flow rates through, and pressure drops across, each vessel using Kirchoff’s laws. To calculate the radii, we begin by assuming that all vessels have the same radius, but assume that vessels undergo structural adaptation. We follow the work of Pries *et al.*⁹ by assuming that the radius $R(t)$ of a vessel, is modified as follows:

$$R(t + \Delta t) = R(t) + R\Delta t \left(\log \left(\frac{\tau_w}{\tau(P)} \right) + k_m \log \left(\frac{\dot{Q}_{ref}}{\dot{Q}H} + 1 \right) - k_s \right) \quad (1)$$

where Δt is the time scale, \dot{Q} is the flow rate, \dot{Q}_{ref} , k_m and k_s are constants, H is the haematocrit (red blood cell volume), $\tau_w = R\Delta P/L$ is the wall shear stress acting on a vessel of length L . P is the transmural pressure, and $\tau(P)$ the magnitude of the corresponding “set point” value of the wall shear stress obtained from an empirical fit to experimental data. The second term on the right-hand side represents the response to mechanical or haemodynamic stimuli. The third term on the right-hand side is the metabolic stimulus and increases with decreasing red blood cell flux. The constant k_s represents the so-called shrinking tendency, that is, without the mechanical and metabolic stimuli, the vessel would atrophy.

Blood viscosity is a complex function of H and R and this is taken from empirical studies, while the distribution of haematocrit at branch points is assumed to be proportional to the flow velocity along each adjoining vessel¹⁰. Pries *et al.* found that for efficient structural adaptation a third stimulus (the so-called conducted stimulus) was required. We omit this

from our model because it is well-known that tumour vasculature does not adapt as well as normal vasculature.

With the above equation we can now iterate our scheme until we reach a steady state and a vascular network with a distribution of different radii. We now use this to conduct nutrient into the tissue. Assuming, for simplicity, that the only nutrient is oxygen, we calculate the nutrient distribution by solving the diffusion equation with the cells as sinks for uptake (we take the adiabatic approximation) with internal boundary conditions representing diffusion of oxygen out of the blood vessels. We impose zero flux boundary conditions at the edge of the tissue.

To model the cell dynamics we assume that if the oxygen level is sufficiently high then cells will divide if there is space (or die otherwise) while if the oxygen level is too low then cells die. However, we assume that for intermediate values of oxygen, cancer cells can undergo quiescence and survive for a certain amount of time, whereas normal cells cannot (see Section 4). We further assume that the threshold levels of oxygen below which cells die is dependent on cell type and on the type of neighbouring cells. For example, if a normal cell is surrounded by cancer cells, then we raise the threshold level (that is, the cell is more likely to die). This is a very crude attempt to model the effects of pH (see Section 5).

A typical solution for the resultant oxygen profile is shown in Figure 1. One sees regions of very high oxygen levels interspersed with regions of hypoxia (low oxygen). Clearly, the system has not adapted well and this is reminiscent qualitatively of oxygen distributions within tumours.

Figure 2 shows the spatio-temporal and temporal evolution of cancerous cells for the case above, compared with the case where we do not assume any structural adaptation but instead impose the condition that the oxygen is distributed uniformly throughout the tissue. We see that spatial inhomogeneity has a significant effect on tumour dynamics by actually lowering the total cancer cell population. This is because there is not an efficient use of nutrient.

Furthermore, we see that the shape of tumour predicted has “finger-like” protrusions similar to those observed in some spreading cancers. This structure has arisen in this model simply because of the spatial heterogeneity in the nutrient distribution. Indeed, closer inspection reveals that one or two parts of the tumour have almost “broken away”. This cannot actually happen in this model because we have not included cell motion but we can imagine that if we did include motion towards areas of high nutrient concentration, then this may be a mechanism for metastasis (Alarcón *et al.*

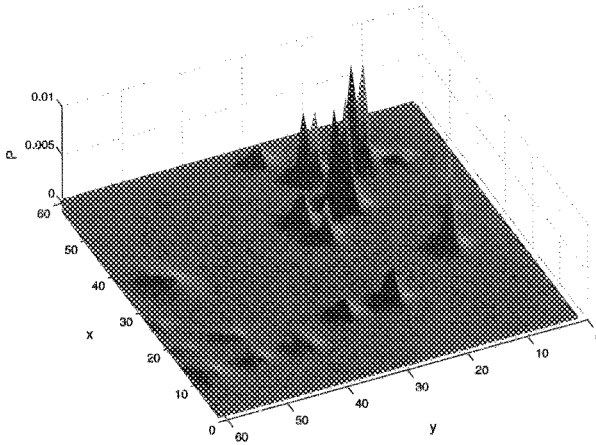


Figure 1. First 3 normalised frequencies versus release location for clamped simply supported beam with internal slide release.

in prep.).

4. Effects of hypoxia on cell cycle dynamics

In the above model we assumed that in hypoxic conditions, cancer cells can undergo quiescence whereas normal cells cannot (in fact, they undergo hypoxia-induced arrest leading to apoptosis). Whereas in the above we simply included this as a rule, here we aim to understand what is the mechanistic underpinning of this phenomenon.

The cell cycle is composed of 4 stages, G1, S, G2, M, with occasionally a G0 phase (see, for example, Alberts *et al.*¹¹). There have been a number of models proposed to account for the G1/S and for the G2/M transitions. The G1/S transition is particularly important because once a cell has passed through this checkpoint it is almost certain to divide. We chose to focus on this transition because some experimentalists felt that cells under hypoxic conditions may be inhibited from making this transition¹².

The cell cycle is controlled by a complex series of coordinated molecular events, with the central components of this interacting network being the two families of proteins, the cyclin-dependent kinases (CDKs) and the cyclins. During G1, the cyc-CDK complexes have low activity, which becomes high after transition. Coupled to this is the activity of the anaphase protein complex (APC) and the protein Cdh1 which both begin at high levels in

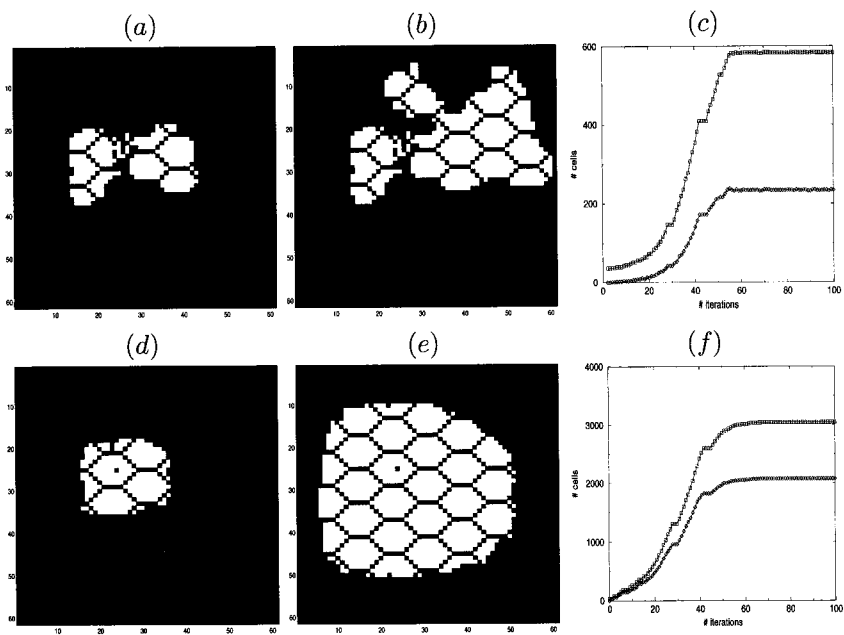


Figure 2. Series of images showing the evolution of the spatial distribution of cells for growth in inhomogeneous (panels a and b), and homogeneous environments (panels d and e). In panels (a), (b), (d), and (f) white spaces are occupied by cancer cells, whereas black spaces are either empty or occupied by vessels. Panels (c) and (f) show the time evolution of the number of (cancer) cells for the heterogeneous and homogeneous cases, respectively. Squares represent the total number of cancer cells (proliferating + quiescent). Diamonds correspond to the quiescent population.

G1 but fall to low levels of activity after the G1/S transition. There are a number of models of this process spanning a large range of detail (from 2 equations to over 60) but for our purposes we consider the model of Tyson and Novak¹³, which captures the essence of the problem. The model takes the form

$$\frac{dx}{dt} = \frac{(k'_3 + k''_3 A)(1-x)}{J_3 + 1 - x} - \frac{k_4 m y x}{J_4 + x}, \quad (2)$$

$$\frac{dy}{dt} = k_1 - (k'_2 + k''_2 x)y, \quad (3)$$

$$\frac{dm}{dt} = \mu m \left(1 - \frac{m}{m_*}\right), \quad (4)$$

where $x \equiv [\text{Cdh1}]$ is the concentration of active Cdh1/APC complexes,

$y \equiv [\text{Cyc}]$, is the concentration of cyclin-CDK complexes^a, and m is the mass of the cell. The parameters k_i ($i = 1, 2, 3, 4$) and J_i ($i = 3, 4$) are positive constants. A represents a generic activator. In Eq. (4), μ is the cell growth rate and m_* is the mass of an adult cell. We refer the reader to Tyson and Novak¹³ for full details.

The above model can exhibit mono- and bi-stability, with the cell mass m as a bifurcation parameter. For low values of m there is a single stable steady state with a high value of x and a low value of y - this would correspond to G1. As m increases, we enter the bistable regime, with a new stable steady state arising at a high value of y and a low value of x . For a critical value of m the latter becomes the only stable steady state and the system switches to this state, corresponding to the S phase. After the cell divides, m decreases, and the system is set back to the “G1 phase steady state”.

We take this as our base model and, together with the experimental results in Gardner *et al.*¹² and the hypothesis that under hypoxic conditions the expression of the regulator p27 increases (in fact due to decreased degradation), which in turn inhibits Cdh1 activity, we derive the (non-dimensionalised) model (see Alarcon *et al.*¹⁴ for full details):

$$\frac{dx}{d\tau} = \frac{(1 + b_3u)(1 - x)}{J_3 + 1 - x} - \frac{b_4mxy}{J_4 + x}, \quad (5)$$

$$\frac{dy}{d\tau} = a_4 - (a_1 + a_2x + a_3z)y, \quad (6)$$

$$\frac{dm}{d\tau} = \eta m \left(1 - \frac{m}{m_*} \right), \quad (7)$$

$$\frac{dz}{d\tau} = \chi(m) - c_2 \frac{P}{B + P} z, \quad (8)$$

$$\frac{du}{d\tau} = d_1 - (d_2 + d_1y)u, \quad (9)$$

where P is the oxygen tension, z is the p27 concentration and u is the concentration of phosphorylated retinoblastoma (RB).

We make the following assumptions: for normal cells, p27 activity is regulated by cell size, that is, $\chi(m) = c_1(1 - \frac{m}{m_*})$, but for cancerous cells, this size-regulation is lost, that is $\chi(m) = c_1$. We make the further assumption

^aIn Tyson and Novak¹³, $[\text{Cyc}]$ corresponds to the concentration of the specific complex cyclinB-CDK. Here we simply consider a generic cyclin-CDK complex in order to keep our model as simple as possible.

that c_1 (maximum rate of synthesis of p27) is larger in normal cells than in cancer cells – this we do to account for the observation of low p27 levels in cancer cells compared to normal cells (see, for example, Philipp-Staheli *et al.*¹⁵). Using other parameter values from Tyson and Novak¹³ we find that assuming these two phenomena characterise the differences between the regulation of p27 in cancer and in normal cells is sufficient to account for hypoxia-induced quiescence in the former, and hypoxia-induced arrest in the latter. Our numerical simulation results are supported by an analytic study of the bifurcation structure of the model (see Alarcon *et al.*¹⁴ for details).

5. The role of acidity

In the cellular automaton model of Section 2 we imposed a rule in which the fate of cells depended on their neighbours. This was motivated by the work of Gatenby and Gawlinski^{16,17}. They proposed a reaction-diffusion model for interaction between tumour cells and normal cells and hypothesised that when tumour cells undergo anaerobic metabolism (which they do even under normoxic conditions) the by-product of lactic acid lowers the pH into a regime where the tumour cells can “over-power” the neighbouring normal cells and invade the tissue simply because of their ability to tolerate more acidic conditions. Their model predicted that there should be a gap between the advancing tumour front and the regressing normal tissue and, indeed, they later observed this phenomenon experimentally.

A drawback in their model was that it predicted either a travelling wave of tumour invasion, or total clearance of tumour cells. It could not predict the formation of a benign tumour. This problem can be overcome if one considers a very simple model in which tumour cells produce acid and the tumour grows but also loses cells via necrosis if the acid level is too high. The resultant coupled system of ordinary differential equations yields three different types of behaviour: saturated (benign) growth of avascular tumours; benign growth of vascular tumours which can become invasive (malignant) as a key dimensionless parameter passes through a critical value (see Smallbone *et al.*¹⁸ for full details).

6. Discussion

We have presented results from our recent research into the growth of vascular tumours. Our approach to incorporating processes occurring on very different length scales is to use a hybrid cellular automaton framework^{19,20}.

Our very preliminary work in this area has already revealed some experimentally testable predictions. Our model shows that nutrient heterogeneity can have a significant effect on the spatio-temporal dynamics of tumour growth. In particular, it shows that it may be the cancerous cells' exploitation of high nutrient sources that causes an initial homogeneously growing tumour to begin to break up. We are in the process of incorporating cell movement into our model to see if this can lead to metastasis. We have recently shown that in some cases anti-angiogenesis treatment could actually enhance tumour growth due to the modified vasculature being more efficient at delivering nutrient²¹.

Our modelling framework allows for detailed sub-models to be included for processes occurring on a specific scale. Thus, for example, our simple rule for the signal for cell division can be expanded to incorporate a model of this process. In doing so, we have generated a hypothesis as to how cancerous cells can undergo hypoxia-induced quiescence while normal cells undergo hypoxia-induced arrest. We propose that p27 plays a key role in this but we must be aware that this is still controversial²². An important point here is that if we were to include a full model for the cell cycle into the cellular automaton model, the resultant model would require a huge amount of computational power to solve and would be so complicated that it would be difficult to gain insight into the phenomena observed from the model. Therefore we must reduce the model and indeed one can do this by taking a caricature model of only a few equations which aims to capture the essence of the full cell cycle model. In this case, however, the question of whether our results are artifacts because of the simplifications we made arises and this is a crucial problem facing all theoreticians working in the Life Sciences, namely, how robust are the models that we generate?

The simple model presented in Section 3 proves inadequate if we want to use it to explore the effects of drug treatment where a drug acts on cells in a certain part of the cell cycle. In this case, we need to incorporate cell cycle models of the form proposed in Section 4, or we can use a probabilistic approach based on empirical data to determine the probability that a certain cell is in a certain phase of its cell cycle at a particular time. The latter approach was used to examine the effects of Doxorubicin treatment on non-Hodgkin's lymphoma to determine the optimal dosage protocol²³.

In Section 5 we explored in more detail the effects of acidity. This simple model isolates a single nondimensional bifurcation parameter which determines whether or not a tumour will grow in an uncontrolled fashion. This raises a number of possible control mechanisms, including the counter-

intuitive prediction that increasing the acidity may eliminate the tumour. This model prediction remains to be tested.

Future work in this area must address the underlying biochemistry of many of the processes we mentioned above and incorporate the mechanical aspects involved in tumour growth. We have recently incorporated rules for production of the growth factor VEGF in response to hypoxic conditions, computed its spatio-temporal distribution by solving a reaction-diffusion model, and modified the vessel structural adaptation equation accordingly²⁴. While this allows us to capture the initial vessel dilation in response to VEGF, it only in a very crude way accounts for the angiogenic response. We are presently incorporating growth of new vasculature into the model.

A crucial aspect of all this work will be model reduction so that the resultant model is computationally tractable and understandable. Only then can mathematical modelling gain useful insights to help direct medical research.

Acknowledgments

TA thanks the EPSRC for financial support under grant GR/509067. HMB thanks the EPSRC for funding as an Advanced Research Fellow. This work has been supported in part by NIH grant CA 113004. The authors wish to acknowledge the support provided by the funders of the Integrative Biology project: The EPSRC (ref no: GR/S72023/01) and IBM.

References

1. Gatenby, R.A., Maini, P.K. (2003), "Mathematical oncology: Cancer summed up," *Nature* 421, 321.
2. Michor, F., Iwasa, Y., Nowak, M.A. (2004), "Dynamics of cancer progression," *Nature Reviews, Cancer* 4, 197-205.
3. Roose, T., Chapman, S.J., Maini, P.K. (2005), "Mathematical models of avascular tumour growth," (submitted)
4. Folkman, J. (2003), "Fundamental concepts of the angiogenic process," *Curr. Mol. Med.* 3, 643-651.
5. Mantzaris, N., Webb, S., Othmer, H.G. (2004), "Mathematical modeling of tumor-induced angiogenesis," *J.Math.Biol.* 95, 111-187.
6. Adam, J.A. (1996), "Mathematical models of perivascular spheroid development and catastrophe-theoretic description of rapid metastatic growth/tumor remission," *Invasion and Metastasis* 16, 247-267.
7. Araujo, R.P., McElwain, D.L.S. (2004), "A history of the study of solid tumor growth: the contribution of mathematical modelling," *Bull. Math. Biol.* 66, 1039-1091.

8. Alarcón, T., Byrne, H.M., Maini, P.K. (2003), "A cellular automaton model for tumour growth in inhomogeneous environment," *J.theor.Biol.* 225, 257-274.
9. Pries, A.R., Secomb, T.W., Gaehtgens, P. (1998), "Structural adaptation and stability of microvascular networks: theory and simulations," *Am. J. Physiol.* 275, H349-H360.
10. Fung, Y.C. (1993), "Biomechanics," *Springer, New York*.
11. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., Watson, J.D. (1994), "Molecular Biology of the Cell," 3rd edition *Garland Publishing, New York, USA*.
12. Gardner, L.B., Li, Q., Parks, M.S., Flanagan, W.M., Semenza, G.L., Dang, C.V. (2001), "Hypoxia inhibits G₁/S transition through regulation of p27 expression," *J. Biol. Chem.* 276, 7919-7926.
13. Tyson, J.J., Novak, B. (2001), "Regulation of the eukariotic cell-cycle: molecular anatagonism, hysteresis, and irreversible transitions," *J. Theor. Biol.* 210, 249-263.
14. Alarcón, T., Byrne, H.M., Maini, P.K. (2003), "A mathematical model of the effects of hypoxia on the cell-cycle of normal and cancer cells," *J. Theor. Biol.* 229, 395-411.
15. Philipp-Staheli, J., Payne, S.R., Kemp, C.J. (2001), "p27(Kip1): regulation and function of haploinsufficient tumour suppressor and its misregulation in cancer," *Exp. Cell. Res.* 264, 148-168.
16. Gatenby, R.A., Gawlinski, E.T. (1996), "A reaction-diffusion model of cancer invasion," *Cancer Res.* 56, 5745-5753.
17. Gatenby, R.A., Gawlinski, E.T. (1996), "The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models," *Cancer Res.* 56, 5745-5753.
18. Smallbone, K., Gavaghan, D.J., Gatenby, R.A., Maini, P.K. (2005), "The role of acidity in solid tumour growth and invasion," *J. Theor. Biol.* 235, 476-484.
19. Patel, A.A., Gawlinsky, E.T., Lemieux, S.K., Gatenby, R.A. (2001), "Cellular automaton model of early tumour growth and invasion: the effects of native tissue vascularity and increased anaerobic tumour metabolism," *J. Theor. Biol.* 213, 315-331.
20. Moreira, J., Deutsch, A. (2002), "Cellular automaton models of tumor development: A critical review," *Adv. in Complex Systems* 5, 247-267.
21. Alarcón, T., Byrne, H.M., Maini, P.K. (2004), "Towards whole-organ modelling of tumour growth," *Prog. Biophys. Mol. Biol.* 85, 451-472.
22. Green, S.L., Freiberg, R.A., Giaccia, A.(2001), "p21^{Cip1} and p27^{Kip1} regulate cell cycle reentry after hypoxic stress but are not necessary for hypoxia-induced arrest," *Mol. & Cell. Biol.* 21, 1196-1206.
23. Ribba, B., Marron, K., Agur, Z., Alarcón T., Maini, P.K. (2005), "A mathematical model of Doxorubicin treatment efficacy for non-Hodgksin's lymphoma: investigation of the current protocol through theoretical modelling results," *Bull. Math. Biol.* 67, 79-99.
24. Alarcón, T., Byrne, H.M., Maini, P.K. (2005), "A multiple scale model for tumour growth," *SIAM J. Multiscale Mod. & Sim.* 3, 440-475.