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Avascular tumour growth dynamics and the constraints of protein binding for drug transportation

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HIGHLIGHTS

- A new cellular automaton model for the tumour growth using Neural Network.
- An *in vitro* experiment with multicellular tumour spheroids to validate the model.
- Examined the protein binding on bioreductive drug transportation.

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ABSTRACT

The potential for the use of *in-silico* models of disease in progression monitoring is becoming increasingly recognised, as well as its contribution to the development of complete curative processes. In this paper we report the development of a hybrid cellular automaton model to mimic the growth of avascular tumours, including the infusion of a bioreductive drug to study the effects of protein binding on drug transportation. The growth model is operated within an extracellular tumour microenvironment. An artificial Neural Network based scheme was implemented that modelled the behaviours of each cell (proliferation, quiescence, apoptosis and/or movement) based on the complex heterogeneous microenvironment; consisting of oxygen, glucose, hydrogen ions, inhibitory factors and growth factors. To validate the growth model results, we conducted experiments with multicellular tumour spheroids. These results showed good agreement with the predicted growth dynamics. The outcome of the avascular tumour growth model suggested that tumour microenvironments have a strong impact on cell behaviour. To address the problem of cellular proteins acting as resistive factors preventing efficient drug penetration, a bioreactive drug (tirapazamine) was added to the system. This allowed us to study the drug penetration through multicellular layers of tissue after its binding to cellular proteins. The results of the *in vitro* model suggested that the proteins reduce the toxicity of the drug, reducing its efficacy for the most severely hypoxic fractions furthest from a functional blood vessel. Finally this research provides a unique comparison of *in vitro* tumour growth with an intelligent *in silico* model to measure bioreductive drug availability inside tumour tissue through a set of experiments.

1. Introduction

Cancer is a complex set of diseases resulting from the deregulation of cell growth. Cancer is characterised by genetic mutations which alter cellular physiology such that malignant growth occurs, as characterised by a lack of response to signals inhibiting apoptosis, angiogenesis and metastasis (Hanahan and Weinberg, 2011). Small tumours are able to get sufficient oxygen and nutrients from the surrounding blood vessels to allow for cell division to occur. When tumours reach a critical size of approximately 10^6 cells, the diffusion of nutrients starts to limits tumours growth. To grow beyond 10^6 cells, a tumour must establish its own blood supply by stimulating angiogenesis (Ferrara and Kerbel, 2005). The blood vessels resulting from abhorrent tumour angiogenesis are inadequate when compared to those in normal tissues (Hicks et al., 2006). They are typified by a heterogeneity that reduces oxygen saturation (hypoxia), as well as nutrient, glucose and energy availability, concomitant with high lactate levels, and extracellular acidosis (Vaupel, 2004). Harsh micro-environmental parameters such as

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hypoxia are to blame for the resistance shown to most of currently used conventional anti-cancer treatments including radiotherapy and chemotherapy, and are known as one of the major causes of cancer treatment failure.

Chemotherapy was developed to kill rapidly dividing abnormal cells and target the aerobic fraction of cancer cells (Brown, 2000). Radiotherapy kills cells by damaging their DNA, while oxygen molecules keep this damage permanent resulting in cell death (Siim et al., 1997). Oxygen is needed for the cytotoxic effects of radiation and cancer chemotherapeutic drugs. With low oxygen levels, hypoxic cells show the chemoresistance and radioresistance (Shannon et al., 2003). Hypoxia occurs in 50–60% of solid tumours (Cowen et al., 2008). Bioreductive drugs such as Tirapazamine (TPZ) have shown promising activity in both preclinical and clinical trials, with preferential activity against hypoxic cells in comparison to normoxic cells (Patterson and Mckeown, 2000). Despite its activity against hypoxic cells the reductive and rapid metabolism of TPZ effects its effective penetration and results in lower drug exposure for hypoxic regions. It is worth mentioning that the TPZ is currently in phase III clinical trials (Hicks et al., 1998; Kyle and Minchinton, 1999).

A limitation of the therapeutic use of TPZ is that it inadequately penetrates the extravascular compartment (Jain, 1998). Heterogeneous tumour vasculature and distance from functional blood vessels combine to reduce the circulating drug concentrations at distal areas; this is recognised as a potential barrier against effective drug delivery (Carins et al., 2006). During drug penetration a significant amount of drug binds to surrounding proteins, reducing the quantity of circulating drug. TPZ experiences reduction into its radical enzyme; a cytotoxic agent called hydroxyl radical that damages cellular DNA in the absence of molecular oxygen *in vivo*. Studies have shown that 30–60% of initial drug mass disappeared as known metabolites, 3-amino-1,2,4-benzotriazine 1-oxide and 3-amino-1,2,4-benzotriazine. This compound was identified as a TPZ metabolite from its one electron activation due to the xanthine/xanthine oxidase enzyme and NADPH: cytochrome P450 oxidoreductase (Fuchs et al., 2001). Inefficient drug diffusion to the most severely hypoxic regions of solid tumour results in a failure to kill all of the cancer cells and results in tumour recurrence. Several approaches have been used to address drug resistance. The classification of recent bioreductive drugs and agents, assessment of liposomal and nanoparticle production will standardise the vasculature of tumour is under development (Palo and Bocci, 2009). The literature shows the use of cellular aggregate models in experimental cancer research for several decades. These spherical aggregates of malignant cells are commonly known as multicellular tumour spheroids. These spheroids serve as *in vitro* models of the tumour microenvironment and allow study of avascular tumour growth (Mueller-Klieser, 1987); this will impact on the future of cancer research (Freyer and Sutherland, 1986; Sutherland, 1988).

An *in silico* model was developed to study the impact of complex tumour microenvironments on the growth of tumours at the cellular level using an artificial intelligence technique namely the neural network. The main idea was to explore the behaviour of the bioreductive drug TPZ inside the tumour tissue so the growth model was integrated with the drug transportation model. The drug transportation model calculates the circulating drug binding to tissue proteins and measures the available drug concentration, drug metabolism, penetration and cytotoxicity at the cellular level. The fundamental goal is to develop an *in silico* model to assist the drug innovation process by reforming preclinical drug development.

This paper is sectionalised as follows; Section 2 defines previous work related to our current study. Section 3 defines the proposed model for solid tumour growth, validation of growth model with experimental data and bioreductive drug TPZ transport and its binding to protein. A brief analysis of the parameters of the model is presented in Section 4. Section 5 discusses the results of the proposed *in silico* hybrid model. The conclusion and future work is presented in Section 6.

2. Related work

Computational models of cancer are being developed to demonstrate both biological discovery and clinical medicine. The development of *in silico* models are helped by quickly advancing experimental and analytical tools that generate information-rich and high-throughput biological data. Many statistical models of cancer at the genomic, transcriptomic and pathway levels have already proven effective in developing diagnostic and prognostic molecular signatures.

A cellular automaton (CA) is a decentralised computing model providing a dynamic platform for performing complex computation in different modalities. The reason behind the popularity of cellular automata can be traced to their simplicity, and to the enormous potential they hold in modelling complex systems, in spite of their simplicity. CA can be viewed as a simple model of a spatially extended decentralised system made up of a number of individual components (cells). CA models have been used immensely in mathematical modelling of biological systems (Deutsch and Dormann, 2005). The first work using cellular automata in cancer modelling was done by Duchting and Vogelsaenger (1984), who used it to investigate the effects of radiotherapy.

Various cellular automaton models are being used to model biological systems (Deutsch and Dormann, 2005). A huge range of new policies, experimental and theoretical techniques are being developed to beat cancer. Mathematical models have the capability to avoid excessive experimentation. They are allowing biologists to develop valuable insight into the development of solid tumours which will help in controlling the spread of this disease (Byrne, 1999). Hill observed the neoplastic tissue and diffusion in tissues and inspired later mathematicians to develop mathematical models of solid tumours (Hill, 1928). CA models represent cancer cells as discrete entities of fixed location and scale, interacting with one another and external factors in discrete time intervals according to predefined rules (Deisboeck et al., 2008).

Mathematical modelling of avascular tumours contributes considerably to solid tumour growth modelling. One of the most important piece of work in this context was reported by Folkman (1974). He discovered hidden avascular lumps in his *in vivo* experiments and demonstrated that the circulation of tumour growth inhibitory factors between the cells is not constant. The loss of coupling between these tumour cells was found to be a reason behind this (Anderson et al., 1999). Byrne and Chaplain (1995) modelled tumour growth activity and discussed the impact of the supply of nutrient and growth inhibitory factors to the tumour by the circulation and by the initiation of angiogenesis.

A mathematical model was presented that discussed a nonlinear and spatially-dependent circulation constant. They defined the growth inhibitory factors by using the circulation constant (Chaplain et al., 1994). A hybrid CA model captured the dynamics of avascular tumour growth processes and the evolutionary aspects of glycolytic phenotype (Smallbone et al., 2007). A model captured the dynamics of vascular tumour growth (Angelis and Preziosi, 2000). The impact of proliferating quiescent and necrotic cells with varying densities, and growth factors on the growth of avascular tumour was studied (Sherratt and Chaplain, 2001). A micro-scale model of vascular tumour showed that cell proliferation and cell death establish gradients of pressure that open