An Agent-based Model of Avascular Tumour Growth

Ana Victoria Ponce Bobadilla[1], René Doursat[2] and François Amblard[3]

[1]Ecole Polytechnique, Paris, France
[2]BioEmergences Lab - CNRS, Gif-sur-Yvette, France
[3]Institut Curie, Paris, France
ana-victoria.ponce-bobadilla@polytechnique.edu

Abstract

We developed a simplified agent-based model for an avascularised tumour. The model takes into account a healthy tissue in which blood vessels introduce nutrients that diffuse. In this tissue, cells move/proliferate/die according to their energy and the available space for their offspring. They can mutate to cancerous with a certain probability and can also change mutated which means that they are affected by their neighbours states. First, we describe briefly the background of the problem and previous models made for the particular avascular state. Then, we describe in detail the rules and actors our model takes into account and comment on the particular choices. Next, we present our results from the parameter exploration. We were interested in finding the critical values that define the transition between a majority of cancerous cells and normal cells. We studied the outcome of varying the effect of neighbours, probability of division and mutation probability. We discovered a phase transition in the effect of neighbours parameter space and the effects of the other parameters in this space. Finally, we discuss the relevance of the model as well improvements one could make.

Introduction

Cancer is among the leading causes of death worldwide (8.2 million deaths in 2012) (Organization, 2014). Mathematical models are being created to help understand the underlying mechanisms of tumour growth and they have the potential to create a framework to perform virtual experiments and simulations. This will enable scientists to efficiently observe the effects of different treatments and improve them or suggest new ones (Roose et al., 2007).

Cancer can be generally defined as the uncontrolled growth and spread of cells. Other terms that are used to refer to it, are malignant tumours and neoplasms. There are more than 100 types of cancers; lung, liver, stomach, colorectal and breast cancers are among the ones that cause the most deaths each year (Organization, 2014). However, they all have certain characteristics in common: the tumour mass grows beyond its typical boundaries, and can invade neighbour parts of the body and spread to other organs.

It is still debated how exactly cancer is initiated. The general consensus is that several gene mutations are required to turn a normal cell into a cancer cell. The factors that trigger these mutations are largely unknown, but are thought to include both environmental and hereditary properties (Roose et al., 2007).

Once some tumour cells have appeared, the tumour growth passes through three different stages: *Avascular growth* This stage is characterized by proliferation of tumour cells. The tumour becomes a solid mass growing by mitosis, there is no invasion of healthy tissue and the tumour mass growth depends largely on the nutrients available. Once there are not enough nutrients, the tumour cells die (necrosis) and this creates a *necrotic core*. In this phase, the tumour tends to have a spherical shape where only cells on the outer perimeter continue to proliferate. The ones in the middle are in a rest state (quiescent) and in the centre of the tumour, a necrotic core appears, an accumulation of death cells. Necrosis and proliferation balance each other and then the tumour reaches a limit size (Diameter $\approx$ 1-3 mm).

Tumour-Induced Angiogenesis In this state, tumour cells from the avascular mass modify the existing *vascular structure* to create new vessels that would feed them. The tumour overcomes its limit size, grows much faster and invades the surrounding tissue. Tumour cells affect nearby blood vessels to steer new vessels towards them, creating new vascular structure.

Vascular growth /Invasive tumour This is the more complex stage. The tumour becomes diffusive and it is not a solid anymore. The nutrients they feed on is not only the one available in the perimeter. The formation of necrotic regions is much more complex, tumours do not have a limit size and they can grow indefinitely.

In this work, we focused on the modelling of the avascular stage of the tumour and the factors that provoke the initial growth. We build a simplified model that takes into account the key mechanisms determined by a literature review of previous models. We investigated how the parameters of the model affect the proportion of cancerous cells. Are there any key parameters that determine a transition from a majority of cancerous cells to normal ones? Before explaining in detail our model and our results, we mention previous works

on avascular tumour growth that have inspired the design of our model.

Previous work

The amount of models of tumour growth is immense, the vast diversity of models being due to the different scales and to the different questions one can pose about tumour growth. Byrne (Byrne, 2010) provides a timeline of the most representative models for each phase of tumour growth. Since in this work we focused on the avascular stage, we are only going to mention a few of the models made in this area. It is important to say that this summary of previous efforts is not trying to give an overview of past work but rather to highlight the parameters that have been identified to be important. An extensive review of models of avascular tumour growth is given by Roose et al. Roose et al. (2007).
Most of the models fall into two categories:

- **Continuum mathematical models** are expressed in partial differential equations and assume space averaging.
- **Discrete cell population models** consider a single cell-scale and cell-cell interactions.

Continuum Cell Populations Models usually describe the interaction between the cell number density and chemical species that provide nutrients. Typically these models consist of reaction-diffusion-convection equations.

The earliest spatio-temporal models of avascular tumour growth consider a tumour to be a three dimensional multicellular spheroid (MCS). Greenspan was the first to propose a biomechanical model of this kind Greenspan (1972). The growth of the tumour was considered to be regulated by a single diffusible chemical (oxygen or glucose) that was supplied externally. The distribution of the chemical predicted an underlying structure in the spheroid: regions of cell proliferation, quiescence and necrosis. However, there were also several simplifications that gave them little applicability: The spheroids were assumed to grow radially symmetric, to compromise of just a single population of cells and stochastic effects were ignored.

Several modifications and extensions were made to the model of Greenspan: relaxing the assumption of radially symmetric, distinguishing different cell populations within the spheroid, introducing cell movement and pressure. (Araujo and McElwain, 2004). One of the most representative models that extended the model of Greenspan, is the one due to Casciari, Sotirchos, and Sutherland (Casciari et al., 1992). This model considers a spherical tumour and the effects of some chemical substances (oxygen, glucose, lactate, carbon dioxide, and bicarbonate, chloride, and hydrogen ions) on the cell growth and on the metabolism of the cell. The basic principle is the fact that diffusion and nutrient consumption limit the growth. This model also takes into account changes in the rate of cell proliferation in different chemical environments and cell movement described by a law of mass conservation.

Continuum models share several features: they do not distinguish between individual cells, they see tumours as continuous masses, stochastic effects are usually neglected and subcellular phenomena is ignored. A good reference of continuum models and techniques to analyse treatments is (Perthame, 2014).

Discrete Cell Population Models There are several techniques to create discrete models: cellular automata, lattice Boltzmann methods, agent-based, extended Potts, and stochastic approach (Roose et al., 2007). All of these models characterizes the state of a cell to be determined by a vector variable $\mathbf{w} = \{x, v, u\}$, where x is the position of the cell, v is its velocity, and u is a vector characterizing the cells biological state, which may incorporate its position in the cell cycle, its interaction with the local biochemical environment, etc.

One of the first discrete models was the one of Duchting and Vogelsaenger Düchting and Vogelsaenger (1985) that consider a complex cell cycle model in a three dimensional model. Another important one is the one of Qi Qi et al. (1993) which uses cellular automata rules to reproduce Gompertz law of cancer growth. Kansal et al. developed a three-dimensional cellular automaton model Kansal et al. (2000) that doesn't include nutrients or mechanical interaction explicitly but rather set the proliferation and death rates to be known functions of position. A Cellular Potts model approach (Turner and Sherratt, 2002), where each biological cell is made up of several lattice points, has been used to take into account cell membrane tension, cell-cell and cell-matrix adhesion, chemotaxis, etc.

Recently a new kind of models have begun to develop which are the hybrid models, some of them consist of continuum equations for nutrients concentrations and are linked to cellular automata models of cell cycle and cell migration. A good reference for this type of models is (Trucu and Chaplain, 2014).

The model

We constructed a model for the initial stages of an avascular tumour. The underlying space where our model takes place is a tissue where nutrients are diffusing. In this tissue, we consider cells that can mutate to cancerous with a certain probability and move/proliferate/die according to the amount of nutrients in the underlying patch where they are located, the available space for their offspring and their internal energy, which is increased by the nutrients in the

patches they are located.

The underlying space for the dynamics of our model is a 2D grid, which is made of squared patches. This 2D grid represents the tissue where the cells interact. The cells are represented as circles and they are assigned a position, a level of energy, a status that can be either normal, normal mutated and cancerous. A patch can be occupied by one cell only. There are special patches that are coded in red and represent blood vessels. All these elements along with the setup and updating rules are explained in this section. It is important to mention that even when in our model, there are blood vessels, we are still simulating an avascular tumour since we are not assuming that the tumour is producing its own blood supply.

Actors and Variables

There are two actors in the model: the cells and the patches. The cells can be either normal, normal-mutated or cancerous and the patches can be either normal tissue or blood vessels. A mutated cell is a cell that follows the dynamics of a normal cell but its state is affected by its neighbours. At the beginning of the model, one sets the initial number of cells in the system (n) and the number of patches that are going to be blood vessels (b). Each cell i is assigned a vector of four values (x_i, e_i, s_i, a_i) which are its position, its level of energy, its state (normal, mutated or cancerous) and its available space for their offspring, respectively. The position can take values in any of the patches in the 2D grid, the level of energy is consider to be normalized ($0 \leq e_i \leq 1$) and the available space for the offspring is different if it is cancerous, normal or mutated. If it is cancerous, we consider it to be any available patch in the Moore neighbourhood, or "8 neighbours", and if it is normal or mutated we consider it to be any available patch in the von Neumann neighbourhood, "4 neighbours" (Fig. 1)

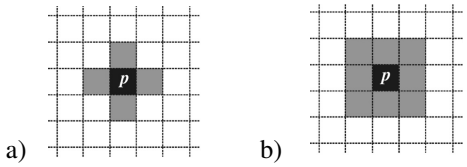

Figure 1: Sketch of the two possible neighbourhoods a) von Neumann neighbourhood, "4 neighbours" b) Moore neighbourhood, or "8 neighbours"

Each patch p_j has assigned a level of nutrients (n_j) and a state that can be either normal tissue or blood vessel. In the following rules we will only make a distinction if the is cancerous or not because mutated cells are consider to be normal cells whose state is affected by its neighbour states.

Parameter	Symbol
Initial number of cells	n
Number of blood vessels	b
Diffusion coefficient	d
Consumed nutrients	c
Energy to divide of normal cells	E_N
Energy to divide of cancerous cells	E_C
Prob for divide of normal cells	p_N
Prob for divide of cancerous cells	p_C
Prob for mutate from normal to cancerous	m
Effect of normal neighbours	ϵ_N
Effect of cancerous neighbours	ϵ_C

Table 1: Parameters of the model

Setup

1. First, we set the values of the parameters listed in Table 1
2. Each patch is assigned as its nutrients level a uniform random value in $[0, 1]$ and a green-scaled colour based on its nutrient value.
3. n cells are created and located randomly in the patches. All cells have "normal" status and are assigned an internal energy drawn uniform-randomly in $[0, 1]$.
4. m patches are selected uniform-randomly to be blood vessels and their colour is set to red.

Update Rule

In each step of the simulation, the update rule of the system can be divided in three types of updates: cell update, patches update and status update (Figure 2).

Cell Update

1. First, cells check if there are nutrients in the patch they occupy, if not; they die with probability $1 - e_i$. If there are nutrients, then they go to step 2.
2. Cells consume nutrients and at most the amount determined by c (i.e. $e_i = e_i + \min(c, n_j)$). The level of nutrients of the patch is decreased by the same value that the cell consumed (i.e. $n_j = n_j - \min(c, n_j)$).
3. Then, each cell is checked if it is cancerous or not. If it is cancerous then it checks if there is space for the offspring in its Moore neighbourhood and if it has enough energy to divide ($e_i > E_C$). There are four different scenarios for this:
   Space available in the 8 neighbours and enough nutrients: The cell divides with a probability $e_i * p_C$ and if it divides, it reduces its energy by the amount that was needed to divide, $e_i = e_i - E_C$. It splits this energy in half and stays with half of its value and gives the other half to its daughter.

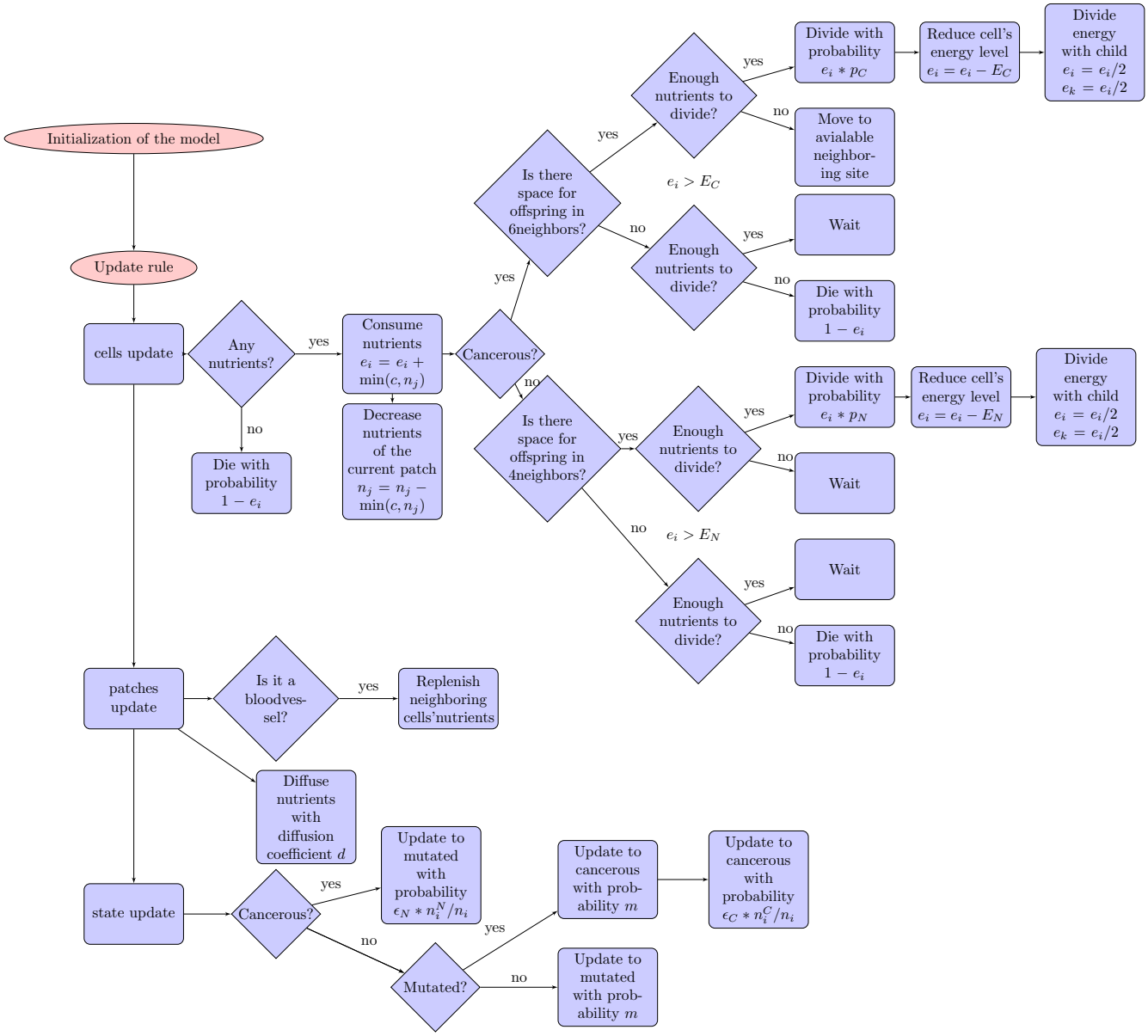

Figure 2: Flowchart of the initialization and update rule

Space available in the 8 neighbours and not enough nutrients: The cell moves randomly to one of the available 8 neighbours.
No space available in the 8 neighbours and enough nutrients: The cell waits.
No space available in the 8 neighbours and not enough nutrients: The cell dies with probability $1 - e_i$.
If the cell is not cancerous, then it checks again if there is space for the offspring in its 4 neighbours and if it has enough energy to divide ($e_i > E_N$) There are analogously four different scenarios for this:
Space available in the 4 neighbours and enough nutrients: The cell divides with a probability $e_i * p_N$ and if it divides, it reduces its energy by the amount that was needed to divide $e_i = e_i - E_N$.
Space available in the 4 neighbours and not enough nutrients: The cell waits.
No space available in the 4 neighbours and enough nutrients: The cell waits.
No space available in the 4 neighbours and not enough nutrients: The cell dies with probability $1 - e_i$.

Patches update
If the patch is a blood vessel, then it replenishes its 8 neighbouring patches. That is, if p_j is a blood vessel and Φ_j is the set of its 8 neighbours, then $n_l = 1$ for all $l \in \Phi_j$. For all patches it diffuses its level of nutrients with diffusion coefficient d.
Status update
Each cell checks if its state; if it is normal then with probability $\epsilon_N * \frac{\#\text{ ofNormal neighbour cells}}{\text{Total}\#\text{ of neighbouring cells}}$, it will turn into a mutated cell. If it is a mutated cell then it changes to cancerous with probability m. If it is cancerous cell it changes to mutated cell with probability $\epsilon_C * \frac{\#\text{Cancerous neighbour cells}}{\text{Total}\#\text{ of neighbouring cells}}$.

Comments on the rules and variables

The variables and rules were chosen to reflect in a realistic while also intuitive way the dynamics of tumour development. First, we chose to ignore the chemical details in the cell in order to avoid a robust model and instead just consider the key features that affect tumour growth.
In order to reflect the state of advantage that cancerous cells have over normal cells, we consider their probability to divide to be bigger than the one for normal cells ($p_C > p_N$), their energy needed to divide smaller than for normal cells ($E_C < E_N$) and the space for their offspring is any available patch in its 8 neighbours while for normal cells is just the 4 neighbours. Another advantageous behaviour cancer cells have in the model is that in the case in which there is space for offspring and no nutrients, cancerous cells move to an available patch in its 8 neighbours while normal cells just wait.

The introduction of mutated cells is to represent an intermediate state between normal and cancerous cells. Mutated cells that are carriers of the disease but still behave as normal cell. Also, experimentally it is possible that a cell is cancerous and then goes back to being normal. Since realistically these cells don't return to the original normal state, the mutated state are normal cells affected by their neighbours. These mutated cells represent the hypothesis of dormant avascular tumour (Udagawa et al., 2002).

Nutrients in the model are oxygen and other chemical substances that the cell needs to divide or move. Every time a cell consumes nutrients, it "eats" at the most the value α or what it can find in the patch. Nutrients diffuse in the tissue to give a realistic dynamic.

In the probability to divide, we include an energy factor: $e_i * p$ where $p = p_C$ or $p = p_N$ in order to give a greater probability of dividing to those cells that have more energy or to those cells that had enough energy for a while and were just waiting for space.

In the rules, we included the nutrient diffusion, we need to clarify that what we are referring as *diffusion coefficient* is a number $\gamma \in [0, 1]$ such that whenever the nutrients diffuses, then each patch gives equal shares of (γ * 100) percent of its nutrient value to its eight neighbouring patches. This number γ is not the diffusion coefficient in the typical sense, however in the classic sense what we have is a diffusion coefficient (in the physical sense) 1 for (γ * 100) percent of the nutrient and 0 for the other part.

It is important to notice that for all simulations that for a fixed random mutation probability (< 0.1), if the effect of normal neighbours is 1 and the effect of cancerous neighbours is 0, then in the end, there are only normal cells, whereas if the effect of normal neighbours is 1 and the effect of cancerous neighbours is 0, there are only cancerous cells. It means that a positive feedback for the proliferation of tumour cells is directly given by the effect of cancerous neighbours and a negative feedback is given by increasing the effect of normal neighbours.
A snapshot of a run of this model is shown in Figure 3.

Results

Measurements

In order to analyse the dynamics of the model, we measure the percentages of cancerous, clustered normal cells and clustered cancerous cells. A cancerous cell is said to be clustered if there are at least 4 other cancerous cells in its 8 neighbours and the same for normal cells.

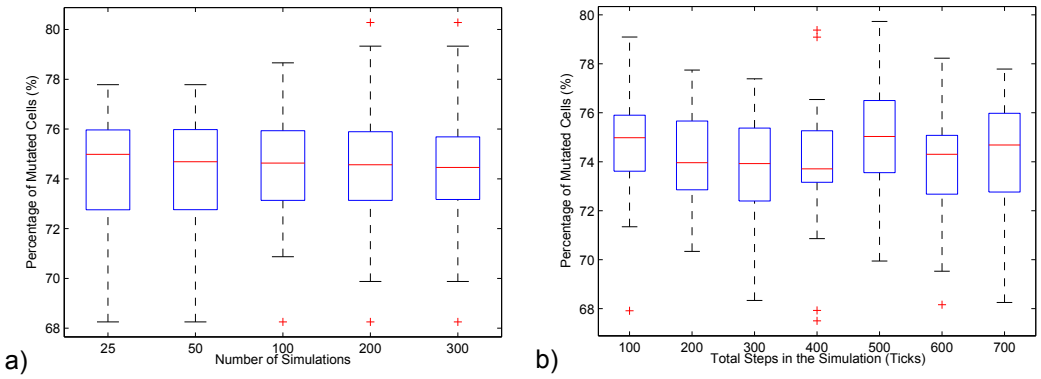

Figure 4: a) Boxplots of percentage of mutated tells considering different number of simulations each time and running 700 ticks. b) Boxplots of percentage of mutated tells considering different total steps and considering 100 simulations each time. All of these experiments were considering the parameters in Table 2.

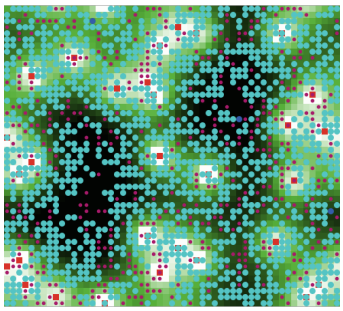

Figure 3: Snapshot of the model after 100 steps under the parameters values of Table 2.. Normal, mutated and cancerous cells are coloured in blue, cyan and magenta respectively. The patches are coloured in a green scaled manner, white being the patches with the highest value and black the lowest. The snapshot shows a majority of mutated cells.

Sensitivity analysis

Since the model is stochastic, first we need to determine how many simulations and how many steps we need to perform to be able to conduct a parameter exploration representative of the general situation. To perform this analysis, we consider the model with the parameters in Table 2. First, to determine how many simulation are sufficient, we run the simulation 700 steps and perform 300 simulations. Then, we derive the boxplot for 25, 50, 100, 200 and 300 simulations and the statistical information for the Mutated cells (4). Next, by performing 200 simulations we get similar boxplot. Now to determine the total number of steps in the simulations, we run 200 simulations varying the total steps from 100 to 700 (4. We observe that means do not vary that much, and adopt 500 time steps as our default value. For every set of parameters, the results were obtained with 100 simulations and 500 steps.

Parameters	Value
n	200
m	30
d	0.8
c	0.02
E_N	0.7
E_C	0.6
p_N	0.7
p_C	0.8
m	0.05
ϵ_N	0.8
ϵ_C	0.4

Table 2: Typical parameters values used in this model

Parameter exploration

For state space exploration we selected the following parameters, expected to be of particular importance in the outcome of the model:

- Effect of neighbours
- Probability of division
- Random mutation probability

Effect of neighbours We use the parameters of Table 2 except E_N and E_C. These two parameters were varied in the interval $[0, 1]$ with increments of 0.1. In Figure 5, the mean and variance of the percentage of mutated and cancerous cells are plotted. The mean value of normal cells in this parameter configuration is less than 3% so we don't include the respective graph.

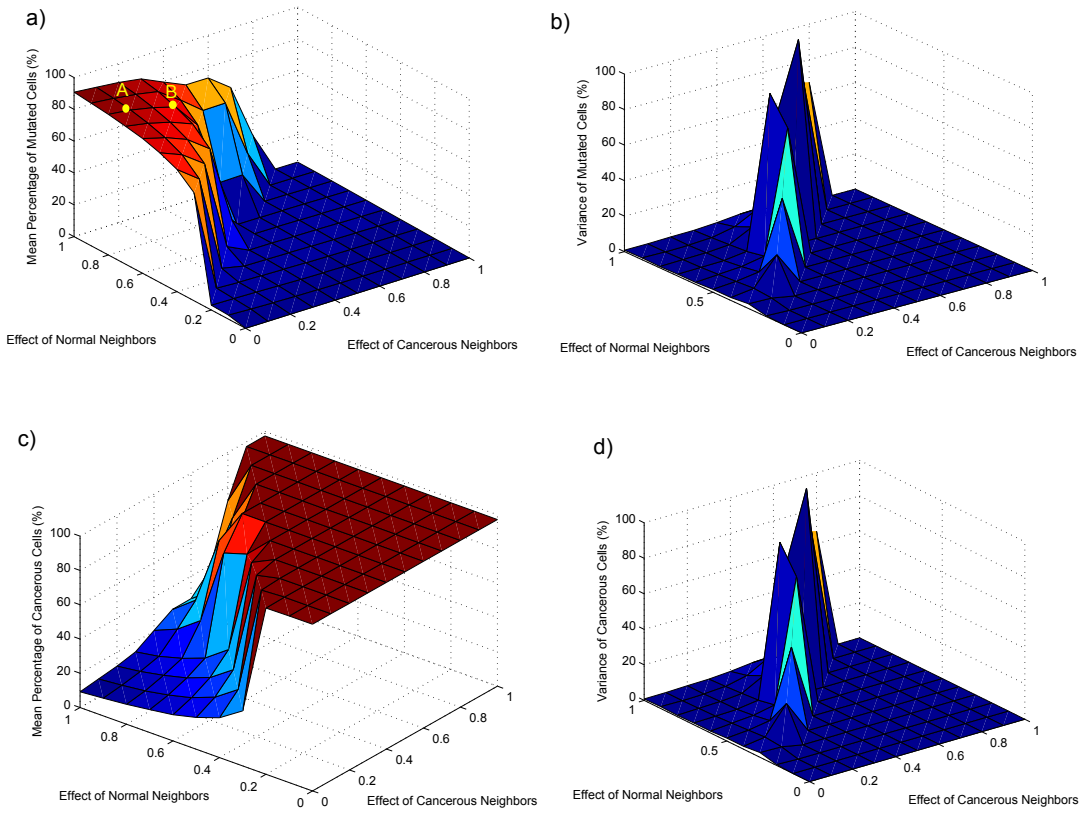

Figure 5: Statistical results of varying the effect of normal neighbours (E_N) and the effect of cancerous neighbours (E_C) and considering the parameters of Table 2 a) Mean percentage of mutated cells b)Variance of the percentage of mutated cells c) Mean percentage of cancerous cells d) Variance of the percentage of cancerous cells. The four graphs show a phase transition for a majority of mutated cells to cancerous cells. There is only a significant variance value where the phase transition happens. In a) it is also shown the location of points $A = (\epsilon_N, \epsilon_C) = (0.8, 0.1)$ and $B = (\epsilon_N, \epsilon_C) = (0.8, 0.4)$ which are of interest when we vary the probability to divide of normal cells.

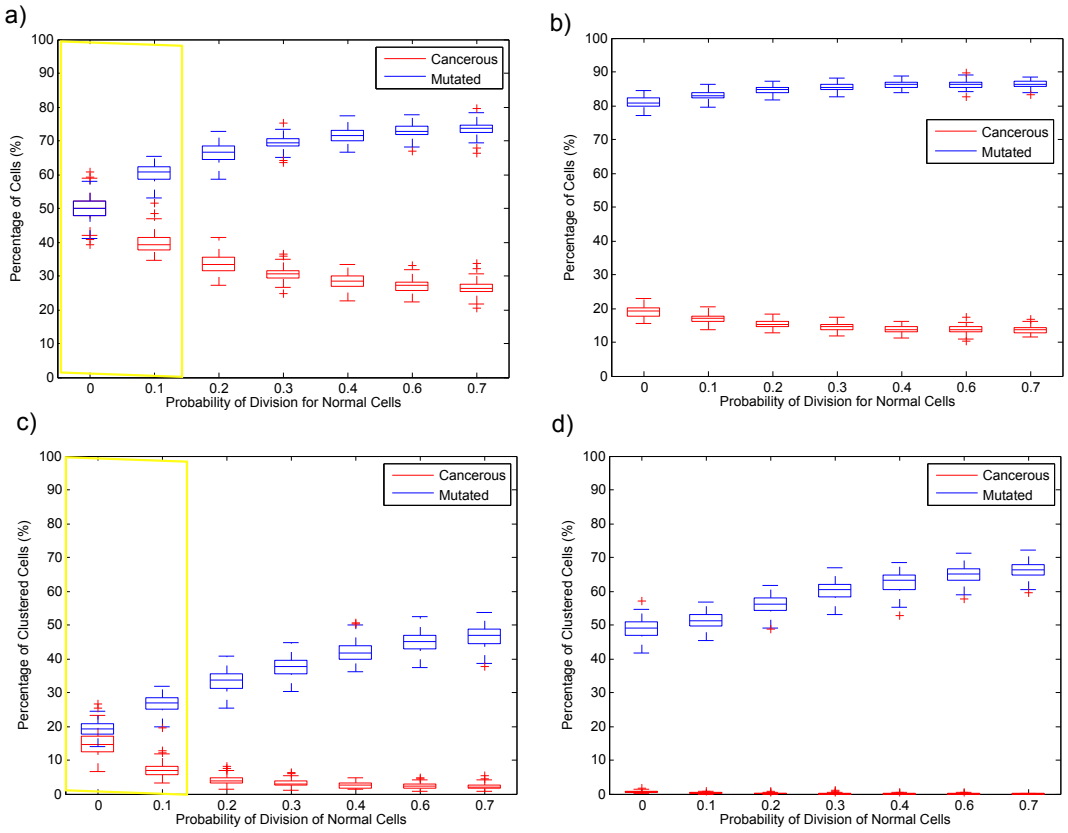

Figure 6: Statistical results of varying the division probability of normal cells (p_N) and fixing the division probability of cancerous cells to 0.8 for points in the effect of neighbour space: $A = (E_N, E_C) = (0.8, 0.1)$ and $B = (E_N, E_C) = (0.8, 0.4)$ a) Boxplot of Percentage of Mutated and Cancerous Cells in point B b) Boxplot of Percentage of Mutated and Cancerous Cells in point A c) Boxplot of Percentage of Clustered Mutated and Cancerous Cells in point B d) Boxplot of Percentage of Clustered Mutated and Cancerous Cells in point A. We can see that in point A, changing the value of p_N does not affect the dynamics. However in point B, lowering the value of p_N makes the percentage of mutated and cancerous cells comparable.

Probability of division We choose two points in the "effect of neighbours" parameter space and vary the probability of division of normal cells from 0 to 0.7 with a step of 0.1. The two points are $A = (E_N, E_C) = (0.8, 0.1)$ and $B = (E_N, E_C) = (0.8, 0.4)$ and they are shown in the surface plot of the percentage of cells considering the effect of neighbour (Figure 5 a)). The statistical results are shown in Figure 5.

Random mutation probability Under the same parameters as 1), we performed experiments varying the random mutation probability over three values $m = 0.05$, 0.15 and 025. It is important to notice that to explore the effect of neighbours we set $m = 0.05$. The statistical results of these experiments are found in Figure 7

Discussion

We have presented a simple agent-based model of tumour growth which is able to give rise to two different situations: one where the majority of cells is cancerous and other one where it is mutated. We have shown the outcomes of varying the effects of neighbours, probability of division and mutation probability. One key characteristic is that normal cells disappear and only mutated and cancerous cells remain in the model. The biological conclusion is that in the end, all normal cells become influenced by the state of their neighbours. We were expecting for more normal cells to survive however the rules of the agent based model gives an advantage to cancerous cells in all dimensions (more space for the offspring, less energy to divide, smaller probability of dying) which leads to the depletion of normal cells.

The exploration of the effect of neighbours, as we can see in Figure 5 reveals a phase transition in the two dimensional parameter space. The variance is larger than 5% only when the simulations are performed close to the transition. This creates two types of points in the model: ones far away from the phase transition, which are stable, and ones close to the phase transition which are unstable.

By varying the probability of division in two different points A and B, we can see that in the case of a small probability of dividing for normal cells, then the percentage of mutated cells significantly decreases and is comparable to the amount of cancerous cells in the model (Figure 6, yellow square). This can be explained by the fact that B is a point close to the phase transition while A is further away. While varying the probability of division is unrealistic, one can think of this analysis as studying the different ratios of division of cancerous and mutated cells. The final variable that we consider to vary was the random mutation probability, we wanted to see if above a certain value for the random mutation probability, we always have cancerous cells. We have seen that at least for the parameters of Table 2, we indeed have the case that for $m = 0.25$, there remains only cancerous cells. It will be important as well to consider other points in the parameter space as well.
By varying these parameters, we researched both the internal and external factors for tumour growth. However, another interesting parameter to vary will be the diffusion coefficient to see that similarly to the earliest models of avascular tumour growth we have a growth limited by diffusion. In this model we tried to describe the dynamics of initial tumour growth using only key principles and concepts without taking into account the chemical and mechanical point of view. A natural extension of this model would consider both aspects as well as the cell cycle. However as simplified as this model may be, it still showed typical behaviour of avascular tumour growth: tumour proliferation in rich nutrient region, a tumour limit size and phase transition in the parameters.

Acknowledgements

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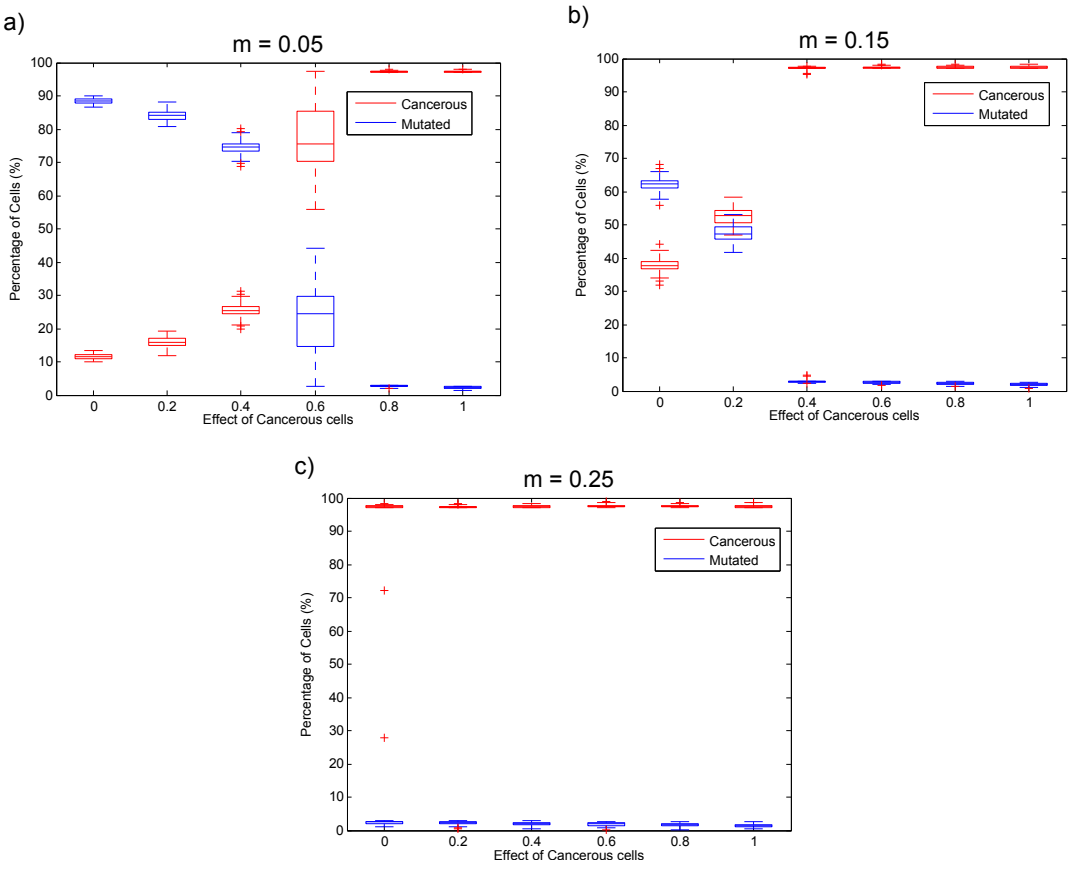

Figure 7: Statistical results of varying the mutation probability (m) for different values of effect of cancerous cells and keeping all the other parameters of Table 2 a) $m = 0.05$, b) $m = 0.15$ and c) $m = 0.25$ In a) we can see the behaviour of Figure 5 a) where there is a transition from a majority of mutated cells to a majority of cancerous cells by increasing the ϵ_C. In b) we can see that the transition happens in lower values of ϵ_C while in c) we can see that the m is so big that there is no transition.

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