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Computer simulation of tumour spheroid behaviour as a platform for understanding cancer in silico

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Overview

An advanced three-dimensional (3D) Monte Carlo simulation model of both the avascular development of multicellular tumour spheroids and their response to radiation therapy is presented. The proposed approach is based on a discrete time cell cycle model, which is applied to each one of the cells constituting the tumour. The discrete time and space character of such a model allows the imposition of arbitrary boundary conditions such as the spatial profile of the oxygen and glucose supply. Moreover, all apparently possible pathways in the cellular level leading to cell death have been incorporated including interphase cell death via either spontaneous or radiation induced apoptosis, as well as mitotic cell death through either necrosis or apoptosis. Such a model can provide an efficient platform for gaining insight into the (radio)biological mechanisms involved in tumour growth *in vitro* as well as during the avascular stages of *in vivo* tumour evolution. Optimisation of dose fractionation during radiation therapy by performing *in silico* experiments before the actual delivery of the radiation dose to the patient is the main practical target.

Model Description

In brief, the model [1][2] is based upon a number of fundamental biological principles such as the transition between the cell cycle phases, the diffusion of oxygen and nutrients and the cell survival probabilities following irradiation. The statistical character of specific biological phenomena during cell proliferation and irradiation is simulated applying a pseudo-random number generator. The model simulates spheroidal growth of avascular tumours as the most satisfactory *in vitro* models of solid tumours seem to be the multicellular tumour spheroids characterized by the emergence of cellular heterogeneity. The absence of vasculature is a plausible hypothesis for both tumour growth in cell culture and the non-vascularized tumour or metastatic nodule growth *in vivo*.

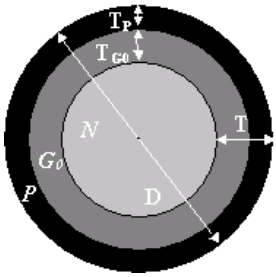

Figure. 1**.** Parameter definition in the spheroid model. ***P***: Proliferating cells, G_0: Quiescent cells, ***N***: Necrosis, **T**: thickness of the viable cell layer, $\mathbf{T_P}$: thickness of the proliferating cell layer , $\mathbf{T_{G0}}$: thickness of the hypoxic cell layer, $T_{G0}= T-T_P$, **D**: spheroid diameter.

The tumour spheroid formation starts with the placement of either a single tumour cell at the stage of mitosis or a small tumour spheroid at the centre of a three-dimensional (3D) discretizing mesh. The tumour cell proliferates passing through the cell cycle (gap 1, DNA synthesis, gap 2 and

mitosis). A cell enters the resting G_0 phase if oxygen and nutrient supply in its current position is not adequate (Fig. 1). The thickness of the viable rim decreases linearly as a function of the spheroid diameter [3]. In addition to necrosis there is always a probability that each cell residing in any phase dies with some probability per hour due to both ageing and spontaneous apoptosis. The cell lysis and apoptosis products are gradually diffused towards the outer environment of the tumour leading to tumour shrinkage due to the exertion of external pressures. Cell shifting due to expansion or shrinkage can take place along any random direction in the 3D space. This has been shown to lead to a remarkable preservation of the spheroidal shape of the tumours in accordance with experimental observations.

The response of each cell to irradiation is described by the LQ model. The parameters of the LQ model modelling the radiosensitivity of the tumour cells depend on the type of tumour, the genetic data as well as on the cellular metabolism. The radiobiological properties of the well oxygenated tumour cells are obtained from the literature, whereas the radiation response under hypoxia is estimated taking into account the oxygen enhancement ratio (OER). The cell death due to irradiation is simulated by three mechanisms: the radiation induced interphase death, the radiation induced mitotic necrotic death and the radiation induced mitotic apoptotic death.

Results

The model has been applied to the specific case of EMT6 tumour spheroids (Fig.2). Nevertheless, it can simulate the basic growth pattern characteristics that have been established for spheroids of many other cell types and cell lines, i.e. volume growth saturation, development of an outer proliferating rim and a growing necrotic centre. Both the numerical stability and the statistical behaviour of the simulation model have been studied and evaluated for the case of EMT6/Ro spheroids in [2]. Predicted histological structure and tumour growth rates have been shown to be in agreement with published experimental data.

Moreover the response to irradiation according to some common fractionated radiotherapeutic schemes is simulated (Fig.3).

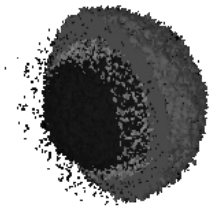

Figure 2. A 3D representation of the internal structure of an EMT6 tumour spheroid using AVS/Express™ 4.2 The proliferating (gray) and hypoxic cell rim (light gray) enclose the necrotic core (black).

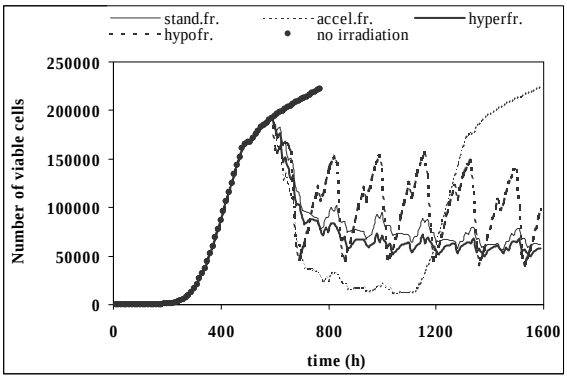

Figure. 3. The number of viable cells of an EMT6 tumour spheroid as a function of time if untreated as well as after the application of the standard fractionation, accelerated fractionation, hyperfractionation and hypofractionation schemes. Irradiation begins 600 h after the placement of a tumour cell at the centre of the discretizing mesh.

References

[1] G.S. Stamatakos, D.D. Dionysiou, E.I. Zacharaki, N. Mouravliansky, K.S. Nikita and N.K. Uzunoglu, *In Silico* Radiation Oncology: Combining Novel Simulation Algorithms with Current Visualization Techniques, Proceedings of the IEEE 90 (2002) 1764-1777.

[2] E.I. Zacharaki, G.S. Stamatakos, K.S. Nikita and N.K. Uzunoglu, "Simulating growth dynamics and radiation response of avascular tumour spheroids – Model validation in the case of an EMT6/Ro multicellular spheroid," *Computer Methods and Programs in Biomedicine*, in press.

[3] J.P. Freyer and R.M. Sutherland, Regulation of growth saturation and development of necrosis in EMT6/Ro multicellular spheroids by the glucose and oxygen supply, Cancer Research 46 (1986) 3504-3512.