In Silico Growth of Cancer Cells & Tumour Angiogensis

COMP90083 Assignment 2 Report (Group 18)
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Introduction

Question

How important is successful angiogenesis in the survival and growth of cancer?

Cancer is one of the leading causes of mortality and morbidity worldwide [1]. This is largely attributed to the ability of cancer to arise through many different mutations, the aggressiveness of its spread and its resistance to chemoradiotherapy treatment. To better understand the characteristics of cancer cells, Hanahan and Weinberg [2] postulated six key hallmarks of cancer cells that provide them with their unique ability to proliferate and survive. These six (phenotypic) hallmarks are: sustained angiogenesis, evading apoptosis, sustaining growth signalling, resistance to anti-growth signals, tissue invasion and limitless replicative potential; they are believed to be common amongst almost all malignancies. These hallmarks have since been expanded to include even more phenotypic traits that have been recognised as aiding the survival of cancer cells [3]. This holistic approach to understanding cancer cells greatly differs from conventional molecular biology studies which typically focused on single genotypes causing cancer, instead of targeting the cancer phenotype as a whole. The benefits of such an approach are twofold. First, they offer an insight into the collective cellular mechanics that cause disease and how they could be exploited to control tumours and prevent metastatic spread. Second, they allow for the incorporation of big-data, which is quickly becoming the gold-standard for real-world analyses. Some of the most popular computational models are differential equation-based models (EBMs) [4]. However, with EBMs, it is virtually impossible to capture the heterogeneity and complexity inherent in cancer cells and their disease dynamics. To this end, agent-based models (ABMs) are an increasingly popular alternative that are able to capture the emergent properties of the tumour/normal cell microenvironment [5], including that of the targeted chemotherapies. Further, ABMs are able to easily capture the multi-scale nature of biological systems, where crucial events on the molecular and cellular scales leads to large-scale tissue and phenotypic outcomes. These events are modelled as agents interacting with other agents and the environment to effectively capture these simultaneous events as models of cancer growth and metastasis progress.

Among the aforementioned cancer hallmarks, sustained angiogensis signalling is of particular interest. Angiogenesis is the growth of new blood vessels and, as well as being a cancer hallmark, is incredibly important to tumour formation (Figure 1). Angiogenesis provides an increased blood supply to the tumour which facilitates ample sugar and oxygen (nutrient) cycling. Further, angiogenesis brings the tumour bulk closer to the circulatory system and thus increases the chance and propensity for tumours to metastasize and spread throughout the body [6]. To facilitate angiogenesis, cancer cells release a growth hormone, VEGF (vascular endothelial growth factor), that binds to the receptors of artery vessel cells to promote cell division and vascular truncation. Following metastasis, the mortality rate for cancer drastically increases as it becomes more difficult to treat and disease recurrence is much more likely [7]. As a result, understanding the processes and dynamics of cancer angiogenesis is crucial to improved cancer outcomes and particularly for designing efficacious, targeted and safe chemotherapies for cancer angiogenesis.

Previous research efforts have used ABMs to capture the hallmarks of cancer [9], as well as more recent efforts honing in on specific hallmarks such as tumour angiogenesis using three-dimensional

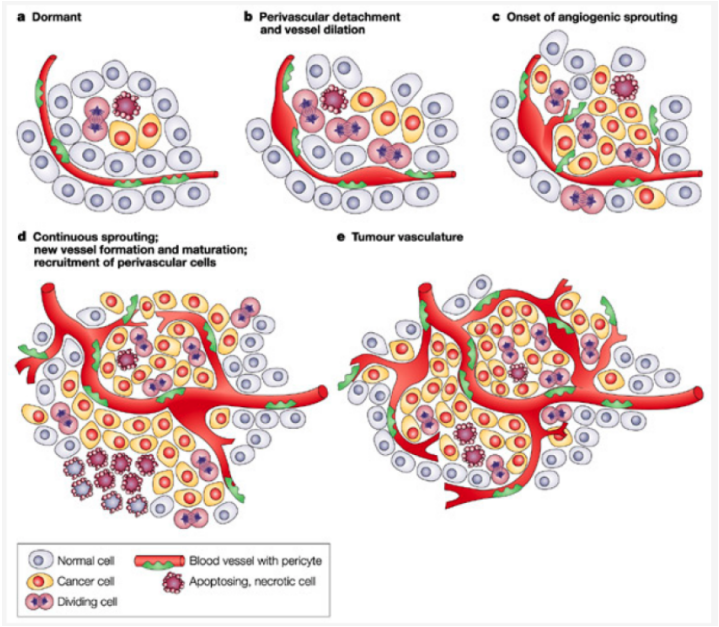

Figure 1: Schematic diagram of angiogenesis promotion and sustained growth in a cancer microenvironment (a-e). Cancer cells stimulate the underlying vasculature to proliferate and truncate towards the growing tumour bulk. Image from: Loizzi et al. [8]

ABMs that focused on model simplifications to the existing EBMs of angiogenesis whilst still capturing the innate behaviours and heterogeneities of cancer cells and the interactions with their environment [10]. Olsen and Siegelmann [10] concluded that despite their model abstractions and simplifications, their model needs to be expanded to better capture the discrete processes driving tumour angiogenesis. Three-dimensional models of *in silico* cancer growth and tumour angiogenesis are incredibly important research tools for understanding cancer growth and disease dynamics for several reasons. First, they provide the closest recreation of the actual 3D tissue environment in a model. Second, they simulate dynamics and processes of cancer growth and angiogensis that can neither be captured *in vitro* nor in human studies. Finally, 3D models are inherently visual models that are easy to understand and more compelling than than 2D and many mathematical models. This research project will work to expand on the work of Olsen and Siegelmann [10] by further simplifying the process of tumour angiogenesis and hormone cycling in an ABM. We propose two key simplifications to current ABM models of tumour angiogenesis:

1. Angiogenesis can only occur in a contiguous manner. That is, new vasculature can only be induced in areas with surrounding artery cells and proliferate in a restricted neighbourhood (to simulate the truncation of artery vessels during angiogenesis).

2. VEGF release and cycling is modelled implicitly as an attribute of cancer cells, instead of releasing a VEGF agent and explicitly modelling VEGF diffusion and concentration. Angiogenesis is initiated at an artery if a threshold of viable cancer cells are close enough to that artery.

To this end, our research hopes to answer the question:

> How important is successful angiogenesis in the survival and growth of cancer?

We hypothesise that:

I angiogenesis is crucial to the growth of tumour bulks and that without sufficient angiogenesis, cancer cells will struggle to maintain their high demand for nutrient and competition for nutrient with somatic cells, even with the ability to become quiescent for a time and evade apoptosis.

II these two key model simplifications will improve upon the ABM proposed by Olsen and Siegelmann [10], furthering our understanding of cell and tissue dynamics and the discrete processes involved in tumour angiogenesis.

Model design

Overview

Purpose

To expand upon the previous work of Abbott, Forrest, and Pienta [9] who used ABMs to model the hallmarks of cancer and in particular the work of Olsen and Siegelmann [10] who modelled angiogenesis and cancer growth with multi-scale ABMs. This model simulates the growth of a nascent solid tissue tumour, how it interacts with the local tissue (somatic cells and artery vasculature) and how the tumour responds to angiogenesis. Further, the purpose of this model is to provide an insight into the discrete steps that can lead to angiogenesis, whether or not it is always necessary for tumour growth and survival and finally some of the possible emergent behaviours of tumour cells as they interact with somatic cells and tissue vasculature in a complex tumour/soma environment.

Entities, State Variables & Scales

Agents are *Tumour (cancer) & Somatic (normal) Cells.*[1] The cell agents have the following state variables:

1. Malignancy, a boolean determiner. If **True**, agents are cancer cells and will have distinct agent behaviours from normal cells.
2. Age, measured in days (i.e. ticks spent alive).
3. Quiescence, the number of days spent in a quiescent state. Only cancer agents can have a nonzero value for quiescence. This is a stem cell-like property of cancer cells and enables cancer cells to evade apoptosis.
4. Proliferation chance (i.e. replicative potential), ranges between 0% and 100%. Cancer cells move through the cell cycle faster and thus have a higher proliferation chance.
5. Energy, a measure of how much nutrient (oxygen and sugar) the cells have absorbed from the ECM. Energy is crucial for the proliferation of all cell agents.
6. Local artery density, the number of artery patches in a daughter cell's radius. Used to quantify the effect of a local neighbourhood of vasculature on cell proliferation rates.
7. Low energy duration, the number of days a cell has spent in a low-energy, apoptosis inducing, stress state.

Environment A three-dimensional cube of 31 x 31 x 31 patches represents the local solid tissue of interest (ECM, somatic and cancerous cells and arteries, Figure 1). The patches of this grid represent the ECM and local vasculature of the solid tissue. Cell agents can proliferate into the ECM patches, but not over the artery patches.[2]. Patches have two variables: the nutrient level on a patch and whether it is an artery or not. Artery patches exclusively replenish their nutrient level as they are part of the systemic vasculature of cycling nutrients. Nutrient is diffused across a *concentration gradient* in the environment.

Scale

- Temporal: The time scale is discrete; each tick represents a day of time.
- Spatial: The spatial scale is continuous and not spatially explicit.

[1] NB: Tumour and Somatic cells (due to their similar state variables) are collectively referred to as "cell agents".

[2] If an ECM patch is at a threshold value of tumour VEGF then it will become a vascular patch and tumour agents can no longer proliferate into that patch

Process Overview and Scheduling

As the model progresses, the patches (ECM and arteries) and the cell agents change as they interact with one another. First, the *normal cell agents* uptake nutrient, proliferate, age and die in an equilibrium until they have to compete for space and resources with the tumour cell agents in the ECM. The *tumour cell agents* uptake nutrient, proliferate faster, age and die as well as promote angiogenesis. Further, in the absence of adequate nutrient, cancer cells can become quiescent to evade apoptosis and survive for longer until nearby angiogenesis increases the diffusion of nutrients into the ECM. Cancer cells continue to proliferate, promote angiogenesis and compete with the normal cell agents for nutrient and normal cell agents and artery patches for space. This control flow is given by Algorithm 1.

Algorithm 1 Scheduling

Initialise (environment, agents)
$t \leftarrow 0$ {set clock}
while t $\leq$ 300 **and not** cell count = 0 **do**
  $Diffuse$(nutrient), $Replenish$(artery patches)
  for all artery patches **do**
    if VEGF threshhold met **then**
      $Angiogenesis$(patch)
    end if
  end for
  for all cells **do**
    $BecomeQuiescent$(cell)
    Die(cell)
    $Proliferate$(cell)
    $AbsorbNutrient$(cell)
  end for
  $t \leftarrow t + 1$
end while

Design Concepts

The following outlines how each concept is either relevant or endemic to the model and under what circumstances they might arise.

Basic Principles

1. The Hallmarks model of cancer [3] (see Introduction). Some of the key design principles of the cancer cells, how they behave and what differentiates them from normal cells are based upon these hallmarks.

2. Stem cell theory of cancer [11]. This model captures a feature of the cancer stem cell theory, namely, that some cancer cells display stem cell-like properties. These cells will have some of the features of stem cells that will protect them from apoptosis and reduce their reliance on nutrient to survive; they can become quiescent.

3. The cell cycle. The main phases of the cell cycle have been modelled, to add realistic cell growth and division behaviours to the cell agents. All cells implicitly a undergo growth (G1) phase by absorbing nutrient and a cell division phase (M or Mitosis). Cancer cells undergo a different cell cycle than normal cells, in that they enter and exit G1 and M phases faster and have a chance to exit into the G0 phase (and become quiescent).

4. Competition for nutrients/space in the tumour bulk.

5. Nutrient requirements of cells and their diffusion from the artery to the extracellular matrix

6. Cancer cells proliferate according to the tumour spheroid model. This is generally expected to occur regardless any emergent angiogenesis at the tumour/soma boundary [12].

∗ Many of the above will interact with one another. 1. will interact with 5. as the tumour cells release growth hormones to promote sustained angiogenesis and the increase of nutrients to the tumour bulk. It is also expected that 3. and 4. will interact as cancer cells will be able to move through the cell cycle much faster and thus will likely out-compete the normal cells for nutrients.

Emergence

- Cell population dynamics. E.g. Cell population collapse (large scale apoptosis) and exponential tumour growth. Depending upon the proliferation rate, acquired mutations and angiogenesis we may see several different population dynamics. Further, this emergent behaviour could be influenced by the quiescent cancer cells, which may be able to re-initiate tumorigenesis after a cell population collapse.

- Angiogenesis-independent vs -dependent tumour survival and growth. The survival of the tumour bulk will depend upon the ability of tumour cells to promote and sustain angiogenesis. Cancer cells may also evade apoptosis for a time (e.g. the ability to become quiescent), which will allow them to survive absent a sufficient vasculature.

- Stable tumour sub-population formation. We may see different populations of tumours forming from tumour cells that have been able to successfully initiate angiogenesis at different sites.

Adaptation

- Cells adapt to properties of their environment, e.g. nutrient levels, neighbouring cells and emergent angiogenesis. That is, under certain starvation conditions, cancer cells may undergo a transformation to a quiescent state, to prolong their life until more favourable conditions arise.

- The vasculature adapts to the presence of cancer cells: if more than `angiogenesis-min-demand` cancer cells are present within a radius of `angiogenesis-signal-radius` patches around existing vasculature, then angiogenesis triggers, causing a new artery patch to sprout, increasing the supply of nutrients to the tumour bulk.

Objectives

The adaptive traits above may help the cancer cells to achieve the following objectives:

- Successful proliferation into stable and/or growing tumour populations.

- Successful stimulation of angiogenesis.

- Changing the ratio of normal to cancer cells in favour of cancer cells. That is, out-competing normal cells for nutrients and space in the environment (tissue).

Interaction

Cancer cells directly interact with the vasculature to promote angiogenesis. Indirect interaction is also present in our model:

- All cells compete for nutrients that are diffusing across the patches of the environment.

- Vasculature (including via angiogenesis) prevents the proliferation of new cells on that patch.

- All cells compete with one another for space.

Stochasticity

Stochasticity determines (or features in) several modalities of this cancer simulation model. Because this model simulates a biological system and in particular cancer, stochasticity is used to model the inherent variability of these systems. Some of the key features that have stochastic elements are:

- Cell proliferation rate and location. All cells proliferate and move to a random neighbour patch (if there is space). Cancer cells proliferate with a higher likelihood
- Cell death. Cells die once they reach a certain age.
- Locations of angiogenesis. Given that cancer cell proliferation is in a random patch in the parent cell neighbourhood, this means that the locations of angiogenesis can change according to the movement of the developing tumour bulk.

Collectives

Collectively, the cancer cells (including any surviving normal cells) may form one or more tumours. These tumours will likely grow until their space it limited by the emergent vasculature and cell population. Further, if angiogenesis successfully occurs, then the vasculature will become part of the tumour collective.

Observation

Several variables and model parameters (and their emergent dynamics) are collected across simulation runs. The purposes of their collection are for: testing the hypotheses of our research question, for justifying our conclusions with statistical rigor and for analysing the robustness of our model.

- Whether stable tumour subpopulations develop? How many are there? What is their size and ratio of normal to cancer cells.
- How angiogenesis affects the the growth of cancer cells/tumours. Is it always required? Does it always occur? How many patches undergo angiogenesis before the tumour bulk runs out of space?

Details

Initialisation

At initialisation the arteriolar structure is initialised by creating a series of connected artery segments tracing a cubic honeycomb (Figure 2). The rest of the patches are ECM patches where the initial cells can spawn. The `initial-cells` parameter specifies how many ECM patches are initially occupied by somatic cells and tumour cells; the `disease-ratio` controls the initial proportion of tumour cells. The state variables of each cell are initialised with the values outlined in Table 1.

`age`	uniform at random between 1 and `min-age-apoptosis`
`energy`	uniform at random between 3 and 8
`malignant`	true with probability `disease-ratio`
`proliferation-chance`	equal to `malignant-proliferation-chance` if `malignant`, `normal-proliferation-chance` otherwise

Table 1: Initial state variables for cell agents

Input Data

No input data is required for this model; however, this model could be expanded to incorporate real spatial data for generating the patches of the tumour/soma environment.

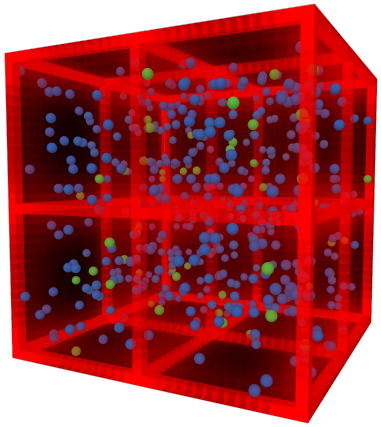

Figure 2: ECM at initialisation, including arteries (red), nutrient levels (semi-transparent red), somatic cells (blue) and cancer cells (green)

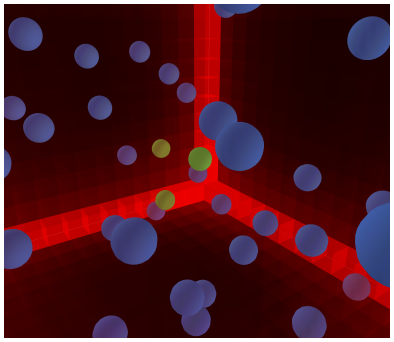

Figure 3: Close-up view showing cancer cells in close proximity to the existing vasculature at the beginning of the simulation

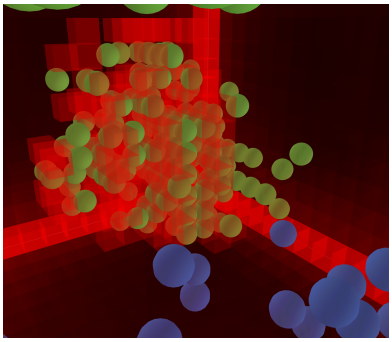

Figure 4: Close-up view of emergent neovasculature, which has evolved in symbiosis with proliferating cancer cells

Submodels

Proliferate Only cells with at least `energy-req-prolif` are eligible to proliferate. If there are no empty adjacent patches, the cell does not proliferate. With probability `proliferation-chance`, a new cell is created on an empty adjacent patch. The cell's `age` is initialised to zero; its initial energy is provided by absorbing 50% of the energy of the parent cell. All other state variables, including whether it is `malignant`, are inherited from its parent cell.

Die There are two conditions that could trigger cell death (i.e. undergo apoptosis):

1. Old age: cells that have been alive for longer than `min-age-apoptosis` have a 50% chance of dying.
2. Low energy: Cells that have low energy, are not quiescent and have been in this state for longer than the `max-low-energy-duration` always die.

Angiogenesis At each tick, ECM patches that meet the following criteria undergo angiogenesis:

- The patch is not already vascular.
- At least one of the six directly adjacent patches is vascular
- At most three of the 26 neighboring patches are vascular
- There are at least `angiogenesis-min-demand` cancer cells within `angiogenesis-signal-radius`

- New vascular patches have their nutrient level set to the mean nutrient level of the neighboring vasculature.

Methods

Approach

We designed our model using a pattern-oriented approach described by Grimm and Railsback [13]. Guided by the modelling cycle, we first began by using NetLogo to create a 3D model of the artery/tumour environment; shown in Figure 2. Before formulating our research question and hypotheses, we created this baseline model by adding basic functionalities such as the cell cycle (growth

in the form of nutrient uptake, proliferation and death) and creating a vascular network. Given that the interaction for nutrients and space is competitive, we scheduled our model updating in a discrete, asynchronous manner. After a baseline model was created we developed our research question and subsequent hypotheses I and II. As well as a null theory that angiogenesis is not essential for tumour survival and formation. We implemented the *Angiogenesis* submodel and updated any associated agent interactions in order to address our research question.

Given the incredibly complicated dynamics and heterogeneities associated with modelling biological systems we decided to embrace the reflections of Abbott, Forrest, and Pienta [9] and Olsen and Siegelmann [10] and further simplify cell agent behaviours. Importantly, we decided not to explicitly program all of the hallmarks of cancer cells. Instead we considered what essential patterns of cancer cells (cancer hallmarks and cell behaviours) were required in order to make the model *structurally realistic* and have sufficient scope to address our research question. (These cell agent behaviours and design principles are described in detail in the *Model design* section). Over several simulation runs we observed the emergence of patterns of neovasculatures and tumour spheroids forming at the site of angiogenesis. To further explore the effect of angiogenesis on cancer cells and the tumour environment we devised a series of experiments (discussed below) to properly calibrate the model parameters, statistically test the sensitivity and uncertainty inherent to our model and quantify the extent to which we can assert or refute our hypotheses and answer our research question.

Technical Implementation

In order to test our hypotheses, we implemented our ABM of cancer using NetLogo 3D [14]. We chose to use a three-dimensional representation in order to achieve structural realism; real cancer does not occur on a two-dimensional grid, but in a complex three-dimensional tumour microenvironment. Further, many conventional *in vitro* models of cancer development are unable to grow tumour spheroids and so cannot test the 3D dynamics at plays as a tumour spheroid grows. Given that the gold standard *in vitro* model of cancer is a 3D model [15], we felt it was also important, for the relevance and the usefulness of our research to the scientific community, that we pursued a 3D ABM environment. Finally, to be certain of our choice to model in a 3D environment, we also implemented baseline models of tumour angiogenesis in NetLogo (2D) as well as a 2D Mesa model, an ABM package for Python [16]. In both cases, we found that these models made it more difficult to capture realistic angiogenesis and tumour patterns of growth and interaction.

Many of the values used in the model are either fixed, or stochastic. These values are based upon the scientific literature and the current best approximates of their real values. For instance, cells with low nutrient levels undergoing hypoxic stress conditions have a 50% chance of dying via apoptosis every day. Finally, to calibrate our model parameters and test our hypotheses we used NetLogo's BehaviourSpace feature to conduct our experiments and exported the resultant observations in comma-separated value (CSV) format. We explored the parameter ranges given in Table 2 and analysed the results using R [17]. We used Jupyter [18], pandas [19], Matplotlib [20] and Seaborn [21] to generate plots.

Experimental Design

Our timing and model updating was guided by the biological literature. Since almost all nascent, malignant tumours promote angiogenesis within the space of less than a year, we decided there was no need to run the simulation for longer than 300 days [6]. That is, a realistic ABM simulation of tumour angiogenesis should have angiogenesis occurring within that time frame, or not at all.
In order to explore our research question and address our hypotheses, we designed three sets of experiments:

1. Our *timeseries* experiments were intended to discover emergent model dynamics over time by capturing model outputs at every step. We varied the parameters `angiogenesis-signal-radius` and `angiogenesis-min-demand`, held all other parameters fixed and measured the number of

malignant cells, number of somatic cells and number of vascular patches at every time step. We expected the number of somatic cells to remain relatively stable throughout each simulation run; in contrast, we anticipated that malignant cells would display an exponential growth pattern, and that their number would correlate with the number of vascular patches. We also expected higher levels of `angiogenesis-signal-radius` and lower levels of `angiogenesis-min-demand` to result in an accelerated exponential growth pattern in the number of vascular patches.

2. The purpose of our *variation A* experiments was to measure model sensitivity with respect to the angiogenesis-relevant parameters. We varied parameters `angiogenesis-signal-radius`, `angiogenesis-min-demand`, and the number of initial cells; we held all other parameters fixed, and measured the number of times a cell entered the quiescent state, the number of somatic and malignant cells, and the number of vascular patches.

3. On the other hand, our *variation B* experiments served to measure model sensitivity with respect to the cell proliferation parameters. We varied parameters `normal-proliferation-chance`, `malignant-proliferation-chance` and `energy-req-prolif`; we held all other parameters fixed, and measured the same outputs as for *variation A*.

To analyse the effects of angiogenesis on tumour growth with statistical significance we used R to perform a multi-way ANOVA on the mean differences on the *timeseries* data. We then performed Tukey's Honestly Significant Difference test on the categorical variables of interest, `angiogenesis-signal-radius` and `angiogenesis-min-demand` to analyse the pairwise statistical significance of these variables on the final number of malignant cells. This test performed one-way and pairwise comparisons with significance of every combination of `angiogenesis-signal-radius` and `angiogenesis-min-demand` values in the time series runs. (Note, given the number of simulation runs that were performed, the assumption of normality in the ANOVA and Tukey's HSD and reliance on the F-distribution is reasonable).

Parameter	**Values**		
	timeseries	variation A	variation B
`quiescent-duration`	15 days		
`angiogenesis-signal-radius`	1–4 patches	2 and 4 patches	3 patches
`initial-cells`	500 cells	250 and 500 cells	500 cells
`max-low-energy-duration`	10 days		
`malignant-proliferation-chance`	2.25%	2.25%	2.15% and 2.35%
`angiogenesis-min-demand`	1-5 cells	1, 3, and 5 cells	3 cells
`energy-req-prolif`	2		1 and 3
`normal-proliferation-chance`	2%		1.9% and 2.1%
`disease-ratio`	2%		
number of runs per combination	100	50	30

Table 2: Parameter ranges. We conducted three groups of experiments in order to qualitatively and quantitatively evaluate the behaviour of our model. We configured the *timeseries* experiments to report model outputs at every time step in order to capture model dynamics over time. We configured the remaining experiments to report model outputs at the end of each run. *variation A* was used to investigate model sensitivity to the angiogenesis-related parameters and number of initial cells, whereas *variation B* was used to investigate model sensitivity with respect to cell proliferation parameters.

Results

The results described herein are a series of violin plots, time-series graphs and statistical tests that are generated according to the experimental design (see Methods) discussed above, using the parameter calibrations given in 2. Their significance and the conclusions made upon hypotheses I and II are

given in the Discussion section.

After performing statistical tests of the data shown in Figure 5, the results of the Tukey's HSD test are as follows:

- When evaluating the mean difference in malignant cell numbers at the end of simulation, on all occasions, altering the level of `angiogenesis-signal-radius` resulted in a statistically significant difference of means (p-value < 0.05).
- The same test altering the level of `angiogenesis-min-demand` resulted in all but statistically significant difference of means in all but two tests: `angiogenesis-min-demand` = 2, 3 and `angiogenesis-min-demand` = 2, 4.
- Pairwise tests of the affect of `angiogenesis-signal-radius` and `angiogenesis-min-demand` on malignant cell numbers produced statistically significant difference of means in $160/190 \approx 84\%$ of tests.

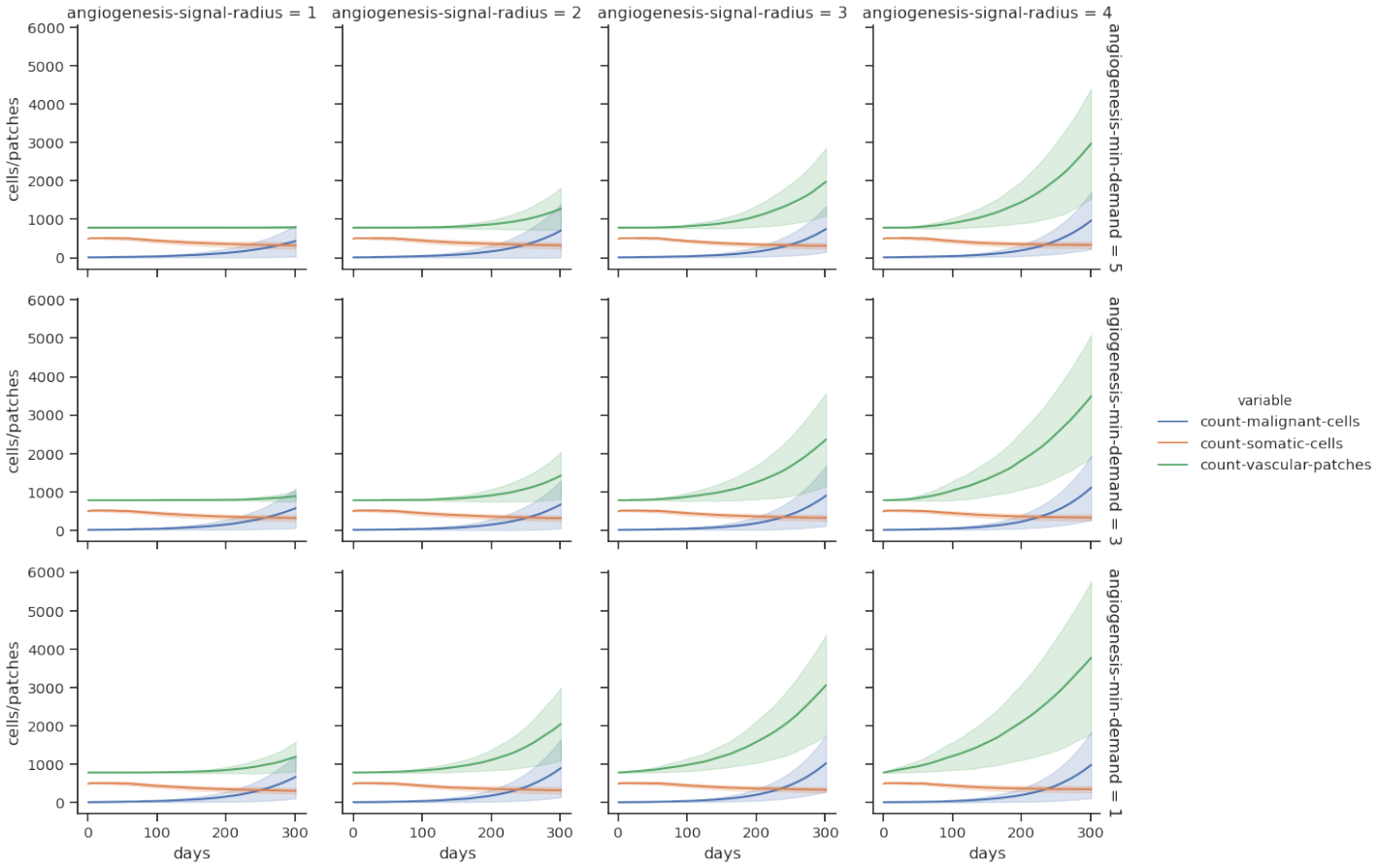

Figure 5: The evolution of cancer cells, somatic cells and vascular patches over time. Lines indicate the mean and shaded areas the standard deviation of each variable over 100 repeated model runs. The number of somatic cells remains near constant with a slight linear decline throughout all scenarios. In contrast, the number of vascular patches increases exponentially during each simulation run, with higher levels of the `angiogenesis-signal-radius` parameter resulting in an increased rate of vasculature growth. The number of cancer cells also follows an exponential growth curve. Higher levels of the `angiogenesis-min-demand` parameter correspond with a reduced standard deviation in model outputs. The green and blue curves follow the same trend, which demonstrates a definitive positive correlation between angiogenesis and tumorigenesis.

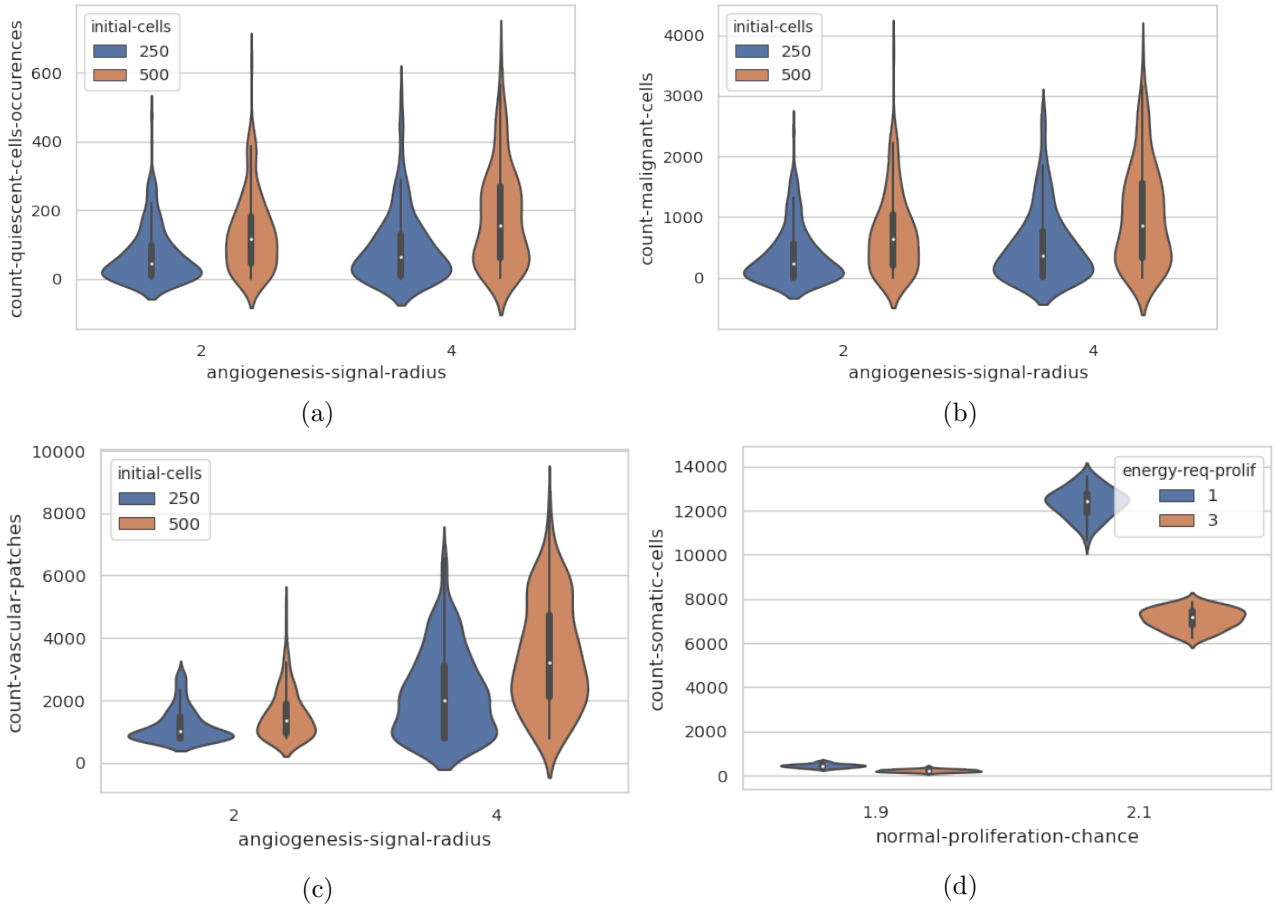

Figure 6: Violin plots of cell and patch populations over varying angiogenesis signal radii (a-c). Plots are generated from experiment *variation A*, using 50 simulation runs to measure the *sensitivity* of the model. (a) Malignant cells entering quiescent states (a), the total number of malignant cells (b) and the total number of vascular patches (c) as the number of cells and the signal for angiogenesis is varied. (d) is generated from experiment *variation B* to measure model *sensitivity* with respect to cell proliferation parameters. Small changes in `normal-proliferation-chance` result in large changes in the final number of somatic cells, moderated somewhat by the minimum energy required to proliferate.

Discussion

We were able to successfully simulate the interplay of angiogenesis and tumorigenesis in a three-dimensional tumour microenvironment. Our results show the significance of certain factors that govern the survival and growth of cancer cells, providing an answer to our research question.

Interpretation of Results

How important is successful angiogenesis in the survival and growth of cancer? Our results show that in the context of our model, angiogenesis plays a vital role in the survival and growth of cancer: When angiogenesis is inhibited by reducing the `angiogenesis-signal-radius` and/or increasing the `angiogenesis-min-demand`, the growth rate in the number of malignant cells is markedly reduced (see figure 5). Further, the output of Tukey's HSD provide preliminary results that the `angiogenesis-signal-radius` is the more important factor of the two in determining the extent of angiogenesis and subsequent tumour growth. This result may indicate that the ability of VEGF signal to permeate through the tumour bulk and the ECM is a key factor in the growth of new tumour vasculature. This observation carries therapeutic implications, demonstrating the potential of therapies that target angiogenesis signalling to slow down and reduce the rate of cancer growth. Thus, in

answering our research question, angiogenesis crucially depends upon the successful diffusion of VEGF into the microenvironment and is instrumental to cancer growth and survival.

Our results support hypothesis I: our model demonstrates that cancer cells must stimulate angiogenesis in order to be successful. Without angiogenesis, the nascent cancer cells are unable to uptake enough nutrient to proliferate rapidly.
Our results provide insufficient evidence to support hypothesis II. Whilst our model tests some novel abstractions for discretely modelling tumour angiogenesis in simpler manner than Olsen and Siegelmann [10], it has proven to be highly sensitive to small variations in certain parameters (see Figure 6). As a result we cannot support this hypothesis without further model extensions and experimentation to decrease model sensitivity and increase robustness.

Model Limitations

The primary limitation of this model is its sensitivity to small variations in the parameters governing cell proliferation. Small variations to these parameters lead to rapid decline or growth in cell populations (see Figure 6). We have attempted to address this limitation by carefully calibrating these parameters to achieve a relatively stable population of somatic and malignant cells (in the absence of angiogenesis).
Further, there are some fixed parameters used in the model, e.g. `energy-req-prolif` (the energy level of a cell required for proliferation) amongst a few others, that have conflicting quantitative results in the literature. This is particularly true for cancer cells, because tumours can be very different their disease dynamics can vary drastically. As a result, there are some parameters of the model that are "best-guess" parameters based upon a qualitative knowledge in the literature. These include the nutrient diffusion rate as well as the quantity of the nutrient.
Another limitation is that due to computational constraints, we were only able to model an initial population of up to 1000 cells. In real tumours, angiogenesis is not a significant factor until the tumour reaches a size of approximately 10^6 cells [22]. More powerful hardware could be used to run our model with a more realistic cell population.
Finally, for the sake of simplicity we did not explicitly model genetic mutations, which are known to play an important role in cancer formation.

Future Directions & Model Extensions

Whilst our model captures several different patterns of cell behaviour and interaction, it could be expanded to more accurately model the innate heterogeneities of tumour populations. Given that tumours are a collection of heterogenous cancer cells with different mutations and properties, including cancer stem cells and genetically unique subpopulations, adding these features to the cell agents would make the model more realistic and possibly less sensitive to changes in the parameters governing cell proliferation.
Addressing one of the model limitations, a possible extension of this model would be to greatly increase the size of the environment and the number of cell agents at initialisation. Finally, we could incorporate input data based upon real computerised topographic images of a tissue in order two create a realistic initial vasculature. This could be used to explore the effect of differing vascular geometries on the model outputs, e.g. in order to investigate heterogeneity in the tissue of interest (e.g. lung cancer vs breast cancer). The combination of these model extensions could provide the agents with an opportunity to display more emergent behaviour which may provide a deeper insight into how cancer cells organise themselves to grow into tumours and successfully stimulate tumour angiogenesis.

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