

A Cellular Model for Avascular Tumor Growth

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ABSTRACT

We model the first step of tumor formation, the growth of an avascular benign tumor. Tumor growth always starts from a small number of abnormally proliferating cells. Multicellular tumor spheroids (MTS) are an experimental model system for the study of initial avascular tumor growth. Typically multicellular spheroids consist of a necrotic core surrounded by layers of quiescent and proliferating tumor cells, respectively. Furthermore, after an initial exponential growth phase further spheroid growth significantly slows down and eventually reaches a fixed size, even in the presence of a periodically applied nutrient supply [1].

Our cellular model explicitly takes into account mitosis, mutation and necrosis as well as nutrient consumption and metabolic waste generation. Unlike differential equation models, in the cellular model it is possible to follow the fate of individual cells. Every cell can proliferate, become quiescent or die due to necrosis depending on its microenvironment. Tumor growth requires the transport of nutrients (e.g. oxygen and glucose) from and waste products to the surrounding tissue. These chemicals regulate cell mitosis, cell death, and potentially cell mutation. Multicellular tumor spheroid as an in vitro tumor model has the great advantage of precisely controlling the external environment while maintaining the cells in the spheroid microenvironment [2,3]. Suspended in culture, tumor cells aggregate and grow into a spheroid, in a process that closely mimics the growth characteristics of tumors. MTS exhibit three distinct phases of growth: 1) an initial phase during which individual cells form small clumps that subsequently grow quasi-exponentially; 2) a layering phase during which the cell cycle distribution within the spheroid changes, leading to formation of a necrotic core, accumulation of quiescent cells around the core, and sequestering of proliferating cells at the periphery; and 3) a plateau phase during which the growth rate begins to decrease and the tumor ultimately attains a maximum diameter. MTS experiments using EMT6/R₀ mouse mammary tumor spheroids provide high-precision measurements for controlled glucose and oxygen supply [2,3], as well as various inhibition factors and growth factors.

Characterizations of spheroid growth kinetics mostly use classical growth models such as the von Bertalanffy, logistic or Gompertz models [4]. Among these, the Gompertz model showed best fit to experimental data. None of these rate models (empirical ordinary differential equations) can simulate the evolution of tumor structure, or predict the effect of chemicals on tumor morphology. A cellular automaton model simulated a spheroid growth but the cells were represented as single lattice points [5]. The only previous work that included cell geometry reproduced a layered tumor structure, but only by introducing an artificial potential [6].

Models of tumor growth must consider cell-cell adhesion, chemotaxis, cell dynamics including cell growth, cell division and cell mutation, as well as the reaction-diffusion of chemicals, and eventually, angiogenesis factors and hormones. Our discrete cellular model is a lattice Monte Carlo model coupled with reaction-diffusion chemical dynamics [7]. This hybrid model is unique in that it treats cells as individual entities that have a finite volume and a deformable shape, at the same time it includes cell growth and mitosis, cell-cell adhesive, and cell-environment chemotactic interactions.

In the cellular model, each cell is given an identification number S ; its properties included its type τ , adhesion strength to its neighboring cells J , and volume v . For growing cells, the properties also include growth rate, metabolic rate (nutrient consumption rate and waste generation rate). The model partitions a three-dimensional space into domains of cells and cell medium. The total energy of the cell aggregate depends on the cell-cell surface interactions due to adhesion, cell elastic bulk energy due to growth, and a chemical energy due to cell interaction with local chemical environment [8]:

$$H = \sum_{\text{sites}} J \tau(s) \tau(s') (1 - \delta_{s, s'}) + \lambda_v \sum_{\text{cells}} [v_s - V_s]^2 + \sum_{\text{cells}} \mu C_f$$

where J is the coupling between cells, corresponds to the adhesive energy between cell surfaces that is cell type dependent; λ is the volume constraint coefficient, corresponds to the elastic rigidity of cells; μ is the chemical potential referring to the cells' capability of chemotaxis, with C_f being the concentration of the chemoattractant.

Chemicals in the spheroid environment follow a reaction-diffusion dynamics that are described by continuous PDEs.

$$\begin{aligned} \frac{\partial C_o}{\partial t} &= d_o \nabla^2 C_o - a(\bar{x}) \quad (C_o = C_{o0} \text{ at tumor boundary}) \\ \frac{\partial C_n}{\partial t} &= d_n \nabla^2 C_n - b(\bar{x}) \quad (C_n = C_{n0} \text{ at tumor boundary}) \\ \frac{\partial C_w}{\partial t} &= d_w \nabla^2 C_w + c(\bar{x}) \quad (C_w = 0 \text{ at tumor boundary}) \end{aligned}$$

Here C_o , C_n , and C_w are concentrations for oxygen, nutrients (e.g. glucose), and wastes (e.g. lactate), respectively, and d_o , d_n and d_w their diffusion constants in spheroid; a and b are metabolic rates for oxygen and nutrients, and c is the production rate of wastes that depends on a and b . As different nutrients affect cells differently, e.g. glucose and oxygen have drastically different effects on the cell cycle, and the diffusion coefficient of oxygen is two orders of magnitudes higher than that of glucose, we treat oxygen and other nutrients separately. We can include more detailed descriptions of C_n as experimental data become available.

The lattice of cells evolves by a Monte Carlo procedure. At each step, a random change is proposed and if the change increases the total energy, it is accepted with a probability determined by a Boltzmann factor. The chemical dynamics are solved on an evolving irregular 3D grid, each grid point being the center of mass of a cell. The time scale for cell dynamics in the lattice Monte Carlo model is determined by cell cycle period or growth rate, while the time scale for chemical dynamics is determined by chemical diffusion and cell metabolic rate. The simultaneous use of a discrete lattice model and a continuous PDE model to represent cell dynamics in aggregates requires matching two different space and time scales.

Cell growth and division are treated with the volume constraint in the total energy. Each cell follows its own cell cycle, which depends sensitively on the chemical environment. The target volumes for each cell in this model depend not only on time but also the concentrations of nutrients and waste: the growth rate increases with nutrient and decreases with waste. If the nutrient concentration falls below a threshold or the waste concentration exceeds its threshold, the cell can stop growing and become quiescent: alive but not growing. If the nutrient concentration drops lower or waste increases further, the quiescent cell may become necrotic. Only when the cell reaches the end of its cell cycle and its volume reaches a target volume will the cell divide. The mature cell then splits along its longest axis into two daughter cells, which may inherit all the properties of the mother cell or undergo mutation with a defined probability.

Figure 1 shows the cell population as a function of time of a simulated tumor. The growth rate is exponential initially when all cells in the small tumor sufficient nutrients and wastes can easily diffuse out, but slows down after 350 Monte Carlo steps, corresponding to 5–10 days when the quiescent cells start appearing in the center of the tumor. Figure 2 compares the simulation data with EMT6 spheroid experiments, showing the cell cycle fraction as a function of time. The agreement is remarkable. More detailed comparison between experiments and simulations are undergoing.

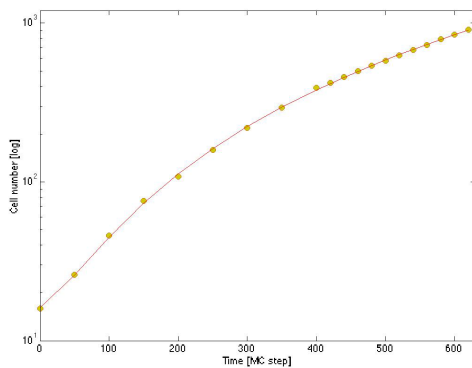


Figure 1. Evolution of cell number in a semilog scale.

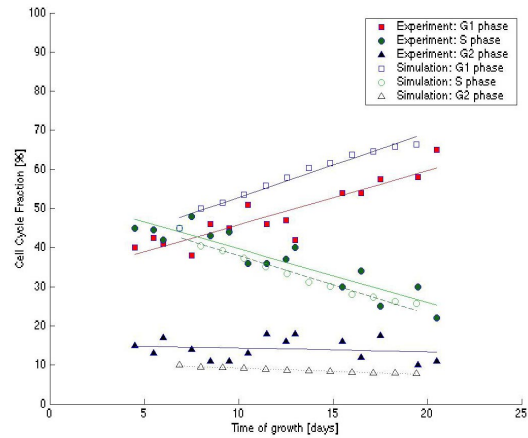


Figure 2. Cell cycle fractions vs. time. Solid symbols are experimental data from EMT6 spheroid grown in 16.5 nM glucose and 20% oxygen [2,3]. Open symbols are from simulation.

REFERENCES

- [1] Folkman, J. and Hochberg, M. Self-Regulation of growth in three dimensions. *J. Exp. Med.* 138, 745-753, 1973.
- [2] Feryer, J.P., Suthreland R.M., Regulation of growth saturation and development of necrosis in EMT6/R0 multicellular spheroids by the glucose and oxygen supply. *Cancer Res.* 46, 3504-3512, 1986.
- [3] Freyer J.P., Sutherland R.M., Proliferative and clonogenic heterogeneity of cells from EMT/Ro multicellular tumor spheroids induced by the glucose and oxygen supply. *Cancer Res.* 46, 3513-3520, 1986.
- [4] Marusic M, Bajzer Z, Freyer JP and Vuk-Pavlovic S, Analysis of Growth of Multicellular Tumour Spheroids by Mathematical Models, *Cell Prolif.* 27, 73, 1994.
- [5] Dormann S and Deutsch D, Modeling of self-organized avascular tumor growth with a hybrid cellular automaton, *In Silico Biology* 2, 35, 2002.
- [6] Stott E, Britton NF, Glazier JA and Zajac M, Stochastic Simulation of Benign Avascular Tumor Growth Using the Potts Model, *Mathematical and Computer Modeling* 30, 183, 1999.
- [7] Jiang Y, Pjesivac J and Freyer JP, A hybrid cellular model for avascular tumor growth, preprint, 2002.
- [8] Jiang Y, Levine H and Glazier JA, Possible collaboration of Differential Adhesion and Chemotaxis Cooperate in Mound Formation of *Dictyostelium*. *Biophys. J.* 75, 2615-2627, 1998.